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CARDIOVASCULAR EFFECTS OF CALCITONIN

BY

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DECLARATION

i) The observations included in this thesis are the work of the author.

ii) During the course of this study the author has not been registered for the award of any other higher degree by any university or the Council for National Academic Awards. No part of this study has been previously submitted for the award of a higher degree.

iii) In partial fulfilment of the degree of Doctor of Philosophy the author has participated in seminars and conferences.

PUBLICATIONS

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ABSTRACT
Cardiovascular Effects of Calcitonin - by J.E. Parker MSc

This thesis has examined the mechanisms underlying the pharmacological effects of salmon calcitonin (sCT), particularly the haemodynamic effects of the peptide in normotensive anaesthetised rats and those rendered hypotensive by haemorrhage, a situation in which sCT may be of potential therapeutic benefit.

Using biochemical techniques, sCT, administered centrally to urethane anaesthetised normotensive rats, was demonstrated to result in a trend for an increase in the concentrations of whole brain 5-hydroxytryptamine (5-HT) or 5-hydroxyindole acetic acid (5-HIAA).

Salmon CT, administered intravenously to anaesthetised rats subjected to endotoxin shock, was demonstrated to have no significant effect on blood pressure, but resulted in a decreased heart rate response in these rats. Conversely, salmon CT (iv) was shown to exert a pressor effect concomitant with an increased heart rate response in urethane anaesthetised rats subjected to haemorrhage, but was devoid of effect in urethane anaesthetised normotensive rats. The pressor effect was abolished and the increased heart rate response attenuated by peripheral sympathetic nervous system blockade. It is concluded that the pressor effect of iv sCT is mediated solely by the peripheral sympathetic nervous system, but that the increased heart rate response is only partly mediated by this system.

Centrally administered sCT has been demonstrated to exert a pressor effect concomitant with an increased heart rate response in both urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. Pharmacological and surgical procedures which interfered with the release of catecholamines from peripheral sympathetic neurones and the adrenal medullae and the function of the renin-angiotensin system were used to investigate these effects.

It is concluded that the release of catecholamines from peripheral sympathetic neurones and the adrenal medullae are equally responsible for the pressor effect of icv sCT in normotensive rats, but that the peripheral sympathetic nervous system is more important than the adrenal medullae in mediating the increased heart rate response to icv sCT. Conversely, the release of catecholamines from peripheral sympathetic neurones and the adrenal medullae as well as the vasoconstrictor effects of angiotensin II are involved in the pressor effect of icv sCT in haemorrhaged rats, although the peripheral sympathetic nervous system appears to play the major role. The peripheral sympathetic nervous system and the presence of intact adrenal medullae would appear to mediate the increased heart rate response to icv sCT in these animals.

The sites of action of peripherally and centrally administered sCT are discussed. The possible interaction of calcitonin gene-related peptide with receptors for CT is also discussed.

CONTENTS PAGE

LIST OF ABBREVIATIONS

ACE	- angiotensin converting enzyme
AChE	- acetylcholinesterase
ACTH	- adrenocorticotrophic hormone
AgI	- angiotensin I
AgII	- angiotensin II
ANOVA	- analysis of variance
AUC	- area under the curve
B_{max}	- maximum binding
BSA	- bovine serum albumin
cAMP	- cyclic 3',5'-adenosine monophosphate
cGMP	- cyclic 3',5'-guanosine monophosphate
CGRP	- calcitonin gene-related peptide
CI	- confidence interval
CNS	- central nervous system
CO	- cardiac output
CT	- calcitonin
CTLI	- calcitonin-like immunoreactivity
1,25-DHCC	- 1,25-dihydroxycholecalciferol
5,7-DHT	- 5,7-dihydroxytryptamine
DMI	- desmethylimipramine
DOCA	- desoxycorticosterone acetate
eCT	- eel calcitonin
EEG	- electroencephalogram
GTP	- guanosine triphosphate
HCl	- hydrochloric acid
hCT	- human calcitonin

5-HIAA - 5-hydroxyindoleacetic acid

HPLC - high performance liquid chromatography

5-HT - 5-hydroxytryptamine

i-CT - immunoreactive calcitonin

IC_{50} - concentration to give 50% inhibition of maximum response

ic - intracisternal

icv - intracerebroventricular

im - intramuscular

ip - intraperitoneal

it - intrathecal

iv - intravenous

K_D - dissociation constant

MAO - monoamine oxidase

MAP - mean arterial pressure

mRNA - messenger RNA

6-OHDA - 6-hydroxydopamine

OPT - o-phthaldialdehyde

pCT - porcine calcitonin

PAG - periaqueductal gray matter

pCPA - p-chlorophenylalanine

PGE_2 - prostaglandin E_2

PPQ - p-phenylquinone

PSN - pre-ganglionic splanchnic nerve

PTH - parathyroid hormone

rCT - rat calcitonin

RIA - radioimmunoassay

sc - subcutaneous

sCT - salmon calcitonin

SHR - spontaneously hypertensive rat

TPR - total peripheral resistance

TRH - thyrotropin-releasing hormone

TSST-1 - toxic shock syndrome toxin type-1

VIP - vasoactiveintestinal polypeptide

1. INTRODUCTION

1.1 Discovery of Calcitonin

The level of ionic calcium in the body fluids is maintained and regulated with great precision. This function was thought to be dependent upon the action of the parathyroid glands, which, when stimulated by hypocalcaemia release a hormone called parathyroid hormone (PTH) which restores calcium levels to normality (McLean, 1957). In 1961, however, Copp et al. discovered the existence of a new calcium regulating hormone which they called calcitonin (CT). These workers observed that on infusion of the thyroid-parathyroid apparatus of the anaesthetised dog with hypercalcaemic blood there was a much more pronounced systemic hypocalcaemia than after parathyroidectomy, indicating that inhibition of PTH secretion could not be the mechanism responsible. A few years later, Hirsch and Munson (1963) noticed that parathyroidectomy performed in young rats by cauterisation resulted in a greater degree of hypocalcaemia than parathyroidectomy performed with the scalpel. It was hypothesised that a hypocalcaemic factor had been released from the thyroid tissue as a result of the more extensive trauma following the use of the cautery.

Foster et al. (1964) demonstrated that the thyroid gland was the source of the hypocalcaemic factor by perfusing the goat thyroid and parathyroid. They failed to produce a systemic hypocalcaemia by perfusing the parathyroid with hypercalcaemic blood, but observed a rapid fall in systemic calcium concentration when the thyroid was also perfused. Care (1965) confirmed the thyroid origin of calcitonin by

demonstrating that direct addition of calcium to the thyroid arterial blood of pigs produced a rapid reduction in systemic plasma calcium. Pearse and Calvalheira (1967) showed that calcitonin was not produced by the regular follicular cells, but rather by the parafollicular 'C' cells derived from the ultimobranchial body of the embryo and ultimately from the neural crest. Thus, CT was clearly recognised to be part of the neuroendocrine system.

1.2 Isolation of Calcitonin

The thyroid is the main gland of origin of CT in many mammalian species, the parafollicular 'C' cells being responsible for production of the hormone. In fishes, reptiles, amphibians and birds, the 'C' cells migrate only as far as the last pharyngeal pouch and remain there as the so-called ultimobranchial bodies. These are anatomically accessible and also contain CT in a fairly high concentration (Doepfner, 1983). The 'C' cells also tend to be spread over a wide area including tissues of the neck and lung (MacIntyre, 1983). Thus, in the lizard a significant amount of CT is found in the lung (Galan Galan et al. 1981a).

1.3 Evolution of Calcitonin

Using a combination of high performance liquid chromatography (HPLC) and radioimmunoassay (RIA) human CT (hCT) has been demonstrated in the nervous system of the chordate Ciona intestinalis (Fritsch et al. 1979) and in the brain of the cyclostome Myxine (Girgis et al. 1980). MacIntyre and Craig (1981) suggested that a duplication of the CT gene occurred early in vertebrate evolution and that hCT is the parent peptide from which the other CT's arose. Perez Cano et al. (1982a;

1982b) have demonstrated the co-existence of hCT and teleost CT in mammals, reptiles, fish and birds which is consistent with duplication of the CT gene early in vertebrate evolution. During evolution one of these genes may be fully expressed, while the second gene may be either suppressed or partially expressed. More recently, the demonstration of the co-existence of salmon CT (sCT) and hCT-like peptides in the human thyroid, brain (Fischer et al. 1983b) and serum (Tobler et al. 1984) has been observed.

A hCT-like molecule has also been shown in E.coli, Candida albicans, and Aspergillus fumigatus (MacIntyre and Craig 1981). This molecule is very similar to, but not completely identical with, synthetic hCT on high performance liquid chromatography (HPLC). It was therefore hypothesised that CT had some function in inter-cellular communication in primitive organisms, perhaps as a cell-to-cell messenger, and thus the hormonal nature of CT in advanced organisms is an extension and development of its ancient role.

1.4 Biosysnthesis of Calcitonin

Several laboratories have confirmed that CT is first synthesised as part of a substantially higher molecular weight precursor (see below). In this respect it is like many of the other small polypeptide hormones. The initial precursor synthesised during cell free translation of rat messenger RNA (mRNA) has a molecular weight of 15,000 daltons (Jacobs et al. 1979; Amara et al. 1980). To determine the amino acid sequence of this CT precursor Jacobs et al. (1981a) constructed and cloned, in bacteria, recombinant plasmids containing DNA's complementary to mRNA's of the parafollicular cells from a rat.

The nucleotide sequence includes portions of the 3' and 5' untranslated regions of the CT mRNA and the entire coding region of 136 amino acids of the CT precursor. The sequence coding for the 32 amino acid of CT is located near the carboxyl terminus of the precursor. From results of the above study, Jacobs et al. (1981a) were able to postulate the cellular processes involved in the formation of the 3,500 MW CT molecule from its larger precursor (15,000 MW):

- cotranslational glycosylation and cleavage of a leader sequence from the CT precursor;
- trypsin - like cleavage of proCT;
- subsequent trimming of basic amino acids from the carboxyl terminus of the cleaved products by carboxypeptidase B - like activity;
- enzymatic conversion of the Pro - Gly amino acid sequence to the carboxyl terminal prolinamide found in the mature form of the hormone.

The knowledge that the precursor is glycosylated and contains peptide sequences attached to CT raised the possibility that forms of the hormone detected in the circulation, with apparent molecular weights greater than CT, may result from incomplete processing of the precursor prior to secretion in vivo (Jacobs et al. 1981b).

Calcitonin is flanked on its carboxyl - terminal side by a peptide referred to as katacalcin and previously known as PDN-21 (Craig et al. 1982; Hillyard et al. 1983). This peptide is secreted in equimolar amounts with CT and was initially assumed to have a calcium lowering effect in the rat and in tissue culture systems (MacIntyre et al. 1982). However, katacalcin has no acute calcium lowering effect in

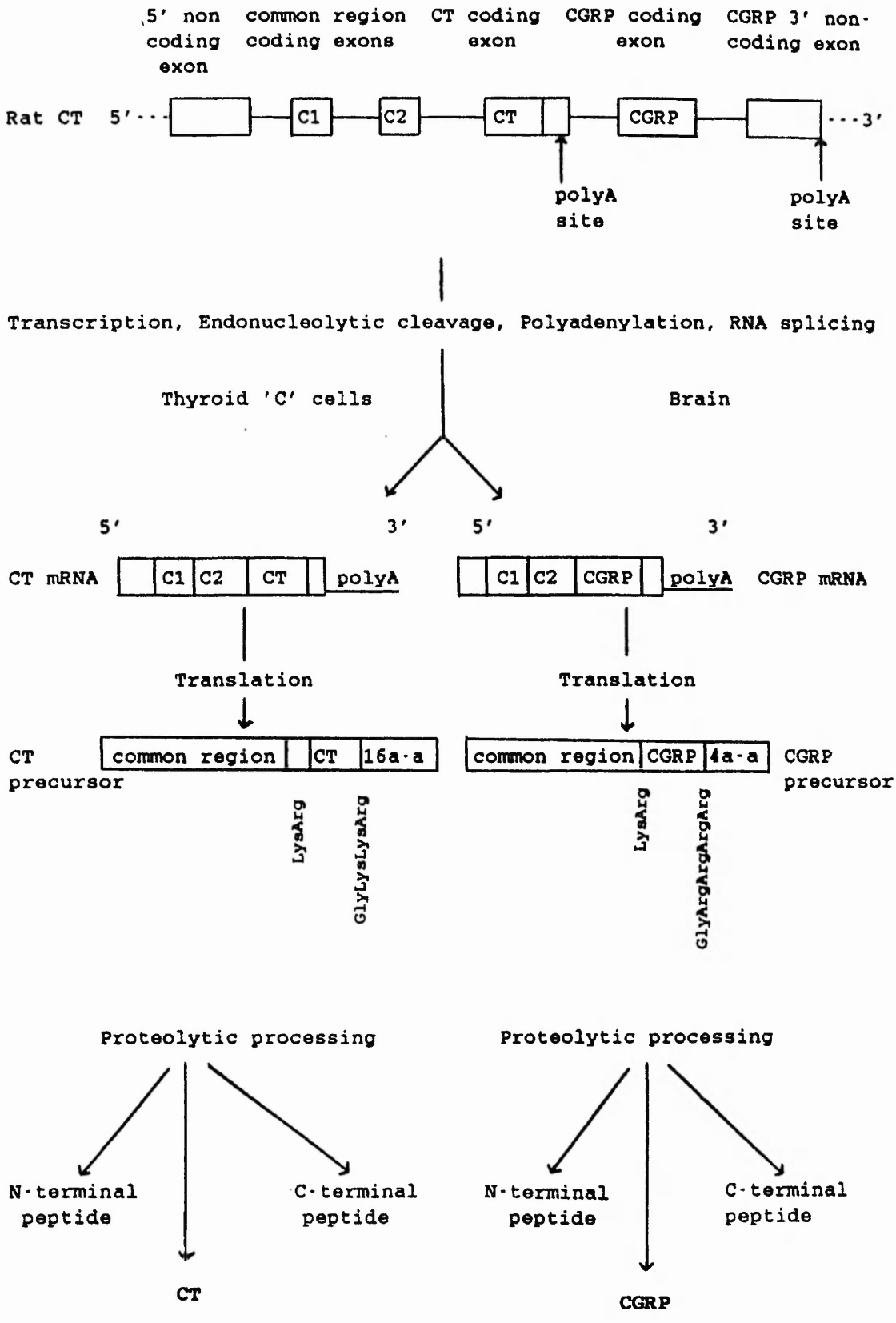
normal man (MacIntyre et al. 1987) and although tissue culture experiments do show some action at high concentrations (MacIntyre et al. 1982), the calcium lowering effects in the rat have proved not to be reproducible (MacIntyre et al. 1984). Thus, the function of katacalcin, if any, is unknown.

Variations in post-transcriptional (pre-translational) processing of the precursor CT gene to produce multiple CT-related mRNA's have been reported. Thus, Rosenfeld et al. (1981) demonstrated the spontaneous and permanent switching of serially transplanted rat medullary thyroid carcinoma lines from states of 'high' to 'low' CT production. Conversion from high to low CT producing states was associated with an altered expression of the CT gene to produce a new cytoplasmic mRNA termed calcitonin gene-related peptide (CGRP) mRNA. CGRP mRNA was shown to be 50 - 250 nucleotides larger than CT mRNA.

Amara et al. (1982; 1984) demonstrated that the CT gene encodes two different mRNA's that share an identical 5' sequence, but have entirely different 3' sequences. Translation of the mRNA's produces either the CT precursor protein, which is proteolytically processed to yield CT and two other peptides, or the CGRP precursor. From the nucleotide sequence of cloned CGRP cDNA it can be predicted that the encoded protein is proteolytically processed to generate three peptides, including the 37 amino acid CGRP (Rosenfeld et al. 1983) (see Fig.1).

Figure 1. Alternative processing pathways in the expression of the calcitonin gene.

(from Rosenfeld et al. 1983)


5' non coding exon
common region coding exons
CT coding exon
CGRP coding exon
CGRP 3' non-coding exon
Rat CT 5'
C1
C2
CT
CGRP
3'
polyA site
polyA site
Transcription, Endonucleolytic cleavage, Polyadenylation, RNA splicing
Thyroid 'C' cells
Brain
5'
3'
5'
3'
CT mRNA
C1
C2
CT
polyA
C1
C2
CGRP
polyA
CGRP mRNA
Translation
Translation
CT precursor
common region
CT
16a-a
LysArg
GlyLysLysArg
common region
CGRP
4a-a
CGRP precursor
LysArg
GlyArgArgArgArg
Proteolytic processing
Proteolytic processing
N-terminal peptide
C-terminal peptide
CT
N-terminal peptide
C-terminal peptide
CGRP

Thyroidal 'C' cells predominantly produce mRNA encoding the precursor of CT, whereas in the brain, spinal cord and cranial nerve ganglia mRNA encoding the precursor of CGRP is the product of CT gene expression. The polyadenylation sites for CT and CGRP mRNA's are located at the end of exons 4 and 6 respectively. Termination of transcription after the CT exon does not dictate the production of CT mRNA, because transcription proceeds through both CT and CGRP exons irrespective of which mRNA is ultimately produced. In isolated nuclei, both polyadenylation sites appear to be utilised; however, the CT site is preferentially used in nuclei from tissues producing CT mRNA. These data suggest that the mechanism dictating production of each mRNA involves the selective use of alternative polyadenylation sites (Amara et al. 1984).

More recent evidence indicates that there is a second CT gene, usually referred to as the β-gene (Amara et al. 1985). It is proposed that proteolytic processing of the predicted protein precursor generates a 37 amino acid peptide product that is amidated at the carboxyl-terminal and differs by only a single amino acid from the CGRP described above (sometimes referred to as α-CGRP). The RNA encoding β-CGRP appears to be the only mature transcript of the β-CGRP gene. β-CGRP RNA appears to be expressed in the brain, sensory ganglia, and the thyroid gland, in a pattern similar to that of α-CGRP mRNA, however the level of expression of β-CGRP mRNA is less than 20% that of α-CGRP mRNA.

1.5 Structure/Activity of Calcitonin

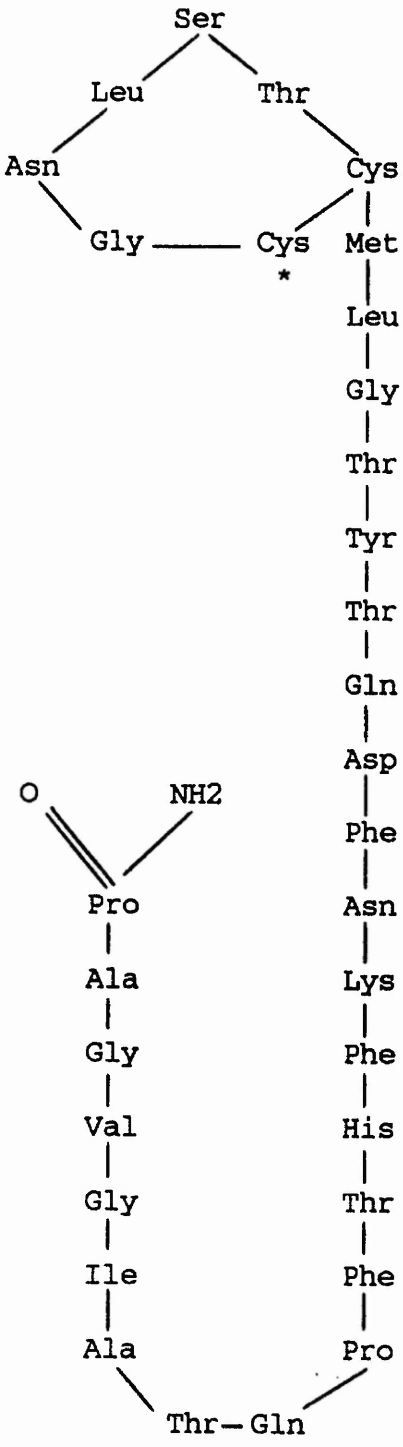
Examination of the naturally occurring CT's reveals certain common structural features together with a considerable amount of apparent variability. Based on structural similarities three major groups of CT's have been identified:

- mammalian (human, rat);
- teleost (salmon, eel);
- artiodactyl (pig, sheep, ox).

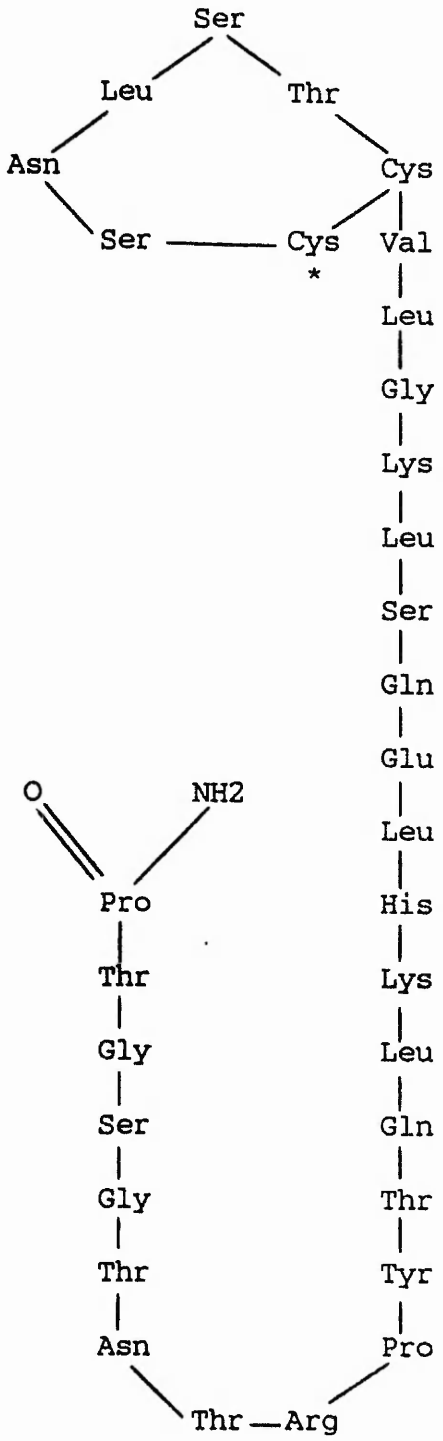
By 1970, the amino acid sequence of 8 CT's had been determined, namely porcine, bovine, ovine, human, eel and salmonI,II and III (Copp, 1983). These all have in common a 32 amino acid length, a disulphide bridge between the cysteine residues at positions 1 and 7 that forms a ring at the amino terminus of the molecule and a carboxyl terminal prolinamide (MacIntyre and Craig, 1981) (see Fig. 2). In addition to glycine at position 28 the other residues common to all of the CT's include those at positions 1, 9 and 32 as well as the polypeptide sequence at positions 3 - 7 (see Fig. 3). Rat CT (rCT) most closely resembles hCT, differing by only 2 amino acids and sCT has more amino acids in common with hCT than does porcine CT (pCT). It might have been expected to find greater similarities within the group of mammals which are phylogenetically closer (Doepfner, 1983).

The relations of the structure and activity of CT are of theoretical and practical importance. Salmon CT and other CT's of ultimobranchial gland origin are much more potent than other forms; indeed they are 10 - 100 times as potent as the mammalian hormones (Copp, 1983). Several groups have used CT analogues in the study of structure/activity relations. Maier et al. (1974) noted that one of the conspicuous

Figure 2. Amino acid sequence of human and salmon calcitonins.



Human Calcitonin



Salmon I Calcitonin

* = position 1

Figure 3. Amino acid sequence of several calcitonins and calcitonin analogues.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Human	Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp	Phe	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro
Rat																Leu										Ser						
Salmon II		Ser									Lys	Leu	Ser			Leu	His		Leu	Gln				Arg		Asn	Thr		Ala		Val	
Salmon III		Ser						Val			Lys	Leu	Ser			Leu	His		Leu	Gln				Arg		Asn	Thr		Ala		Val	
Salmon I		Ser						Val			Lys	Leu	Ser		Glu	Leu	His		Leu	Gln		Tyr		Arg		Asn	Thr		Ser		Thr	
Eel		Ser						Val			Lys	Leu	Ser		Glu	Leu	His		Leu	Gln		Tyr		Arg		Asn	Thr		Ala		Val	
Porcine		Ser						Val		Ser	Ala		Trp	Arg	Asn	Leu		Asn			Arg		Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	
Bovine		Ser						Val		Ser	Ala		Trp	Lys		Leu		Asn	Tyr		Arg		Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	
Ovine		Ser						Val		Ser	Ala		Trp	Lys		Leu		Asn	Tyr		Arg	Tyr	Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	
MCT-I								Leu		Gln	Gln	Trp	Gln	Lys	Leu	Leu	Gln		Leu	Lys	Gln	Leu		Arg		Asn	Thr		Ser		Thr	
MCT-II		Ser						Leu		Gln	Gln	Leu	Gln	Lys	Leu	Leu	Gln		Leu	Lys	Gln	Tyr		Arg		Asn	Thr		Ser		Thr	

differences between hCT and sCTI is the more hydrophilic character of the C-terminal region of sCTI. These workers substituted Val^{29} for Ala and Ala^{31} for Val in hCT and produced a synthetic hormone with less than 10% of the hypocalcaemic activity of hCT and a much shorter duration of effect. They also replaced Val^{29} and Ala^{31} with the corresponding amino acids of sCTI, Ser and Thr, and produced a synthetic analogue which demonstrated a five-fold greater activity than natural hCT with a slightly longer duration of action.

In 1975, Maier et al. introduced either Val in place of Met^{8} or Tyr in place of Phe^{22} of the hCT molecule and yielded analogues 4 - 5 times as potent and nearly twice as long-lasting as hCT. The doubly substituted peptide [Val^{8},Tyr^{22}]-hCT displayed properties closely similar to those of Val^{8}-hCT and Tyr^{22}-hCT. Findlay et al. (1983) prepared synthetic analogues of sCT and hCT, namely [des-Leu^{16}]-sCT and [des-Phe^{16}]-hCT, in which the amino acid sequence from position 16 was omitted. A substantial loss of biological activity, as demonstrated by the rat hypocalcaemic assay, <u>in vitro</u> binding of [^{125}I]-CT and stimulation of adenylate cyclase was observed indicating the importance for a hydrophobic residue at position 16 of the CT molecule.

Moe et al. (1983) observed that when the amino acid sequences of natural variants of CT in the region 8 - 22 were viewed as axial projections of α-helices the segregation of residues into opposing hydrophilic and hydrophobic faces of cylindrical segments was a feature common to all of them. They therefore proposed a model for CT structure in which there are 3 structural regions:

- a "loop" involving the disulphide bridge between 1 and 7;
- an amphiphilic α-helical segment 8 - 22;
- a hydrophilic random coil sequence from 23 - 32.

They synthesised a model peptide, MCT-I in which the residues 8 - 22 had little sequence homology to the natural CT's but retained the same balance of charge and hydrophobicity as sCTI. In addition, amino acids having a high helix forming potential were chosen to constitute the helical region of MCT-I and residues 1 - 7 corresponded to those of hCT whilst residues 23 - 32 corresponded to sCTI. It was demonstrated that MCT-I had chemical and biological properties similar to those of sCTI, but was approximately 10-fold less potent. The biological activity of MCT-I was comparable to that of pCT. It was suggested that the region from residues 8 - 22 of calcitonin has a primarily structural role, providing an amphiphilic surface in the α-helical conformation for binding interactions with its receptor.

Another model peptide, MCT-II, was synthesised by Moe and Kaiser in 1985. This analogue is nearly identical with MCT-I having the potential to form an amphiphilic α-helix from residues 8 - 22 with a continuous surface of aliphatic Leu side chains on the hydrophobic face of the helix. Since in the *in vitro* and *in vivo* assays used to compare MCT-II with sCTI, MCT-II was indistinguishable from sCTI,it was confirmed that the amphiphilic α-helical structure is important for binding to CT receptors. After comparing MCT-I with MCT-II it was hypothesised that, in addition to differences in amphiphilicity and α-helix forming potential, the lower biological activity of the mammalian hormones compared to those of the ultimobranchial gland origin may be due to the presence of one or more aromatic residues at

positions 12, 16 and 19 on the hydrophobic face of the mammalian hormones.

Thus, it would appear that conservation of the amphipathic helix along with the overall tertiary structure of CT are more important for potency than chain length per se. A greater understanding of the structure-activity relationships will lead to the synthesis of CT analogues which are more stable and intrinsically at least as potent as the parent molecule.

1.6 Calcitonin receptors/binding sites

In 1978, Deftos et al. demonstrated, by a specific immunoperoxidase procedure, the presence of immunoreactive CT (i-CT) in the cells of the intermediate lobe, but not the posterior lobe, of the rat pituitary gland. This was subsequently confirmed by Watkins et al. (1980) and Cooper et al. (1980). The latter workers suggested that it was not authentic CT, but rather a peptide related sufficiently in structure to react with certain antisera to CT. The location of central i-CT was later extended to include the hypothalamus and other brain regions. Thus, Flynn et al. (1981) demonstrated CT-like immunoreactivity (CTLI) in extracts of hypothalami from rats (0.21 ng/hypothalamus). This amount of CTLI was shown to be much less than that in either the anterior lobe (1.16 ng) or neurointermediate lobe (0.81 ng) of the pituitary. Galan Galan et al. (1981b) observed that extracts of pigeon hypothalamus contained more sCTLI than midbrain or brainstem. Conversely, no sCTLI was found in the pigeon pituitary gland. CTLI has also been detected in the cerebrospinal fluid of humans (Pavlinac et al. 1980) and in the endocrine cells of human lungs (Cutz et al. 1981).

As early as 1980 evidence for the presence of central CT binding sites was presented. Koida et al. (1980), using [^{125}I]-sCTI, demonstrated CT binding sites in the hypothalamus, cerebellum and cortex of rat brain. Scatchard plot analysis of whole brain homogenate indicated at least two types of binding sites; K_D and B_{max} for the low affinity site were 10 nM and 440 fmole/mg protein respectively and those for the high affinity site were 0.57 nM and 56 fmole/mg protein respectively.

Fischer et al. (1981a) investigated the binding of [^{125}I]-sCT to a crude membrane fraction prepared from rat brains. They observed that specific binding to membranes of the hypothalamus was highest followed by the brainstem and midbrain-thalamus. Binding was comparable in the midbrain-thalamus and the striatum and lower in the cortex. Binding was negligible in the hippocampus, cerebellum and spinal cord. These results were confirmed by Rizzo and Goltzman (1981) and Nakamuta et al. (1981). Nakamuta et al. (1981) demonstrated that maximal binding occurred in the physiological pH range of 7.25-7.50 and that the binding reaction proceeded in a temperature-dependant manner. Binding of [^{125}I]-sCT was also shown to be inhibited by unlabelled sCT, [Asu^{1,7}]-eel CT and pCT in a dose-dependant manner with IC_{50}s of 2, 8 and 30 nM respectively. [Asu^{1,7}]-eCT is a synthetic derivative of eCT in which the paired cysteine residues at positions 1 and 7 of the amino acid sequence and linked by a sulphide bridge have been substituted by a 2,7-diamino-octanedioic acid residue. These authors suggested that the binding sites may not exist as the receptor for the thyroidal CT, but rather as receptors for a CT-like substance which may be of brain origin, since the IC_{50}s quoted above are much higher than normal plasma levels of CT (0.01 - 0.03 nM).

Later studies concentrated on the techniques of in vitro autoradiography. Thus, Olgiati et al. (1983) observed that sCT did not bind in the cortex or cerebellum, but that labelling was striking in the reticular formation, the amygdaloid complex, in some thalamic nuclei and in the caudal part of the striatum. Moreover, the hypothalamic region, the acumbens and the raphe nuclei contained the highest density of binding sites.

The evidence presented above indicating the presence of CTLI and CT binding sites in the brain strongly indicates a physiological role for CT in the CNS. This is supported by evidence suggesting a role for CT in pain perception (Pecile et al. 1975) and anorexia (Freed et al. 1979) in which the hypothalamus, thalamus and brainstem are involved (Fischer et al. 1981a; Olgiati et al. 1983). [^{125}I]-sCT has also been shown to bind specifically to renal and skeletal receptors (Marx et al. 1973). The degradation of [^{125}I]-sCT by renal plasma membranes was inhibited by agents that did not block the high affinity binding to receptors. High affinity, low capacity sites were also found in membranes prepared from skeletal tissue (Marx et al. 1973).

1.7 Secretion of Calcitonin

Since the introduction of the initial concept of a hypocalcaemic hormone the importance of the calcium ion on CT secretion has been well defined. It has been shown in man and experimental animals that the secretion of CT is directly related to blood calcium concentration; an acute increase in blood calcium results in an increase in plasma CT and, conversely, an acute decrease in blood calcium produces a corresponding decrease in plasma CT (Deftos, 1978).

Silva et al. (1974) observed that plasma CT concentration responded to induced and spontaneous hypercalcaemia and induced hypocalcaemia.

Minerals related to calcium also appear to have an acute stimulatory effect on CT secretion. Pento et al. (1974) infused young pigs with solutions of magnesium and strontium and observed that a several-fold increase in plasma concentration of magnesium or strontium was followed by a rise in plasma CT. The effects of magnesium and strontium, however, are probably not physiologically important since elevations of each, sufficient to influence CT release, probably do not occur naturally.

Sex and age appear to play a significant role in modulating the secretion of CT. Heath and Sizemore (1977) demonstrated that normal men have more plasma CT than normal women. Furthermore, 90% of the men studied responded to induced hypercalcaemia with increases in plasma CT, whereas only 56% of the women examined had similar responses. They also observed a similar response after pentagastrin infusion.

Several gastrointestinal factors can regulate the secretion of CT, namely pancreozymin, cerulein and pentagastrin, as demonstrated in animal models (Care et al. 1971). However, the concentration of hormones is probably higher than those observed even in the post-prandial period and therefore the physiological importance of such effects is unknown.

1.8 Metabolism of Calcitonin

Calcitonin disappears from plasma in a multi-exponential manner with an early half-life measured in minutes and a later half-life measured in hours (Deftos, 1982). The kidney appears to be the most important organ of clearance for CT. Ardaillou et al. (1970) demonstrated that patients with renal failure show a marked decrease in clearance rate of [^{125}I]-hCT compared with normal subjects. Furthermore, in vitro studies have demonstrated rapid destruction of CT by renal slices and single renal cells (Hsu and Haymovitz, 1974).

DeLuise et al. (1972) have shown that the kidney is the chief organ involved in the breakdown of sCT and hCT in the rat, whereas the liver is the major site of breakdown of pCT. These workers also demonstrated that the metabolism of sCT is markedly resistant to digestion by nearly all other tissues and thus suggested that this contributes to the very high specific biological activity of sCT. This finding was confirmed by Singer et al. (1972). Clark et al. (1974) confirmed that the kidney plays the major role in the clearance of hCT by infusing the hormone into anaesthetised dogs and collecting plasma samples from the aorta, hepatic and renal veins. They calculated that the kidneys remove 8% of the circulating hormone/min. It was also demonstrated that removal of the kidneys prolonged the disappearance of CT.

Major criticisms of the above studies are that most of them have been performed with either pharmacologic amounts or radioiodinated species of CT. Many of the studies have also been performed with non-homologous species of CT and many have assessed metabolism by

measuring the immunological activity of CT rather than biological activity.

1.9 Physiological and pharmacological effects of calcitonin

1.9a Effect of calcitonin on plasma calcium

The concentration of ionised calcium within cells is approximately 10^{-7} M and variations in this concentration are of critical importance to numerous biological functions including muscle contraction and relaxation, endocrine and exocrine secretion and neuronal function. In the short term, rapid regulation of intracellular ionised calcium depends largely on the activity of intracellular organelles, however, in the long term the continuous influx of calcium must be counteracted by ejection through plasma membranes. Since both influx and efflux are concentration dependent, the efficiency of intracellular calcium regulation is ultimately dependent on the calcium concentration of extracellular fluid.

Calcium homeostasis is achieved by the coordinated effects of CT, PTH and 1,25-dihydroxycholecalciferol (1,25-DHCC). Briefly, 1,25-DHCC, produced by the renal tubules when plasma calcium is low, acts on the small intestine to promote active absorption of calcium. Plasma calcium is also elevated by actions on bone, muscle and proximal tubules of the kidney. Similarly, PTH secreted from the parathyroid glands in response to hypocalcaemia increases calcium reabsorption by the renal tubules and indirectly facilitates intestinal absorption of calcium by promoting 1,25-DHCC production in the kidney.

The major effect of CT is considered to be inhibition of bone resorption, as discussed below (1.9b). Prevention of calcium resorption from bone cannot, however, be considered as the sole physiological effect of CT towards lowering plasma calcium levels, since the hormone also increases renal excretion of calcium (see 1.9c) and has been suggested to be secreted postprandially in order to protect against hypercalcaemia (see 1.9d). Thus, CT can be considered to exert coordinated effects on kidney, gastrointestinal tract and bone which serve to prevent variations in plasma calcium and to protect bone from excessive calcium resorption.

At the cellular level CT has been shown to depress cytoplasmic calcium activity and the cellular transport of calcium by regulating the mitochondrial calcium controller mechanism (Borle, 1983).

1.9b Effect of calcitonin on bone

Calcitonin protects the skeleton during periods of high bone turnover, namely growth, pregnancy and lactation. The basis of the plasma calcium - lowering action of CT is inhibition of bone resorption as shown by Friedman and Raisz (1965). These investigators demonstrated that CT inhibits the release of ^{45}Ca from long bones of embryo rats in tissue culture. Martin et al. (1966) provided strong support for the view that calcitonin acts by inhibition of bone resorption after the observation that sCT decreased hydroxyproline excretion in rats concomitant with a decrease in plasma calcium.

Chambers and Magnus (1982) suggested that calcitonin may inhibit bone resorption by inhibiting motile processes in the resorptive cell. The

effect of calcitonin on incubated rat osteoclasts was investigated with the observation that calcitonin caused immotility of osteoclasts followed by gradual fragmentation and retraction of pseudopodial processes. This quiescent state was reversed when calcitonin was removed from the medium. Since the effect was abolished by prior treatment of osteoclasts with trypsin the authors concluded that quiescence is induced by the interaction of CT with a trypsin-sensitive CT receptor located on osteoclasts. No effect of CT on osteoblasts was observed.

Nicholson et al. (1986) have reported that the rat osteoclast is the most abundant cellular source of CT receptors yet recognised. These receptors, demonstrated by autoradiography and biochemical methods, are specific and of a high affinity and have been shown to respond to CT with a dose-dependent increase in cyclic 3',5'-adenosine monophosphate (cAMP). Greater than 80% of specific binding was associated with multinucleate osteoclasts and the remainder was associated with mononuclear cells (not osteoblasts) which may be osteoclast precursors. It is of interest to note that chick osteoclasts are less sensitive than rat osteoclasts to sCT and that this may explain why CT has generally not been found to evoke an acute hypocalcaemic response in birds (Arnett and Dempster, 1987).

It is clear that CT inhibits bone resorption *in vitro* and *in vivo*, but Farley et al. (1988) were interested to investigate whether the hormone has direct effects on bone formation indices *in vitro*. It was demonstrated that sCT has direct dose - dependent actions on embryonic chicken skeletal tissues *in vitro* to increase calvarial cell proliferation, alkaline phosphatase activity in bone and synthesis and

deposition of bone matrix collagen. Salmon CT was also shown to have cell proliferative effects in primary cultures of cells prepared from newborn mouse calvaria.

CGRP has been demonstrated to inhibit bone resorption by active rat osteoclasts and to lower plasma calcium when injected into young rats (Zaidi et al. 1988). In both respects CGRP was found to be 100 - 1000-fold less potent than hCT.

The major therapeutic use of CT is in the treatment of Paget's disease, a disorder characterised by increased bone resorption, in which CT reduces the elevated levels of plasma alkaline phosphatase and urinary hydroxyproline and new bone is observed replacing Pagetic tissue (Deftos and First, 1981).

1.9c Effect of calcitonin on kidney

Calcitonin has been shown to modify the renal clearance of several ions. Thus, Haas et al. (1971) demonstrated that CT and PTH act independently on the human kidney to promote phosphate, sodium and potassium excretion whilst CT alone increases calcium and magnesium clearance. This study was extended by Paillard et al. (1972) who showed sCT to produce a direct renal effect demonstrated by an increase in sodium, phosphate, calcium and magnesium excretion followed by an indirect effect, due to the release of endogenous PTH, leading to a secondary decrease of calcium and magnesium excretion and, in part, a sustained increase of phosphate excretion. However, Kawamura and his group (1978) were not able to show a natriuretic effect of CT (3 U by iv infusion) in intact, uraemic or

parathyroidectomised rats. Plasma concentrations of calcium and phosphate decreased following CT infusion in both intact and parathyroidectomised rats indicating that the calciuric and phosphaturic effects of CT are secondary to a direct effect of CT on the renal tubule. Although serum calcium concentrations were reduced in uraemic rats, the rate of urinary calcium excretion was not altered which suggests an antagonistic action of the high levels of PTH present in uraemia to the hypocalcaemic effect of CT.

1.9d Effect of calcitonin on the gastrointestinal tract

Calcitonin would appear to be secreted postprandially in order to prevent an undue rise in plasma calcium. This is demonstrated in young thyroidectomised pigs after a meal, which show an increase in plasma calcium compared with a decrease in plasma calcium observed in normal pigs. Furthermore, exposure of the pig thyroid gland to gastrin, pancreozymin/cholecystokinin or to enteroglucagon, at concentrations of the same order as those found after ingestion of a meal, results in an increased secretion of CT (Swaminathan et al. 1973). The hormone has also been shown to inhibit basal and food stimulated serum gastrin concentrations in man at doses that have no effect on total and ultrafilterable concentrations of serum calcium. Moreover, since sCT reduced mainly gastrin-17 and gastrin-13 and since gastrointestinal tissue releases immunoreactive gastrin mainly as gastrin-17, it was hypothesised that sCT acts directly on the gastrin cells in the gastric antrum and duodenum (Fahrenkrug et al. 1975). Calcitonin has also been demonstrated to cause a profound inhibition in secretion of insulin, pancreatic glucagon, motilin and pancreatic polypeptide in man (Stevenson et al. 1985). The physiological

significance of the action of CT on gastrointestinal regulatory peptides remains to be established.

Bueno et al. (1983) investigated the effect of centrally administered sCT on intestinal motility in the conscious rat. Doses of sCT as low as 0.083 pmol.rat^{-1} restored the "fasted" motility pattern of the small intestine in fed rats, an effect which was blocked by calcium gluconate (10 μg.rat^{-1} icv). It was suggested that sCT acts centrally to control the pattern of intestinal motility by inhibiting the digestive influences responsible for the "fed" pattern.

The most intensively studied effect of CT on the gastrointestinal tract is that of inhibition of gastric acid secretion. Many investigators have demonstrated that CT inhibits both basal and stimulated gastric acid secretion in many species. Thus, Morley et al. (1981a) observed that iv administration of sCT (10 U.kg^{-1}) and icv administration of sCT (0.2 mU - 2 U) significantly suppressed basal gastric acid secretion. Moreover, centrally administered sCT was approximately 1000-fold more potent than parenterally administered sCT. Intravenous CT has been shown to inhibit gastric acid secretion stimulated by histamine, pentagastrin and calcium in cats (Becker et al. 1973) and to inhibit pentagastrin - stimulated gastric acid secretion in man (Beblinger et al. 1988). Intraperitoneal administration of CT inhibits thyrotropin - releasing hormone (TRH) - and 2-deoxy-D-glucose - stimulated gastric acid secretion in rats (Maeda-Hagiwara and Watanabe, 1985) and central administration has been shown to inhibit TRH - induced (Maeda-Hagiwara and Watanabe, 1985; Morley et al. 1981a) and insulin - induced (Morley et al. 1981a) gastric acid secretion in rats.

The mechanisms by which CT inhibits gastric acid secretion are not completely understood, but various hypotheses have been put forward. CT may interact with acid producing cells (Beblinger et al. 1988), but the presence of CT receptors on gastric cells has not yet been demonstrated. Calcitonin may interact with TRH in the hypothalamus (Maeda-Hagiwara and Watanabe, 1985) or suppress the elevation of gastrin in response to calcium (Becker et al. 1973). Calcitonin may even have a direct effect on the CNS by altering neuronal calcium fluxes (Morley et al. 1981a). This is supported by the observation that CT reduces $^{45}Ca^{2+}$ uptake in hypothalamic explant cultures at doses that suppress gastric acid secretion (Levine and Morley, 1981).

Whatever the mechanism(s) of action, the physiological significance of CT in the gastrointestinal tract remains obscure, especially since many of the studies performed were using pharmacological concentrations of CT. It is of interest to note that CGRP has also been reported to suppress basal (Lenz et al. 1985a) and pentagastrin-, 2-deoxy-D-glucose-, histamine-, bethanecol-, and bombesin-stimulated gastric acid secretion (Beblinger et al. 1988; Lenz et al. 1985b; Tache et al. 1984; Pappas et al. 1986). This response has been shown to depend on intact vagal cholinergic fibres and is perhaps mediated by inhibition of parasympathetic output within the CNS (Lenz et al. 1985a).

1.9e The anorectic effect of calcitonin

CT has been shown by several investigators to be a potent and long-lasting anorectic agent. Thus, Gaggi et al. (1985) demonstrated that sCT (20 $U.kg^{-1}$ sc) inhibits spontaneous feeding in rats, an

effect which is independent of the hypocalcaemic activity of the hormone. Centrally administered CT also inhibits spontaneous feeding for up to 32 h as shown by Freed et al. (1979) and by Levine and Morley (1981). Parenterally and centrally administered CT have been shown to inhibit stress-induced eating in rats. Levine and Morley (1981) observed that icv CT (0.002 - 2 $U.kg^{-1}$) was approximately 1000-fold more potent than iv CT (10 - 40 $U.kg^{-1}$). CT was therefore suggested to exert its anorectic effect on the CNS. Freed and de Beaurepaire (1984) extended this observation and named the hypothalamus as the site of action of centrally administered CT. These workers showed that the hormone was most effective in inhibiting eating in the paraventricular nucleus (89% inhibition), perifornical area (87% inhibition) and supraoptic nucleus (89% inhibition). A substantial inhibition of eating was also produced by injections in the nucleus reuniens, ventromedial hypothalamus, the vertical limit of the diagonal band and the nucleus accumbens.

Morley et al. (1981b) demonstrated that CT (2 U icv) results in 69% inhibition of muscimol-induced feeding. These workers suggested that a number of substances in the ventromedial hypothalamus interact with an inhibitory substance, possibly CT, which, when its concentration increases, is capable of inhibiting the dopamine-enkephalinergic feeding mechanism in the lateral hypothalamus.

Centrally administered CGRP (0.5 - 20 μg icv) has also been shown to diminish spontaneous food intake, but is less effective than CT in this action (Tannenbaum and Goltzman, 1985; Krahn et al. 1984).

However, Krahn et al. (1986) suggested that CGRP decreases feeding via aversive mechanisms, an action that has not been demonstrated for CT.

1.9f Effect of calcitonin on inflammation and ulceration

CT has been shown to have anti-inflammatory actions in acute and chronic animal models of inflammation. Bobalik et al. (1974) demonstrated that sCT (10 $U.kg^{-1}.day^{-1}$ for 49 days sc) resulted in a significant inhibition of paw oedema and a reduction in bone deterioration in a rat adjuvent arthritis model. When combined with such anti-inflammatory agents as phenylbutazone, hydrocortisone acetate and adrenocorticotrophic hormone (ACTH), the combination produced greater degrees of inhibition of paw oedema than the individual agents alone. Salmon CT (10 - 50 $U.kg^{-1}$ im) was also shown to be effective in an acute model of inflammation, namely carrageenin-induced oedema of the rat foot (Abdullahi et al. 1975). These investigators suggested that the inhibition of oedema may be based upon partial suppression of prostaglandins due to a decrease of calcaemia. Strettle et al. (1980), however, hypothesised that CT may have a direct action on oedema after the observation that pCT (4 $mU.kg^{-1}$ - 120 $U.kg^{-1}$ iv) resulted in a dose-related inhibition of the inflammatory response to histamine in mice, but did not affect the response to 5-hydroxytryptamine (5-HT), whereas plasma calcium concentrations were reduced in both cases.

CT has also been shown to significantly decrease restraint-induced gastric ulceration (Bates and Barlett, 1974), acetic acid-induced chronic gastric ulceration (Bates et al. 1980) and indomethacin-induced gastric ulceration in rats (Bates et al. 1979). Erosions produced by the latter model were unaffected by cimetidine,

atropine and an antacid at doses which markedly inhibit gastric acid secretion suggesting that CT must be acting by a previously undescribed mechanism to inhibit indomethacin-induced erosion formation (Bates et al. 1979).

1.9g Antinociceptive effects of calcitonin

The first demonstration of an antinociceptive effect of CT was put forward by Pecile et al. (1975) who reported that sCT (icv) increases the nociceptive threshold in rabbits. This antinociceptive effect has since been confirmed by several groups in different models of pain perception and in several species, however, the fundamental mechanism of action is still unclear.

Braga et al. (1978) observed that central injection of sCT (12 $U.kg^{-1}$) into rabbits induced an analgesia not reversible by naloxone (1 $mg.kg^{-1}$ sc). Furthermore, this analgesia could be repeatedly elicited for up to 5 days whilst tolerance developed to repeated injections of morphine (19 $\mu g.kg^{-1}$ icv). It was concluded that opiate receptors are not involved in central CT analgesia. This was confirmed by Yamamoto et al. (1979) who showed that the antinociceptive effect of pCT (10 - 60 $U.kg^{-1}$ icv) in the paw pressure test was not influenced by pretreatment with levallorphan, a partial opiate antagonist. Bates et al. (1981b) observed that the antinociceptive effect of sCT (0.04 - 10 $U.kg^{-1}$ icv) in the mouse acetic acid-induced writhing test was antagonised by naloxone only at doses 10 - 1000-fold greater than that required to reverse the effects of morphine in the mouse suggesting that CT may not interact directly at the opiate receptor; such high doses of naloxone could elicit

non-specific effects. In a later study, Bates et al. (1982a) demonstrated that sCT does not interact with the μ or δ opiate receptors in the rat colon. Since opiate-induced analgesia in the abdominal constriction test is mediated via the μ receptor, it was concluded that the central analgesic activity of CT is not mediated by direct interaction with opiate receptors of the μ type.

More recently, Welch et al. (1986) demonstrated that centrally administered sCT (1 μg icv) resulted in antinociception in mice which was reversed by naloxone (1 $mg.kg^{-1}$ sc) using the hot-plate test and tail-flick models. Naloxone did not have any effect on sCT - induced antinociception in the p-phenylquinone (PPQ)-writhing test, however, this test is much less specific for opiates.

Calcitonin may well act as an endogenous modulator of opiates centrally via alterations in calcium fluxes in the brain. Satoh et al. (1979) investigated the effect of calcium on CT-induced analgesia in mice. They demonstrated that the dose-dependent antinociception resulting from pCT (1.5 - 6 U intracisternally; ic) was reduced by simultaneous administration of $CaCl_2$ (0.1 μmol ic). This was confirmed by Bates et al. (1981a) using the acetic acid - induced abdominal constriction test in mice. In subsequent experiments, Bates et al. (1981c) observed that analgesia induced by sCT (2 $U.kg^{-1}$ icv) was antagonised by calcium ionophore, A23187 (117 $nmoles.kg^{-1}$ icv), providing further evidence that the analgesia may be due to calcium fluxes in the brain. Bates et al. (1982b) also demonstrated that simultaneous administration of sCT (0.1 $U.kg^{-1}$ icv) and the calcium antagonist, nifedipine (0.33 $\mu moles.kg^{-1}$ icv) produced an additive

effect on inhibition of acetic acid-induced abdominal constrictions in mice.

Later evidence suggests that the catecholaminergic and/or the tryptaminergic system(s) may be involved in the mechanism of action of centrally administered sCT-induced antinociception. Bates et al. (1983a) and Clementi et al. (1984; 1985) put forward evidence that the central tryptaminergic system may be involved, whereas Guidobono et al. (1985; 1986a) discounted the role played by 5-HT suggesting that the integrity of the catecholaminergic system is required. This work is discussed in detail in chapter 3.

Several groups have correlated CNS binding sites with effects on pain perception after central administration of CT. Fabbri et al. (1985) demonstrated by autoradiography, that sCT binding in the rat mesencephalon reveals an exceptionally high concentration of receptors in the ventral and ventrolateral segments of the periaqueductal grey matter (PAG) extending along the entire rostrocaudal axis. Injection of sCT (0.3 - 10 nmol) into the PAG induced a dose-dependent increase in hot-plate latencies. Furthermore, all rostrocaudal levels of these brain regions appeared to be equally responsive. Guidobono et al. (1986b) confirmed that the PAG is an area densely populated with CT binding sites and that eCT was able to enhance the pain threshold in the rat hot-plate test. Morton et al. (1986) also demonstrated that responses induced by noxious skin heating in anaesthetised cats were clearly reduced by microinjection of sCT (0.1 U) into the PAG or the medullary raphe regions. Interestingly, the PAG seems to be one of the sites of action of opiates in inducing analgesia (Yaksh and Rudy,

1977) and the raphe nucleus is the site of tryptaminergic pathway(s) mediating pain perception (Clementi et al. 1985).

CGRP (1 - 10 $nmol.kg^{-1}$ icv) has also been shown to induce antinociception in the mouse acetic acid-induced abdominal constriction test (Bates et al. 1984b) and to produce naloxone-reversible antinociception in the PPQ test and hot-plate test (ED_{50} of 20 μg icv and ED_{35} of 20 μg icv respectively; Welch et al. 1988). It is noticeable, however, that sCT has an almost 55-fold greater antinociceptive potency than CGRP.

1.9h Calcitonin and the cardiovascular system

Total blood flow to the skeleton in Pagets disease may be increased several times above normal, but after treatment with CT skeletal blood flow has been demonstrated to fall (Wootton et al. 1978). This may contribute to the reduction in skeletal calcium turnover and to the CT-induced reduction in the elevated temperature of the skin over Pagetic bone. Driessens and Vanhoutte (1981) observed a dose-related increase in perfusion pressure of vascularly perfused dog tibias with sCT (0.1 $mU.ml^{-1}$ - 1 $U.ml^{-1}$) indicating constriction of bone blood vessels. The vascular effects of CT are not localised to bone nor is vasoconstriction a constant observation. Charbon and Pieper (1972) observed that pCT (0.5 $U.kg^{-1}$ iv) and sCT (1 $U.kg^{-1}$ iv) inhibited the PTH-induced hepatic vasodilatation.

Bates et al. (1983b; 1984a; 1984c) have provided evidence that CT may play a role in mechanisms of cardiovascular regulation by investigating the acute effect of both centrally and peripherally administered sCT in normotensive rats and rats rendered hypotensive by

haemorrhage. Salmon CT (0.1 - 10 $U.kg^{-1}$ iv) exerts a dose-dependent pressor effect in anaesthetised hypotensive rats, but has no effect in normotensive rats (Bates et al. 1983b). This response is not a result of direct peripheral vasoconstriction but may involve an increase in sympathetic tone (Bates et al. 1984a). Centrally administered sCT (0.1 - 10 $U.kg^{-1}$ icv) has also been shown to increase blood pressure in haemorrhaged rats, and also to have a pressor effect, although to a lesser degree, in normotensive rats. This response to icv sCT is not affected by chemical sympathectomy (Bates et al. 1984c). The mechanisms involved in this pressor effect of sCT form the major part of work presented in this thesis.

CGRP has also been demonstrated to exert potent cardiovascular effects. When administered by central injection to conscious rats the peptide was shown to produce dose-related increases in MAP and heart rate along with a rise in noradrenaline levels. Fischer et al. (1983a) suggested that CGRP exerted its effect by stimulation of noradrenergic sympathetic outflow. Gardiner et al. (1988) have demonstrated that centrally administered CGRP has differential effects on sympathetic efferent outflow to different vascular beds after the observation that rats demonstrate a significant pressor response along with renal vasodilatation and mesenteric vasoconstriction following CGRP (0.25 nmol icv).

When administered parenterally to rats CGRP produces rapid dose-related increases in heart rate (Fischer et al. 1983a). The cardiovascular effects observed after peripheral injection of CGRP have been shown to be unaffected by mepyramine or cimetidine

suggesting that neither β-adrenoceptors nor histamine receptors are involved (Marshall et al. 1986). Calcitonin gene-related peptide has been demonstrated to increase coronary flow in the isolated rabbit heart and to have positive inotropic and chronotropic effects on the rat and guinea-pig isolated right atrium (Marshall et al. 1986). These observations are supported by radioligand binding studies which show a high concentration of CGRP binding sites in rat peripheral and mesenteric arteries and in the atria (Wimalawansa and MacIntyre, 1988) and in the intima and media of the aorta, coronary arteries and heart valves (Sigrist et al. 1986).

Brain et al. (1986) reported that CGRP is one of the most potent vasodilators yet discovered after the observation that CGRP applied intradermally in man produces the most persistant local erythema when compared with histamine, substance P, vasoactiveintestinal polypeptide (VIP) and prostaglandin E_2 (PGE_2). Thus, all the evidence indicates that CGRP could be an important regulator of peripheral vascular tone in man.

1.9i Effect of calcitonin on cAMP metabolism

Several investigations are consistent with the view that the physiological effects of CT are secondary to the modulation of cAMP. Care et al. (1970) reported that dibutyryl cAMP (0.2 mM) increased the rate of secretion of CT in isolated porcine thyroid glands perfused *in situ* and that this effect was enhanced by theophylline (0.5 mM). It is of interest to note that glucagon, a known stimulator of the adenylate cyclase system in liver, also stimulated CT secretion. At the same time Murad et al. (1970) reported that CT stimulated

adenylate cyclase activity in homogenates of rat renal cortex to a greater extent than that of rat renal medulla. Furthermore, accumulation of cAMP in incubations of rat kidney cortex slices was increased by 20-60% by CT, and in incubations of rat calvaria cAMP was increased 3-fold by CT. These observations have been confirmed by Marx and Aurbach (1975) who demonstrated that high affinity binding sites for CT in rat renal plasma membranes were distributed where the specific activity of hormone-sensitive adenylate cyclase was greatest and that CT binding and activation of adenylate cyclase occurred at similar hormone concentrations.

It was demonstrated by Rotella et al.(1985) that hCT produced an increase in cyclic 3',5'-guanosine monophosphate (cGMP) accumulation in human kidney cortical cells, but that the hormone was a more efficacious and potent stimulator of cAMP accumulation. Human CT also stimulated human kidney cortical or medullary plasma membrane adenylate cyclase but only in the presence of guanylyl 5'-imidodiphosphate. In contrast, Stock and Coderre (1982) observed that sCT inhibited the accumulation of cAMP in human monocytes stimulated by latex particles.

Calcitonin also has an effect on central cyclic nucleotide levels as shown by Loffler et al. (1972) who reported an increase in cAMP in primary cultures of rat glial cells and McArdle (1984) who demonstrated an increase in cAMP and adenylate cyclase in mouse hypothalamic homogenates. In stark contrast, CT was found to inhibit the activity of adenylate cyclase in mouse hypothalamus and whole brain homogenates, however, this inhibition occurred at doses which

were higher than those required for [^{125}I]-sCT binding activity (Rizzo and Goltzman, 1981). Inhibition of adenylate cyclase has also been demonstrated in different areas of rat brain, namely midbrain, hypothalamus, medulla, pons and caudate nucleus. This inhibitory effect was enhanced by guanosine triphosphate (GTP) (Nicosia et al. 1986).

It may be postulated that there are two different classes of CT receptors that can be coupled to adenylate cyclase either for stimulation or inhibition of cAMP.

1.10 AIMS OF THE PROJECT

It is evident from the preceding sections that administration of CT to animals and man can produce a wide range of biological responses which may or may not be of physiological, pharmacological or therapeutic relevance. The acute effects of CT on cardiovascular parameters in the rat, particularly after haemorrhage, have been the subject of the experiments reported in this thesis. Attention has focused on the mechanism(s) of action by which such effects might be produced, since these effects may be therapeutically relevant to man.

The work presented in this thesis falls into distinct categories:

- biochemical techniques to determine the effect of central administration of CT on the central tryptaminergic system in order to confirm or refute previous reports concerning this effect.
- the determination of the acute pharmacological effect of peripherally administered CT on blood pressure and heart rate of anaesthetised rats subjected to endotoxin shock.

- the determination of the acute pharmacological effect of peripherally and centrally administered CT on blood pressure and heart rate of anaesthetised normotensive rats and those rendered hypotensive by haemorrhage.
- the use of pharmacological and surgical procedures to identify the mechanism(s) of action of the acute cardiovascular effects of CT after peripheral or central administration to urethane anaesthetised normotensive rats and those subjected to haemorrhage.

2. MATERIALS AND METHODS

2.1. Animals

Male Sprague-Dawley rats of 150-350 g were used. Rats were maintained in an ambient temperature of 21-23° C with 12 h light (0600-1800 h) and 12 h dark (1800-0600 h). They were allowed access to food (Heygate and Son Ltd., breeding diet cubes containing 1.42% calcium and 1.09% phosphate) and water ad libitum.

Rats were bred from stock obtained from the University of Nottingham, Sutton Bonnington Breeding Unit.

2.2. Chemicals

Synthetic salmon calcitonin (sCT; Calsynar) was donated by Armour Pharmaceuticals Co. Ltd. U.K. Enalapril maleate was a gift from Merck, Sharp and Dohme Ltd. Guanethidine monosulphate (Ismelin) was obtained from Ciba Geigy Pharmaceuticals U.K. Pentobarbitone sodium B.P. (Sagatal) was obtained from May and Baker Ltd. U.K.

The following were purchased from Sigma Biochemicals Ltd. U.K.:

E. coli lipopolysaccharide (0127:B8)

Naloxone hydrochloride

Urethane (ethyl carbamate)

Eserine sulphate (physostigmine sulphate)

Angiotensin I acetate salt (human sequence)

5-Hydroxytryptamine creatinine sulphate complex

5-Hydroxyindole-3-acetic acid

p-Chlorophenylalanine

Probenecid

Pargyline hydrochloride

o-Phthaldialdehyde

Polyoxyethylene-4-lauryl ether (Brij 30)

The remaining chemicals, which were of 'Analar' grade wherever possible, were purchased from major chemical suppliers.

2.3. Solutions

a) Physiological salt solutions.

1) 0.15M sodium phosphate buffer (pH 7.4)

19 ml of 0.2M NaH_2PO_4 were combined with 81 ml of 0.2M $NaHPO_4$.

75 ml of the resulting solution were combined with 25 ml of distilled water.

2) 50mM tris-buffer (pH 7.4)

$50mmol^{-1}$ tris(hydroxymethyl)aminomethane

$0.1mol.l^{-1}$ NaCl

0.1% BSA (w/v)

The pH was adjusted to 7.4 with a few drops of 1M HCl.

b) Solutions for drug administrations.

Unless stated otherwise, drugs were administered in 0.9% (w/v) NaCl.

1) Vehicles for calcitonin.

Salmon CT was obtained in vials containing 100 $I.U.ml^{-1}$ sCT in 0.2%(w/v) sodium acetate and 0.5% (w/v) NaCl. This was diluted in 0.15M sodium phosphate buffer, pH 7.4, containing 0.1% (w/v) BSA for intravenous (iv) administration, and 50mM tris-buffer, pH 7.4,

containing 0.1% (w/v) BSA for intracerebroventricular (icv) administration.

2) Probenecid.

Probenecid was dissolved in 5 drops of 0.9% (w/v) NaCl, to which 5 drops of 2M NaOH was added. The pH was adjusted to 7.4 with 2N HCl, and the solution brought to volume with 0.9% (w/v) NaCl.
This produced a fine suspension of probenecid.

3) p-Chlorophenylalanine.

This was suspended in a solution of 0.9% (w/v) NaCl and 0.5% Brij 30.

2.4. Intracerebroventricular injection technique

Rats were anaesthetised with 1.6 $g.kg^{-1}$ urethane (50% intraperitoneal; ip and 50% subcutaneous; sc) and placed in a Kopf 900 stereotaxic frame as described by De Groot (1959), after which the skull was exposed. The stereotaxic frame has a vertical racking assembly onto which a syringe or drill may be attached. Using the drill in the racking assembly, two 1mm diameter holes were bored into the skull directly above the right and left lateral ventricles. (De Groot co-ordinates; horizontal + 0.66mm; lateral $\pm$ 0.13mm). A glass Hamilton syringe with a 25 gauge needle was mounted in the racking assembly and lowered vertically through each hole into the lateral ventricles. (De Groot co-ordinate; vertical + 0.15mm).

Salmon CT was administered in a volume of 25 μl; 12.5 μl into each lateral ventricle, each given over a period of one minute and the needle left for 30 sec before removal. At the end of each experiment,

25 μl of Indian ink (10% in 0.9% NaCl) was injected as described for sCT. The brains were rapidly removed and fixed in formol saline before being examined by eye to ensure distribution throughout the ventricular system.

2.5. Assay of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in rat whole brain.

The concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in rat whole brain were assayed by the spectrofluorimetric method of Maickel et al. (1968) as modified by Curzon and Green (1970) and as described below.

Rats were stunned by a blow on the head and decapitated. The brains were rapidly removed onto an ice-cold petri dish where any remaining spinal cord was removed. The brains were weighed and 10 volumes of ice-cold acidified n-butanol (0.83 ml conc. HCl in 1l n-butanol) was added. The tissue was then homogenised with an ultraturrax homogeniser at maximum speed for 5 sec and the homogenate was then centrifuged for 15 min at 3000 rpm and 4^{o} C.

Separation of 5-HT and 5-HIAA

2.5 ml of the supernatant was transferred to a stoppered pyrex tube to which 5 ml n-heptane and 0.4 ml L-cysteine (0.1% in 0.1N HCl) were added. This was then mechanically shaken for 5 min (250 shakes.min^{-1} using an Edmund Buhler SM25 shaker). The aqueous phase containing 5-HT and the organic phase containing 5-HIAA were then separated by centrifugation for 5 min at 3000 rpm and 4^{o} C. They were then assayed as follows:

1) 5-HT

0.2 ml samples of the aqueous phase from the centrifuged extraction layers were transferred to pyrex test-tubes and 1.2 ml o-phthaldialdehyde (0.004% OPT in 10N HCl) added. Blanks of 0.2 ml L-cysteine (0.1% in 0.1N HCl) were similarly treated and carried through the rest of the procedure. The tubes were immediately vortexed, placed in a boiling water bath for 15 min and cooled in running cold water prior to the measurement of fluorescence using a Perkin-Elmer 650-40 spectrophotofluorimeter (excitation wavelength 360 nm, emission wavelength 470 nm, slit width 15 nm).

2) 5-HIAA

5 ml of the organic phase from the centrifuged extraction layers was mechanically shaken with 1.2 ml phosphate buffer (0.5M at pH 7.0) for 10 min. After centrifugating for 5 min at 3000 rpm and 4^{o}C two 0.4 ml aliquots of the aqueous phase were obtained. One aliquot was taken through procedure A below, the other aliquot through procedure B below.

Procedure A: determination of 5-HIAA in samples

0.4 ml aliquots of the aqueous phase were transferred to pyrex test-tubes, to which were added 0.04 ml L-cysteine (1% in deionised water), 0.8 ml conc. HCl, 0.04 ml OPT (0.1% in methanol) and 0.04 ml sodium periodate (0.02% in deionised water). The order of addition was strictly adhered to and all tubes mixed by vortex immediately after each addition. The tubes were left for 30 min at 4^{o} C and then placed in a boiling water bath for 10 min. They were cooled in running cold water prior to the measurement of fluorescence

(excitation wavelength 360 nm, emission wavelength 470 nm, slit width 15 nm).

Procedure B: determination of 5-HIAA in blanks

0.4 ml aliquots of the aqueous phase were transferred to pyrex test-tubes to which were added 0.04 ml sodium periodate (0.02% in deionised water) and 0.8 ml conc. HCl. These tubes were left for 30 min at 4^{o} C after which time 0.04 ml L-cysteine (1% in deionised water) and 0.04 ml OPT (0.1% in methanol) were added. Again, order of addition was strictly adhered to and samples were mixed by vortex immediately after each addition. The tubes were then placed in a boiling water bath for 10 min and cooled in running cold water prior to the measurement of fluorescence (excitation wavelength 360 nm, emission wavelength 470 nm, slit width 15 nm).

Standards

External standards for 5-HT (250 ng base/2.5 ml and 125 ng base/2.5 ml) and 5-HIAA (250 ng/2.5 ml, 125 ng/2.5 ml and 62.5 ng/2.5 ml) were included in all experiments. These were prepared by taking 2.5 ml of 5-HT (1 μg/10 ml and 0.5 μg/10 ml) and 5-HIAA (0.5 μg/10 ml, 0.25 μg/10 ml and 0.125 μg/10 ml) in acidified n-butanol through the extraction procedures as described previously.

All solutions were prepared fresh on the day of use, using deionised water where appropriate. Glassware was washed in 20% nitric acid, followed by rinsing in distilled water. Deionised water was used for a final rinse of the glassware. All tubes used were chilled to 4^{o} C before use.

Preliminary experiments were carried out to determine the efficiency of extraction of 5-HT and 5-HIAA, and the specificity of the assay for both compounds. The effect of various compounds on 5-HT and 5-HIAA levels in rat whole brain was then investigated. All rats were sacrificed at the same time of day, and body temperature was maintained at 37^{o} C $\pm$ 0.5^{o} C by means of heating lamps for rats under anaesthesia.

2.6. Determination of the effects of sCT on blood pressure and heart rate.

2.6.a Anaesthesia

Rats were anaesthetised with urethane (1.6 $g.kg^{-1}$ in 0.9% NaCl; 50% ip and 50% sc) or sodium pentobarbitone (60 $mg.kg^{-1}$ ip; anaesthesia was maintained by sc sodium pentobarbitone as required).

2.6.b Cannulation and drug administration

In rats which were to receive sCT by iv administration, the trachea, jugular vein and carotid artery were cannulated to aid respiration, for the administration of drugs and for the measurement of mean arterial pressure (MAP) and heart rate respectively. Those rats which were to receive sCT by icv administration were placed in a stereotaxic frame throughout the experiment. The trachea was not cannulated since this inhibited respiration due to the position of these animals. The jugular vein and carotid artery were cannulated in those animals to receive iv administered drugs and for the measurement of MAP and heart rate respectively. The jugular cannula and carotid cannula were kept patent with heparinised 0.9% NaCl (50 $U.ml^{-1}$ heparin). Following surgery, animals were allowed a stabilisation period of approximately 20 min before administration of drugs. Rectal temperature, measured

by use of an indwelling rectal thermistor, was maintained at 37° C ± 0.5° C throughout the experiments by means of heating lamps.

All drugs administered iv were followed by 0.1 ml 0.9% NaCl (iv) to flush the cannula; this included sCT or its vehicle (0.15M sodium phosphate buffer containing 0.1% BSA). Salmon CT (icv) or its vehicle (50mM tris-buffer containing 0.1% BSA) was administered as described in 2.4.

2.6.c Measurement of MAP and heart rate

The carotid cannula was connected to a Washington 400 MDI chart recorder via a Washington PT 400 pressure transducer. Mean arterial pressure and heart rate were calculated from the trace obtained. In all experiments, except those involving endotoxin administration, MAP and heart rate were monitored for 60 min. In those experiments involving endotoxin administration MAP and heart rate were monitored for 95 min.

2.6.d Haemorrhage

Approximately 20 min after surgery haemorrhage was induced by the withdrawal of 1-2 ml arterial blood (via the carotid artery) such that MAP was reduced by approximately 20%. Rats were allowed a further 20 min stabilisation period before the administration of drugs (40 min after surgery). Normotensive rats were allowed a 20 min stabilisation period before the administration of drugs (20 min after surgery).

2.6.e Salmon CT in normotensive rats and those subjected to haemorrhage

Salmon CT, iv or icv, or appropriate vehicle was administered to both

urethane anaesthetised normotensive rats (20 min after surgery) and those subjected to haemorrhage (40 min after surgery). Intravenous sCT or appropriate vehicle was also administered to sodium pentobarbitone anaesthetised rats rendered hypotensive by haemorrhage (40 min after surgery).

2.6.f Salmon CT in rats subjected to endotoxin shock.

Sodium pentobarbitone anaesthetised rats were maintained on a SRI constant volume ventilator (50 strokes.min^{-1}; 5 ml volume) throughout the experiment. A SRI slow infusion apparatus was used for the infusion of E. coli lipopolysaccharide (0.03 ml.min^{-1} over 2 h, equivalent to 2 mg.kg^{-1} over 2 h). Twenty five min after the start of endotoxin infusion sCT, naloxone (10 mg.kg^{-1}), or appropriate vehicle, was administered iv.

2.6.g Salmon CT in rats subjected to acute sympathetic blockade.

Guanethidine monosulphate (10 mg.kg^{-1}) was administered iv to urethane anaesthetised normotensive rats (20 min after surgery) and those subjected to haemorrhage (40 min after surgery). Ten min later, sCT or vehicle was administered by iv injection to haemorrhaged rats or by icv injection, as described previously, to normotensive rats and those subjected to haemorrhage.

2.6.h Salmon CT in rats subjected to bilateral adrenalectomy.

An incision was made in the dorsal surface of urethane anaesthetised rats, on each side of the spinal cord, anterior to the position of the hind leg joints. The adrenal glands were located and tied off; they were then removed. Sham adrenalectomy was performed in control animals by locating the adrenal glands, but neither tying off nor

removing. Guanethidine monosulphate (10 $mg.kg^{-1}$) was administered iv, when stated, to normotensive rats (20 min after surgery) and those subjected to haemorrhage (40 min after surgery). Ten min later, sCT or vehicle was administered by icv injection as described previously.

2.6.i Salmon CT in rats subjected to renin-angiotensin system blockade.

Enalapril maleate (2 $mg.kg^{-1}$) was administered iv to urethane anaesthetised normotensive rats (20 min after surgery) and those subjected to haemorrhage (40 min after surgery). Ten min later, sCT or vehicle was administered by icv injection as described previously.

2.7 Statistics

Experimental design was arranged so that test results could be compared with those of a control group. Approximate normality of distribution of the data was checked using a normal probability plot (Statistical Graphics System, 1985; Statistical Graphics Corporation) on an IBM PS/2 model 50 computer. The plot consists of an arithmetic horizontal axis and a vertical axis scaled so that the cumulative distribution function of a normal distribution plots as a straight line. Considerable deviation from a straight line shows that the data are not normally distributed. The data were then analysed by analysis of variance (ANOVA) (Statistical Graphics System, 1985; Statistical Graphics Corporation) on an IBM PS/2 model 50 computer.

Results are expressed as mean difference (95% confidence intervals; 95% CI) where the mean difference is the mean of the treated group

minus the mean of the control group. Results are statistically significant when the 95% CI does **not** contain zero. The CI gives a range of values that are considered to be plausible for the whole population based on a sample of that population and the width of the CI depends partly on the standard deviation and the sample size.

The area under the curve (AUC) was considered to be the appropriate indication of the biological response. This is always expressed as mean difference (95% CI) and is quoted with the significance level (p). P values of less than 0.05 were considered to be significant. AUC values were calculated from time 0 min to the end of the response (usually 60 min) using a program devised by Dr. SE Mireylees, Trent Polytechnic, Nottingham on a DEC 20 computer.

3. CALCITONIN AND THE CENTRAL TRYPTAMINERGIC SYSTEM

3.1 INTRODUCTION

More and more results accord with the theory that CT may play an important role as a central neurotransmitter or neuromodulator. The earliest evidence for an interaction between CT and the central nervous system (CNS) was offered by Pecile et al. (1975) who measured changes in the electroencephalogram (EEG) and in the pain threshold of the dental pulp of rabbits in response to sCT (icv). The EEG studies demonstrated an increase in the electrical activity, throughout all regions of the brain studied, which was parallel to the development of analgesia.

Salmon and human CT-like peptides have been detected in the CNS of several animal species (Deftos et al. 1980) and also in human brain (Fischer et al. 1983b). In addition, binding sites for CT have been demonstrated in human and rat CNS (Fischer et al. 1981b, Olgiati et al. 1983). Furthermore, CT injected centrally has been shown to induce behavioural activity such as decreased feeding (Freed et al. 1979), inhibition of gastric acid secretion (Morley et al. 1981a), pressor effects (Bates et al. 1984c) and analgesia (Pecile 1983). Nakhla and Nandi Majumbar (1978) observed that a single injection of pCT (im) to normal rats produced an immediate decrease in total calcium and free tryptophan in plasma with a concomitant increase in both brain 5-HT and cerebral acetylcholinesterase (AChE) activity. They suggested that enhancement of cerebral AChE activity after CT injection was the result of a CT-mediated increase in 5-HT in the brain. Dupuy et al. (1983) confirmed this increase in brain 5-HT after treating normal rats with sCT (ip). They observed a maximum

increase in 5-HT levels 45 min after administration of sCT.

Several workers have since investigated the effect of CT on brain 5-HT concentrations. Bates et al. (1982c) investigated brain AChE activity in mice and rats after treatment with sCT (sc and iv), eserine, p-chlorophenylalanine (pCPA), 5-HT or tryptophan. They observed no significant change in AChE activity after any of the treatments, except eserine, which was the reference drug for the assay system. In contrast to Nakhla and Nandi Majumbar (1978) they were unable to provide evidence either that agents which modify 5-HT metabolism influence brain AChE activity or that sCT is associated with changes in AChE activity. Guidobono et al. (1984) treated conscious rats with sCT (icv) and measured brain 5-HT and 5-HIAA levels at various times after treatment. They demonstrated a significant increase in 5-HIAA levels in the striatum, hypothalamus, brainstem and spinal cord 4 h after treatment; this increase was still significant after 18 h. In contrast, 5-HT levels remained unaffected. They concluded that 5-HT neuronal activity may be modulated by CT.

The majority of evidence suggests that sCT affects the central tryptaminergic system. Several workers have further investigated this interaction between CT and the central tryptaminergic system in terms of the production of analgesia. Bates et al. (1983a) demonstrated that sCT (icv) resulted in a dose-dependant reduction in abdominal constriction rate in mice which was abolished after pretreatment with pCPA. They suggested that central tryptaminergic systems may be involved in the antinociceptive action of sCT. This finding was confirmed by Clementi et al. (1984, 1985) who demonstrated that methysergide, 5,7-dihydroxytryptamine (5,7-DHT), cyproheptadine and

reserpine pretreatment abolished the analgesic effect of sCT (icv). Furthermore, they concluded that the midbrain raphe dorsalis is an important focus for the analgesic effect of sCT.

In contrast to the above findings Guidobono et al. (1986a) concluded that analgesia induced by sCT injection into the rat brain, either icv or intrathecally (it), does not require the integrity of the central tryptaminergic system. The integrity of the catecholaminergic system, on the other hand, is required. The analgesic effect of sCT was completely abolished after pretreatment with 5,7-DHT at a dose which significantly reduced 5-HT and noradrenaline levels in the spinal cord. This dose of 5,7-DHT was 12.5 times greater than that used by Clementi et al. (1985). When animals were pretreated with 5,7-DHT and desmethylimipramine (DMI; to protect the noradrenergic neurones) the analgesic effects of sCT (icv) were the same as in animals with 5-HT not depleted; the analgesic response to sCT (it) was delayed. After pretreatment with pCPA the analgesic effect of sCT (icv) was not diminished.

The central tryptaminergic system has also been implicated in the cardiovascular effects of centrally administered sCT. It was demonstrated by Clementi et al. (1986) that sCT (1.5 $u.kg^{-1}$ icv) increases blood pressure and plasma renin activity in conscious rats. They suggested that, since an increase in cerebral content of 5-HT enhances renin secretion, then sCT may increase plasma renin activity through an interference with tryptaminergic pathways and in turn enhance blood pressure.

The results presented in this chapter were obtained from a series of

experiments designed to clarify the current thinking on the involvement of sCT with the central tryptaminergic system. The assays used were based on those described by Curzon and Green (1970) in which 5-HT and 5-HIAA are extracted from crude brain homogenates and reacted with o-phthaldialdehyde to produce highly fluorescent products.

3.2 RESULTS

NB. Results are expressed as mean difference, ie treated - control (95% CI)

1) Standard curves and extraction efficiency

The relationship between fluorescence and indoleamine concentration was found to be linear over the range 0-250 ng/2.5 ml, with 5-HT producing the greater degree of fluorescence compared with 5-HIAA. (see figs. R.1a and b). Curzon and Green (1970) reported the efficiency of extraction of both 5-HT and 5-HIAA from acidified n-butanol to be 95% - 100%. However, since the present investigation was concerned with the determination of 5-HT and 5-HIAA in brain tissue, it seemed pertinent to investigate any effect of the tissue itself on the assay. Thus standards were extracted from tissue homogenate. The efficiency of extraction of 5-HT from homogenate did not differ markedly from that in acidified n-butanol. However, the efficiency of extraction of 5-HIAA from homogenate was reduced by 28% compared with that in acidified n-butanol (see fig.R.1b). The reason for this reduction in efficiency is unclear, but possibilities include specific or non-specific binding of 5-HIAA to the tissue. Since n-butanol standards were used in all subsequent experiments the 5-HIAA results obtained have been adjusted for this reduction in extraction efficiency, by increasing absolute values by 28%.

2) Assay specificity

The measurement of both 5-HT and 5-HIAA was carried out at 360/470nm (excitation wavelength/emission wavelength), slit width 15 nm. The assay is therefore dependent on the specificity of the extraction process since fluorescence alone cannot be used to distinguish between these indoleamines. As can be seen in fig.R.2, 5-HIAA added to tissue homogenate prior to assay was not found to interfere with the determination of 5-HT and likewise 5-HT was not found to interfere with the determination of 5-HIAA.

3) Linearity of homogenate concentration

Serial dilution of brain homogenate in acidified n-butanol resulted in a linear decrease in both 5-HT and 5-HIAA concentrations (see Fig.R.3a and b).

4) Effect of pCPA, pargyline and probenecid on rat whole brain 5-HT and 5-HIAA concentrations

Treatment of rats with pCPA (300 $mg.kg^{-1}$ ip 48 h before sacrifice) led to significant reductions in the concentrations of brain 5-HT and 5-HIAA of -297 (-368, -227) $ng.g^{-1}$ wet tissue (mean difference (95% CI); $p< 0.001$) and -207 (-259, -155) $ng.g^{-1}$ wet tissue ($p< 0.001$) respectively relative to control rats (see figs.R.4 and R.5). This represents a decrease in 5-HT concentration of 36% and a decrease in 5-HIAA concentration of 32%. Koe and Weissman (1966) suggested that pCPA inhibits tryptophan hydroxylase after the observation of a dose-dependent depletion in rat brain 5-HT accompanied by a decrease in brain 5-HIAA. Concomitant with this decline in indoleamine concentration was a reduction in liver tryptophan hydroxylating activity. In contrast, brain catecholamine levels were only slightly

lowered.

When rats were treated with pargyline (75 $mg.kg^{-1}$ ip 90 min before sacrifice) a significant increase in brain 5-HT of 150 (75, 225) $ng.g^{-1}$ wet tissue ($p< 0.001$) along with a significant decrease in brain 5-HIAA of -237 (-322, -151) $ng.g^{-1}$ wet tissue ($p< 0.001$) relative to control rats was observed (see figs.R.4 and R.5). This represents an increase in brain 5-HT concentration of 27% and a decrease in brain 5-HIAA concentration of 48%. This is in accord with Tozer et al. (1966) who demonstrated that pargyline treatment caused an almost complete loss of 5-HIAA content and produced a marked rise in that of 5-HT. Pargyline inhibits monoamine oxidase (MAO) thus producing a rapid decline of the acid metabolites due to elimination and/or conjugation processes (Korf 1981).

In rats treated with probenecid (300 $mg.kg^{-1}$ ip 90 min before sacrifice) no significant change in brain 5-HT concentration, but a significant increase of 445 (325, 565) $ng.g^{-1}$ wet tissue ($p< 0.001$) in brain 5-HIAA relative to control rats was observed (see figs.R.4 and R.5). This represents an increase in central 5-HIAA concentration of 129%. Curzon (1981) suggested that probenecid blocks egress of 5-HIAA from the brain leading to an increase in 5-HIAA concentration.

5) Effect of urethane on rat whole brain 5-HT and 5-HIAA concentrations

In order to determine any effect of anaesthetic on brain 5-HT and 5-HIAA levels, rats were sacrificed 90 min after receiving urethane (1.6 $g.kg^{-1}$ 50% ip and 50% sc). Control rats received no injections whatsoever. It can be seen from fig.R.6 that urethane resulted in a

statistically significant increase in both brain 5-HT and 5-HIAA concentrations of 539 (435, 642) $ng.g^{-1}$ wet tissue ($p< 0.001$) and 195 (93, 298) $ng.g^{-1}$ wet tissue ($p< 0.001$) respectively relative to control rats. This represents an increase in brain 5-HT and 5-HIAA concentrations of 112% and 58% respectively. Furthermore, the control values obtained (479 $ng.g^{-1}$ wet tissue 5-HT and 334 $ng.g^{-1}$ wet tissue 5-HIAA) were very similar to those obtained after control injections for pCPA, pargyline and probenecid, for which the rats received no anaesthetic.

6) Effect of icv sCT on rat whole brain 5-HT and 5-HIAA concentrations

In order to assess any effect of sCT on brain 5-HT and 5-HIAA concentrations, rats were anaesthetised with urethane (as above) approximately 30 min before being subjected to central injection of 5 $U.kg^{-1}$ sCT or appropriate vehicle, as described in 2.4. Control rats (no anaesthetic, sCT or vehicle) were also included in order to recognise the participating effects from the anaesthetic. Rats were sacrificed either 1, 2, 4 or 6 h after treatment and brains assayed for 5-HT and 5-HIAA. As can be seen in figs.R.7 and R.8, there were no significant effects of sCT (icv) on brain 5-HT or 5-HIAA levels, however, there was a trend towards an increase both in 5-HT and 5-HIAA levels over the time period investigated. It must be noted that the values obtained for brain 5-HT and 5-HIAA concentrations are very much higher after both sCT (954 to 1443 $ng.g^{-1}$ wet tissue 5-HT and 603 to 957 $ng.g^{-1}$ wet tissue 5-HIAA) and vehicle (834 to 1399 $ng.g^{-1}$ wet tissue 5-HT and 564 to 764 $ng.g^{-1}$ wet tissue 5-HIAA) than those obtained in control rats (479 $ng.g^{-1}$ wet tissue 5-HT and 334 $ng.g^{-1}$ wet tissue 5-HIAA). It is assumed that this is due to the administration of anaesthetic, since urethane treatment was found to

result in higher 5-HT and 5-HIAA concentrations than those found in control rats, i.e. without anaesthetic (see fig.R.6).

3.3 DISCUSSION

The effect of pargyline, probenecid and pCPA on rat whole brain 5-HT and 5-HIAA levels confirmed the effects seen by other workers (Tozer et al. 1966; Curzon, 1981; Koe and Weissman, 1966). It thus appears that the experimental procedures adopted are suitable for a simple and specific spectrofluorimetric assay of brain 5-HT and 5-HIAA levels. No significant changes in brain 5-HT/5-HIAA levels were apparent after central administration of sCT, however there was a trend for an increase in 5-HT and 5-HIAA levels which almost reached significance at 240 min and 360 min respectively.

When rats were treated with the anaesthetic urethane the concentrations of 5-HT and 5-HIAA in whole brain were significantly higher than those of control animals (see fig.R.6). The reason for these increases is unclear, since urethane does not appear to exert effects similar to those seen in the trial experiments after administration of pargyline, probenecid or pCPA in which the rats were not anaesthetised (see figs.R.4 and R.5). The increased levels of 5-HT and 5-HIAA observed suggest that the anaesthetic may be masking any response to sCT.

The results obtained in the present investigation add support to the findings of Guidobono et al. (1984) who demonstrated significant increases in 5-HIAA levels in various regions of the rat brain after central administration of sCT. Guidobono et al., however, were looking at the effect of sCT (icv) on brain 5-HT/5-HIAA levels in

conscious rats. There were, therefore, no interfering effects from anaesthetic as were seen in the present investigation. This is reflected in the control values obtained by Guidobono et al. (400-900 $ng.g^{-1}$ 5-HT; 300-450 $ng.g^{-1}$ 5-HIAA), which are very much lower than those obtained in the above experiments (834-1399 $ng.g^{-1}$ 5-HT; 564-764 $ng.g^{-1}$ 5-HIAA).

It is of interest to discuss the current evidence concerning the contribution of the central tryptaminergic system to calcitonin and analgesia. Guidobono et al. (1986a) concluded that sCT analgesia does not require the integrity of the central tryptaminergic system. The effect on 5-HT metabolism is long-lasting and not correlated with the time course of the analgesic effect that they observed. Clementi et al. (1984, 1985) concluded that the central tryptaminergic system is involved in sCT analgesia on the basis of a reversal of analgesia after administration of 5,7-DHT directly into the dorsal raphe of rats. These workers did not pretreat rats with DMI and therefore lesions of noradrenergic neurones due to diffusion of 5,7-DHT into areas surrounding the dorsal raphe cannot be excluded. This is pertinent since there is evidence for the existence of a noradrenergic control of nociception. Proudfit and Hammond (1981) suggested that tonically active serotonergic and noradrenergic neuronal systems modulate sensitivity to nociceptive stimuli at the level of the spinal cord after the observation that intrathecal administration of either methysergide or phentolamine produced hyperalgesia in rats.

The data of Bates et al. (1983a) contrast with the data of Guidobono et al. (1986a). Bates et al. investigated the effect of pCPA ($300\ mg.kg^{-1}$, 72, 48 and 24 h prior to the analgesic test) on the

analgesic response to sCT (0.003-1.5 U/animal icv) in mice using the abdominal constriction test. Guidobono et al. studied the effect of pCPA (150 $mg.kg^{-1}$,72 and 24 h prior to the analgesic test) on the analgesic response to sCT (approx. 11.25 U/animal icv) in rats using the hot-plate test. Bates et al. observed an inhibition in the analgesic effect of sCT after treatment with pCPA; Guidobono et al. observed no effect of pCPA on the analgesic response to sCT. These discrepancies may be attributed to the differences in animal species, the tests used for measuring the analgesic effect, the doses of pCPA used, and the doses of sCT used. Guidobono et al. employed doses of pCPA 3 times smaller than did Bates et al., and yet used doses of sCT approximately 7.5 times greater than those of Bates et al. The timing of the tests also differ. Bates et al. used the abdominal constriction test 20 min after the administration of sCT in mice; Guidobono et al. used the hot plate test 30-240 min after administration of sCT in rats.

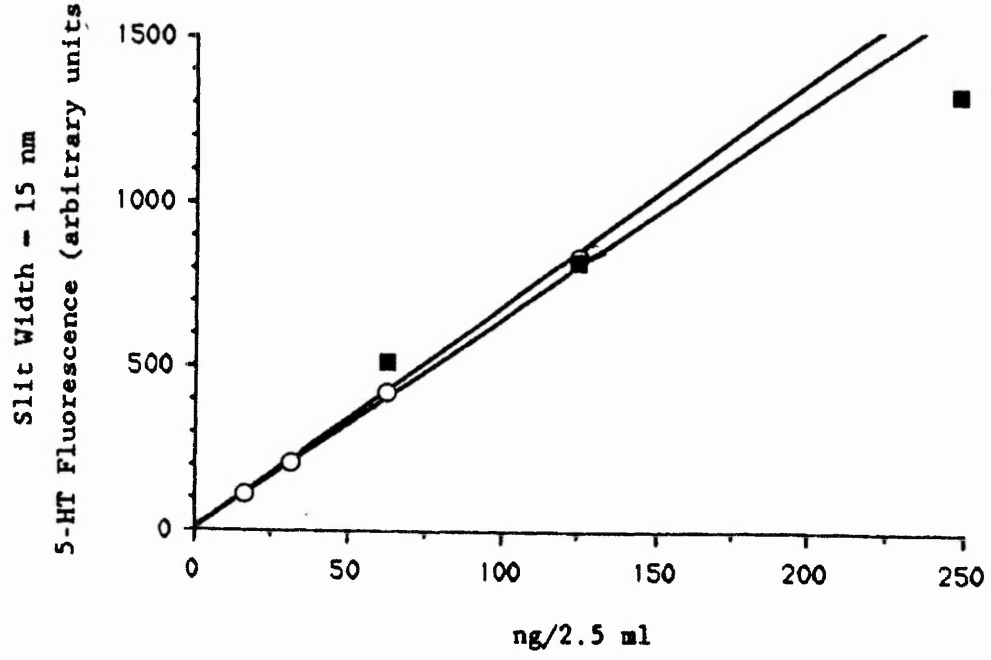
The results presented in this chapter demonstrate that central administration of sCT (5 $U.kg^{-1}$) leads to a trend for an increase in central 5-HT and 5-HIAA levels in urethane anaesthetised rats. Perhaps with a larger sample size or 2 way ANOVA as the statistical analysis these increases in 5-HT and 5-HIAA levels would reach statistical significance. It would be interesting to look at the effect of sCT (icv) on 5-HT/5-HIAA levels in isolated regions of the brain, for example, the hypothalamus, since 5-HT/5-HIAA levels in areas such as the cerebellum may influence the results. Moreover, it would appear that urethane anaesthesia exerts a confounding influence on the results obtained in that central 5-HT and 5-HIAA levels respond to this anaesthetic treatment.

Fig.R.1. Efficiency of Extraction of 5-HT and 5-HIAA

Standards were extracted from acidified n-butanol (■) or from brain tissue homogenate (O).

Graphs show the mean of two determinations

a) 5-HT


Slit Width - 15 nm
5-HT Fluorescence (arbitrary units)
1500
1000
500
0
0
50
100
150
200
250
ng/2.5 ml

b) 5-HIAA

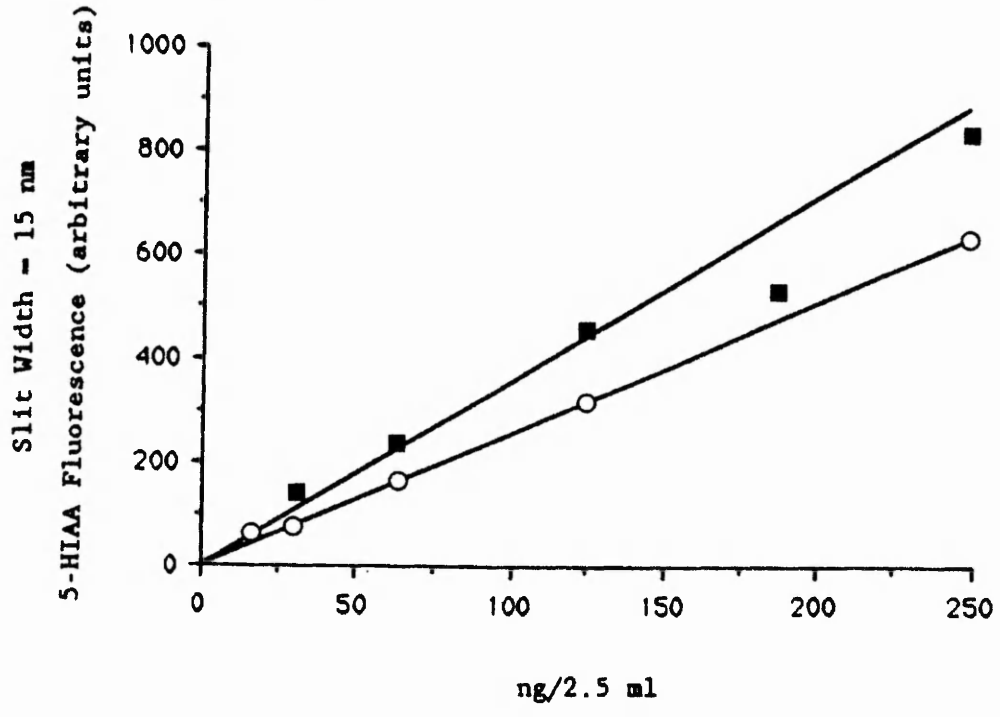

Slit Width - 15 nm
5-HIAA Fluorescence (arbitrary units)
1000
800
600
400
200
0
0
50
100
150
200
250
ng/2.5 ml

Fig.R.2. Specificity of the Assay

5-HT was assayed in tissue homogenate alone and tissue homogenate containing known amounts of 5-HIAA. Likewise, 5-HIAA was assayed in tissue homogenate alone and tissue homogenate containing known amounts of 5-HT.

Graph shows the mean of two determinations

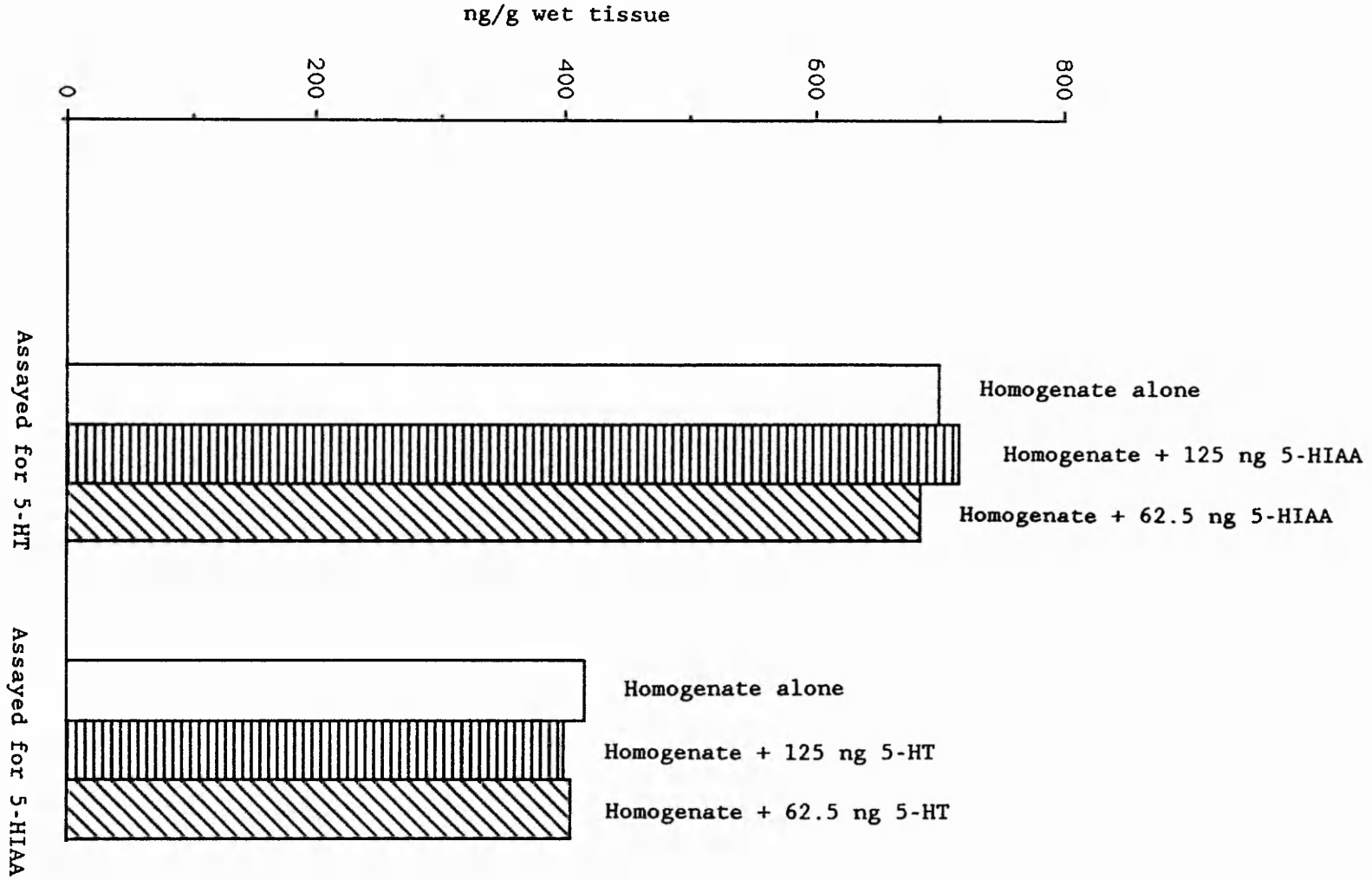
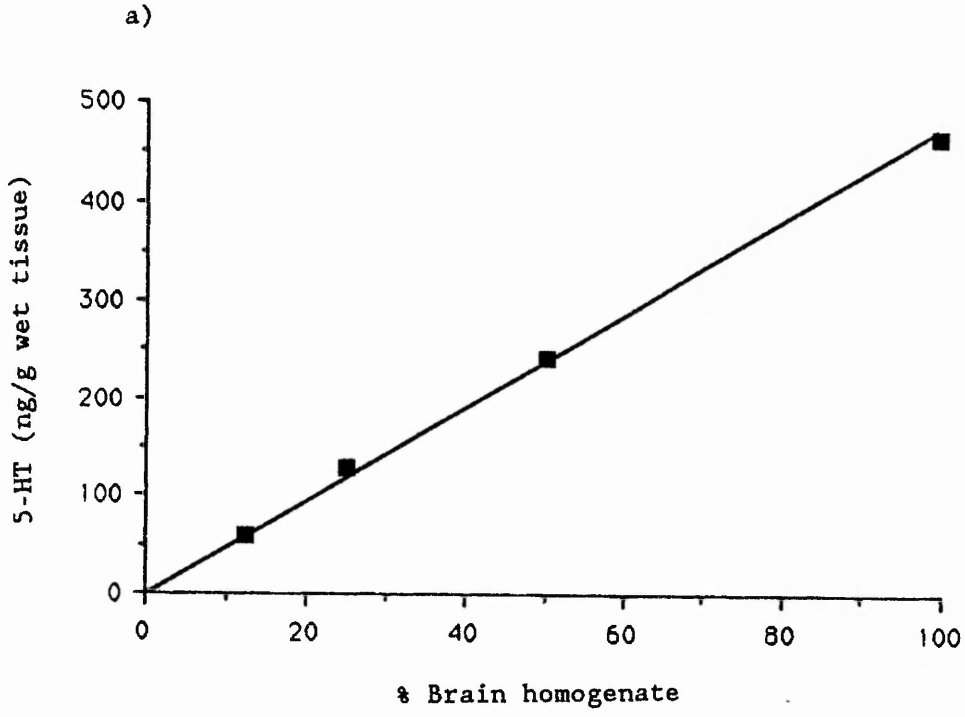

ng/g wet tissue
0
200
400
600
800
Assayed for 5-HT
Homogenate alone
Homogenate + 125 ng 5-HIAA
Homogenate + 62.5 ng 5-HIAA
Assayed for 5-HIAA
Homogenate alone
Homogenate + 125 ng 5-HT
Homogenate + 62.5 ng 5-HT

Fig.R.3. Linearity of Homogenate Concentration

Tissue homogenate was serially diluted prior to the assay of 5-HT (a) and 5-HIAA (b).

Graphs show the mean of two determinations

a)


5-HT (ng/g wet tissue)
500
400
300
200
100
0
0
20
40
60
80
100
% Brain homogenate

b)

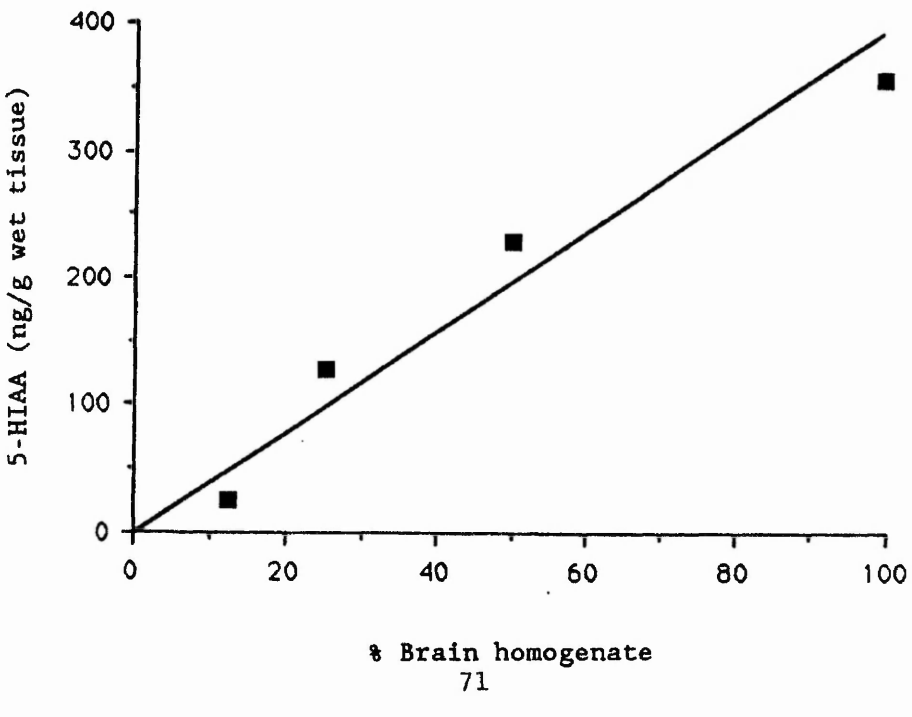

5-HIAA (ng/g wet tissue)
400
300
200
100
0
0
20
40
60
80
100
% Brain homogenate

Fig.R.4. Effect of Treatment with Pargyline, Probenecid or pCPA on Rat Whole Brain 5-HT Levels.

Rats were treated with pargyline (75 $mg.kg^{-1}$ ip 90 min before sacrifice) (treated), probenecid (300 $mg.kg^{-1}$ ip 90 min before sacrifice) (treated), pCPA (300 $mg.kg^{-1}$ ip 48 h before sacrifice) (treated) or the appropriate vehicle (control). The brains were then assayed for the determination of 5-HT concentration.

Graph shows the mean difference (95% CI); see 2.7

n = 5-6

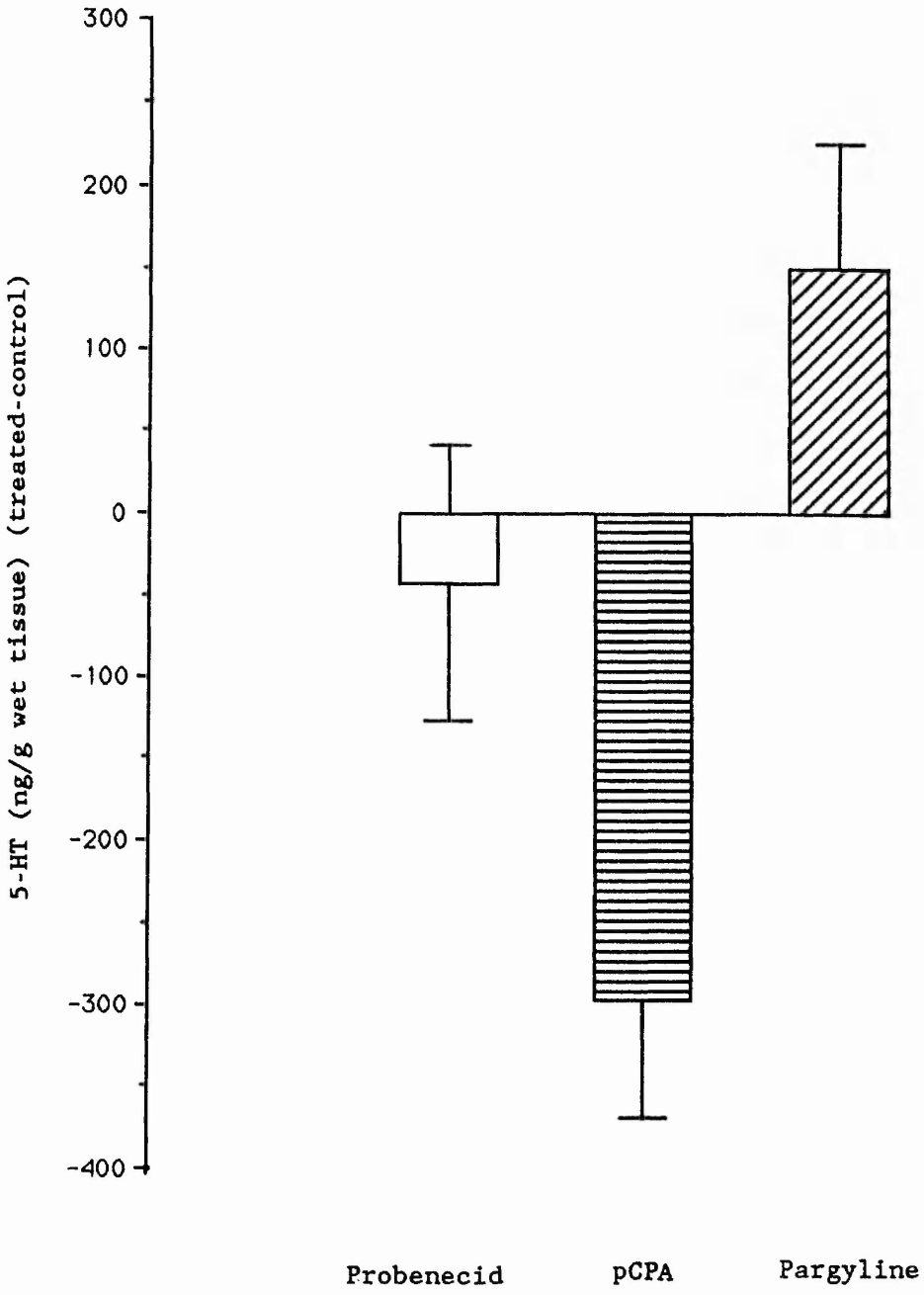

300
200
100
0
-100
-200
-300
-400
5-HT (ng/g wet tissue) (treated-control)
Probenecid
pCPA
Pargyline

Fig.R.5. Effect of Treatment with Pargyline, Probenecid or pCPA on Rat Whole Brain 5-HIAA Levels.

Rats were treated with pargyline (75 $mg.kg^{-1}$ ip 90 min before sacrifice) (treated), probenecid (300 $mg.kg^{-1}$ ip 90 min before sacrifice) (treated), pCPA (300 $mg.kg^{-1}$ ip 48 h before sacrifice) (treated) or the appropriate vehicle (control). The brains were then assayed for the determination of 5-HIAA concentration.

Graph shows the mean difference (95% CI); see 2.7

n = 5-6

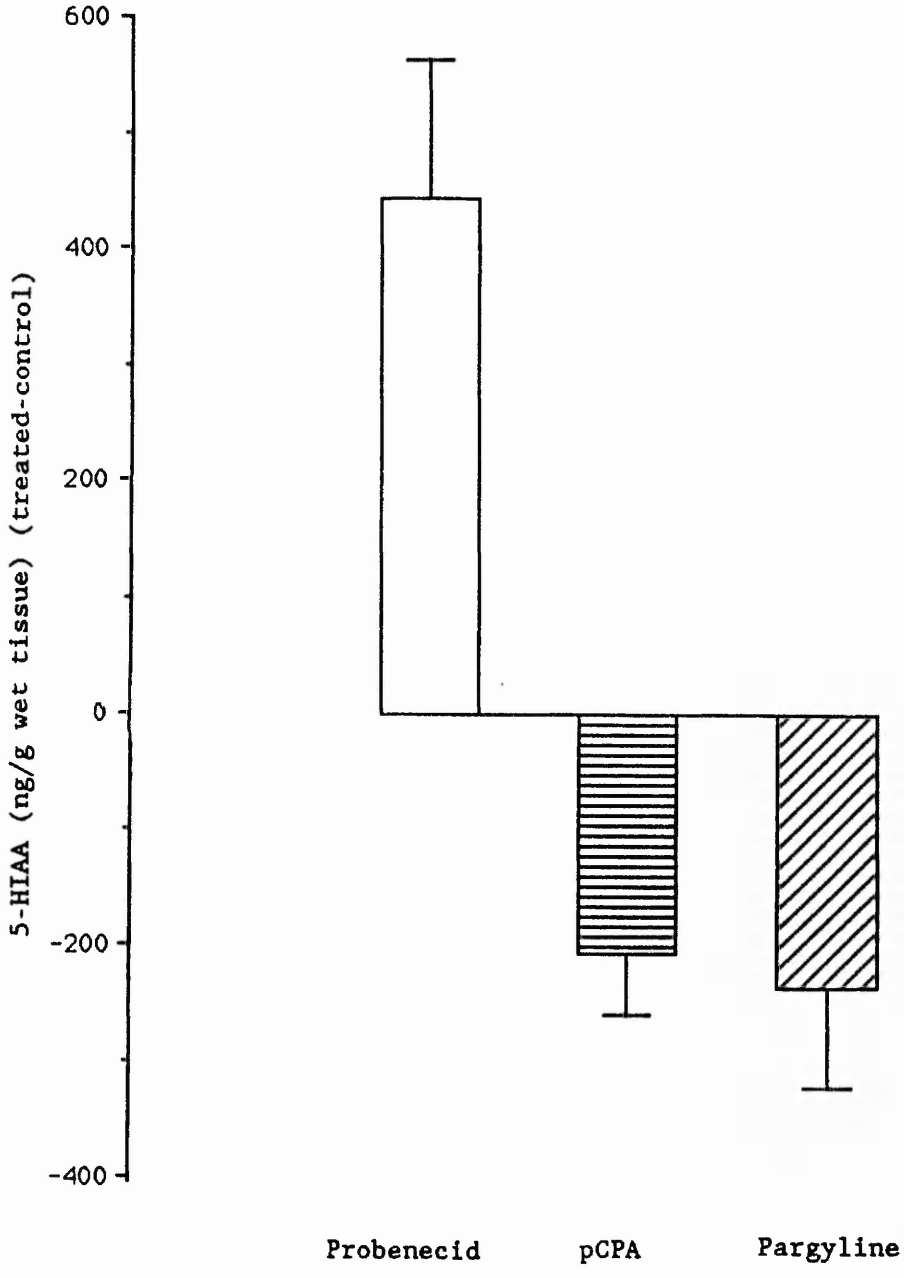

600
400
200
0
-200
-400
5-HIAA (ng/g wet tissue) (treated-control)
Probenecid
pCPA
Pargyline

Fig.R.6. Effect of Urethane Anaesthesia on Rat Whole Brain 5-HT and 5-HIAA Levels.

Rats were treated with urethane (1.6 $g.kg^{-1}$ 50% ip and 50% sc 90 min before sacrifice) (treated). Control rats received no treatment (control). The brains were then assayed for the determination of 5-HT and 5-HIAA concentrations.

Graph shows the mean difference (95% CI); see 2.7

n = 4-29

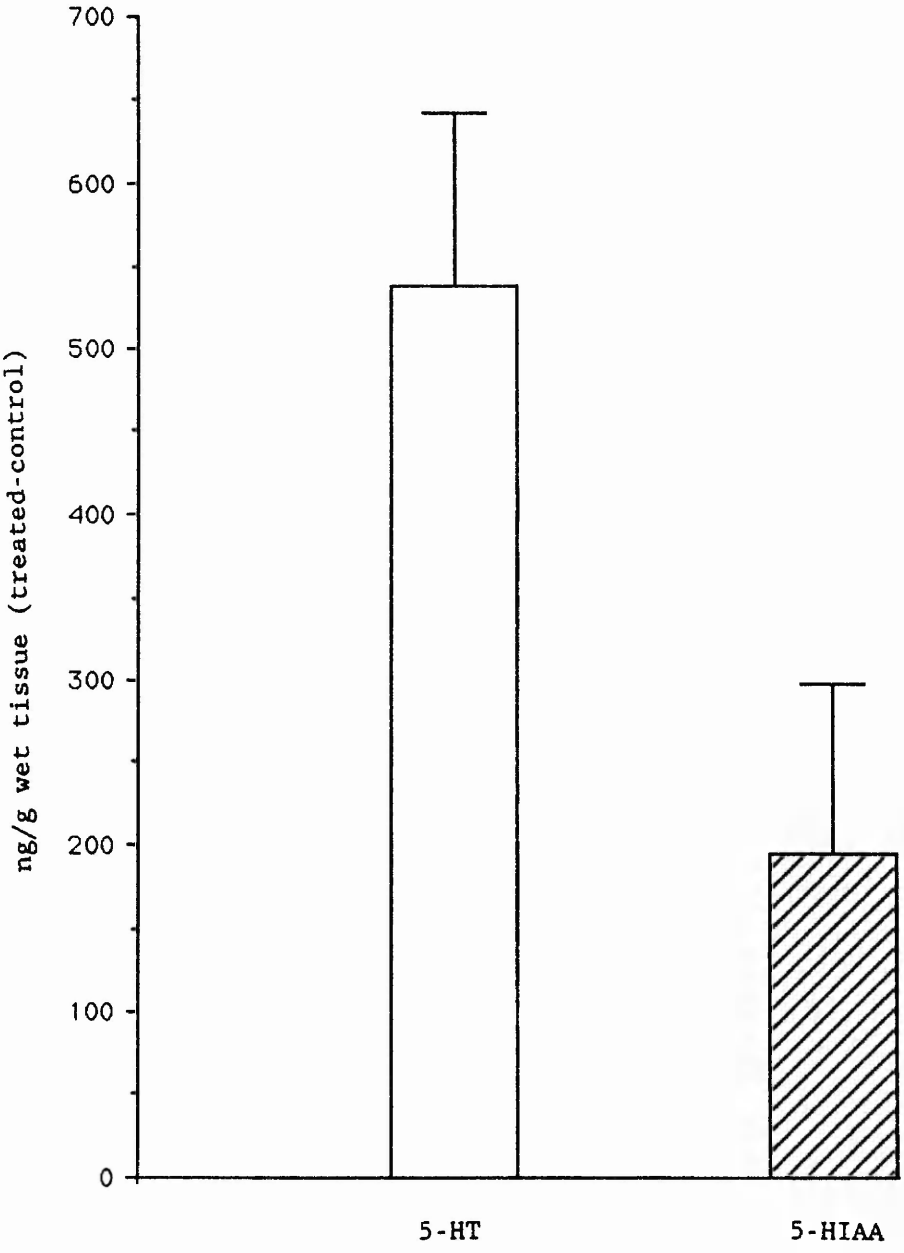

700
600
500
400
300
200
100
0
ng/g wet tissue (treated-control)
5-HT
5-HIAA

Fig.R.7. Effect of Treatment with SCT on Rat Whole Brain 5-HT Levels.

Urethane anaesthetised rats were treated with sCT (5 $U.kg^{-1}$ icv) (treated) or the appropriate vehicle (control) for varying times before sacrifice. The brains were then assayed for the determination of 5-HT concentration.

Graph shows the mean difference (95% CI); see 2.7

n = 5-6

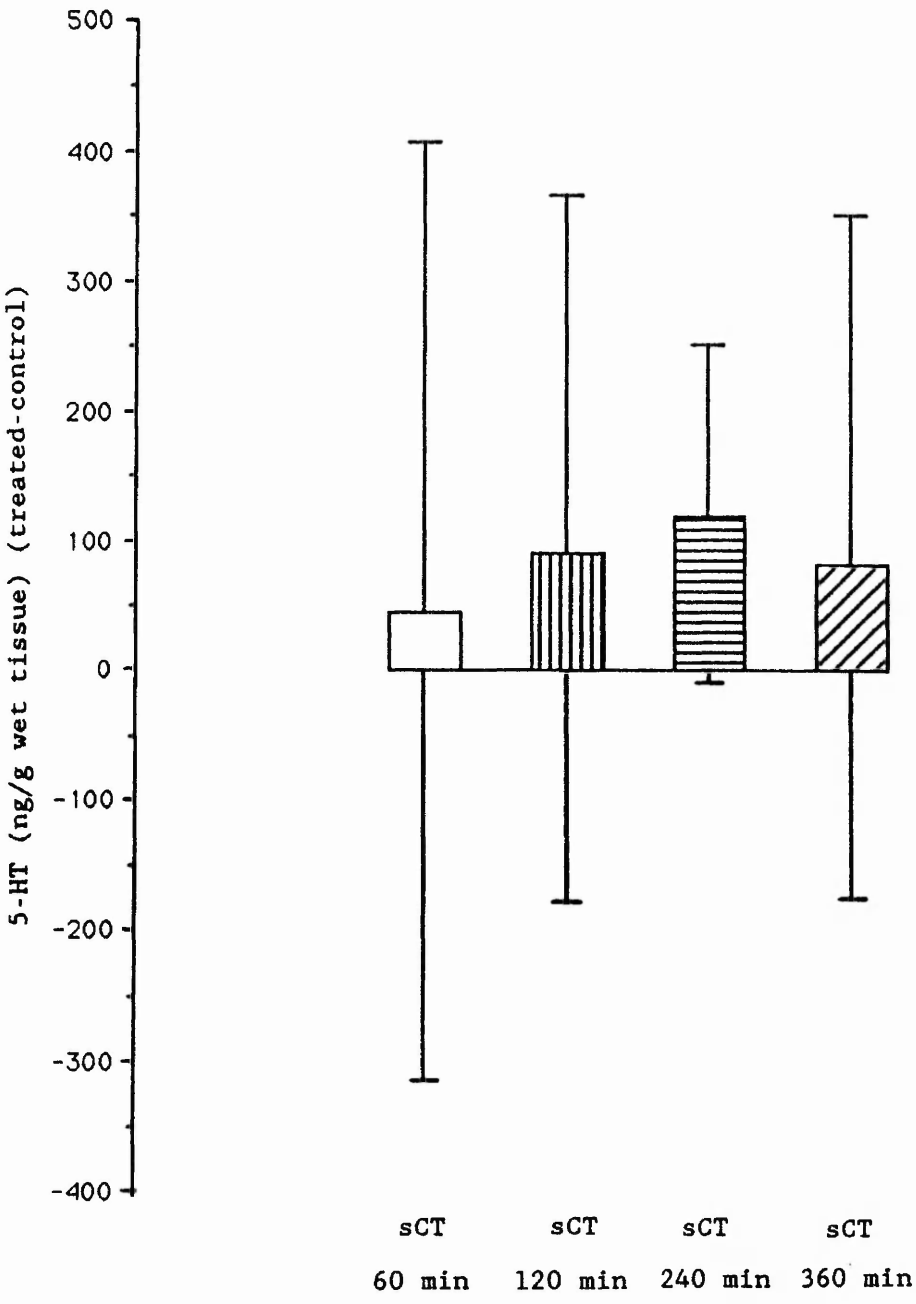
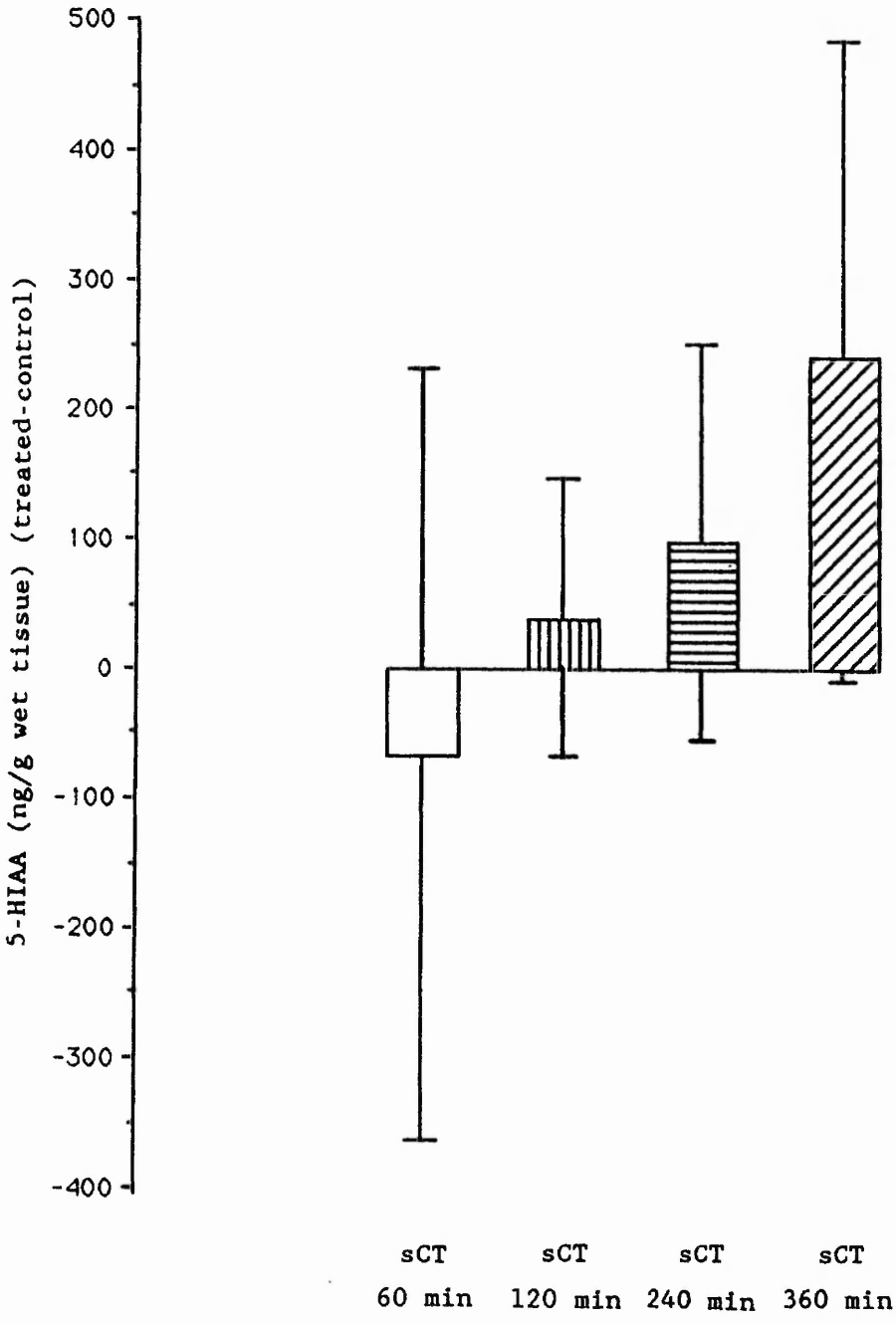

500
400
300
200
100
0
-100
-200
-300
-400
5-HT (ng/g wet tissue) (treated-control)
sCT
60 min
sCT
120 min
sCT
240 min
sCT
360 min

Fig.R.8. Effect of Treatment with SCT on Rat Whole Brain 5-HIAA Levels.

Urethane anaesthetised rats were treated with sCT (5 $U.kg^{-1}$ icv) (treated) or the appropriate vehicle (control) for varying times before sacrifice. The brains were then assayed for the determination of 5-HIAA concentration.

Graph shows the mean difference (95% CI); see 2.7

n = 5-6


500
400
300
200
100
0
-100
-200
-300
-400
5-HIAA (ng/g wet tissue) (treated-control)
sCT
60 min
sCT
120 min
sCT
240 min
sCT
360 min

4. CARDIOVASCULAR EFFECTS OF CALCITONIN

4.1 INTRODUCTION

4.1a Calcitonin in Endotoxin Shock

It has been recognised for many years that a profound state of systemic hypotension and shock will accompany the existence of a significant concentration of gram negative E.coli endotoxin in the blood of mammals. The precise mechanism(s) responsible for this hypotensive episode are not well understood, but many vasoactive mediators have been implicated in the pathophysiology of endotoxin shock. These include the opioids, prostaglandins, histamine, kinins, 5-HT, and VIP. (Bond, 1985)

Opioid peptides and receptors are widely distributed in sites important for cardiovascular function and homeostasis. Furthermore, endorphins have been implicated in various shock states, since opiate receptor antagonists have improved haemodynamics, metabolism, and survival. Holaday and Faden (1978) observed that naloxone (10 $mg.kg^{-1}$ iv) restored MAP and pulse pressure to pretreatment values in conscious rats subjected to endotoxin (4 $mg.rat^{-1}$ iv). Naloxone pre-treatment was also shown to abolish the decline in blood pressure after endotoxin administration. In a later study, Faden and Holaday (1980) demonstrated the stereospecificity of naloxone's effect after endotoxin administration. (-)Naloxone significantly increased MAP whereas (+)naloxone had no effect, thus leading to the conclusion that the blood pressure changes are mediated, in part, through opiate receptors.

Many other workers have confirmed the above results in dogs (Reynolds et al. 1980; Raymond et al. 1981; Toth et al. 1984). In a more elaborate study, Janssen and Lutherer (1980) looked at the effect of continuous administration of naloxone (approx. 1 $\mu g.kg^{-1}.min^{-1}$) in anaesthetised dogs, by ventriculocisternal perfusion, on the changes in blood pressure observed with endotoxin challenge. Naloxone treatment resulted in a significant increase in MAP and pulse pressure. The authors suggested that naloxone can exert beneficial effects after endotoxin via a central mechanism since ventriculocisternal perfusion excludes most of the drug from the circulation.

It has been demonstrated that sCT and naloxone exert a pressor effect in rats rendered hypotensive by haemorrhage (Bates et al. 1983b). Since, as previously stated, naloxone exerts a pressor effect in rats subjected to endotoxin shock it was of interest to investigate whether sCT exerts a similar pressor effect.

4.1b Cardiovascular Effects of Calcitonin

It has been demonstrated that central administration of sCT to mice produces antinociception which resembles that produced by opiates (Bates et al. 1981b). It was, therefore, of interest to determine if sCT produces opiate-like effects in other biological models known to respond to opiate agonists and antagonists.

The cardiovascular system is extremely sensitive to both exogenous and endogenous opiate agonists. This is demonstrated by iv infusion of morphine (Evans et al. 1952) or β-endorphin (Lemaire et al. 1978)

which results in a profound systemic hypotension and bradycardia. The opiate antagonist, naloxone, has been shown to reverse the hypotension observed in rats after endotoxin administration (Holaday and Faden, 1978) and after haemorrhage (Faden and Holaday, 1979). Thus, naloxone (1 $mg.kg^{-1}$ iv) significantly increased both blood pressure and survival in conscious rats subjected to hypovolaemic shock. This has subsequently been confirmed in the rat and other species (Schadt and York. 1981; Feuerstein et al. 1981; Toth et al. 1982).

In contrast to the findings in shock, it has been demonstrated that naloxone (10 $mg.kg^{-1}$ iv) has no effect on blood pressure in the normotensive rat (Holaday and Faden, 1978). Conversely, Schadt and York (1981) demonstrated that naloxone (5 $mg.kg^{-1}$ iv) resulted in a short, but significant, increase in blood pressure in normotensive conscious rabbits. Differences in results can be attributed to species variation, the anaesthetic or recuperative state of the animal, and the doses of naloxone used (5 $mg.kg^{-1}$ iv compared with 10 $mg.kg^{-1}$ iv).

Calcitonin has been shown to display cardiovascular effects (see below) and it has thus been suggested that the hormone may play a role in the mechanism of cardiovascular regulation (Bates et al. 1984c). Aldred et al. (1976) observed an antihypertensive effect of sCT in rat chronic hypertension induced by desoxycorticosterone acetate (DOCA) administration and a high salt intake. Takayangi et al. (1983) also reported that eel calcitonin (eCT) (sc) lowers the blood pressure in DOCA/saline-hypertensive and spontaneously hypertensive rats, but not in normotensive Wistar rats. Calcitonin has also been shown to

antagonise PTH-induced vasodilator responses in the hepatic and renal circulation of the dog (Charbon and Pipier, 1972) and to reduce skeletal blood flow in patients with Paget's disease (Wootton et al. 1978).

Bates et al.(1983b) described the acute effects of sCT on MAP. They demonstrated that iv administered sCT (0.1, 1 and 10 $U.kg^{-1}$) exerts a dose-dependent pressor effect in urethane anaesthetised rats rendered hypotensive by haemorrhage, but not in anaesthetised normotensive rats. Clementi et al.(1986) confirmed the lack of effect of iv sCT (1,2 and 4 $U.kg^{-1}$) on blood pressure of conscious normotensive rats and, in addition, observed no effect of intra-muscularly administered sCT (5,10 and 20 $U.kg^{-1}$) in the same model. The pressor effect of sCT was absent in pithed rats which led Bates et al. (1983b) to conclude that the response is probably not a result of direct peripheral vasoconstriction. In a later study, Bates et al. (1984a) investigated the effect of iv sCT (10 $U.kg^{-1}$) on MAP in urethane anaesthetised haemorrhaged rats after chemical sympathectomy, bilateral vagotomy and vasopressin antagonism. The latter two treatments had no effect on the response to sCT, but chemical sympathectomy significantly attenuated the response, thus suggesting that an increase in sympathetic tone, rather than modification of vagal afferents or the secretion of vasopressin, may be involved.

Bates et al. (1984c) extended their investigations to the effect of centrally administered sCT (0.1, 1 and 10 $U.kg^{-1}$) on MAP of urethane anaesthetised rats, both normotensive and those subjected to haemorrhage. Salmon CT (icv) was found to exert a dose-dependent

pressor effect in both models, however the response was greater in haemorrhaged rats. Unlike the response to iv sCT, the response to icv sCT was not affected by chemical sympathectomy. Clementi et al. (1986) confirmed the increase in blood pressure after icv sCT (0.4 $U.rat^{-1}$) in normotensive conscious rats.

4.1c Influence of Anaesthetics on the Cardiovascular System

Anaesthesia is often required for cardiovascular research. However, general anaesthetic agents exert a variety of influences on circulation, respiration, and metabolism. These effects are evident in normal laboratory animals but appear to be magnified when shock is present. It is therefore not surprising that conflicting results have been obtained by investigators studying the cardiovascular effects of anaesthesia. The anaesthetics used in the following experiments are urethane (1.6 $g.kg^{-1}$, 50% ip and 50% sc) and sodium pentobarbitone (60 $mg.kg^{-1}$ ip).

Carruba et al. (1987) demonstrated that urethane (1.2 $g.kg^{-1}$ ip) had no effect on MAP or heart rate in rats, but was associated with an immediate and sustained increase in both plasma adrenaline and noradrenaline concentrations. They concluded that urethane activates both the neuronal and adrenomedullary components of the sympathetic nervous system. Urethane administration has also been associated with an increased haematocrit (Van der Meer et al. 1975), hypersecretion of ACTH from the pituitary gland (Spriggs and Stockham, 1964), reductions in renal blood flow and glomerular filtration rates (Gumbleton et al. 1987a), and hyperglycaemia (Reinart, 1964).

Gilmore (1965) showed that pentobarbitone (30 $mg.kg^{-1}$ iv) had no effect on MAP, but was associated with tachycardia and decreased cardiac output, haematocrit, and rectal temperature in normotensive dogs. Best et al. (1984) observed that iv administration of pentobarbitone (30 $mg.kg^{-1}$ followed by an infusion of 0.1 - 0.2 $mg.kg^{-1}.min^{-1}$) in normotensive dogs was associated with a consistent fall in plasma noradrenaline due to a decline in release rate into plasma. The effect of pentobarbitone (25 $mg.kg^{-1}$ iv) on the response to progressive haemorrhage in dogs was investigated by Zimpfer et al. (1982). It was shown that anaesthetised dogs were less able to maintain MAP than were conscious dogs, and that this was consistent with a defect in the baroreceptor reflex system. Anaesthesia also resulted in a greater reduction in cardiac output. Anaesthetised dogs responded to haemorrhage with a smaller rise in plasma noradrenaline and no increase in plasma adrenaline, which the authors suggested pointed to a defect in the response of the adrenal medulla. An increase in plasma renin activity was also observed after anaesthesia, which had previously been illustrated in normotensive rats by Pettinger et al. (1975).

Several investigators have compared the cardiovascular effects of urethane and pentobarbitone. Buelke-Sam et al. (1978) demonstrated that urethane anaesthesia (1 $g.kg^{-1}$ ip) produced minimal physiological variation in normotensive rats. Conversely, pentobarbitone anaesthesia (40 $mg.kg^{-1}$ ip) resulted in a noticeable and progressive decrease in heart rate, systolic blood pressure, diastolic blood pressure, and pulse pressure. Gumbleton et al. (1987b) showed that urethane anaesthesia (1.75 $g.kg^{-1}$ ip) in normotensive rats resulted in a lower MAP when compared to pentobarbitone anaesthetised rats (67 $mg.kg^{-1}$ ip). However, plasma renin activity was increased after

urethane compared with pentobarbitone. Finally, Folle and Levesque (1976) illustrated a significant increase in systolic blood pressure and PCO_2 and a significant decrease in respiration rate and pH after pentobarbitone anaesthesia (50 $mg.kg^{-1}$ ip) in rats. However, after urethane (1.25 $g.kg^{-1}$ ip) they observed no change in systolic blood pressure and respiration rate, but an increase in PCO_2 and a decrease in pH.

The effects of opioid peptides on blood pressure and heart rate can be severely modified by anaesthetic agents. Thus, Sander et al. (1982) reported that leucine-enkephalin (35 $\mu g.kg^{-1}$ iv) increases cardiovascular parameters in the conscious dog, whereas after pentobarbitone-induced anaesthesia, the cardiovascular parameters are depressed. This suggests that barbiturate anaesthesia can reverse the cardiovascular stimulatory activity of iv administered leucine-enkephalin.

It would appear that naloxone exerts a pressor effect in animals subjected to haemorrhage and those subjected to endotoxin shock. Since sCT has also been shown to exert a pressor effect in rats rendered hypotensive by haemorrhage, the following experiments were designed to investigate whether sCT exerts a similar effect in rats during endotoxin shock. Experiments were also conducted to confirm the aforementioned pressor effects of sCT during haemorrhage and to extend these observations to the effect, if any, on heart rate. It was also of interest to determine the involvement of the anaesthetic agents used in the above experiments by comparing results from urethane anaesthetised rats with those from pentobarbitone anaesthetised rats.

4.2 RESULTS

NB. Results are expressed as mean difference, ie treated - control (95% CI)

4.2a Salmon CT in Endotoxin Shock

1) Effect of iv endotoxin infusion on MAP and heart rate of pentobarbitone anaesthetised rats.

Intravenous infusion of endotoxin (1 $mg.kg.^{-1}$ hr^{-1}) resulted in a rapid, statistically significant decrease in MAP. MAP then remained at this hypotensive level for the duration of the experiment. AUC was significantly reduced after endotoxin treatment relative to control by -2615 (-4427, -803) mmHg.min (mean difference (95% CI); $p< 0.01$) (see fig.R.9). Heart rate was significantly increased from 20 to 30 min of endotoxin infusion. Although the AUC was not significantly increased, it can be seen by the 95% CI that there is a trend for an increase in heart rate during endotoxin infusion in pentobarbitone anaesthetised rats (see fig.R.10).

2) Effect of iv naloxone on MAP and heart rate of pentobarbitone anaesthetised rats during endotoxin infusion.

Intravenous naloxone administration to rats subjected to endotoxin shock produced a statistically significant increase in MAP, which was maintained for the duration of the experiment (see fig.R.11a). This is demonstrated in the increase in AUC, relative to control, of 1766 (316, 3216) mmHg.min ($p< 0.05$). There were no clear effects on heart rate after naloxone (see fig.R.12a).

3) Effect of iv sCT on MAP and heart rate of pentobarbitone anaesthetised rats during endotoxin infusion.

The administration of sCT (iv) to rats subjected to endotoxin shock

resulted in a significant decrease in MAP 10 and 25 min post administration. The AUC, relative to control, of -590 (-1747, 568) mmHg.min was not statistically significant, however, it can be seen by the 95% CI that there is a trend for an overall decrease in MAP after sCT administration (see fig.R.11b). Salmon CT resulted in a statistically significant decrease in heart rate which is reflected in the decrease in AUC, relative to control, of -3181 (-5698, -663) beats ($p< 0.05$) (see fig.R.12b).

4.2b Salmon CT in Normotensive Rats and those Subjected to Haemorrhage

1) Effect of iv sCT on MAP and heart rate of pentobarbitone anaesthetised rats subjected to haemorrhage.

As can be seen in fig.R.13a, the administration of 20 $U.kg^{-1}$ sCT (iv) to pentobarbitone anaesthetised haemorrhaged rats had no significant effect on MAP as shown by the AUC, relative to control, of -208 (-702, 286) mmHg.min. There were no significant effects on heart rate (see fig.R.14a). The administration of 10 $U.kg^{-1}$ sCT (iv) also had no significant effect on MAP (see fig.R.13b) or heart rate (see fig.R.14b).

2) Effect of iv sCT on MAP and heart rate of urethane anaesthetised rats subjected to haemorrhage.

Intravenous administration of sCT to urethane anaesthetised rats subjected to haemorrhage resulted in a dose-dependent increase in MAP lasting for 40 min, 20 min and 5 min after 20 $U.kg^{-1}$ sCT, 10 $U.kg^{-1}$ sCT and 1 $U.kg^{-1}$ sCT respectively (see figs.R.15a, b and c). Only the administration of 20 $U.kg^{-1}$ sCT (iv), however, resulted in a

statistically significant increase in AUC, relative to control, of 577 (264, 889) mmHg.min ($p< 0.01$), although the AUC relative to control after 10 $U.kg^{-1}$ sCT (iv) almost reached significance. Heart rate, as demonstrated by the AUC, was not statistically significantly increased after sCT (1 $U.kg^{-1}$, 10 $U.kg^{-1}$ or 20 $U.kg^{-1}$), however, it can be seen from the 95% CI that there is a clear trend for an increase in heart rate after sCT (iv) (see figs.R16a, b and c).

3) Effect of iv sCT on MAP and heart rate of urethane anaesthetised normotensive rats.

The administration of iv sCT to urethane anaesthetised normotensive rats resulted in a statistically significant increase in MAP 5 min post administration. The AUC's, however, were not statistically significantly increased (see figs.R.17a and b). There were no significant effects on heart rate after iv sCT in urethane anaesthetised normotensive rats (see figs.R.18a and b).

4) Effect of icv sCT on MAP and heart rate of urethane anaesthetised rats subjected to haemorrhage.

Intracerebroventricular administration of sCT (5 $U.kg^{-1}$) to urethane anaesthetised rats rendered hypotensive by haemorrhage resulted in an immediate and dramatic, statistically significant increase in MAP which was maintained for the duration of the experiment (see fig.R.19a). There was a statistically significant increase in AUC, relative to control, of 1492 (937, 2047) mmHg.min ($p< 0.001$). The lower dose of sCT (1 $U.kg^{-1}$ icv) had no significant effects on MAP, however, there was a trend for an increase in MAP (see fig.R.19b). 5 $U.kg^{-1}$ sCT (icv) in urethane anaesthetised haemorrhaged rats led to a

gradual, statistically significant increase in heart rate (see fig.R.20a). The increase in AUC, relative to control, of 3411 (2508, 4313) beats after sCT was statistically significant ($p< 0.001$). 1 $U.kg^{-1}$ sCT (icv) had no significant effects on heart rate (see fig.R.20b).

5) Effect of icv sCT on MAP and heart rate of urethane anaesthetised normotensive rats.

The administration of sCT (5 $U.kg^{-1}$ icv) to urethane anaesthetised normotensive rats resulted in an immediate and statistically significant increase in MAP as shown by the AUC, relative to control, of 1476 (791, 2160) mmHg.min ($p< 0.001$)(see fig.R.21a). As in haemorrhaged rats, 1 $U.kg^{-1}$ sCT (icv) had no significant effect on MAP in normotensive rats (see fig.R.21b). Figs.R.22a and b show the effect of sCT (icv) on heart rate in urethane anaesthetised normotensive rats. 5 $U.kg^{-1}$ sCT led to a gradual, statistically significant increase in heart rate. This increase in heart rate was maintained for the duration of the experiment. Heart rate was also statistically significantly increased after 1 $U.kg^{-1}$ sCT (icv). The AUC, relative to control, was significantly increased after both 5 $U.kg^{-1}$ sCT (3538 (2233, 4843) beats; $p< 0.001$) and 1 $U.kg^{-1}$ sCT (1073 (292, 1859) beats; $p< 0.01$).

6) Effect of haemorrhage on the cardiovascular response to sCT, both iv and icv

The administration of 20 $U.kg^{-1}$ sCT (iv) and 1 $U.kg^{-1}$ sCT (icv) resulted in a statistically significantly greater increase in MAP after haemorrhage than in normotensive rats. The response to 10 $U.kg^{-1}$

sCT (iv) and 5 $U.kg^{-1}$ sCT (icv) was not significantly different between rats subjected to haemorrhage and normotensive rats (see fig.R.23). On the other hand, the administration of 10 $U.kg^{-1}$ sCT (iv), 1 $U.kg^{-1}$ sCT (icv) and 5 $U.kg^{-1}$ sCT (icv) led to a greater increase in heart rate in rats rendered hypotensive by haemorrhage than in normotensive rats; only after 20 $U.kg^{-1}$ sCT (iv) was the difference in response not significantly different (see fig.R.24).

4.3 DISCUSSION

4.3a Calcitonin in Endotoxin Shock

Infusion of endotoxin resulted in a profound hypotension and indeed this is true in all mammalian species studied. However, the time of its occurrence and the degree of response vary in different species. Even within the same species the pattern of induction of hypotension following endotoxin can be markedly variable (Stephen and Pietrowski, 1981). This is reflected in the large 95% CI's presented in fig.R.9. The mean response in MAP was an initial rapid fall followed by a sustained reduction. When looking at the individual data, however, some animals exhibited the above response, some exhibited abrupt falls followed by partial recovery followed by sustained falls and still others exhibited a slow gradual reduction in MAP. Holaday and Faden (1978) demonstrated two hypotensive episodes 10 and 45 min after the administration of a bolus iv injection of endotoxin into conscious rats; the blood pressure had returned to baseline values at 30 min and showed a gradual increase from 45 - 120 min. These workers also reported that the heart rate data tended to follow the blood pressure data (results not shown). This is not true in the present study in which no significant effect on heart rate after endotoxin was observed.

It would appear that sCT (iv) exerts no significant effect on MAP during endotoxin infusion,however, there is a clear trend towards a decrease in MAP. Salmon CT did exert a significant decrease in heart rate.

Quimby and Resnick (1985) studied the effect of toxic shock syndrome toxin type-1 (TSST-1, 100-140 $\mu g.kg^{-1}$ iv) on the blood pressure of baboons. They observed a reproducible hypotension within 3 h concomitant with decreased serum calcium ion levels and increased iCT levels. Baboons which had undergone bilateral thyroidectomy and supplementation with thyroxine were no longer susceptible to TSST-1-induced hypotension. When these baboons were given sCT (9-11 U slow iv infusion over 60 min) followed by iv TSST-1 they demonstrated a rapid and profound hypotension. The authors therefore concluded that iCT plays a role in the pathphysiology of TSST-1-induced toxic shock syndrome.

The effects on MAP after naloxone administration confirmed the results of other workers (Holaday and Faden, 1978; Faden and Holaday, 1980) suggesting an involvement of endorphins in the pathophysiology of endotoxin shock. MAP was not restored to pre-treatment values as was the case with Holaday and Faden (1978), but these workers administered a bolus iv injection of endotoxin of approximately 13 $mg.kg^{-1}$, as opposed to an iv infusion of 1 $mg.kg^{-1}.h^{-1}$ in the experiments presented in this thesis. Moreover, Holaday and Faden were using conscious rats, whereas the rats were anaesthetised in the experiments presented here. Naloxone had no effect on heart rate during endotoxin infusion. This is in agreement with Faden and Holaday (1980). In an earlier study (1978), these workers suggested that the heart rate

changes seen after endotoxin were blocked by pre-treatment with naloxone; the results were not published.

The mechanism of naloxone's action in endotoxin shock is unclear. In the study of Reynolds et al. (1980) an increased cardiac output (CO) without a change in total peripheral resistance (TPR) was observed. In the study of Toth et al. (1984) naloxone improved MAP transiently by increasing TPR. Naloxone in other shock models has been shown to increase TPR rather than by affecting other parameters (Schadt et al. 1982). This suggests that naloxone may have multiple effects in different species and in different shock models.

D'Amato and Holaday (1984) suggested that administration of naloxone for the treatment of circulatory shock may enhance pain by antagonising endogenous or exogenous opioid analgesia. These workers therefore looked at the effect of two selective opioid receptor antagonists, β-funaltrexamine (μ-antagonist) and M154,129 (δ-antagonist), administered centrally, on MAP during endotoxin administration and the antagonistic action on morphine analgesia. The μ-antagonist reversed morphine analgesia, but failed to alter the typical pattern of MAP changes during endotoxin shock whereas the δ-antagonist resulted in a significant and sustained increase in MAP during shock but failed to block morphine analgesia. They concluded that selective δ-antagonists may be of therapeutic value in reversing circulatory shock without altering the analgesic actions of endogenous or exogenous opioids.

It would appear that sCT has no therapeutic value in the treatment of endotoxin shock since the effects of peripherally administered sCT

differ from those of peripherally administered naloxone. Salmon CT administration results in a tendency for a decrease in blood pressure, and a statistically significant decrease in heart rate, whereas naloxone exerts a pressor effect with no effect on heart rate.

4.3b Calcitonin in Normotensive Rats and those rendered Hypotensive by Haemorrhage

The results presented above show that peripherally administered sCT (1, 10, and 20 $U.kg^{-1}$) exerts a statistically significant pressor effect in urethane anaesthetised rats rendered hypotensive by haemorrhage, but is devoid of effect in normotensive rats. This pressor response is accompanied by a tendency for an increase in heart rate. Conversely, central administration of sCT (5 $U.kg^{-1}$) leads to a pressor effect in both urethane anaesthetised normotensive rats and those subjected to haemorrhage, the response being maintained for at least 60 min. This pressor response is associated with an increase in heart rate. The pressor responses observed are in general agreement with those of Bates et al. (1983b; 1984a; 1984c).

A number of peptides (see below) are localised in brain areas that are known to be involved in blood pressure control. They have been demonstrated to exert different cardiovascular effects after central and peripheral administration. Thus, peripheral administration of bradykinin and substance P leads to vasodilator effects, whereas $angiotensin_2$ exerts a hypertensive effect. When administered centrally, bradykinin, substance P, $angiotensin_2$, renin, and vasopressin all exert pressor effects (Palkovits et al. 1981). The response to centrally administered sCT in normotensive rats would appear to be of comparatively long duration and potency. Calcitonin

gene-related peptide, the major proposed product of CT gene expression within the CNS, has also been found to be localised in areas of the brain involved in cardiovascular regulation. Fischer et al. (1983a) reported that icv CGRP increases MAP, heart rate, and plasma noradrenaline levels in conscious rats leading to the suggestion that CGRP evokes a centrally mediated increase in sympathetic tone. However, when administered systemically, CGRP evokes sustained falls in MAP in both conscious (Fischer et al. 1983a) and anaesthetised rats (Marshall et al. 1986). Fischer et al. (1983a) suggested that the underlying hypotensive effect of CGRP may be by generalised vasodilatation since marked hyperaemia was observed. The reason for the contradictory effects of iv sCT and iv CGRP is unclear.

In order to support the arterial pressure during haemorrhage the activity of the sympathoadrenal system is generally considered to increase giving increases in heart rate and blood levels of catecholamines (Hess et al., 1983). However, it is evident from the results presented in this thesis that haemorrhage leads to a decrease in heart rate both in urethane anaesthetised rats and pentobarbitone anaesthetised rats (see tables R.14(ii), R.16(ii) and R.20(ii)). This bradycardia may be due to the anaesthetic since urethane and pentobarbitone have been reported to attenuate baroreceptor reflex control of heart rate as judged by a decrease in the bradycardia seen after the pressor response to phenylephrine (Fluckiger et al. 1985). The attenuation of the baroreceptor reflex is more marked with urethane than with pentobarbitone. It would be of interest to investigate the effects of haemorrhage alone and sCT administered to normotensive- and haemorrhaged-conscious, freely moving rats, thus excluding anaesthetic effects.

It is noticeable that icv sCT resulted in increases in heart rate concomitant with pressor responses in both normotensive rats and those subjected to haemorrhage. This has also been observed after intracerebroventricular leucine-enkephalin; Schaz et al. (1980) demonstrated a marked attenuation of the vagal component of the baroreceptor reflex in enkephalin-treated animals. Moreover, several other neuropeptides have been reported to attenuate the baroreceptor reflex. Unger et al. (1981) observed an increase in heart rate with an increase in blood pressure after central administration of bradykinin in conscious normotensive and spontaneously hypertensive rats (SHR). The pressor responses to centrally injected substance P were dramatically increased in SHR when compared to normotensive Wistar Kyoto rats. However, differences in the blood pressure-increase associated heart rate responses to substance P in SHR and Wistar Kyoto rats, namely tachycardia in the hypertensive animals and bradycardia in the Wistar Kyoto rats were observed. This led Unger et al. (1981) to conclude that the central pressor action of substance P is buffered by the baroreceptor reflex in the normotensive Wistar Kyoto rats, but is not buffered in the SHR. This is confirmed by baroreceptor deafferentiation experiments (Unger et al. 1981). The increase in heart rate concomitant with the increase in blood pressure seen after sCT may be due to several factors:

- impaired baroreceptor reflex function after anaesthesia;
- an effect of sCT itself on baroreceptor reflex function;
- increased sympathetic output;
- resetting of the baroreceptors such that higher blood pressures elicit the appropriate heart rate response.

These would also explain the sustained increase in heart rate observed after sCT when the pressor response is declining. It would be of

interest to determine the effect of sCT in conscious rats and those subjected to sino-aortic denervation.

The pressor response and tachycardia observed after sCT administration is greater in urethane anaesthetised rats subjected to haemorrhage than in normotensive rats; indeed iv sCT has no effect in normotensive rats. Since during haemorrhage there is an increase in sympathetic discharge, as the reflexes strive to maintain arterial pressure, it may be hypothesised that sCT potentiates existing neuronal activity resulting in a lack of effect or a reduced effect in normotensive rats. The fact that icv sCT exerts an effect in normotensive rats suggests some central component of the response.

When rats anaesthetised with sodium pentobarbitone are rendered hypotensive by haemorrhage, iv sCT exerts no effect on either MAP or heart rate. On the contrary, there is a trend towards a depressor effect with a decrease in heart rate, although this is not statistically significant. It appears that the cardiovascular response to sCT is modified by the anaesthetic agent administered.

It is noticeable that administration of sodium pentobarbitone results in higher normotensive (pre-haemorrhage, pre-treatment) MAP's and heart rates (148-153 mmHg and 411-447 bpm; see tables R.13(i) and R.14(i) respectively) than those seen after urethane (89-116 mmHg and 339-404 bpm; see tables R.15(i), R.17, R.19(i), R.21 and tables R.16(i), R.18, R.20(i), R.22 respectively). Haemorrhage leads to a decrease in MAP and heart rate with the result that post-haemorrhage (pre-treatment) MAP's and heart rates are still higher after pentobarbitone (123-133 mmHg and 377-403 bpm; see tables R.13(ii) and

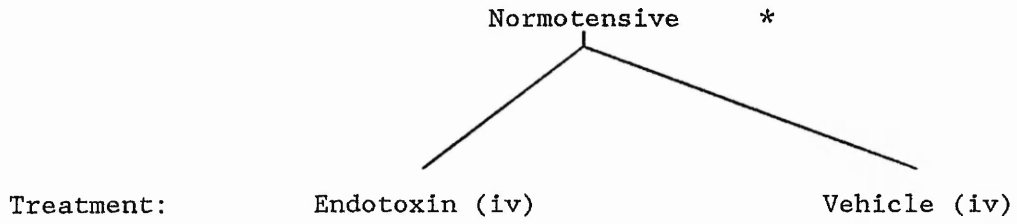
R.14(ii) respectively) compared with normotensive (pre-treatment) urethane anaesthetised rats. Gumbleton et al. (1987b) observed a decrease in MAP in normotensive rats after urethane compared to pentobarbitone. In contrast, Buelke-Sam et al. (1978) demonstrated that pentobarbitone lowers blood pressure in rats to a greater degree than urethane. Normotensive blood pressures and heart rates of conscious Sprague-Dawley rats would appear to lie between 100 and 120 mmHg and 400 and 450 bpm respectively (Farnebo et al. 1979; Aldred et al. 1976; Faden and Holaday, 1979). These MAP values are thus lower than those observed after sodium pentobarbitone anaesthesia, but higher than those seen after urethane anaesthesia. The heart rate values are very similar in conscious normotensive rats and those anaesthetised by sodium pentobarbitone, but are lower in those rats anaesthetised by urethane. Indeed Farnebo et al. (1979) found large differences between conscious rats and rats anaesthetised with pentobarbitone, both as regards circulatory adjustment and catecholamine release following haemorrhagic shock; the anaesthetised rats had much lower levels of plasma noradrenaline. Best et al. (1984) suggested that pentobarbitone can suppress sympathetic nervous system activity, an observation confirmed by Baum (1985). Conversely, Carruba et al. (1987) reported that urethane activates the sympathetic nervous system. Interestingly, the results presented here would indicate that the sympathetic nervous system is more active during pentobarbitone anaesthesia than urethane anaesthesia.

Since iv sCT has no effect in normotensive rats anaesthetised with urethane - a situation where the pre-treatment MAP is lower than that observed after haemorrhage (pre-treatment) in pentobarbitone

anaesthetised rats, and since iv sCT has no effect in haemorrhaged rats anaesthetised with pentobarbitone, it may be possible that pentobarbitone is masking the response to sCT suggesting that peripherally administered sCT only exerts a pressor effect and tachycardia at a sufficiently low MAP and heart rate, such as that seen after urethane. It is interesting that Aldred et al. (1976) observed no effect of sCT (sc) in DOCA-induced hypertension where the MAP ranged from 113-120 mmHg and the heart rate ranged from 314-333 bpm. However, at starting pressures of 166-191 mmHg and heart rates of 394-450 bpm, sCT resulted in a hypotensive response.

The effect of sCT on cardiovascular parameters during endotoxin shock and haemorrhage would appear to differ. Intravenous sCT exerts a pressor effect in urethane anaesthetised rats subjected to haemorrhage. Intracerebroventricular administration of sCT results in a pressor effect and an increase in heart rate in both urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. In pentobarbitone anaesthetised rats subjected to endotoxin shock, peripherally administered sCT results in a tendency for a decrease in MAP and a significant decrease in heart rate. It should be recognised, however, that the two models are not interrelated, since, during haemorrhage there is physical loss of blood and during endotoxin shock there is redistribution of blood. Furthermore, it has been shown that the administration of anaesthetics can have a profound effect on the host response to shock and administration of drugs.

Fig.R.9. Effect of Endotoxin Infusion on MAP of Pentobarbitone Anaesthetised Rats.



* denotes the stage of the experiment at which the MAP values depicted in table R.9 overleaf were determined.

Fig.R.9. Effect of Endotoxin Infusion on MAP of Pentobarbitone Anaesthetised Rats.

E.coli lipopolysaccharide (1 $mg.kg^{-1}.h^{-1}$) (treated) or vehicle (0.9% NaCl) (control) was continuously administered iv at a rate of 0.03 $ml.min^{-1}$ over 2 h.

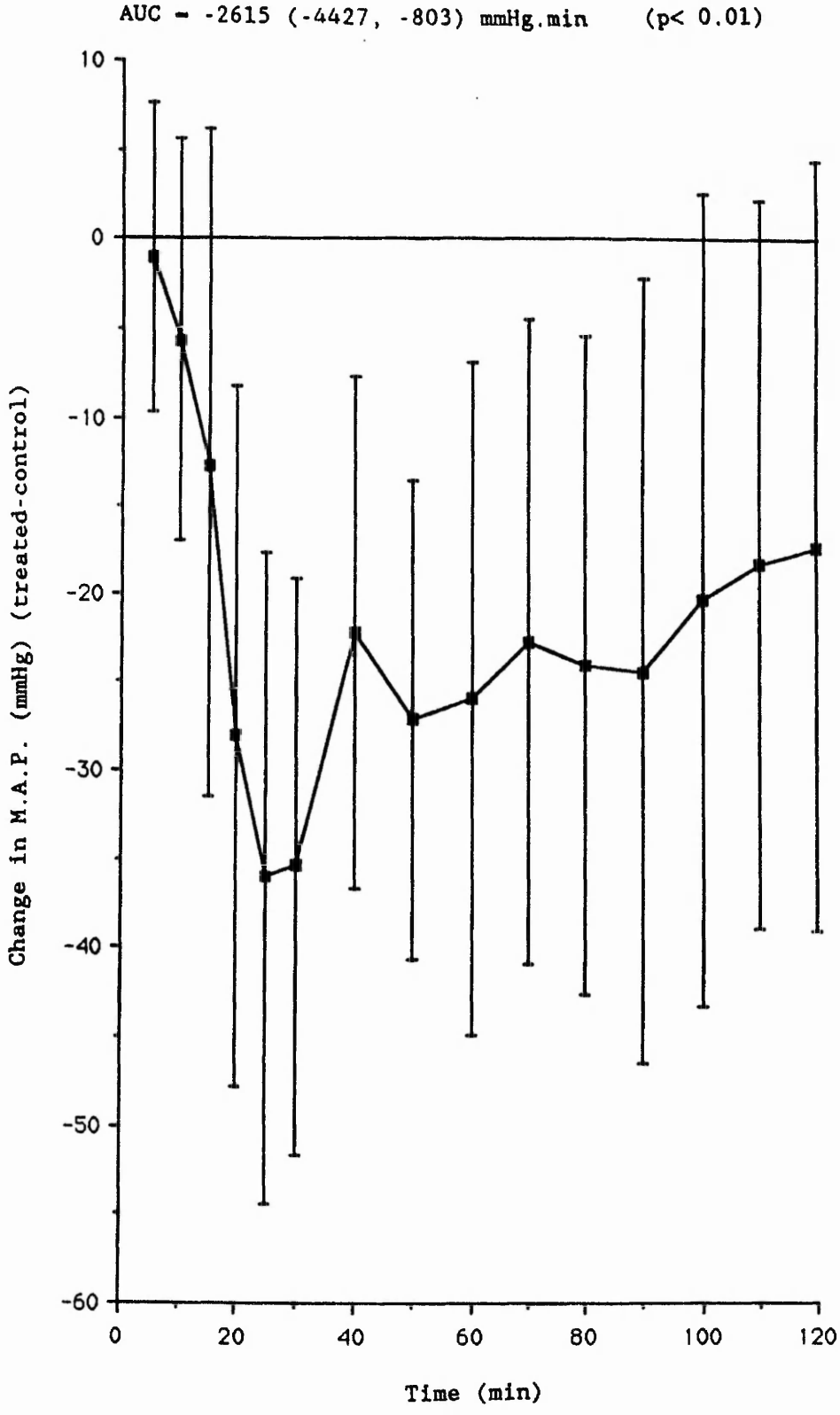
Table R.9 Pre-endotoxin/vehicle MAP's

Normotensive (t = 0 min)	Treatment:	Endotoxin	Vehicle
	MAP (mmHg):	171 (6)	161 (6)
	Mean difference (95% CI): 10 (-10, 30) NS		

MAP = mean (n)

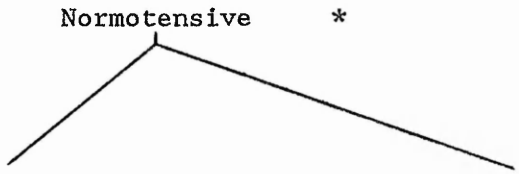
NS = not statistically significant

Graph shows mean difference (95% CI); see 2.7



Endotoxin/vehicle administered at t=0 min

Fig.R.10. Effect of Endotoxin Infusion on Heart Rate of Pentobarbitone Anaesthetised Rats.



Treatment: Endotoxin (iv) Vehicle (iv)

* denotes the stage of the experiment at which the heart rate values depicted in table R.10 overleaf were determined.

Fig.R.10. Effect of Endotoxin Infusion on Heart Rate of Pentobarbitone Anaesthetised Rats.

E.coli lipopolysaccharide (1 $mg.kg^{-1}.h^{-1}$) (treated) or vehicle (0.9% NaCl) (control) was continuously administered iv at a rate of 0.03 $ml.min^{-1}$ over 2 h.

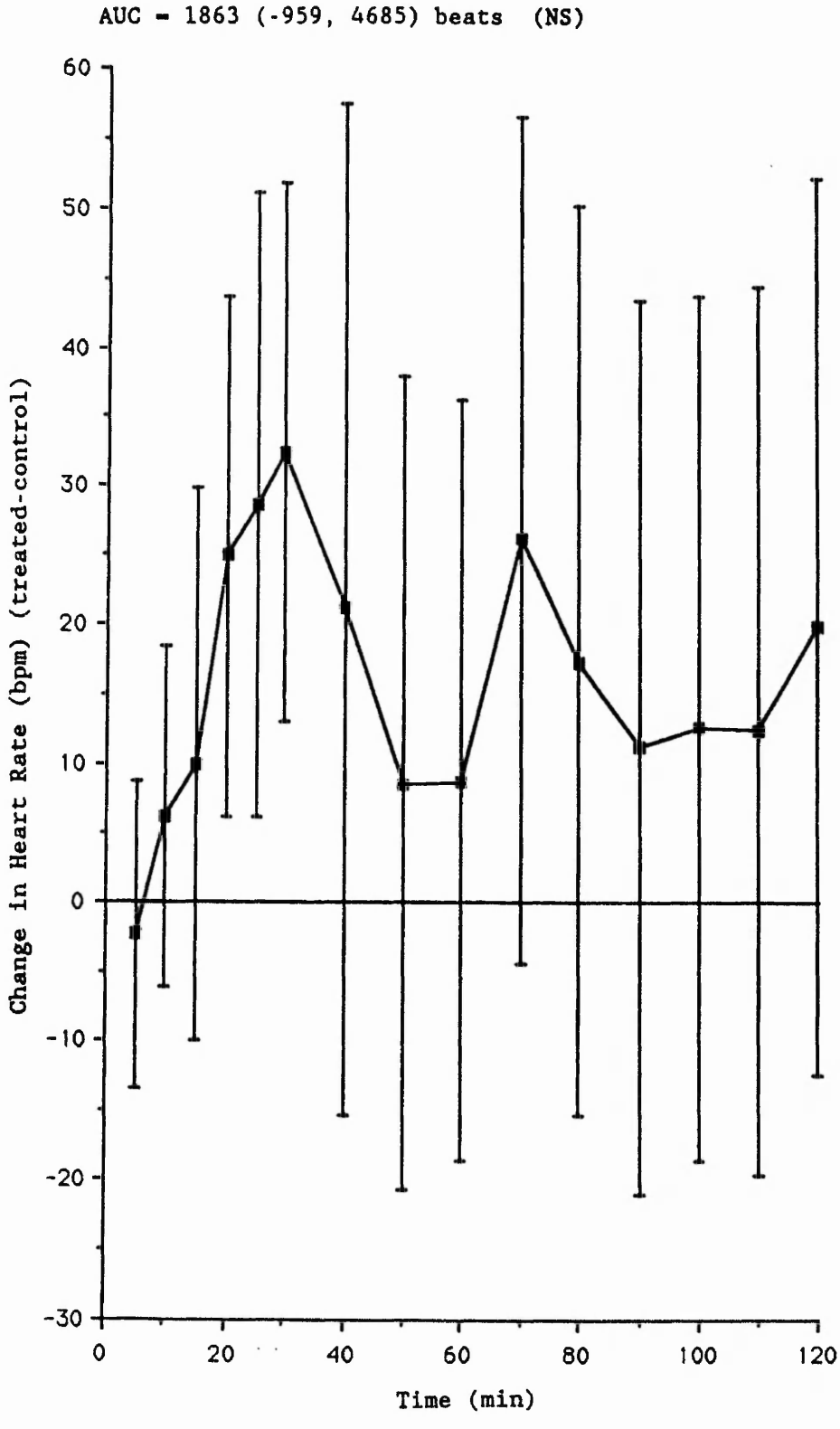
Table R.10 Pre-endotoxin/vehicle heart rates

Normotensive (t = 0 min)	Treatment:	Endotoxin	Vehicle
	Heart rate (bpm):	428 (6)	438 (6)
	Mean difference (95% CI): -10 (-39, 19) NS		

Heart rate = mean (n)

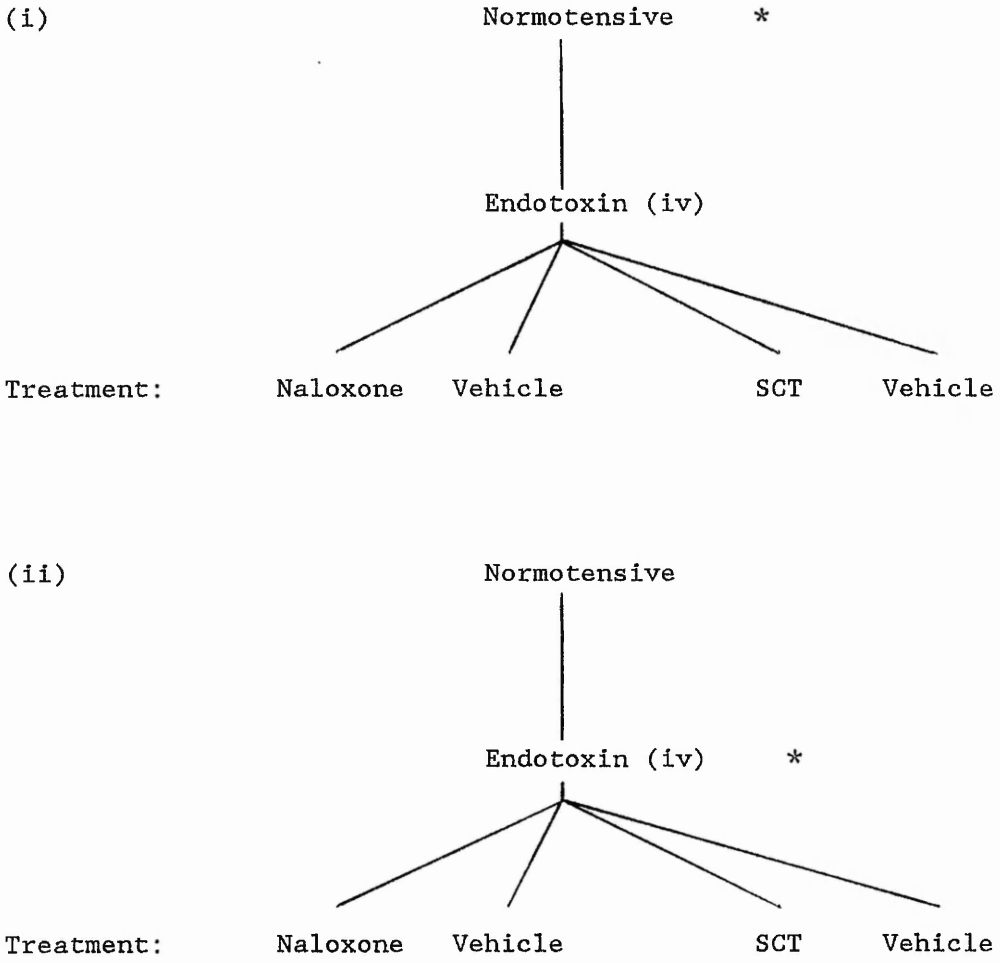
NS = not statistically significant

Graph shows mean difference (95% CI); see 2.7



Endotoxin/vehicle administered at t=0 min

Fig.R.11. Effect of Naloxone (iv) or SCT (iv) on MAP during Endotoxin Infusion in Pentobarbitone Anaesthetised Rats.



* denotes the stages of the experiment at which the MAP values depicted in tables R.11(i) and (ii) overleaf were determined.

Fig.R.11. Effect of Naloxone (iv) or SCT (iv) on MAP during Endotoxin Infusion in Pentobarbitone Anaesthetised Rats.

Naloxone (10 $mg.kg^{-1}$) (treated) (a), sCT (10 $U.kg^{-1}$) (treated) (b), or appropriate vehicle (control) was administered iv 25 min after the start of endotoxin infusion (1 $mg.kg^{-1}.h^{-1}$).

Table R.11(i) Pre-endotoxin, pre-treatment MAP's

Normotensive (t = -25 min)	Treatment:	Endotoxin + Naloxone	Endotoxin + Vehicle
	MAP (mmHg):	153 (6)	150 (6)
	Mean difference (95% CI): 3 (-21, 28) NS		
Normotensive (t = -25 min)	Treatment:	Endotoxin + SCT	Endotoxin + Vehicle
	MAP (mmHg):	159 (6)	163 (6)
	Mean difference (95% CI): -4 (-20, 13) NS		

Table R.11(ii) Post-endotoxin, pre-treatment MAP's

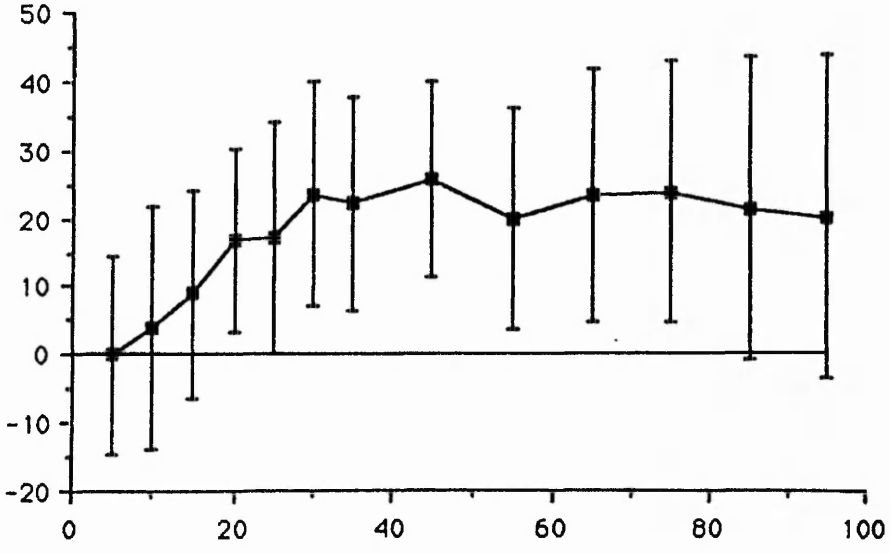
25 min post-endotoxin (t = 0 min)	Treatment:	Endotoxin + Naloxone	Endotoxin + Vehicle
	MAP (mmHg):	96 (6)	106 (6)
	Mean difference (95% CI): -10 (-35, 15) NS		
25 min post-endotoxin (t = 0 min)	Treatment:	Endotoxin + SCT	Endotoxin + Vehicle
	MAP (mmHg):	110 (6)	103 (6)
	Mean difference (95% CI): 7 (-7, 22) NS		

MAP = mean (n)

NS = not statistically significant

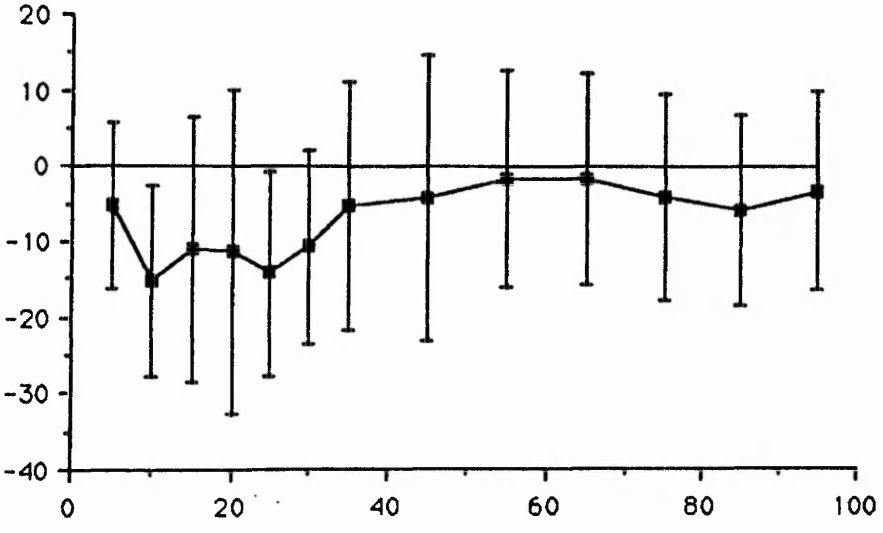
Graphs show mean difference (95% CI); see 2.7

a) AUC = 1766 (316, 3216) mmHg.min ($p < 0.05$)



Naloxone/vehicle administered at t=0 min

b) AUC = -590 (-1747, 568) mmHg.min (NS)

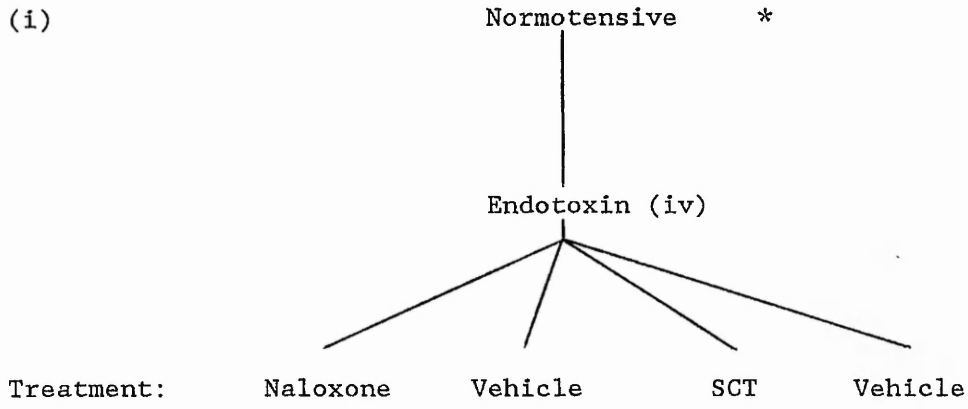


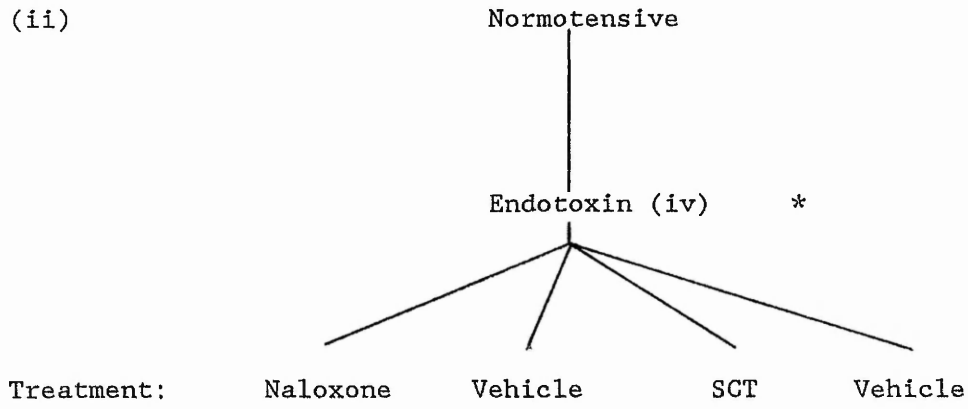
Change in M.A.P. (mmHg) (treated-control)

Time (min)

SCT/vehicle administered at t=0 min

Fig.R.12. Effect of Naloxone (iv) or SCT (iv) on Heart Rate during Endotoxin Infusion in Pentobarbitone Anaesthetised Rats.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.12(i) and (ii) overleaf were determined.

Fig.R.12. Effect of Naloxone (iv) or SCT (iv) on Heart Rate during Endotoxin Infusion in Pentobarbitone Anaesthetised Rats.

Naloxone (10 $mg.kg^{-1}$) (treated) (a), sCT (10 $U.kg^{-1}$) (treated) (b) or appropriate vehicle (control) was administered iv 25 min after the start of endotoxin infusion (1 $mg.kg^{-1}.h^{-1}$).

Table R.12(i) Pre-endotoxin, pre-treatment heart rates

Normotensive (t = -25 min)	Treatment:	Endotoxin + Naloxone	Endotoxin + Vehicle
	Heart rate (bpm):	448 (6)	447 (6)
	Mean difference (95% CI): 1 (-48, 51) NS		
Normotensive (t = -25 min)	Treatment:	Endotoxin + SCT	Endotoxin + Vehicle
	Heart rate (bpm):	461 (6)	440 (6)
	Mean difference (95% CI): 21 (-35, 77) NS		

Table R.12(ii) Post-endotoxin, pre-treatment heart rates

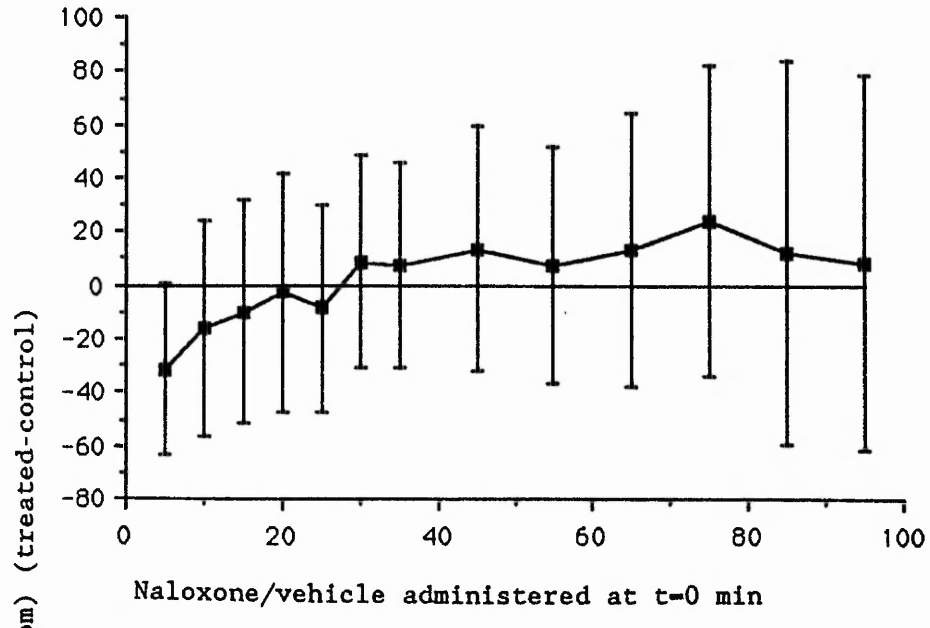
25 min post-endotoxin (t = 0 min)	Treatment:	Endotoxin + Naloxone	Endotoxin + Vehicle
	Heart rate (bpm):	416 (6)	431 (6)
	Mean difference (95% CI): -15 (-64, 34) NS		
25 min post-endotoxin (t = 0 min)	Treatment:	Endotoxin + SCT	Endotoxin + Vehicle
	Heart rate (bpm):	459 (6)	440 (6)
	Mean difference (95% CI): 19 (-28, 66) NS		

Heart rate = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

a) AUC = 489 (-3834, 4812) beats (NS)



b) AUC = -3181 (-5698, -663) beats ($p<0.05$)

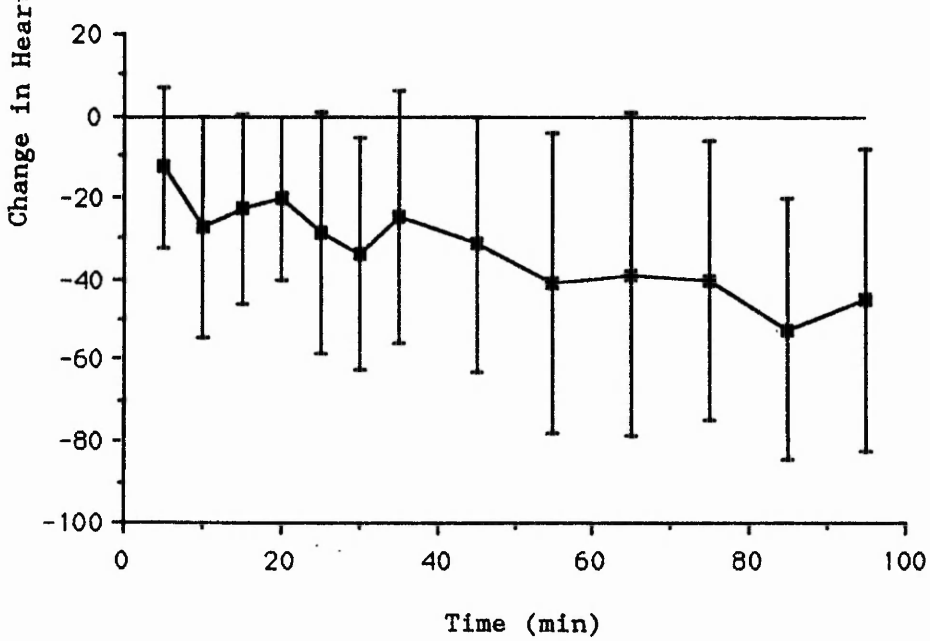
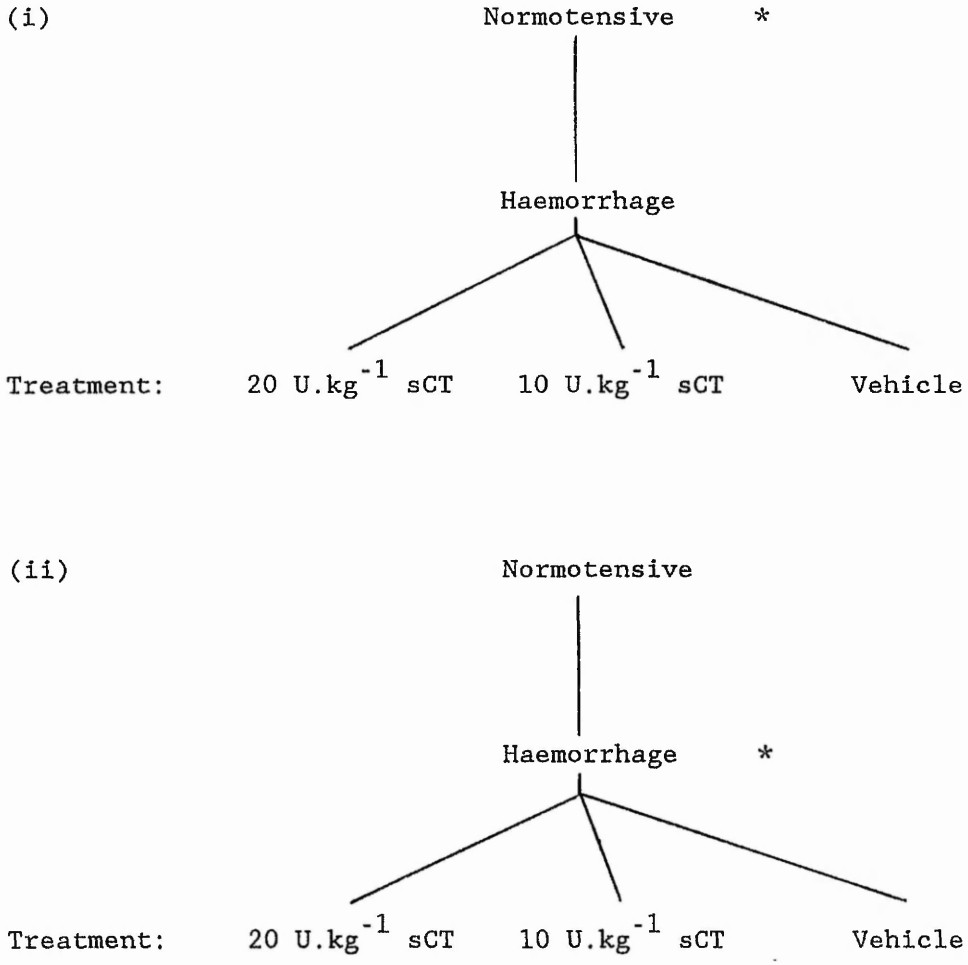


Fig.R.13. Effect of SCT (iv) on MAP of Pentobarbitone Anaesthetised Rats subjected to Haemorrhage.



* denotes the stages of the experiment at which the MAP values depicted in tables R.13(i) and (ii) were determined.

Fig.R.13. Effect of SCT (iv) on MAP of Pentobarbitone Anaesthetised Rats subjected to Haemorrhage.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered iv after haemorrhage.

Table R.13(i) Pre-haemorrhage, pre-treatment MAP's

Normotensive	Treatment:	20 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	148 (5)	153 (10)
	Mean difference (95% CI): -5 (-18, 9) NS		
Normotensive	Treatment:	10 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	161 (7)	153 (10)
	Mean difference (95% CI): 8 (-2, 18) NS		

Table R.13(ii) Post-haemorrhage, pre-treatment MAP's

Post-haemorrhage (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	123 (5)	127 (10)
	Mean difference (95% CI): -4 (-19, 11) NS		
Post-haemorrhage (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	129 (7)	127 (10)
	Mean difference (95% CI): 2 (-11, 15) NS		

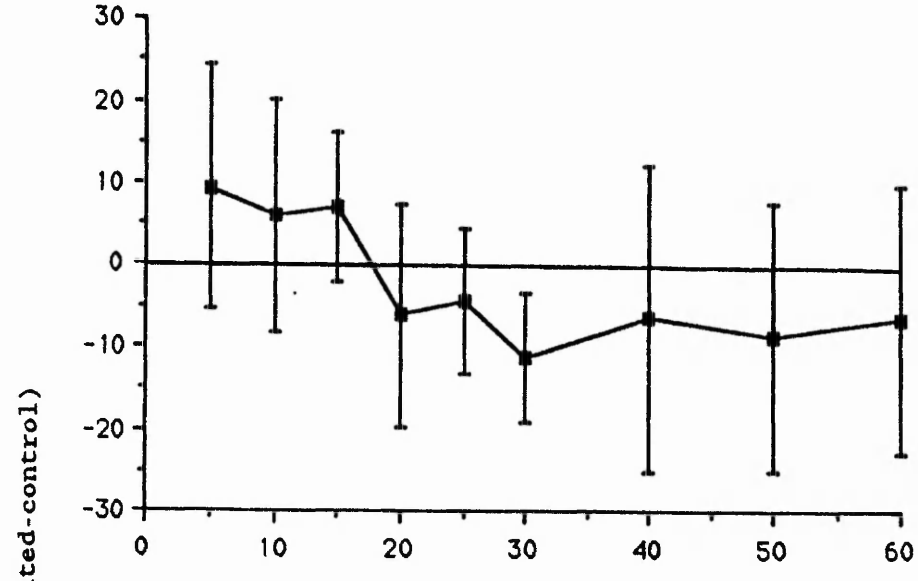
MAP = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

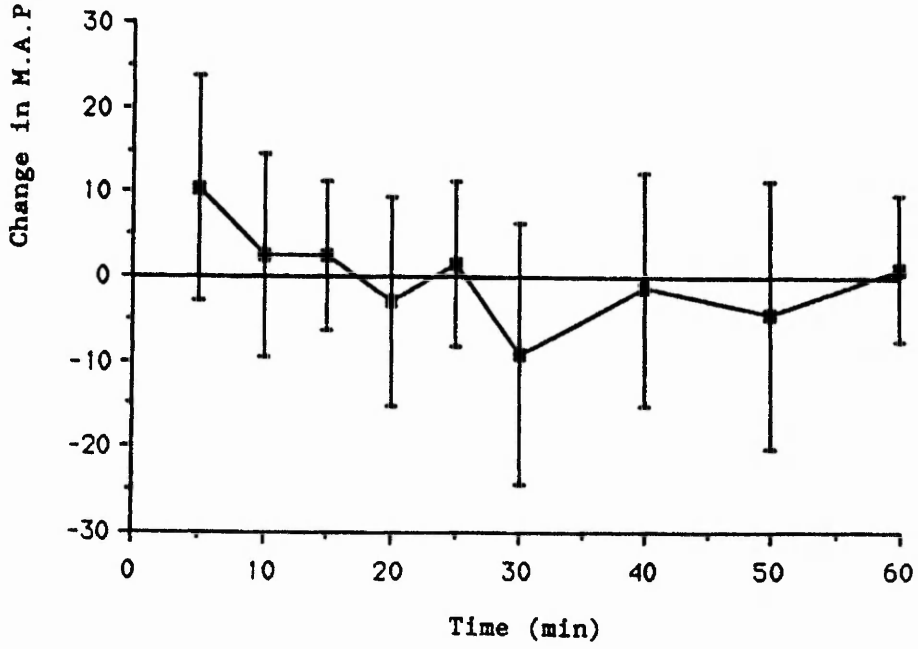
Vehicle results were pooled

a) AUC = -208 (-702, 286) mmHg.min (NS)



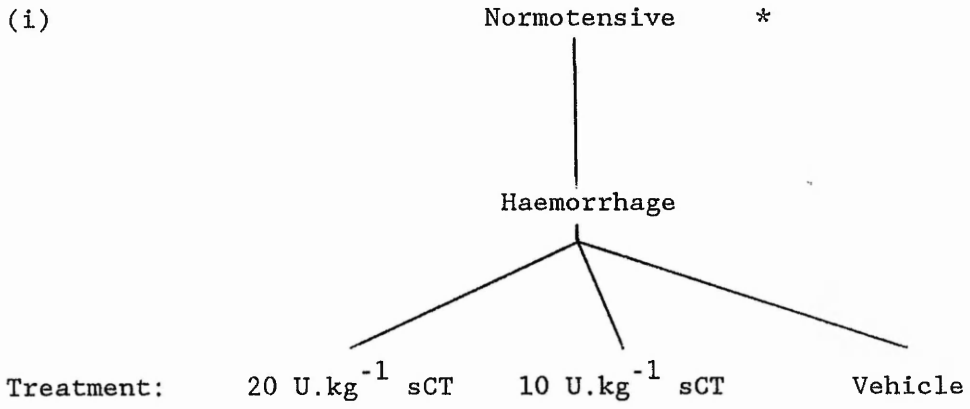
20 U.kg^{-1} sCT/vehicle administered at t=0 min

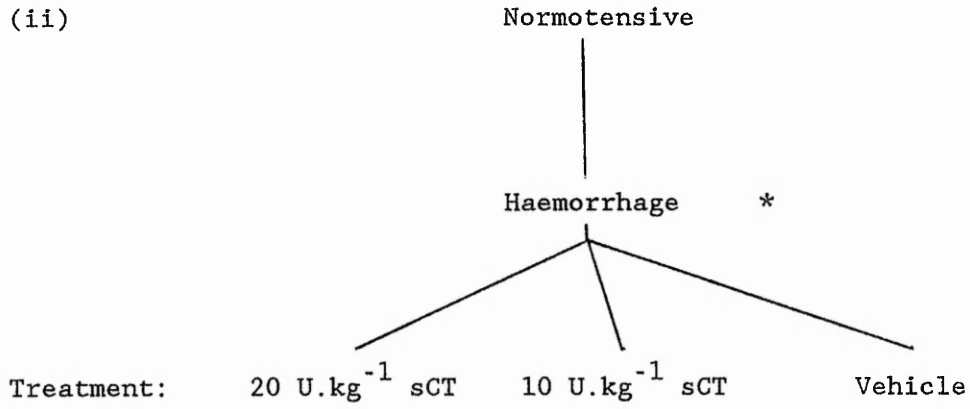
b) AUC = -49 (-555,457)mmHg.min (NS)



10 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.14. Effect of SCT (iv) on Heart Rate of Pentobarbitone Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.14(i) and (ii) overleaf were determined.

Fig.R.14. Effect of SCT (iv) on Heart Rate of Pentobarbitone Anaesthetised Rats subjected to Haemorrhage.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered iv after haemorrhage.

Table R.14(i) Pre-haemorrhage, pre-treatment heart rates

Normotensive	Treatment:	20 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	411 (5)	436 (10)
	Mean difference (95% CI): -25 (-53, 3) NS		
Normotensive	Treatment:	10 $U.kg^{-1}$	Vehicle
	Heart rate (bpm)):	447 (7)	436 (10)
	Mean difference (95% CI): 11(-17, 39) NS		

Table R.14(ii) Post-haemorrhage, pre-treatment heart rates

Post-haemorrhage (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	377 (5)	388 (10)
	Mean difference (95% CI): -11 (-52, 30) NS		
Post-haemorrhage (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	403 (7)	388 (10)
	Mean difference (95% CI): 15 (-27, 57) NS		

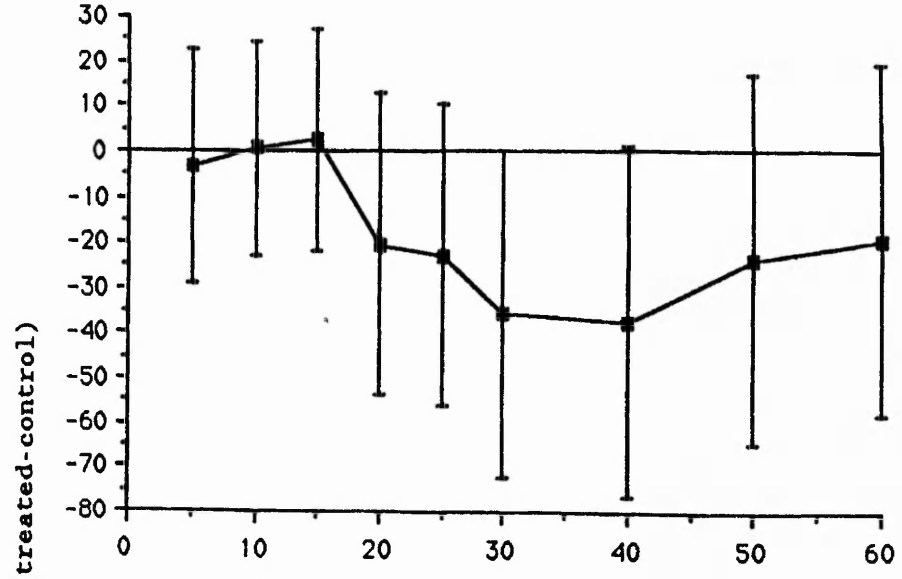
Heart rate = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

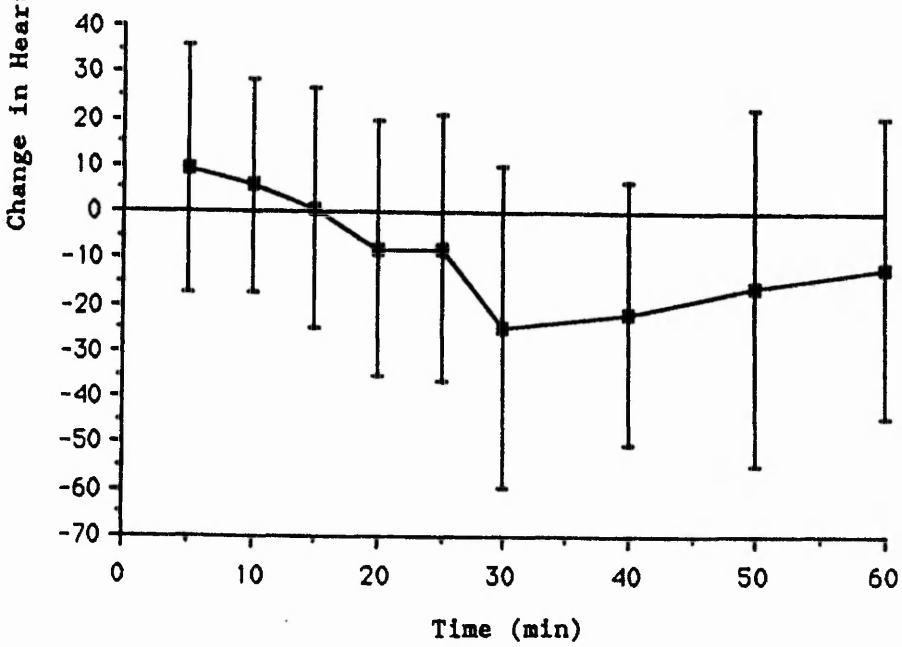
Vehicle results were pooled.

a) AUC = -1178 (-2706, 350) beats (NS)



20 U.kg^{-1} sCT/vehicle administered at t=0 min

b) AUC = -666 (-2107, 774) beats (NS)

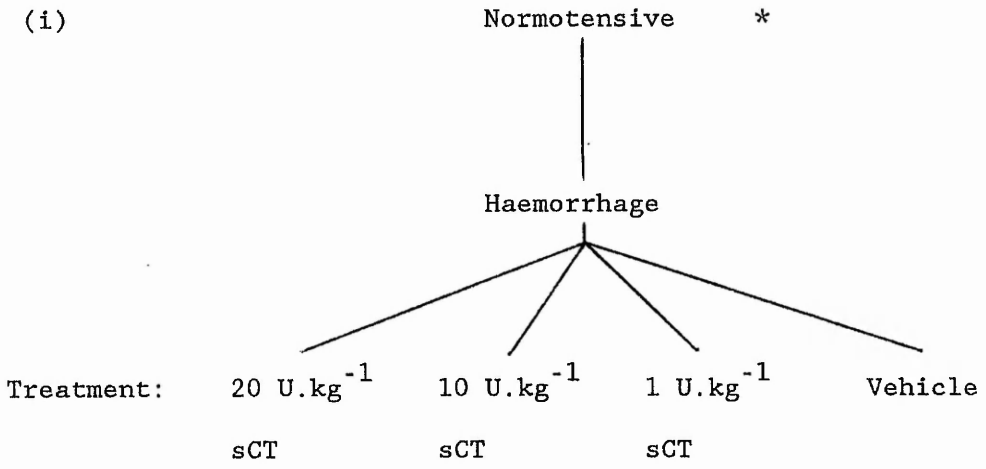


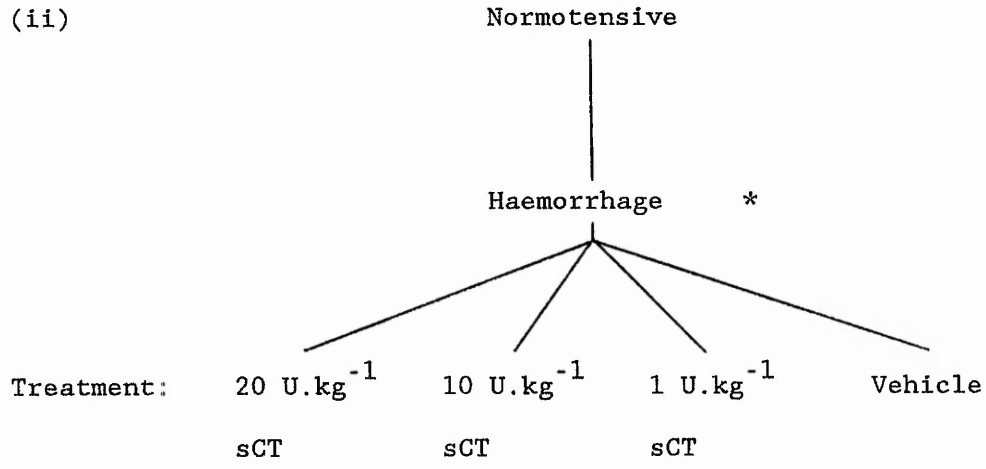
Change in Heart Rate (bpm) (treated-control)

Time (min)

10 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.15. Effect of SCT (iv) on MAP of Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.15(i) and (ii) overleaf were determined.

Fig.R.15. Effect of SCT (iv) on MAP of Urethane Anaesthetised Rats subjected to Haemorrhage.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b), 1 $U.kg^{-1}$ sCT (treated) (c) or appropriate vehicle (control) was administered iv after haemorrhage.

Table R.15(i) Pre-haemorrhage, pre-treatment MAP's

Normotensive	Treatment:	20 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	91 (7)	100 (23)
	Mean difference (95% CI): -9 (-18, 1) NS		
Normotensive	Treatment:	10 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	103 (9)	100 (23)
	Mean difference (95% CI): 3 (-8, 14) NS		
Normotensive	Treatment:	1 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	102 (8)	100 (23)
	Mean difference (95% CI): 2 (-7, 11) NS		

Table R.15(ii) Post-haemorrhage, pre-treatment MAP's

Post-haemorrhage (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	72 (7)	78 (23)
	Mean difference (95% CI): -6 (-13, 1) NS		
Post-haemorrhage (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	82 (9)	78 (23)
	Mean difference (95% CI): 4 (-5, 13) NS		
Post-haemorrhage (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	75 (8)	78 (23)
	Mean difference (95% CI): -3 (-10, 4) NS		

MAP = mean (n)
NS = not statistically significant
Graphs show mean difference (95% CI); see 2.7
Vehicle results were pooled.

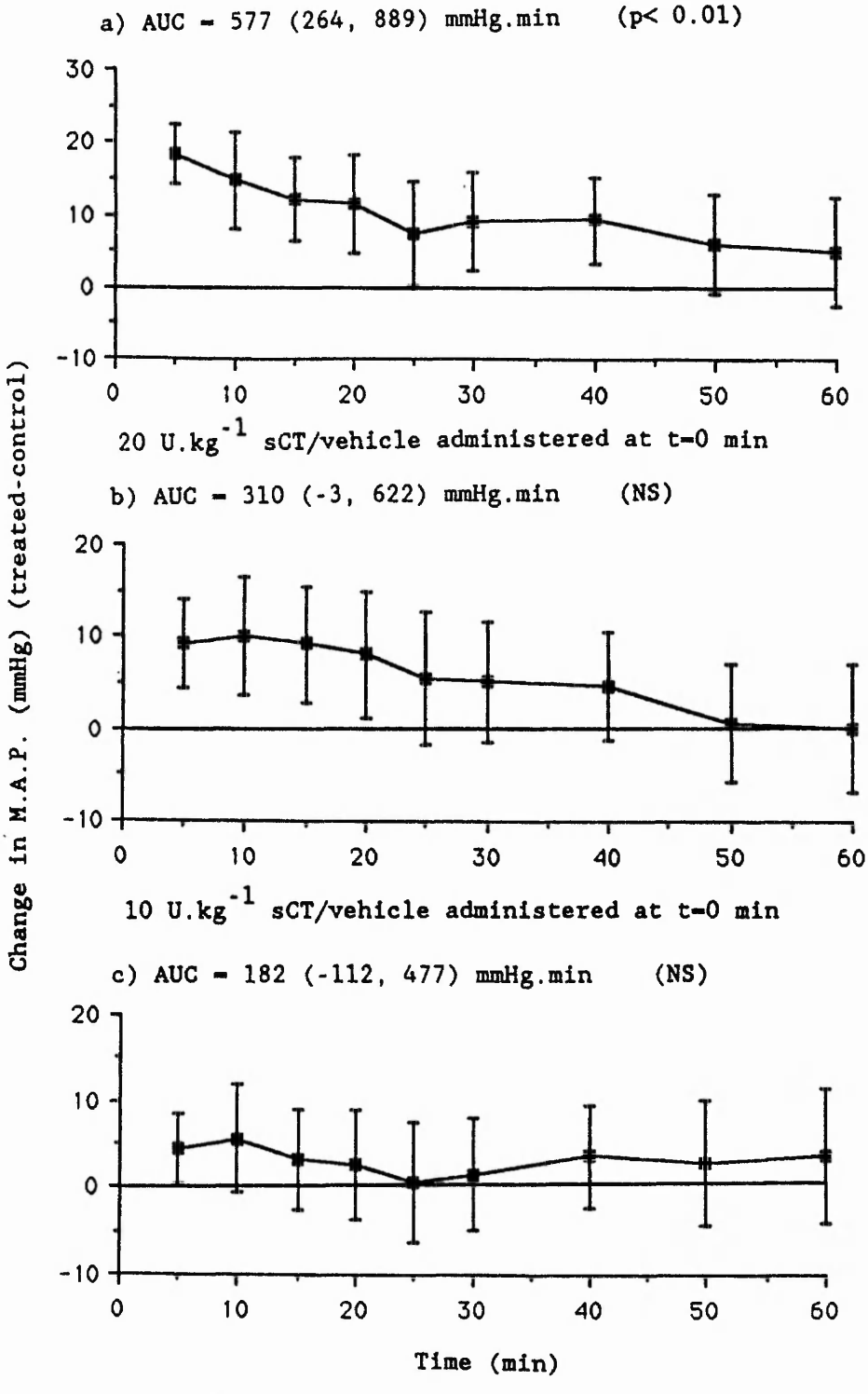
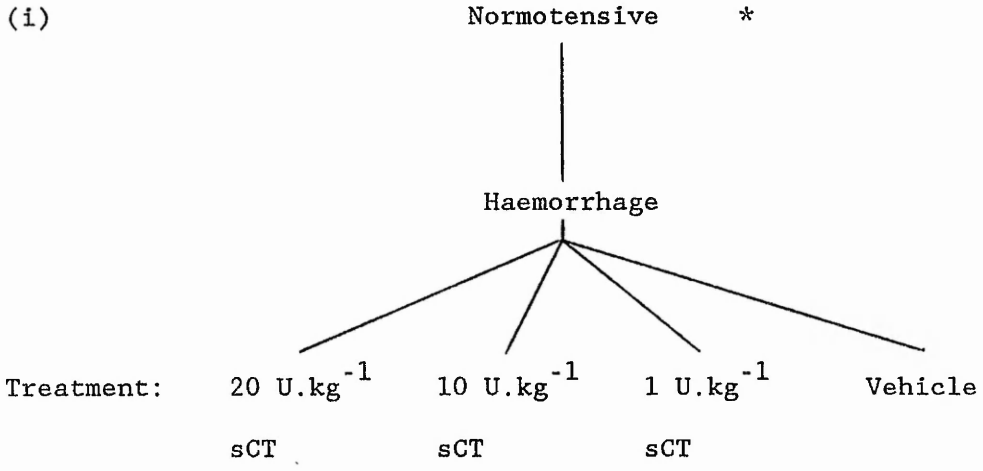
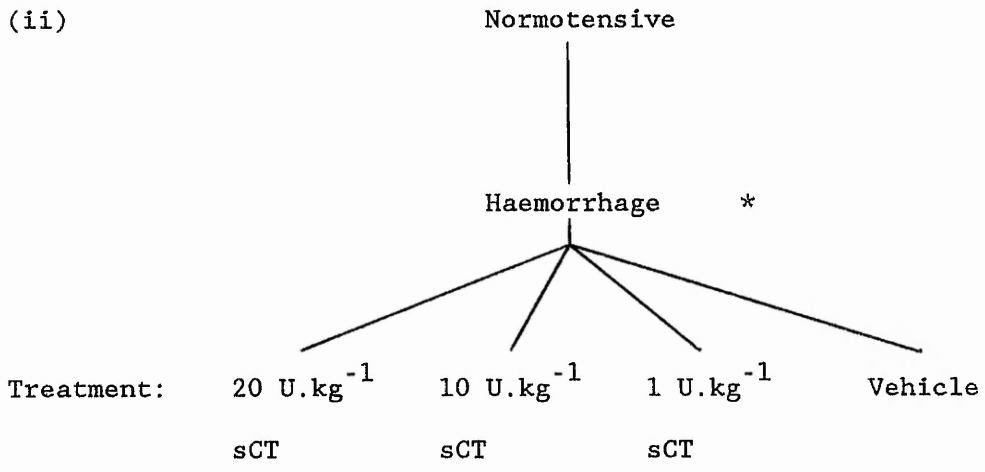

a) AUC = 577 (264, 889) mmHg.min (p< 0.01)
30
20
10
0
-10
0
10
20
30
40
50
60
20 U.kg-1 sCT/vehicle administered at t=0 min
b) AUC = 310 (-3, 622) mmHg.min (NS)
20
10
0
-10
0
10
20
30
40
50
60
10 U.kg-1 sCT/vehicle administered at t=0 min
c) AUC = 182 (-112, 477) mmHg.min (NS)
20
10
0
-10
0
10
20
30
40
50
60
Time (min)
1 U.kg-1 sCT/vehicle administered at t=0 min
Change in M.A.P. (mmHg) (treated-control)

Fig.R.16. Effect of SCT (iv) on Heart Rate of Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.16(i) and (ii) were determined.

Fig.R.16. Effect of SCT (iv) on Heart Rate of Urethane Anaesthetised Rats subjected to Haemorrhage.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b), 1 $U.kg^{-1}$ sCT (treated) (c) or appropriate vehicle (control) was administered iv after haemorrhage.

Table R.16(i) Pre-haemorrhage, pre-treatment heart rates

Normotensive	Treatment:	20 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	342 (7)	363 (23)
	Mean difference (95% CI): -21 (-56, 14) NS		
Normotensive	Treatment:	10 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	365 (9)	363 (23)
	Mean difference (95% CI): 2 (-32, 36) NS		
Normotensive	Treatment:	1 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	372 (8)	363 (23)
	Mean difference (95% CI): 9 (-28, 46) NS		

Table R.16(ii) Post-haemorrhage, pre-treatment heart rates

Post-haemorrhage (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	318 (7)	327 (23)
	Mean difference (95% CI): -9 (-41, 23) NS		
Post-haemorrhage (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	332 (9)	327 (23)
	Mean difference (95% CI): 5 (-23, 33) NS		
Post-haemorrhage (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	327 (8)	327 (23)
	Mean difference (95% CI): 0 (-33, 33) NS		

Heart rate = mean (n)
NS = not statistically significant
Graphs show mean difference (95% CI); see 2.7
Vehicle results were pooled.

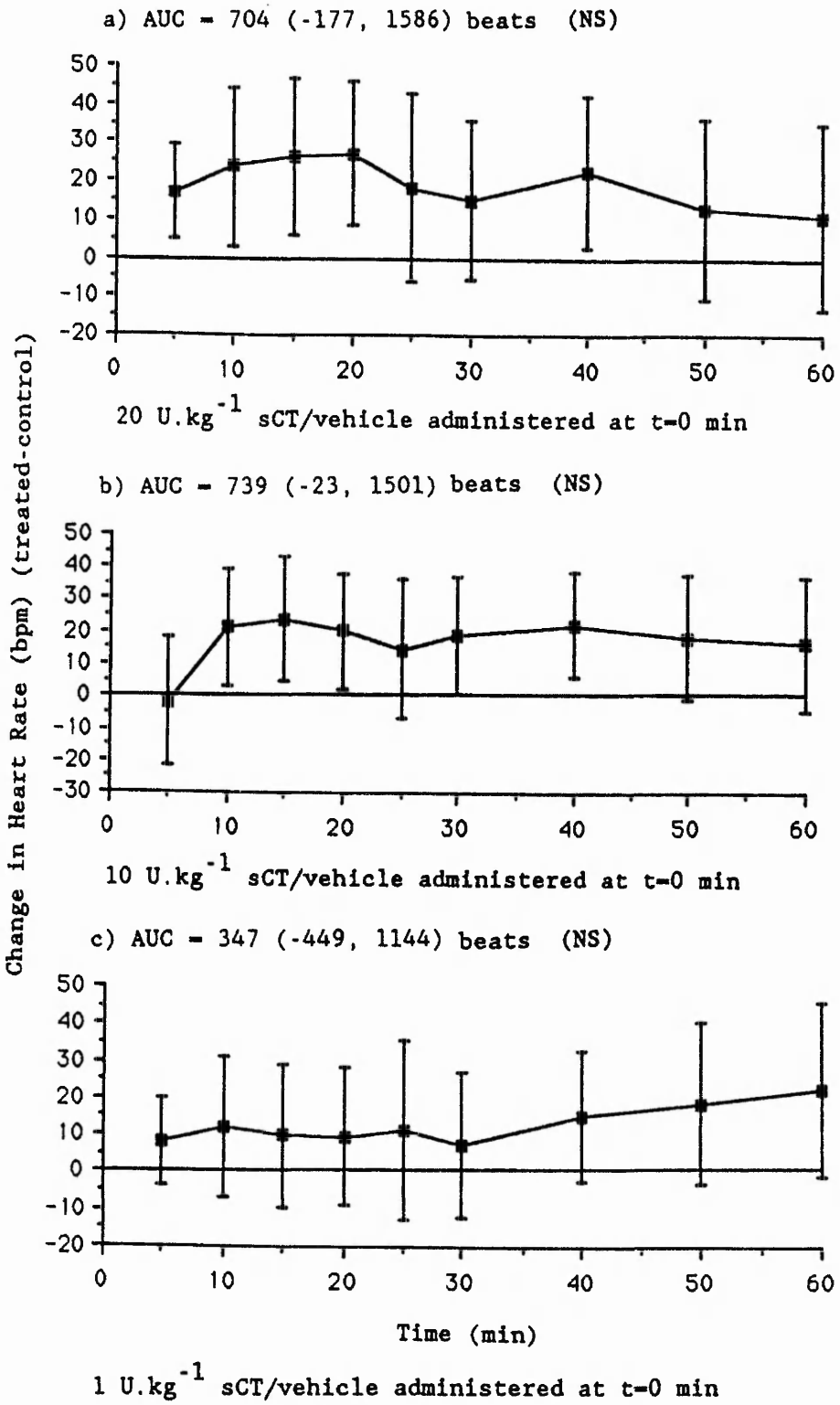
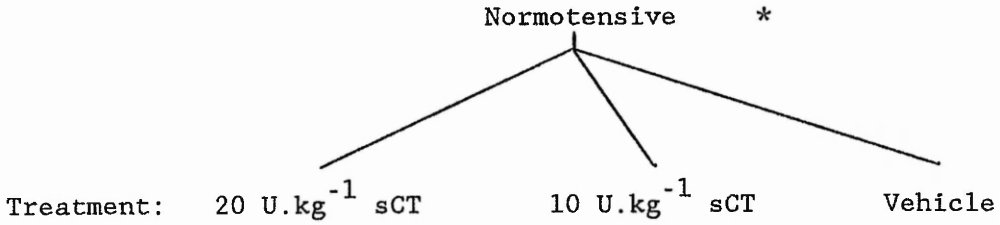

a) AUC = 704 (-177, 1586) beats (NS)
50
40
30
20
10
0
-10
-20
0
10
20
30
40
50
60
20 U.kg-1 sCT/vehicle administered at t=0 min
b) AUC = 739 (-23, 1501) beats (NS)
50
40
30
20
10
0
-10
-20
-30
0
10
20
30
40
50
60
10 U.kg-1 sCT/vehicle administered at t=0 min
c) AUC = 347 (-449, 1144) beats (NS)
50
40
30
20
10
0
-10
-20
0
10
20
30
40
50
60
Time (min)
1 U.kg-1 sCT/vehicle administered at t=0 min
Change in Heart Rate (bpm) (treated-control)

Fig.R.17. Effect of SCT (iv) on MAP of Urethane Anaesthetised Normotensive Rats.



* denotes the stage of the experiment at which the MAP values depicted in table R.17 overleaf were determined.

Fig.R.17. Effect of SCT (iv) on MAP of Urethane Anaesthetised Normotensive Rats.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered iv

Table R.17 Normotensive, pre-treatment MAP's

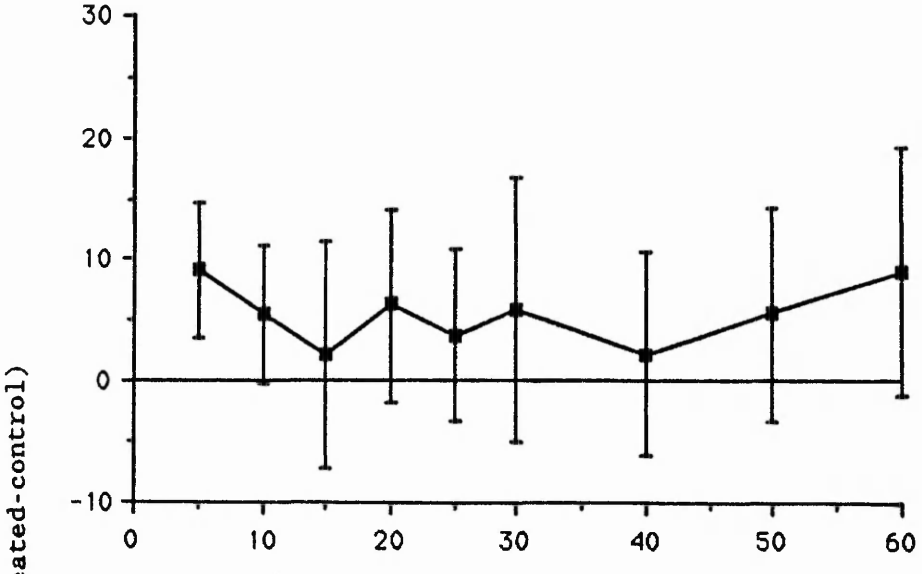
Normotensive (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	97 (6)	105 (6)
	Mean difference (95% CI): -8 (-20, 4) NS		
Normotensive (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	101 (6)	105 (6)
	Mean difference (95% CI): -4 (-15, 7) NS		

MAP = mean (n)

NS = not statistically significant

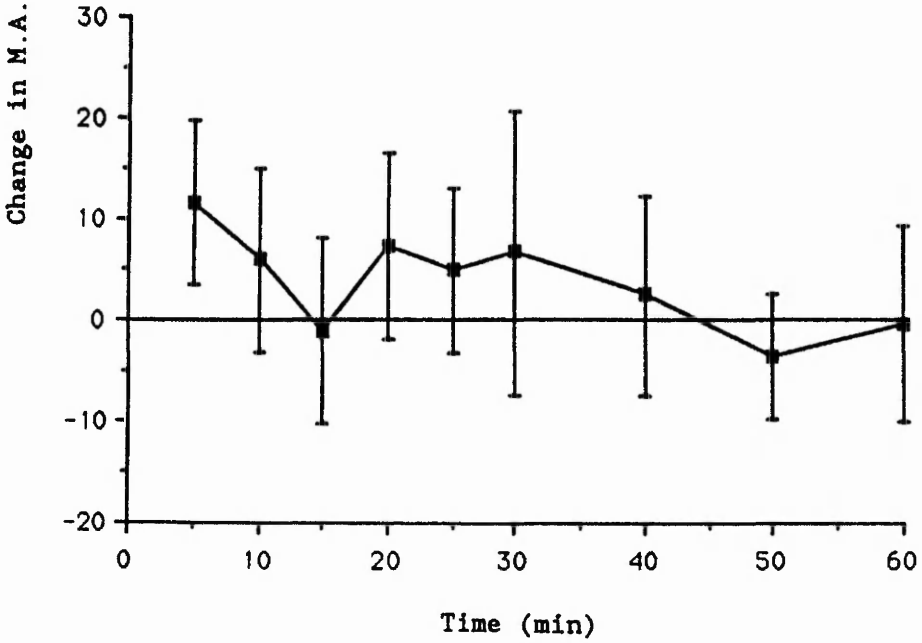
Graphs show mean difference (95% CI); see 2.7

a) AUC = 309 (-54, 673) mmHg.min (NS)



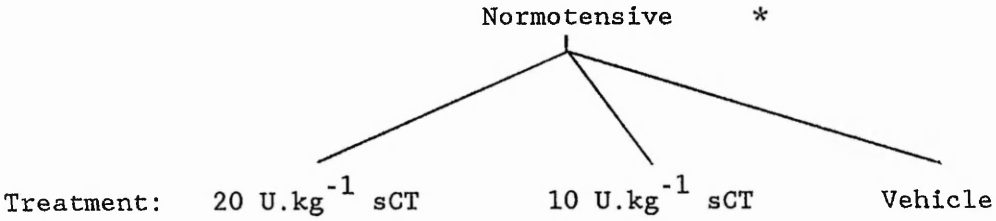
20 U.kg^{-1} sCT/vehicle administered at t=0 min

b) AUC = 186 (-257, 629) mmHg.min (NS)



10 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.18. Effect of SCT (iv) on Heart Rate of Urethane Anaesthetised Normotensive Rats.



* denotes the stage of the experiment at which the heart rate values depicted in table R.18 overleaf were determined.

Fig.R.18. Effect of SCT (iv) on Heart Rate of Urethane Anaesthetised Normotensive Rats.

20 $U.kg^{-1}$ sCT (treated) (a), 10 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered iv

Table R.18 Normotensive, pre-treatment heart rates

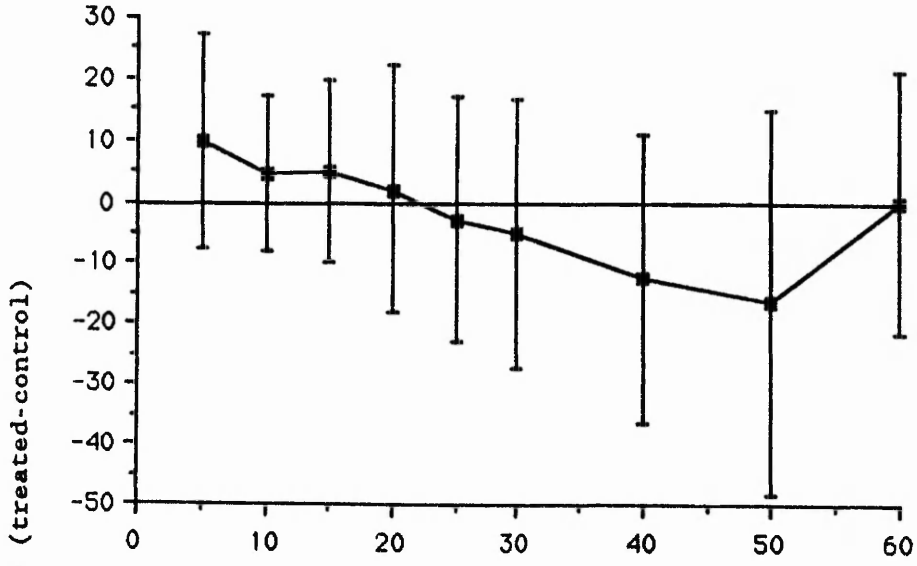
Normotensive (t = 0 min)	Treatment:	20 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	374 (6)	389 (6)
	Mean difference (95% CI): -15 (-58, 28) NS		
Normotensive (t = 0 min)	Treatment:	10 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	376 (6)	389 (6)
	Mean difference (95% CI): -13 (-62, 36) NS		

Heart rate = mean (n)

NS = not statistically significant

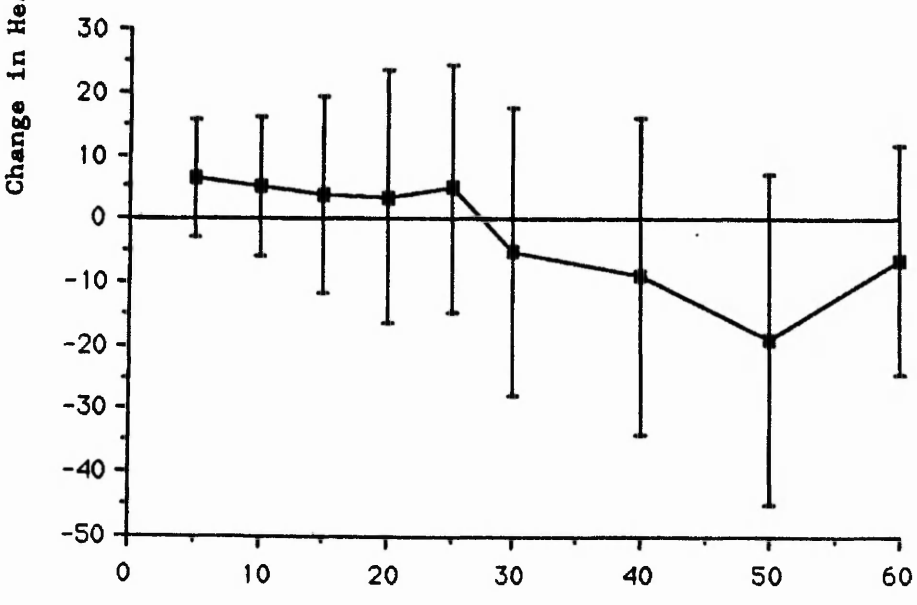
Graphs show mean difference (95% CI); see 2.7

a) AUC = -234 (-1311, 844) beats (NS)



20 U.kg^{-1} sCT/vehicle administered at t=0 min

b) AUC = -225 (-1187, 737) beats (NS)

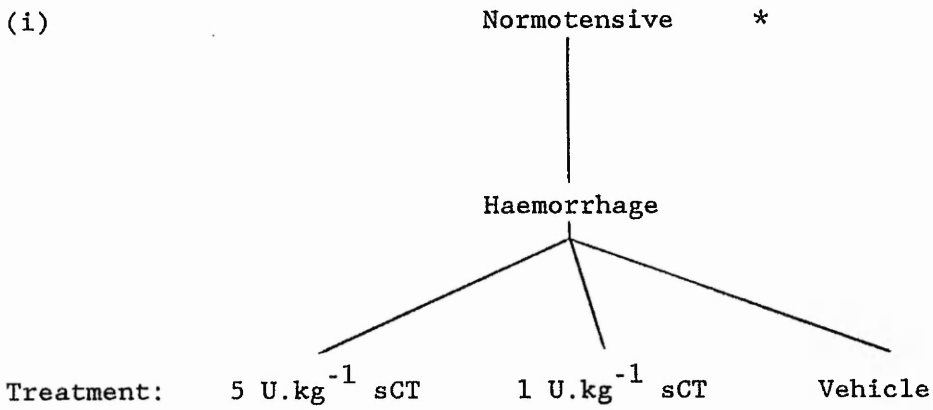


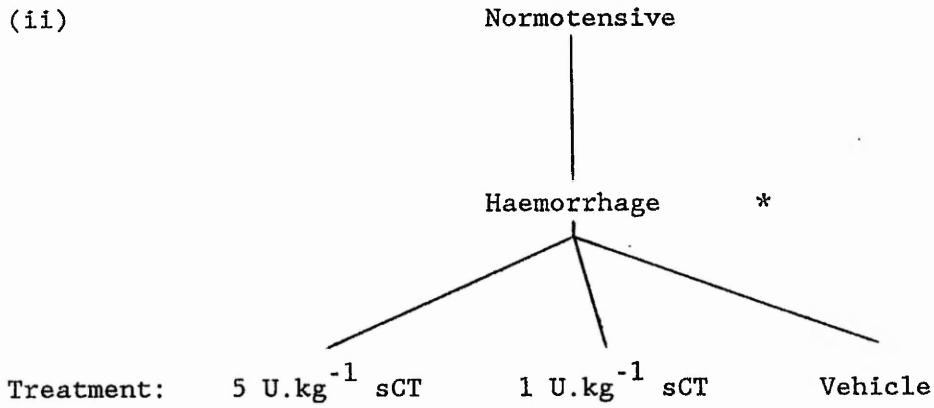
Change in Heart Rate (bpm) (treated-control)

Time (min)

10 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.19. Effect of SCT (icv) on MAP of Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.19(i) and (ii) overleaf were determined.

Fig.R.19. Effect of SCT (icv) on MAP of Urethane Anaesthetised Rats subjected to Haemorrhage.

5 $U.kg^{-1}$ sCT (treated) (a), 1 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered icv after haemorrhage.

Table R.19(i) Pre-haemorrhage, pre-treatment MAP's

Normotensive	Treatment:	5 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	104 (6)	98 (6)
	Mean difference (95% CI): 6 (-11, 23) NS		
Normotensive	Treatment:	1 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	108 (6)	98 (6)
	Mean difference (95% CI): 10 (-5, 25) NS		

Table R.19(ii) Post-haemorrhage, pre-treatment MAP's

Post-haemorrhage (t = 0 min)	Treatment:	5 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	80 (6)	74 (6)
	Mean difference (95% CI): 7 (-6, 19) NS		
Post-haemorrhage (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	84 (6)	74 (6)
	Mean difference (95% CI): 10 (-2, 22) NS		

MAP = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

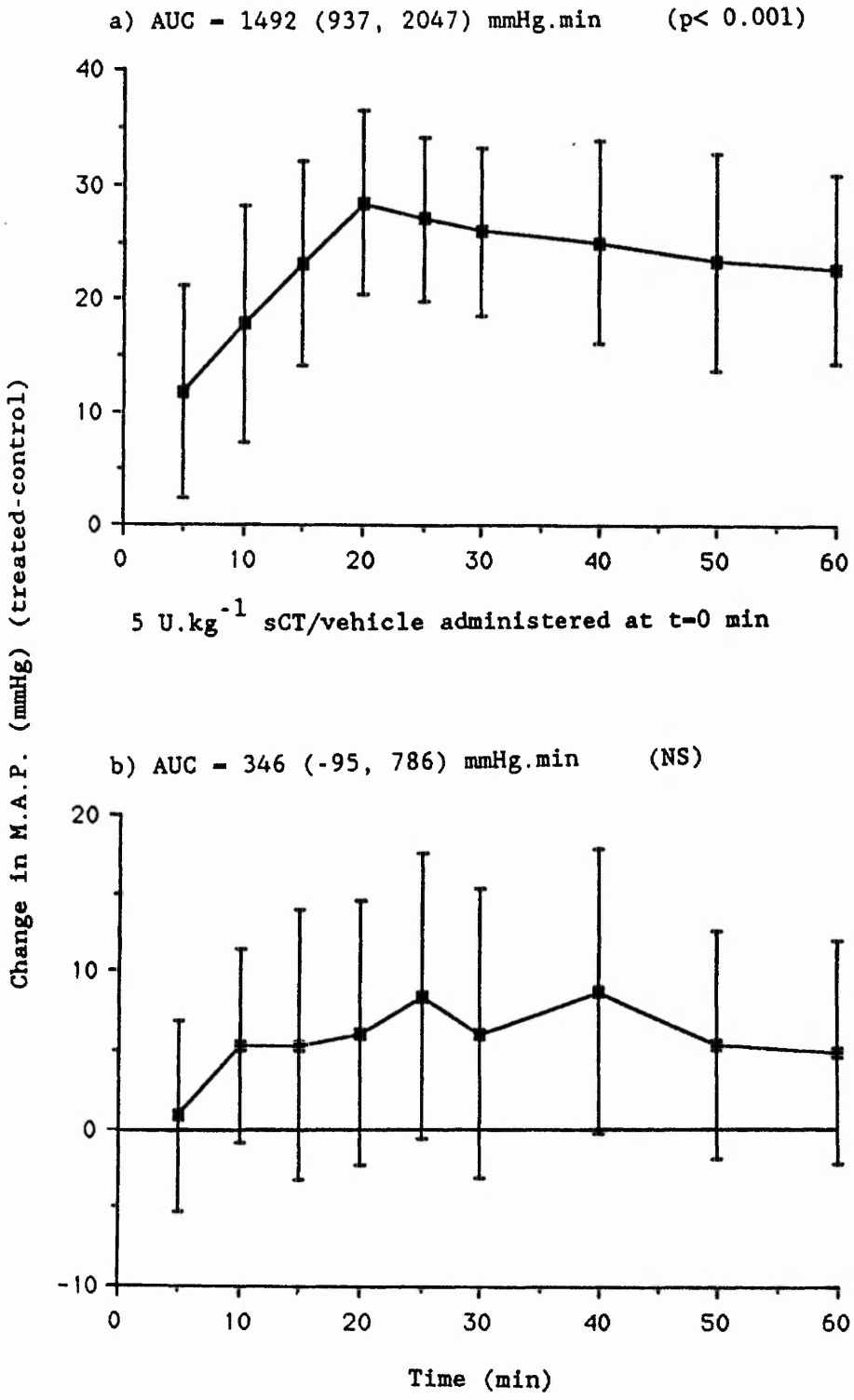
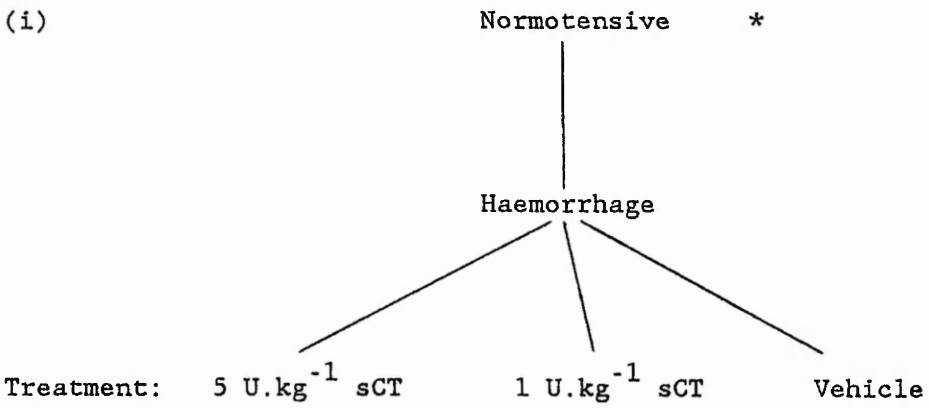
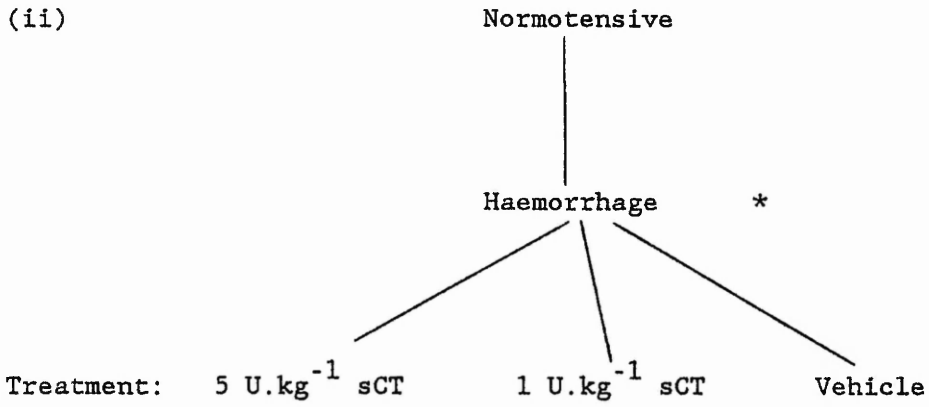

a) AUC = 1492 (937, 2047) mmHg.min (p< 0.001)
40
30
20
10
0
0
10
20
30
40
50
60
5 U.kg-1 sCT/vehicle administered at t=0 min
Change in M.A.P. (mmHg) (treated-control)
b) AUC = 346 (-95, 786) mmHg.min (NS)
20
10
0
-10
0
10
20
30
40
50
60
Time (min)
1 U.kg-1 sCT/vehicle administered at t=0 min

Fig.R.20. Effect of SCT (icv) on Heart Rate of Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.20(i) and (ii) overleaf were determined.

Fig.R.20. Effect of SCT (icv) on Heart Rate of Urethane Anaesthetised Rats subjected to Haemorrhage.

5 $U.kg^{-1}$ sCT (treated) (a), 1 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered icv after haemorrhage.

Table R.20(i) Pre-haemorrhage, pre-treatment heart rates

Normotensive	Treatment:	5 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	369 (6)	339 (6)
	Mean difference (95% CI): 30 (-8, 67) NS		
Normotensive	Treatment:	1 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	365 (6)	339 (6)
	Mean difference (95% CI): 26 (-3, 55) NS		

Table R.20(ii) Post-haemorrhage, pre-treatment heart rates

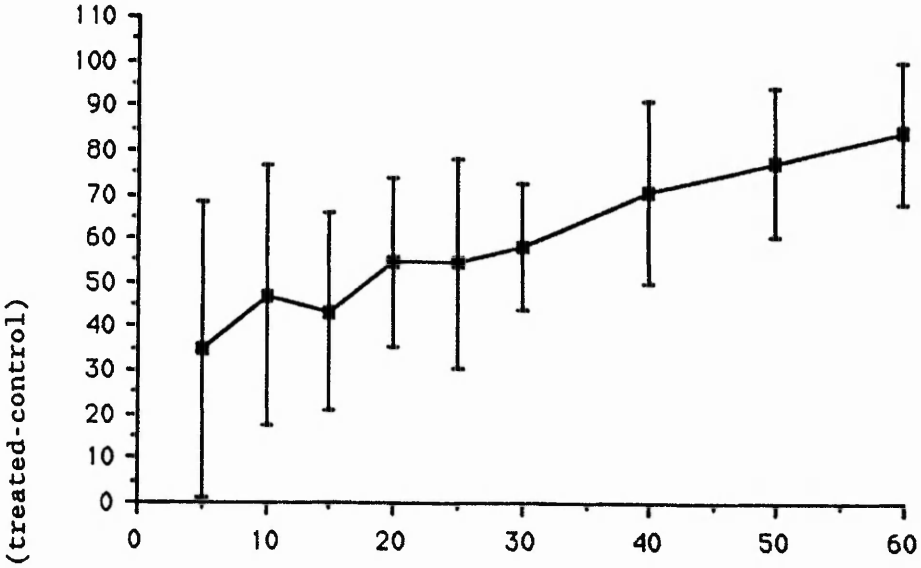
Post-haemorrhage (t = 0 min)	Treatment:	5 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	345 (6)	323 (6)
	Mean difference (95% CI): 23 (-28, 73) NS		
Post-haemorrhage (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	345 (6)	323 (6)
	Mean difference (95% CI): 22 (-13, 57) NS		

Heart rate = mean (n)

NS = not statistically significant

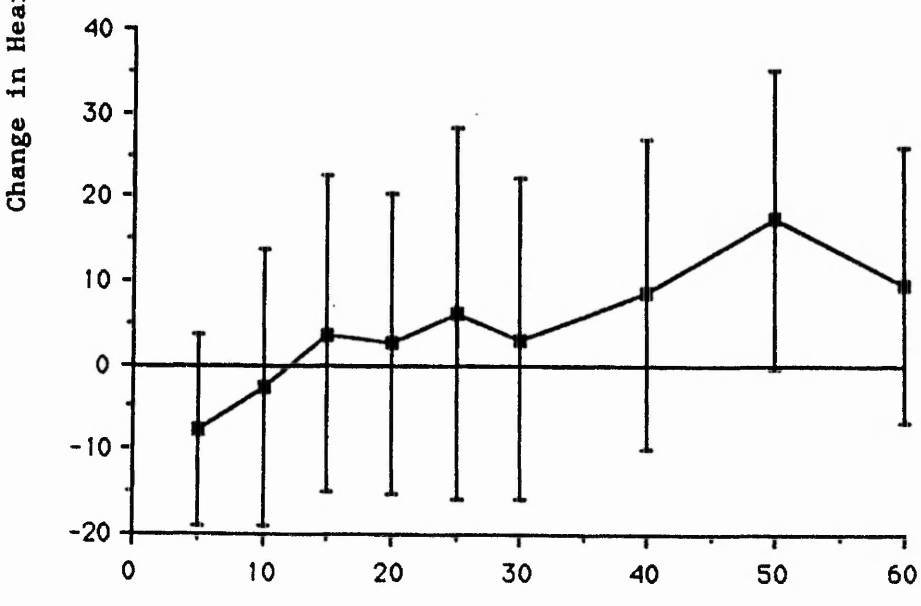
Graphs show mean difference (95% CI); see 2.7

a) AUC = 3411 (2508, 4313) beats ($p < 0.001$)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

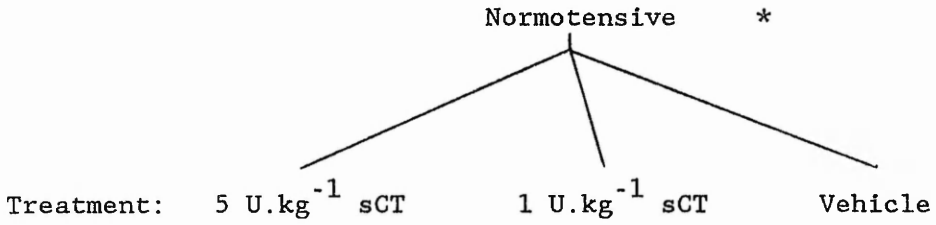
b) AUC = 346 (-585, 1276) beats (NS)



1 U.kg^{-1} sCT/vehicle administered at t=0 min

Change in Heart Rate (bpm) (treated-control)

Fig.R.21. Effect of SCT (icv) on MAP of Urethane Anaesthetised Normotensive Rats.



* denotes the stage of the experiment at which the MAP values depicted in table R.21 overleaf were determined.

Fig.R.21. Effect of SCT (icv) on MAP of Urethane Anaesthetised Normotensive Rats.

5 $U.kg^{-1}$ sCT (treated) (a), 1 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered icv

Table R.21 Normotensive, pre-treatment MAP's

Normotensive (t = 0 min)	Treatment:	5 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	97 (6)	108 (6)
	Mean difference (95% CI): -11 (-32, 9) NS		
Normotensive (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	MAP (mmHg):	105 (6)	108 (6)
	Mean difference (95% CI): -3 (-21, 16) NS		

MAP = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

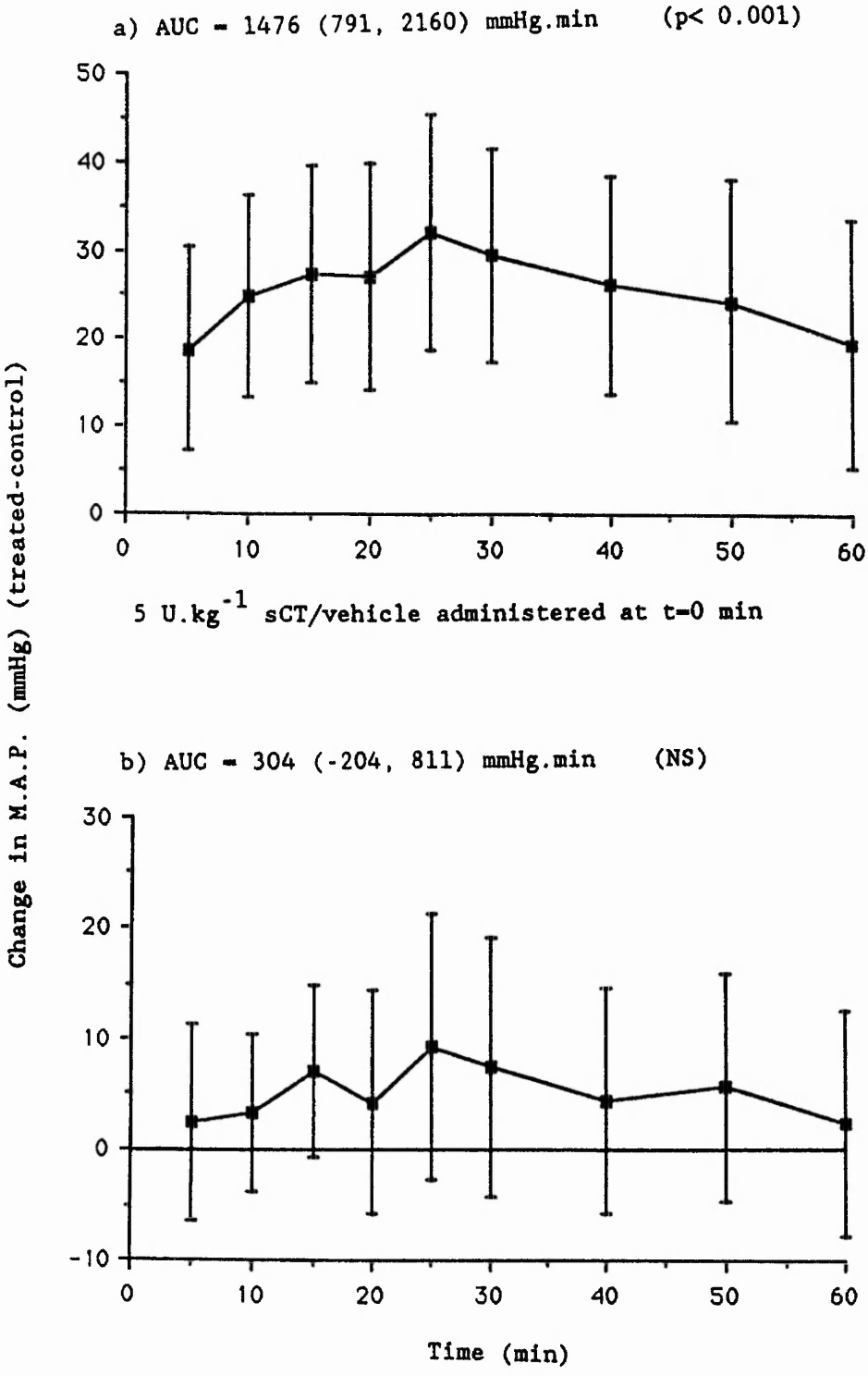
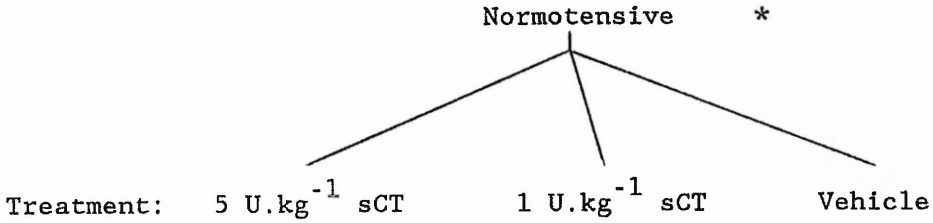

a) AUC = 1476 (791, 2160) mmHg.min (p< 0.001)
50
40
30
20
10
0
0
10
20
30
40
50
60
5 U.kg-1 sCT/vehicle administered at t=0 min
b) AUC = 304 (-204, 811) mmHg.min (NS)
30
20
10
0
-10
0
10
20
30
40
50
60
Time (min)
1 U.kg-1 sCT/vehicle administered at t=0 min
Change in M.A.P. (mmHg) (treated-control)

Fig.R.22. Effect of SCT (icv) on Heart Rate of Urethane Anaesthetised Normotensive Rats.



* denotes the stage of the experiment at which the heart rate values depicted in table R.22 overleaf were determined.

Fig.R.22. Effect of SCT (icv) on Heart Rate of Urethane Anaesthetised Normotensive Rats.

5 $U.kg^{-1}$ sCT (treated) (a), 1 $U.kg^{-1}$ sCT (treated) (b) or appropriate vehicle (control) was administered icv

Table R.22 Normotensive, pre-treatment heart rates

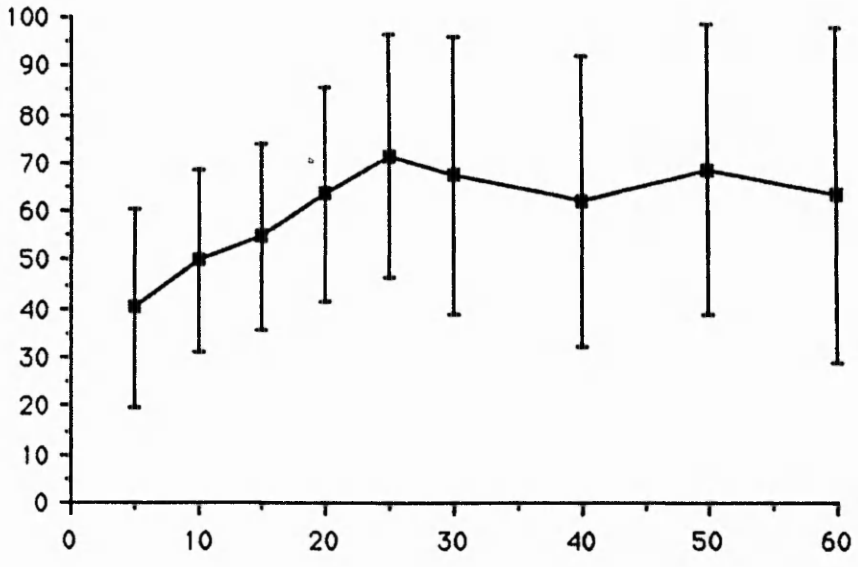
Normotensive (t = 0 min)	Treatment:	5 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	364 (6)	404 (6)
	Mean difference (95% CI): -40 (-94, 4) NS		
Normotensive (t = 0 min)	Treatment:	1 $U.kg^{-1}$	Vehicle
	Heart rate (bpm):	390 (6)	404 (6)
	Mean difference (95% CI): -14 (-73, 45) NS		

Heart rate = mean (n)

NS = not statistically significant

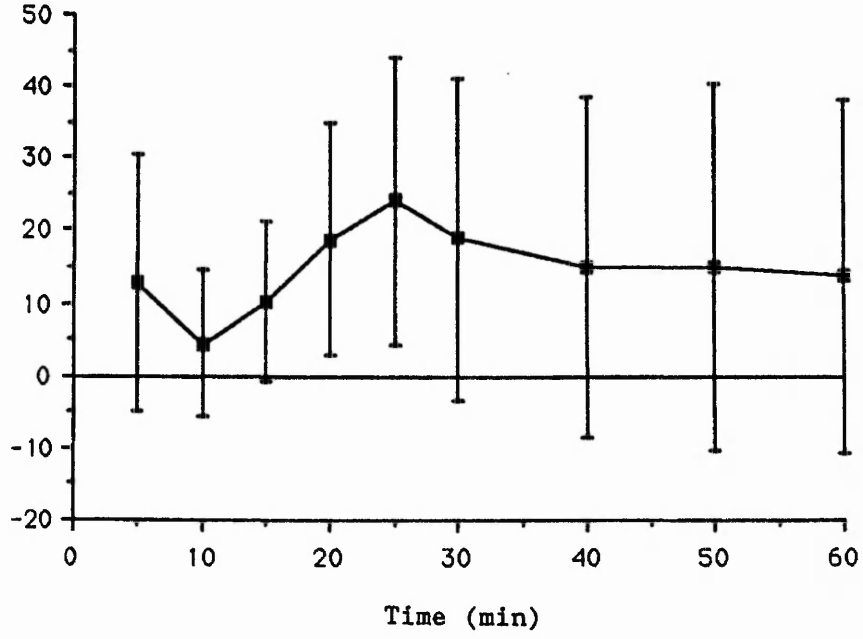
Graphs show mean difference (95% CI); see 2.7

a) AUC = 3538 (2233, 4843) beats ($p < 0.001$)



5 $U.kg^{-1}$ sCT/vehicle administered at t=0 min

b) AUC = 1073 (292, 1859) beats ($p < 0.01$)



1 $U.kg^{-1}$ sCT/vehicle administered at t=0 min

Change in Heart Rate (bpm) (treated-control)

Fig.R.23. Effect of Haemorrhage on the Pressor Response (AUC) after SCT, both iv and icv

Rats subjected to haemorrhage (treated) were compared with normotensive rats (control).

Graph shows mean difference (95% CI); see 2.7

n = 6 - 9

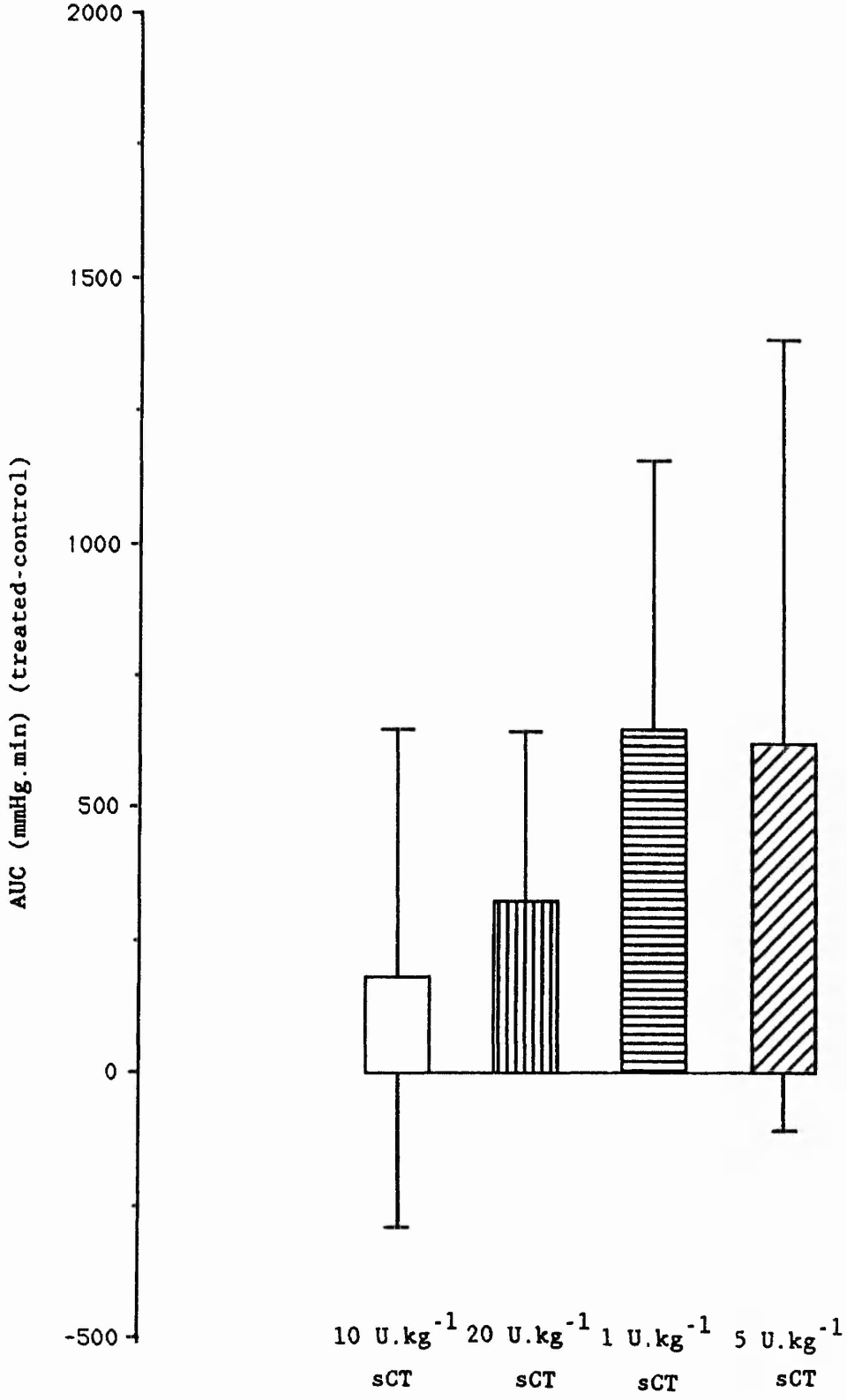
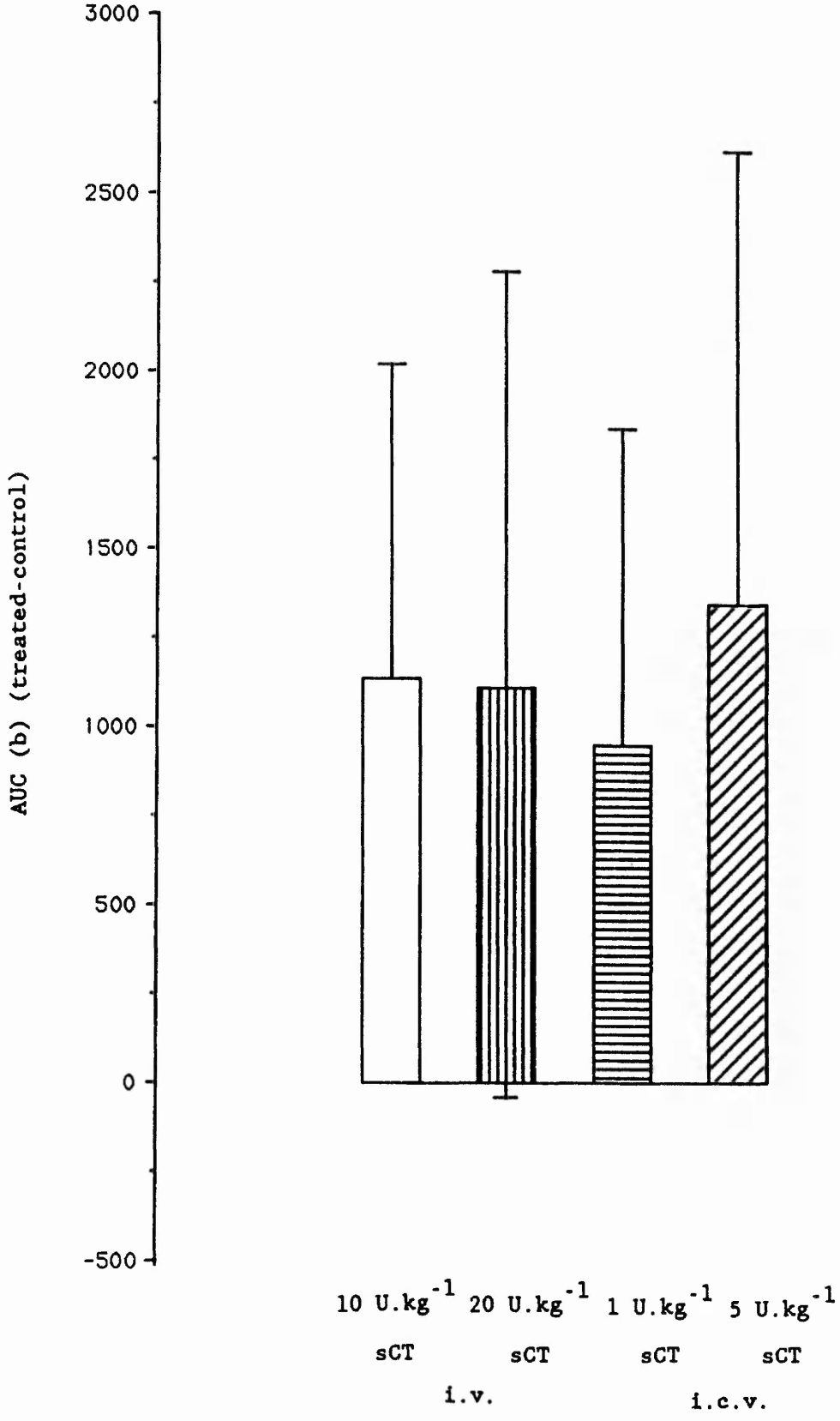

2000
1500
1000
500
0
-500
AUC (mmHg.min) (treated-control)
10 U.kg-1 sCT
20 U.kg-1 sCT
1 U.kg-1 sCT
5 U.kg-1 sCT
i.v.
i.c.v.

Fig.R.24. Effect of Haemorrhage on the Heart Rate Response (AUC) after SCT, both iv and icv

Rats subjected to haemorrhage (treated) were compared with normotensive rats (control).

Graph shows mean difference (95% CI); see 2.7

n = 6 - 9


3000
2500
2000
1500
1000
500
0
-500
AUC (b) (treated-control)
10 U.kg-1 sCT
20 U.kg-1 sCT
1 U.kg-1 sCT
5 U.kg-1 sCT
i.v.
i.c.v.

5. CALCITONIN AND THE PERIPHERAL SYMPATHETIC NERVOUS SYSTEM

5.1 INTRODUCTION

It has been demonstrated in the previous chapter that sCT, administered both peripherally and centrally to urethane anaesthetised rats rendered hypotensive by haemorrhage and administered centrally to urethane anaesthetised normotensive rats, results in a marked pressor effect concomitant with a profound increase in heart rate. One of the explanations put forward for this observation is that sCT may exert its effect on the cardiovascular system by an increase in sympathetic outflow. The present chapter aims to clarify the role of the peripheral sympathetic nervous system in the blood pressure- and heart rate- response to sCT.

Briefly, when haemorrhage is induced, MAP is lowered and the pulse pressure is narrowed which, together, reduce the afferent impulses from the baroreceptors in the carotid sinus and aortic arch. This releases the medullary cardiovascular centre from baroreceptor inhibition and activates the sympathetic efferents to the heart and blood vessels (Chien 1967). Generalised arteriolar vasoconstriction is a prominent response to the diminished baroreceptor stimulation during haemorrhage. In addition, peripheral chemoreceptors may be stimulated, thus enhancing the vasoconstriction associated with baroreceptor reflexes. The increased activation of the sympathetic nervous system is illustrated by the increased blood levels of catecholamines, which are high during and after haemorrhage (Berne and Levy, 1986).

A widely used approach to study the role of the sympathetic nervous system in physiological processes is to compare the responses of animals with intact peripheral sympathetic nervous systems with animals whose sympathetic nervous systems have been destroyed (sympathectomy) or blocked. Several methods of achieving this goal are available:

- immunosympathectomy produced by the administration of antibody to nerve growth factor;
- surgical methods;
- administration of 6-hydroxydopamine (6-OHDA);
- administration of adrenergic-neurone blocking agents.

Immunosympathectomy can only be achieved in newborn animals and has been shown to be relatively incomplete since it only prevents the development of sympathetic fibres which have not already matured at birth (Burnstock et al. 1971; Johnson et al. 1976). Surgical methods are particularly useful for selective sympathectomy of specific organs. Procedures for more generalised sympathectomy, however, involve extensive and traumatic surgery without guarantee of complete denervation (Burnstock et al. 1971; Johnson et al. 1976). Degeneration of adrenergic neurones with 6-OHDA is limited to the terminal varicose region of the nerve and does not seriously damage the cell bodies. Thus, after several weeks, new nerve processes grow out to reinnervate the system. The drug affects various systems differentially; the heart and large vessels are affected first followed by the more peripheral vessels and visceral organs (Burnstock et al. 1971). 6-OHDA administered to newborn animals causes permanent alterations of noradrenergic neurones in the central nervous system

(Johnson et al. 1976). Adrenergic-neurone blocking agents inhibit the function of sympathetic postganglionic adrenergic neurones by blocking the release of noradrenaline from the terminal varicosities in response to the conducted nerve impulse. They also cause a delayed depletion of neuronal noradrenaline. The blockade is specific to adrenergic neurones, with little or no inhibition of sympathetic postganglionic cholinergic transmission. Inhibition of transmission in sympathetic ganglia or block of α- or β-adrenoceptors plays no part in their action; they do not inhibit the release of catecholamines from the adrenal medulla (Boura and Green, 1984).

The involvement of the sympathetic nervous system in the blood pressure response to sCT has previously been investigated by Bates et al. (1984a; 1984c) and McArdle (1984), who treated rats with 6-OHDA (100 $mg.kg^{-1}$ ip, 4, 3 and 2 days prior to experimental use). The effect of haemorrhage followed by iv sCT (10 $U.kg^{-1}$) or icv sCT (0.1, 1 and 10 $U.kg^{-1}$) on cardiovascular parameters in these rats was then investigated. The MAP response to iv sCT was significantly attenuated in sympathectomised animals, whilst the MAP response to icv sCT was unaffected. These workers suggested that the mechanisms underlying the haemodynamic effects of peripherally and centrally administered sCT may differ in that stimulation of sympathetic neuronal activity is not of major importance in the latter response. Although McArdle (1984) made reference to previously published papers suggesting that the dose of 6-OHDA employed was sufficient to selectively destroy adrenergic nerve terminals, it was not determined whether complete chemical sympathectomy had actually been achieved.

In order to investigate the role of the peripheral sympathetic nervous system in the cardiovascular response to sCT observed in Chapter 4, it was considered adequate to block the peripheral sympathetic nervous system rather than achieve widespread sympathectomy. The adrenergic neurone blocking agent, guanethidine, was thus utilised since this produces adequate blockade after acute treatment (see below).

It was demonstrated in 1961 by Cass and Spriggs that a single administration of guanethidine can produce adrenergic blockade immediately. Urethane anaesthetised rats were given 15 $mg.kg^{-1}$ guanethidine sulphate by sc administration. The degree of sympathetic function present was measured by the pressor response to iv administration of 20 μg eserine; Lesic and Varagic (1961) have shown that eserine exerts its pressor effect by central activation of adrenergic nervous elements with impulses passing through the sympathetic ganglia. The liberation of catecholamines from the adrenal glands and blood vessels by eserine is believed to be an insignificant factor in producing the hypertensive response (Lesic and Varagic, 1961). The pressor response to eserine was absent in guanethidine-treated rats; this lack of effect was maintained for at least 6 h. Sympathetic block is induced more rapidly than is amine depletion, after the observation that maximum reductions in tissue noradrenaline levels (80-90% depletion) occurred at 6-18 h (Cass and Spriggs, 1961). This has been confirmed by several other workers including Day (1962) in the rabbit and Fielden and Green (1967) in mice and cats.

The following experiments were designed to investigate the effect of sCT (iv and icv) after acute blockade of the peripheral sympathetic nervous system in urethane anaesthetised normotensive rats and those subjected to haemorrhage. Preliminary experiments were involved with the determination of an appropriate dose of guanethidine (iv) as measured by the abolition of the pressor response to eserine (iv). It was also possible to compare the effects of sCT after guanethidine (acute blockade) with those after 6-OHDA (chronic blockade).

5.2 RESULTS

NB. Results are expressed as mean difference, ie treated - control (95% CI)

1) Effect of iv guanethidine on the cardiovascular response to iv eserine in urethane anaesthetised rats subjected to haemorrhage

The administration of eserine (20 μg/rat iv) to urethane anaesthetised rats subjected to haemorrhage resulted in a statistically significant increase in MAP as shown by the increase in AUC, relative to control, of 491 (37, 946) mmHg.min ($p< 0.05$) (see Fig.R.25a). Guanethidine (10 $mg.kg^{-1}$ iv) abolished the pressor response to eserine, as reflected in the statistically significant decrease in AUC, relative to control, of -602(-1071, -133) mmHg.min ($p< 0.05$) (see Fig.R.25b). The heart rate response after eserine was similar to the blood pressure response seen in fig.R.25a in that there was a significant increase in heart rate lasting 15 min, however, the increase in AUC was not statistically significant (see fig.R.26a). Pre-treatment with guanethidine abolished the heart rate response to eserine, as seen by the statistically significant decrease in AUC, relative to control, of -2340 (-4262, -418) beats ($p< 0.05$) (see fig.R.26b).

2) Effect of iv guanethidine on the cardiovascular response to eserine in urethane anaesthetised normotensive rats

Fig.R.27a illustrates the effect of eserine (20 μg/rat iv) in urethane anaesthetised normotensive rats. A statistically significant increase in MAP lasting 15 min was observed. However, unlike the response in haemorrhaged rats, the increase in AUC, relative to control, was not statistically significant. Pre-treatment with guanethidine (10mg.kg^{-1} iv) resulted in a statistically significant decrease in the pressor effect of eserine 5 min post administration (see fig.R.27b).

Administration of eserine to urethane anaesthetised normotensive rats resulted in a statistically significant increase in heart rate demonstrated in the significant increase in AUC, relative to control, of 2151 (580, 3722) beats ($p< 0.01$) (see fig.R.28a). This heart rate response to eserine was abolished by pre-treatment with guanethidine as demonstrated by the statistically significant reduction in AUC, relative to control, of -2800 (-4601, -999) beats ($p< 0.01$) (see fig.R.28b).

3) Effect of iv guanethidine on the cardiovascular response to iv sCT in urethane anaesthetised rats rendered hypotensive by haemorrhage

The administration of 20 U.kg^{-1}sCT (iv) to haemorrhaged rats pretreated with guanethidine (10 mg.kg^{-1} iv) compared with those pretreated with the appropriate vehicle for guanethidine resulted in a statistically significant reduction in MAP which is demonstrated in the AUC of -569 (-871, -267) mmHg.min ($p< 0.01$) (see fig.R.29a). Since the administration of 20 U.kg^{-1} sCT (iv) to haemorrhaged rats pretreated with guanethidine had no significant effect on MAP when

compared with the administration of the appropriate vehicle for sCT in guanethidine pretreated , haemorrhaged rats it would suggest that pretreatment with guanethidine abolishes the pressor response to peripherally administered sCT (see fig.R.29b).

Administration of 20 $U.kg^{-1}$ sCT (iv) to haemorrhaged rats pretreated with guanethidine (10 $mg.kg^{-1}$ iv) resulted in a statistically significant decrease in heart rate (AUC = -1427 (-2071, -782) beats; $p< 0.001$) when compared with those pretreated with the appropriate vehicle for guanethidine (see fig.R.30a). However, unlike the blood pressure response, administration of sCT (20 $U.kg^{-1}$ iv) to haemorrhaged rats pretreated with guanethidine resulted in a statistically significant increase in heart rate compared with the administration of the appropriate vehicle for sCT to haemorrhaged, guanethidine pretreated rats (see fig.R.30b). This is demonstrated in the AUC of 752 (5, 1498) beats ($p< 0.05$), suggesting that the increased-heart rate response to 20 $U.kg^{-1}$ sCT (iv) is reduced, but not abolished after pretreatment with guanethidine.

4) Effect of iv guanethidine on the cardiovascular response to icv sCT in urethane anaesthetised rats subjected to haemorrhage

Administration of 5 $U.kg^{-1}$ sCT (icv) to haemorrhaged rats pretreated with guanethidine (10 $mg.kg^{-1}$ iv) resulted in a statistically significant decrease in MAP compared with the appropriate vehicle for guanethidine (see fig.R.31a). It can be seen that the decrease in AUC, relative to control, of -889 (-1671, -107) mmHg.min is statistically significant ($p< 0.05$). Moreover, since the administration of 5 $U.kg^{-1}$ sCT (icv) to haemorrhaged rats pretreated

with guanethidine resulted in a statistically significant increase in MAP when compared with the administration of the appropriate vehicle for sCT in guanethidine pretreated haemorrhaged rats (AUC = 579 (119, 1039) mmHg.min; $p< 0.05$) it would suggest that pretreatment with guanethidine attenuates, but does not abolish, the pressor effect of central administration of sCT (see fig.R.31b).

Central administration of sCT (5 $U.kg^{-1}$) to haemorrhaged rats pretreated with guanethidine (10 $mg.kg^{-1}$ iv) leads to a significant reduction in heart rate compared with those rats pretreated with the appropriate vehicle for guanethidine. This is reflected in the AUC, relative to control, of -4473 (-6584, -2363) beats; $p< 0.001$. The increased-heart rate response to sCT (icv) would appear to be reduced, but not abolished, after pretreatment with guanethidine (see fig R.32b). Thus, the administration of sCT (icv) to haemorrhaged rats pretreated with guanethidine resulted in a statistically significant increase in heart rate when compared with the administration of the appropriate vehicle for sCT in guanethidine pretreated haemorrhaged rats. This is shown in the increase in AUC, relative to control, of 1917 (101, 3732) beats; $p< 0.05$ after 5 $U.kg^{-1}$ sCT.

5) Effect of iv guanethidine on the cardiovascular response to icv sCT in urethane anaesthetised normotensive rats

Pretreatment with guanethidine resulted in an attenuation, but not abolition, of both the pressor response and increased-heart rate response to centrally administered sCT in normotensive rats (see figs.R.33a and b and R.34a and b). When sCT (5 $U.kg^{-1}$ icv) was administered to guanethidine pretreated normotensive rats it resulted

in a statistically significant decrease in MAP (AUC = -701(-1260, -141) mmHg.min; $p< 0.05$) and heart rate (AUC = -5295 (-7514, -3075) beats; $p< 0.001$) when compared with those rats pretreated with the appropriate vehicle for guanethidine. However, administration of sCT (5 $U.kg^{-1}$ icv) to guanethidine pretreated normotensive rats compared with administration of the appropriate vehicle for sCT (icv) to guanethidine pretreated normotensive rats resulted in a statistically significant increase in MAP (AUC = 805 (469, 1140) mmHg.min; $p< 0.001$) and heart rate (AUC = 2169 (442, 3895) beats; $p< 0.05$).

5.3 DISCUSSION

Intravenous administration of eserine (20μg/rat) results in a pressor effect lasting 15 min in both urethane anaesthetised normotensive rats and those subjected to haemorrhage. This effect appears to be more marked in hypotensive rats, as demonstrated by the AUC results (see figs.R.25a and R.27a). Eserine has a similar effect on heart rate, increasing the rate for 15 min in haemorrhaged rats and increasing the rate for 30 min in normotensive rats, suggesting that the heart rate response, unlike the blood pressure response, is more pronounced in normotensive rats. The reason for this is unclear. After pre-treatment with guanethidine the pressor response to eserine in haemorrhaged rats is abolished as is the heart rate response. It can be concluded therefore that 10 $mg.kg^{-1}$ guanethidine (iv) is effective in blocking the peripheral adrenergic neurones in urethane anaesthetised rats subjected to haemorrhage.

The effect of guanethidine in normotensive rats is not so clearly defined. Guanethidine led to a significant decrease in the pressor

response to iv eserine in these rats, an effect lasting only 5 min. There was no significant decrease in AUC, however, since a pressor effect was not observed it can be assumed that the peripheral sympathetic nervous system is adequately blocked by 10 $mg.kg^{-1}$ guanethidine (iv). This assumption is supported by the abolition of the increased heart rate response after pre-treatment with guanethidine. These results confirm those of Cass and Spriggs (1961) in which 15 $mg.kg^{-1}$ guanethidine (sc) was administered to urethane anaesthetised rats to block the peripheral sympathetic nervous system, as demonstrated by the lack of effect of eserine. This effect was shown to be maintained for at least 6 h. Furthermore, it was demonstrated that the anaesthetic used can prolong the blocking action of guanethidine; in rats given guanethidine less than 2 h before the anaesthetic there was no response to eserine, but when guanethidine was administered more than 2 h before the anaesthetic there was a small response to eserine. In the experiments described in this chapter, guanethidine was given less than 2 h after urethane anaesthesia was induced.

When comparing the MAP and heart rate results pre- and post-treatment with guanethidine it can be seen that guanethidine leads to a reduction in MAP (which is statistically significant in some instances) when compared with the appropriate vehicle, but results in an increased heart rate, both in urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage (see tables R.25(ii) to R.38(ii)). A number of observers have reported that guanethidine can lead to a transient sympathomimetic effect due to a transient release of noradrenaline from adrenergic nerve endings. Maxwell (1982) demonstrated that guanethidine (1 $mg.kg^{-1}$) injected

into the splenic artery of cats caused an increase in tritiated noradrenaline in the splenic outflow following its release into the splenic artery. It was suggested that release is a result of a direct action of guanethidine at the nerve endings, and is independent of electrical activity in the neurones. In the results presented in this chapter, a sympathomimetic effect is not evident from the blood pressure results, but appears to be evident from the heart rate results.

The pressor response to 20 $U.kg^{-1}$ sCT (i.v.) is abolished after pre-treatment with guanethidine in urethane anaesthetised rats subjected to haemorrhage. This would suggest that the peripheral sympathetic nervous system is solely responsible for the aforementioned response. Bates et al. (1984a) observed a similar effect after chronic pre-treatment with 6-OHDA when a pressor effect after peripherally administered sCT in urethane anaesthetised haemorrhaged rats was only observed 5 min after administration. It was also shown by these workers that the pressor response to iv sCT was not affected by bilateral vagotomy or pre-treatment with a vasopressin antagonist, thus suggesting that modification of vagal baroreceptor afferents or the secretion of vasopressin is not the mechanism of the pressor effect.

The heart rate response to iv administered sCT (20 $U.kg^{-1}$) does not appear to be abolished after pre-treatment with guanethidine, but rather appears to be significantly attenuated leading to the conclusion that the peripheral sympathetic nervous system is only partly responsible for the increase in heart rate observed after peripherally administered sCT.

Blockade of the peripheral sympathetic nervous system in urethane anaesthetised rats rendered hypotensive by haemorrhage leads to a significant reduction in the pressor response to centrally administered sCT (5 $U.kg^{-1}$). Unlike the effect on the response to peripherally administered sCT, however, the pressor response to icv sCT is not abolished. It can thus be concluded that an increase in peripheral sympathetic nervous system activity is not the only mechanism whereby sCT (icv) exerts a pressor effect. This attenuation of the blood pressure response after blockade of the peripheral sympathetic nervous system was not observed by Bates et al. (1984c) who pre-treated rats with 6-OHDA and observed no effect on the response to icv sCT (1 and 10 $U.kg^{-1}$). It would therefore appear that the method of inducing "sympathectomy" exerts some influence on the aforementioned response, perhaps due to differences between acute and chronic sympathectomy. Again, the heart rate response to centrally administered sCT is significantly attenuated but not abolished after pre-treatment with guanethidine.

The response to sCT (icv) in urethane anaesthetised normotensive rats is similar to that observed in rats subjected to haemorrhage in that pre-treatment with guanethidine significantly attenuates, but does not abolish, both the pressor response and the heart rate response indicating again that the peripheral sympathetic nervous system is only partly involved in the response.

The sympathetic nervous system has been implicated in the pressor response to naloxone during haemorrhage. Longnecker and Kettler, (1983) observed an increase in plasma catecholamine levels after

10 $mg.kg^{-1}$ naloxone (iv) in enflurane anaesthetised rats. Koyama et al. (1983) demonstrated a reversal in the reduction of preganglionic splanchnic nerve activity induced by endotoxin (1 $mg.kg^{-1}$ iv) after naloxone (2 $mg.kg^{-1}$ iv) in anaesthetised cats. This occurred in association with the increase in blood pressure. On the other hand, Feuerstein (1981) did not observe an increase in circulating levels of catecholamines after naloxone (7 $mg.kg^{-1}$ intraarterially) in conscious rats subjected to acute haemorrhage.

The peripheral sympathetic nervous system has also been implicated in the pressor response to centrally administered CGRP. Fisher et al. (1983a) observed a significant increase in plasma noradrenaline levels in conscious rats after 2.2 nmol CGRP (icv) but no significant effects on plasma adrenaline. Struthers et al. (1986) demonstrated that iv infusion of human CGRP into normal volunteers resulted in increased plasma noradrenaline and adrenaline levels, concomitant with a decrease in diastolic blood pressure and an increase in heart rate. The increase in catecholamines was suggested to be a reflex action to the vasodilatory effects of CGRP.

It has been demonstrated that the mechanism of action of peripherally and centrally administered sCT differ. The peripheral sympathetic nervous system seems to be solely responsible for the pressor effect observed in urethane anaesthetised haemorrhaged rats after peripheral administration of sCT , but is only partly responsible for the pressor effect seen after central administration of sCT to urethane anaesthetised normotensive and haemorrhaged rats. The other mechanisms of the blood pressure response may be:

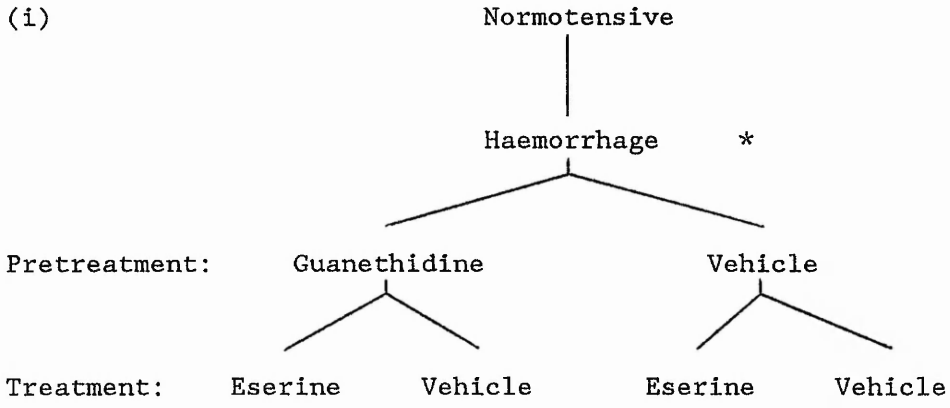
- catecholamines released from the adrenal medulla and acting on α-adrenoceptors;
- activation of angiotensin$_2$ resulting in vasoconstriction;
- activation of other peptides thereby indirectly exerting cardiovascular effects.

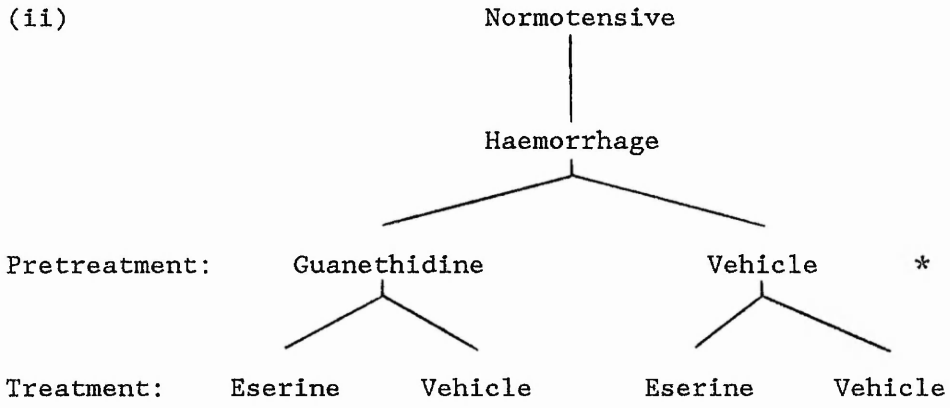
The peripheral sympathetic nervous system also appears not to be the only mechanism of action for the increase in heart rate observed after sCT (iv) in haemorrhaged rats and after sCT (icv) in normotensive and haemorrhaged rats. The residual increase in heart rate may be explained by:

- the release of catecholamines from the adrenal medulla thereby acting on β-adrenoceptors;
- an inhibitory action on the parasympathetic nervous system.

It would be of interest to determine the plasma levels of catecholamines pre- and post-treatment with sCT, both iv and icv, in order to confirm the involvement of the peripheral sympathetic nervous system in the pressor response and increased heart rate response to sCT.

Fig.R.25. Effect of Guanethidine Pre-treatment (iv) on the Blood Pressure Response to Eserine (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.25(i) and (ii) overleaf were determined.

Fig.R.25. Effect of Guanethidine Pre-treatment (iv) on the Blood Pressure Response to Eserine (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) 20 $\mu g.rat^{-1}$ eserine (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with vehicle (0.9% NaCl, iv), to rats rendered hypotensive by haemorrhage.

b) Haemorrhaged rats were pre-treated with 10 $mg.kg^{-1}$ guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, eserine (20 $\mu g.rat^{-1}$ iv) was administered.

Table R.25(i) Post-haemorrhage, pre-pretreatment MAP's

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	91 (5)	79 (4)
	Mean difference (95% CI): 12 (-5, 29) NS		

Table R.25(ii) Post-haemorrhage, post-pretreatment MAP's

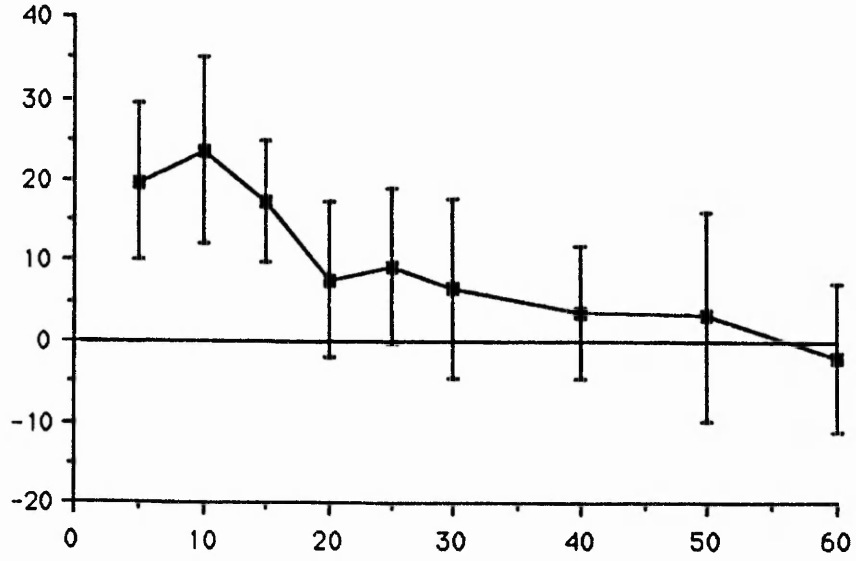
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	78 (5)	81 (4)
	Mean difference (95% CI): -3 (-18, 13) NS		

MAP = mean (n)

NS = not statistically significant

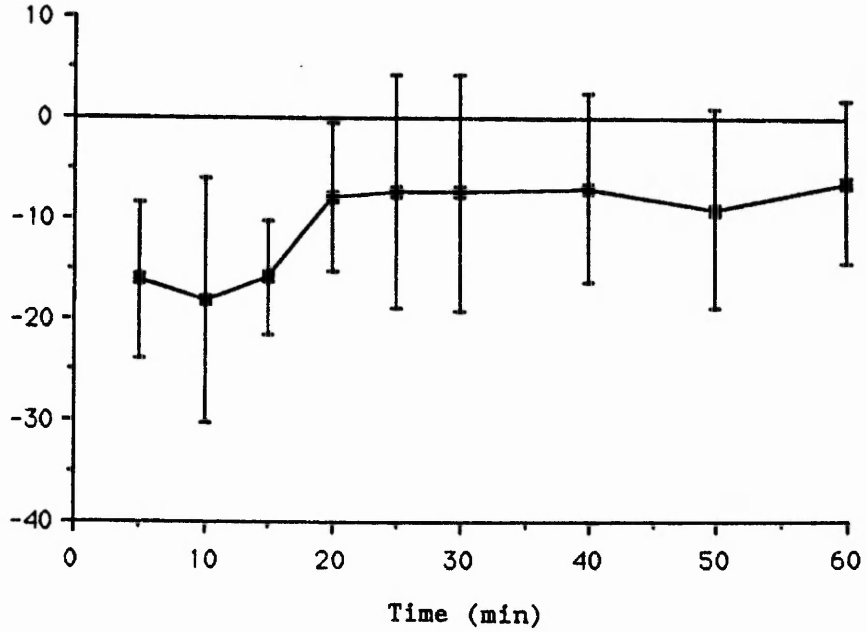
Graphs show mean difference (95% CI); see 2.7

a) AUC = 491 (37, 946) mmHg.min ($p < 0.05$)



Eserine/vehicle administered at t=0 min

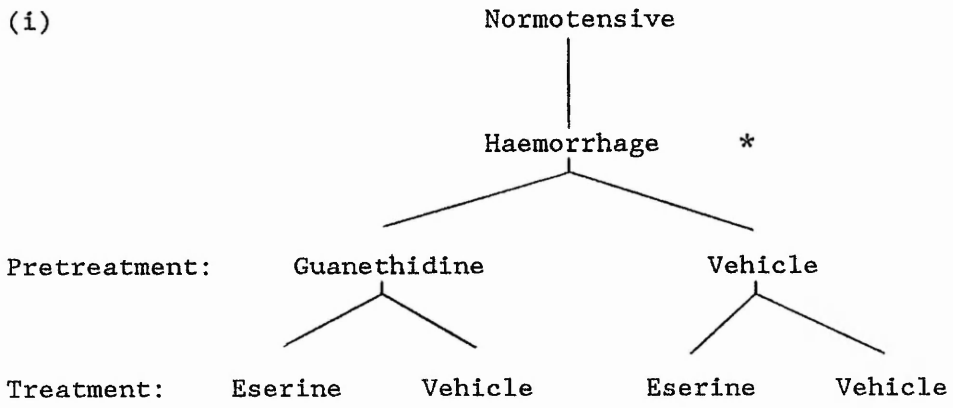
b) AUC = -602 (-1071, -133) mmHg.min ($p < 0.05$)

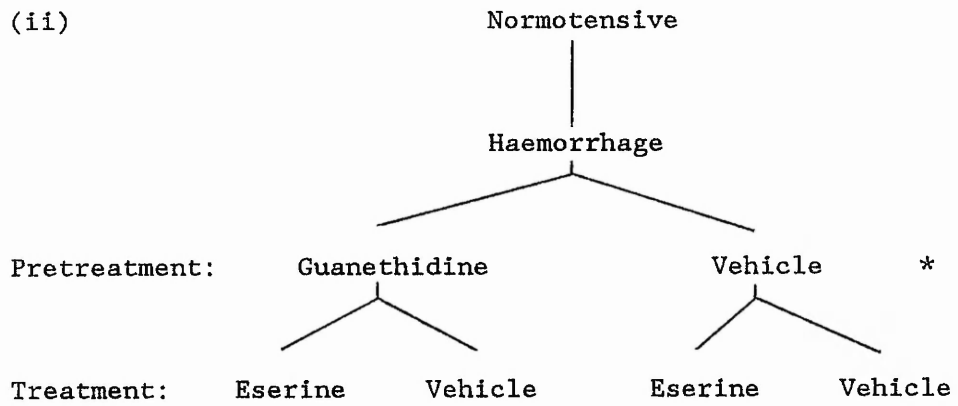


Change in M.A.P. (mmHg) (treated-control)

Eserine administered at t=0 min

Fig.R.26. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to Eserine (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.26(i) and (ii) overleaf were determined.

Fig.R.26. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to Eserine (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) 20 $\mu g.rat^{-1}$ eserine (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with vehicle (0.9% NaCl, iv), to rats rendered hypotensive by haemorrhage.

b) Haemorrhaged rats were pre-treated with 10 $mg.kg^{-1}$ guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, eserine (20 $\mu g.rat^{-1}$ iv) was administered.

Table R.26(i) Post-haemorrhage, pre-pretreatment heart rates

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	347 (5)	357 (4)
	Mean difference (95% CI): -10 (-80, 61) NS		

Table R.26(ii) Post-haemorrhage, post-pretreatment heart rates

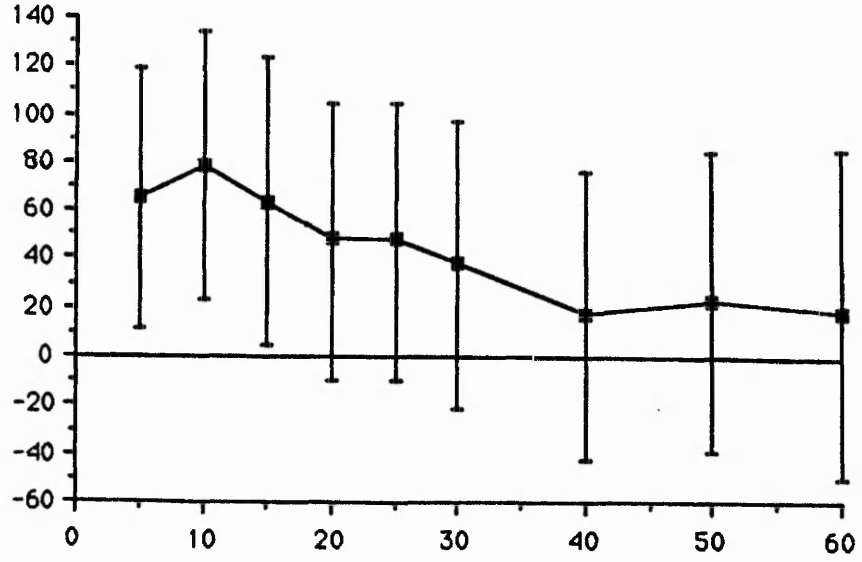
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	416 (5)	364 (4)
	Mean difference (95% CI): 52 (-15, 118) NS		

Heart rate = mean (n)

NS = not statistically significant

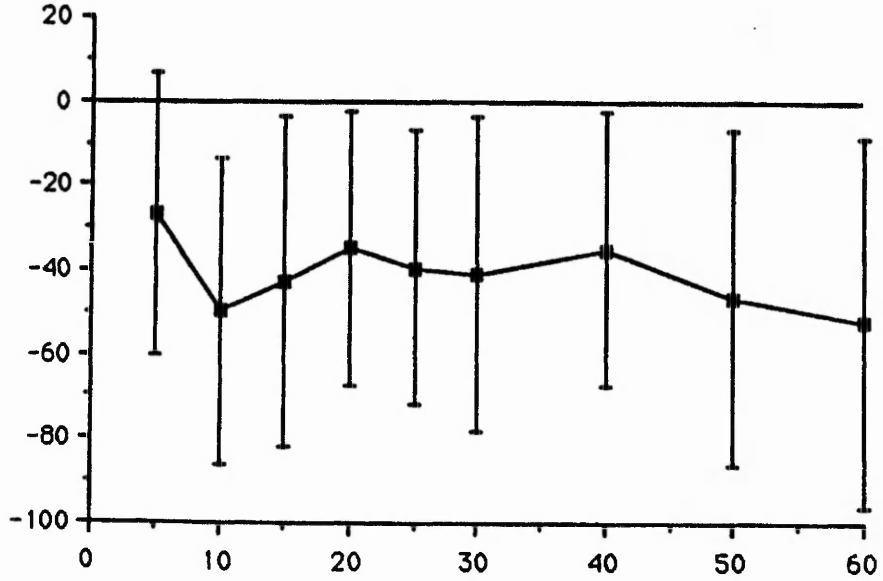
Graphs show mean difference (95% CI); see 2.7

a) AUC = 2296 (-1039, 5630) beats (NS)



Eserine/vehicle administered at t=0 min

b) AUC = -2340 (-4262, -418) beats ($p < 0.05$)

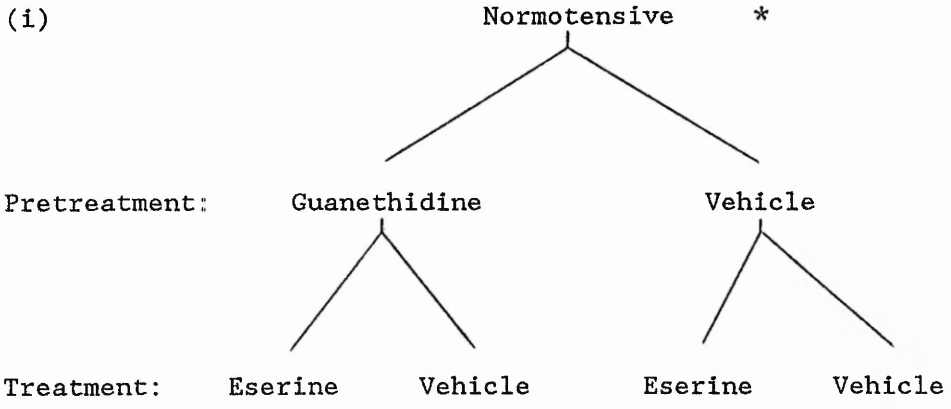


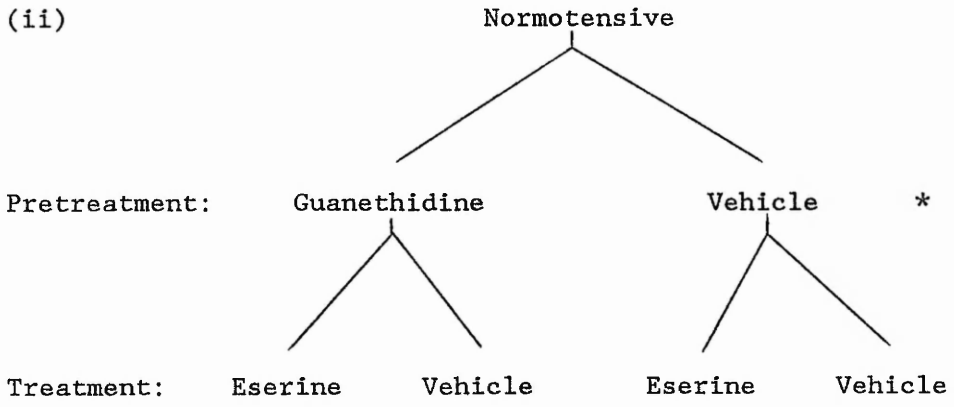
Change in Heart Rate (bpm) (treated-control)

Time (min)

Eserine administered at t=0 min

Fig.R.27. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to Eserine (iv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the MAP values depicted in tables R.27(i) and (ii) overleaf were determined.

Fig.R.27. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to Eserine (iv) in Urethane Anaesthetised Normotensive Rats.

a) 20 $\mu g.rat^{-1}$ eserine (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with vehicle (0.9% NaCl, iv), to normotensive rats.

b) Normotensive rats were pre-treated with 10 $mg.kg^{-1}$ guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, eserine (20 $\mu g.rat^{-1}$ iv) was administered.

Table R.27(i) Normotensive, pre-pretreatment MAP's

Normotensive (t = -10 min)	Pre-treatment: MAP (mmHg):	Guanethidine 104 (5)	Vehicle 105 (5)
	Mean difference (95% CI): -1 (-10, 9) NS		

Table R.27(ii) Normotensive, post-pretreatment MAP's

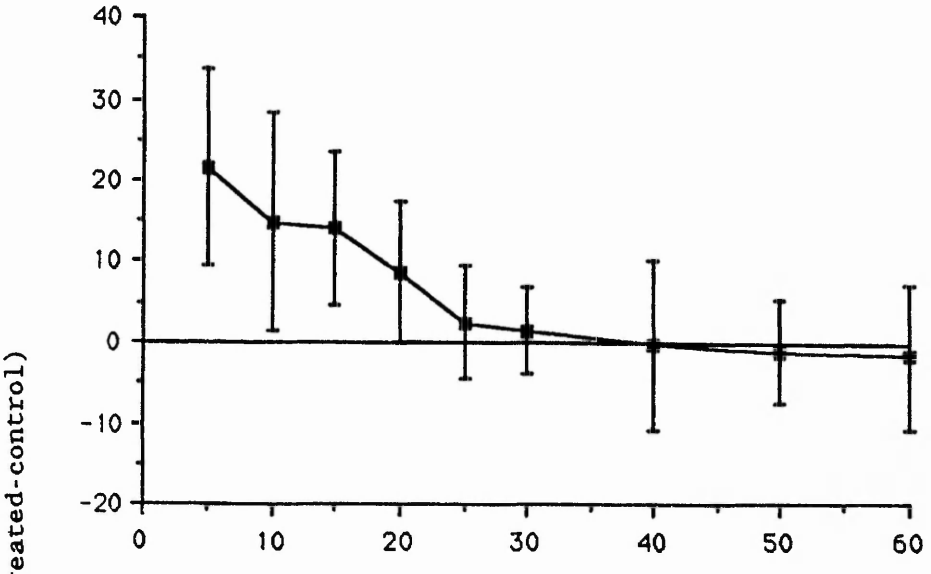
Normotensive + pre-treatment (t = 0 min)	Pre-treatment: MAP (mmHg):	Guanethidine 87 (5)	Vehicle 102 (5)
	Mean difference (95% CI): -15 (-27, -3) $p< 0.05$		

MAP = mean (n)

NS = not statistically significant

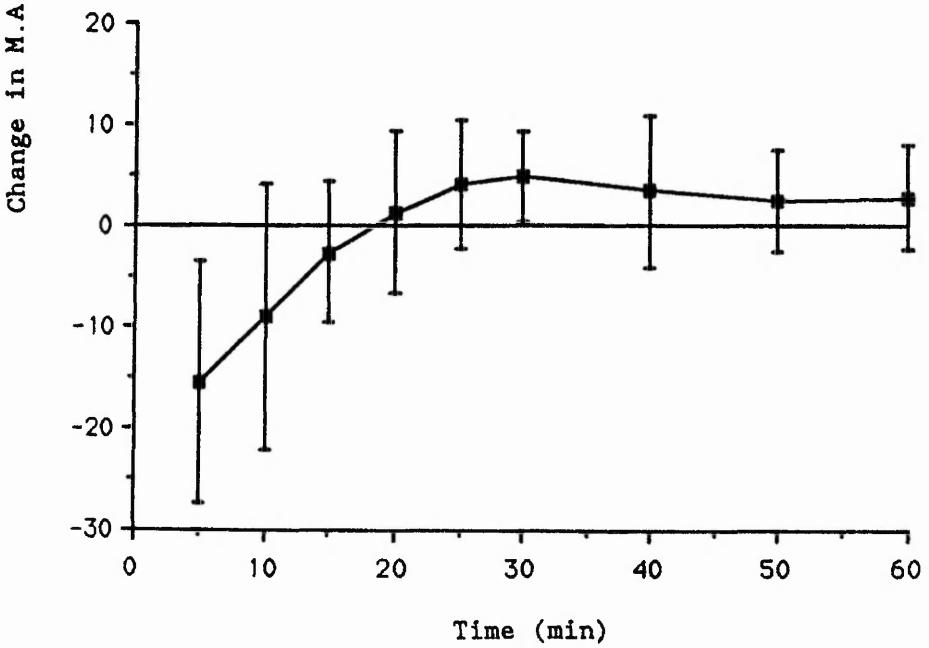
Graphs show mean difference (95% CI); see 2.7

a) AUC = 293 (-130, 715) mmHg.min (NS)



Eserine/vehicle administered at t=0 min

b) AUC = 8 (-361, 376) mmHg.min (NS)

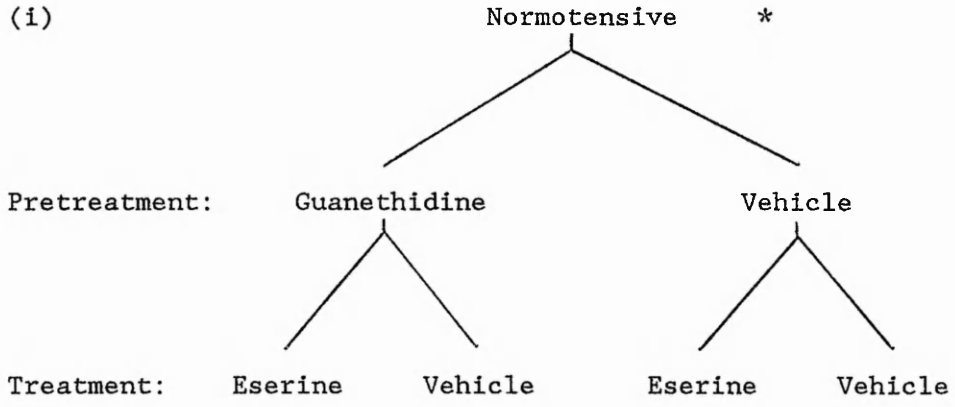


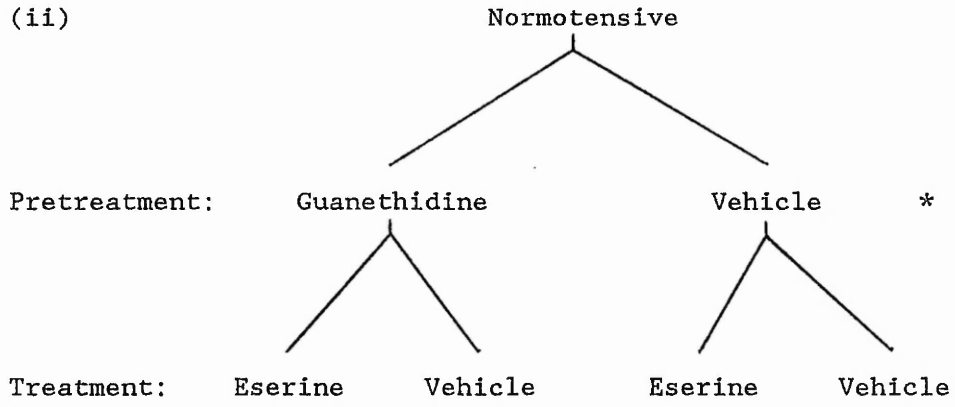
Change in M.A.P. (mmHg) (treated-control)

Time (min)

Eserine administered at t=0 min

Fig.R.28. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to Eserine (iv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.28(i) and (ii) overleaf were determined.

Fig.R.28. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to Eserine (iv) in Urethane Anaesthetised Normotensive Rats.

a) 20 $\mu g.rat^{-1}$ eserine (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with vehicle (0.9% NaCl, iv), to normotensive rats.

b) Normotensive rats were pre-treated with 10 $mg.kg^{-1}$ guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, eserine (20 $\mu g.rat^{-1}$ iv) was administered.

Table R.28(i) Normotensive, pre-pretreatment heart rates

Normotensive (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	375 (5)	405 (5)
	Mean difference (95% CI): -30 (-76, 16) NS		

Table R.28(ii) Normotensive, post-pretreatment heart rates

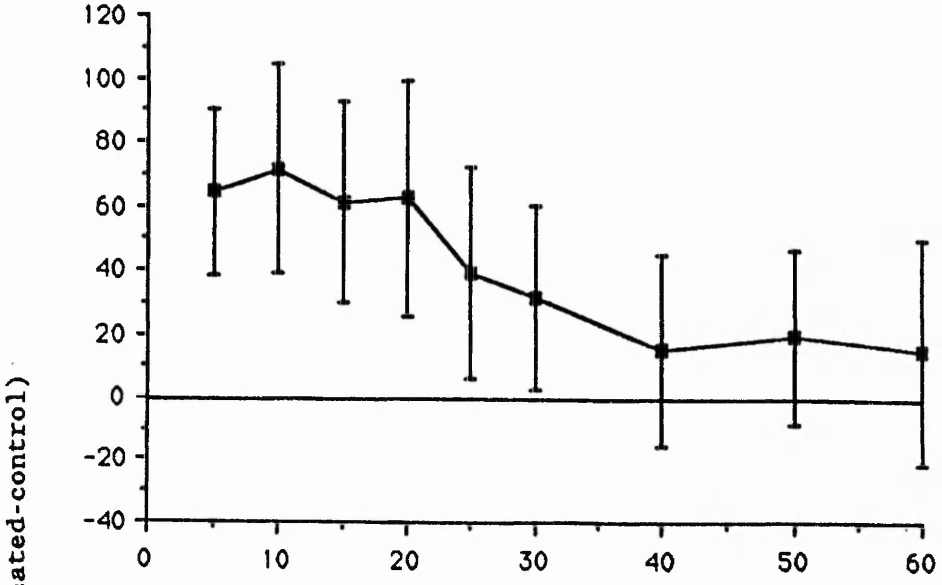
Normotensive + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	446 (5)	392 (5)
	Mean difference (95% CI): 54 (9, 99) $p< 0.05$		

Heart rate = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

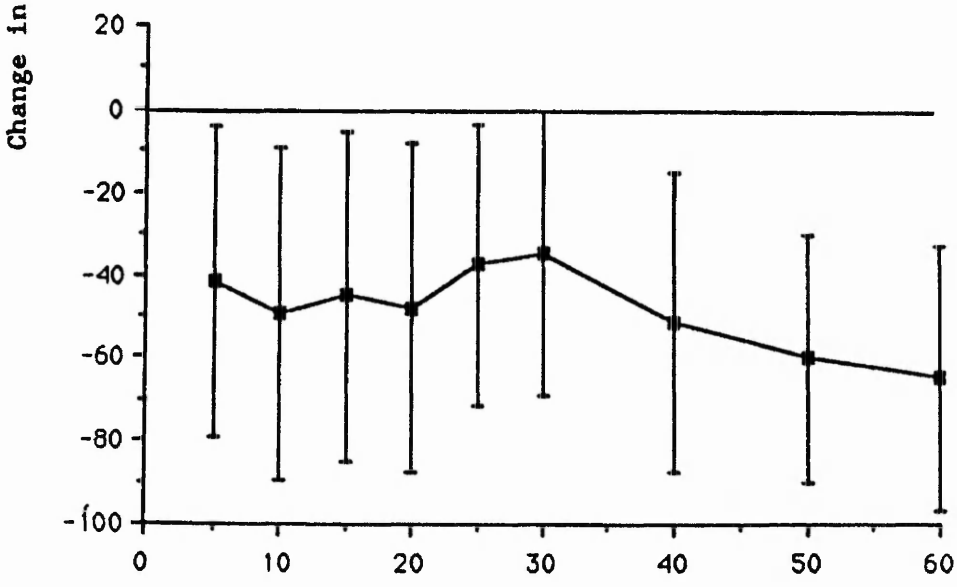
a) AUC = 2151 (580, 3722) beats ($p < 0.01$)



Eserine/vehicle administered at t=0 min

Change in Heart Rate (bpm) (treated-control)

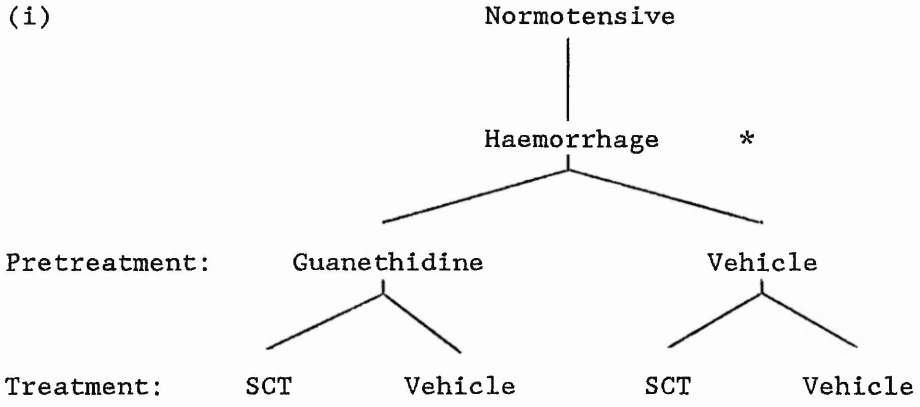
b) AUC = -2800 (-4601, -999) beats ($p < 0.01$)

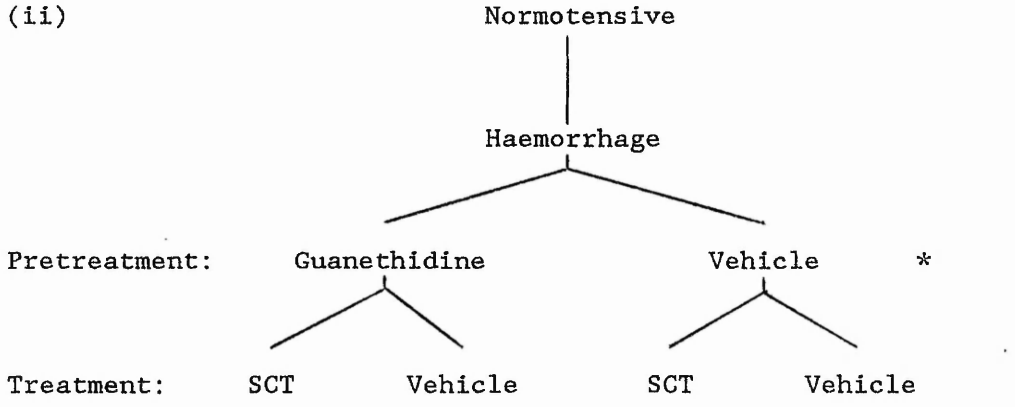


Time (min)

Eserine administered at t=0 min

Fig.R.29. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 20 $U.kg^{-1}$ SCT (iv) in Urethane Anaesthetised Rats subjected to haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.29(i) and (ii) overleaf were determined.

Fig.R.29. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 20 U.kg^{-1} SCT (iv) in Urethane Anaesthetised Rats subjected to haemorrhage.

a) Haemorrhaged rats were pre-treated with 10 mg.kg^{-1} guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (20 U.kg^{-1} iv) was administered.

b) 20 U.kg^{-1} sCT (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with guanethidine (10 mg.kg^{-1} iv), to rats rendered hypotensive by haemorrhage.

Table R.29(i) Post-haemorrhage, pre-pretreatment MAP's

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	80 (6)	83 (6)
	Mean difference (95% CI): -3 (-15, 10) NS		

Table R.29(ii) Post-haemorrhage, post-pretreatment MAP's

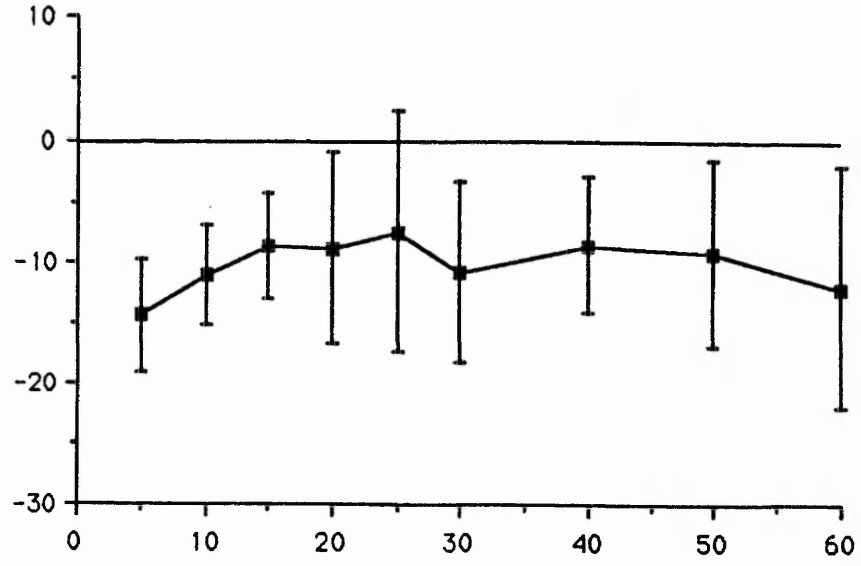
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	73 (6)	85 (6)
	Mean difference (95% CI): -12 (-22, -2) $p< 0.05$		

MAP = mean (n)

NS = not statistically significant

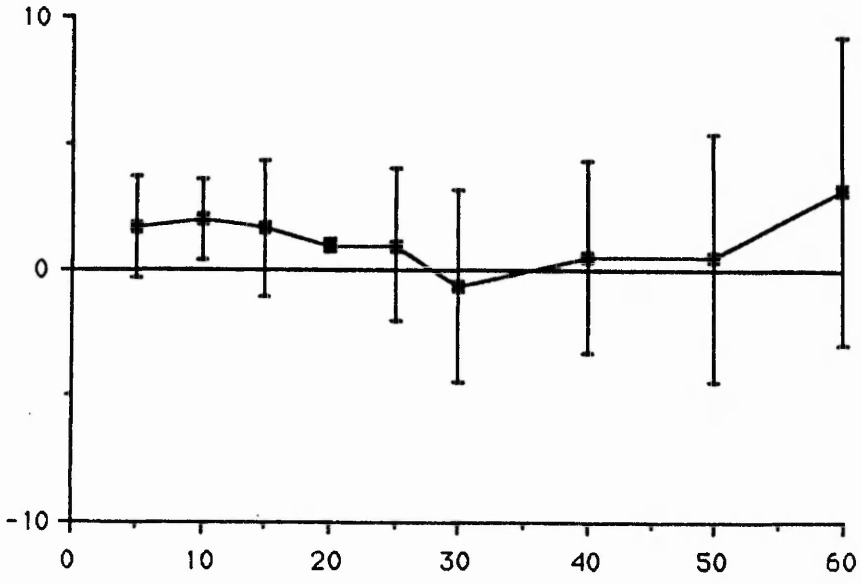
Graphs show mean difference (95% CI); see 2.7

a) AUC = -569 (-871, -267) mmHg.min (p< 0.01)



20 U.kg^{-1} sCT administered at t=0 min

b) AUC = 55 (-119, 229) mmHg.min (NS)

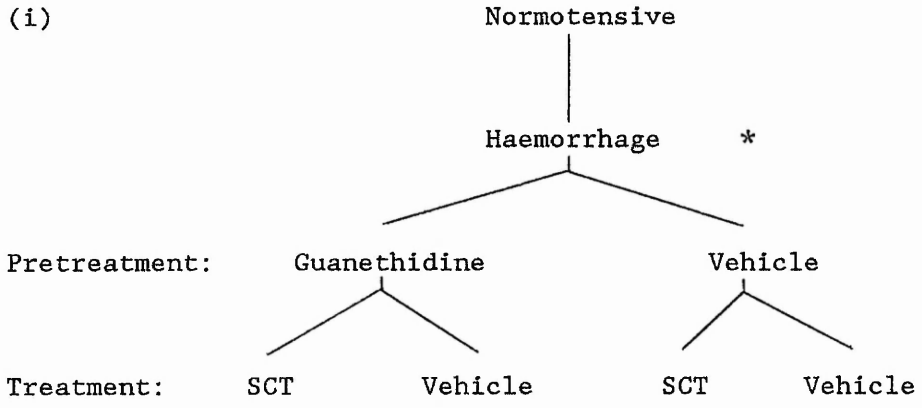


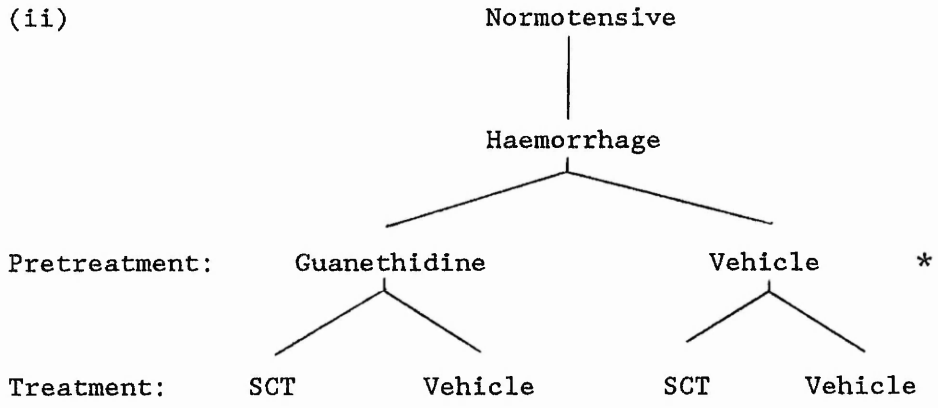
Change in M.A.P. (mmHg) (treated-control)

Time (min)

20 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.30. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 20 $U.kg^{-1}$ SCT (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.30(i) and (ii) overleaf were determined.

Fig.R.30. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 20 $U.kg^{-1}$ SCT (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Haemorrhaged rats were pre-treated with 10 $mg.kg^{-1}$ guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (20 $U.kg^{-1}$ iv) was administered.

b) 20 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered iv, 10 min after pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv), to rats rendered hypotensive by haemorrhage.

Table R.30(i) Post-haemorrhage, pre-pretreatment heart rates

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	352 (6)	340 (6)
	Mean difference (95% CI): 12 (-39, 64) NS		

Table R.30(ii) Post-haemorrhage, post-pretreatment heart rates

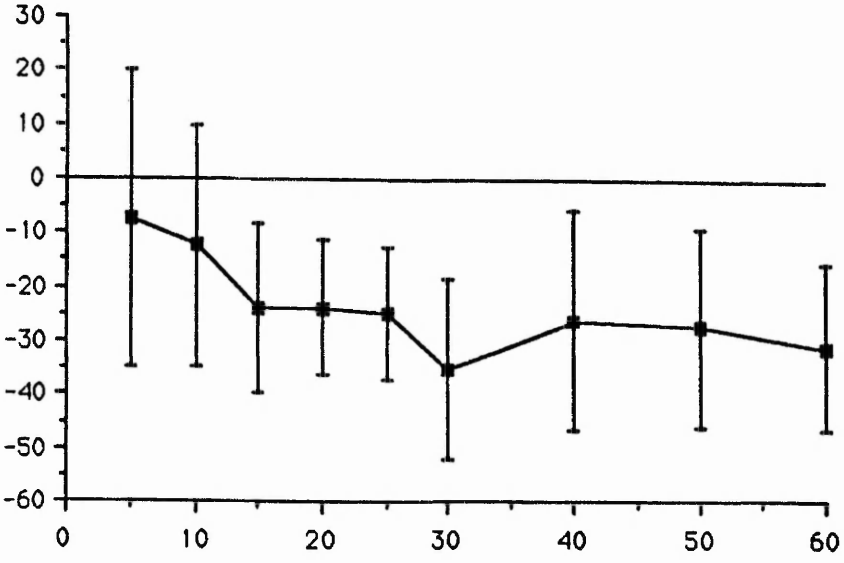
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	422 (6)	339 (6)
	Mean difference (95% CI): 83(23, 143) $p< 0.01$		

Heart rate = mean (n)

NS = not statistically significant

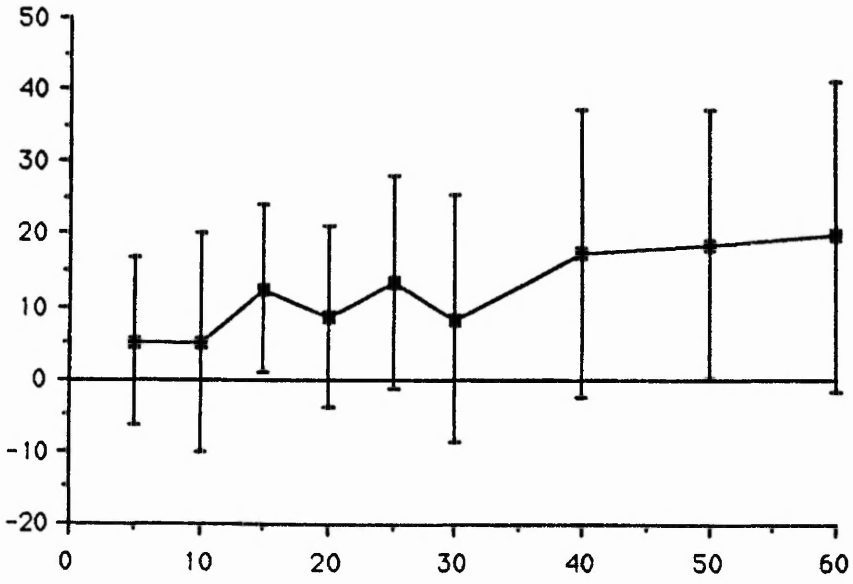
Graphs show mean difference (95% CI); see 2.7

a) AUC = -1427 (-2071, -782) beats ($p < 0.001$)



20 U.kg^{-1} sCT administered at t=0 min

b) AUC = 752 (5, 1498) beats ($p < 0.05$)

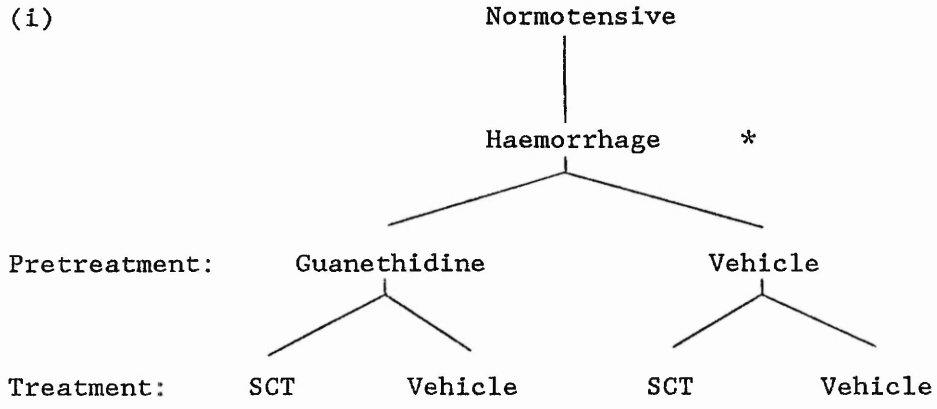


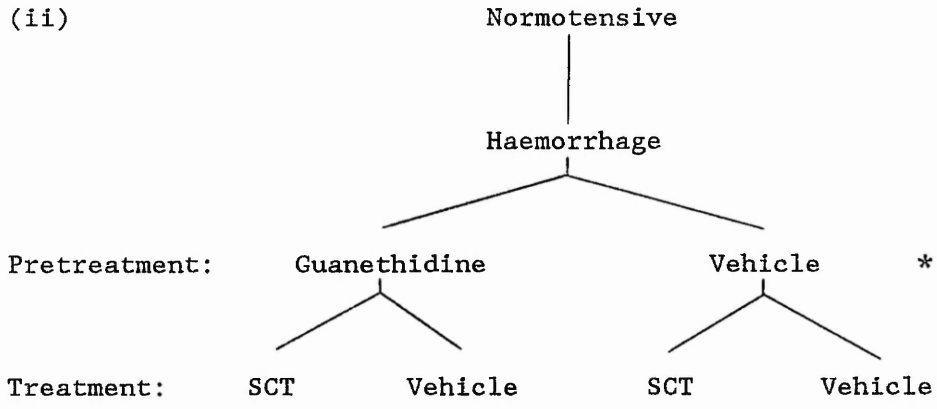
Change in Heart Rate (bpm) (treated-control)

Time (min)

20 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.31. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 5 $U.kg^{-1}$ SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorhhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.31(i) and (ii) overleaf were determined.

Fig.R.31. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 5 U.kg^{-1} SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorhhage.

a) Haemorrhaged rats were pre-treated with 10 mg.kg^{-1} guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 U.kg^{-1} icv) was administered.

b) 5 U.kg^{-1} sCT (treated) or appropriate vehicle (control) was administered icv, 10 min after pre-treatment with guanethidine (10 mg.kg^{-1} iv), to rats rendered hypotensive by haemorrhage.

Table R.31(i) Post-haemorrhage, pre-pretreatment MAP's

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	79 (5)	81 (5)
	Mean difference (95% CI): -3(-18, 13) NS		

Table R.31(ii) Post-haemorrhage, post-pretreatment MAP's

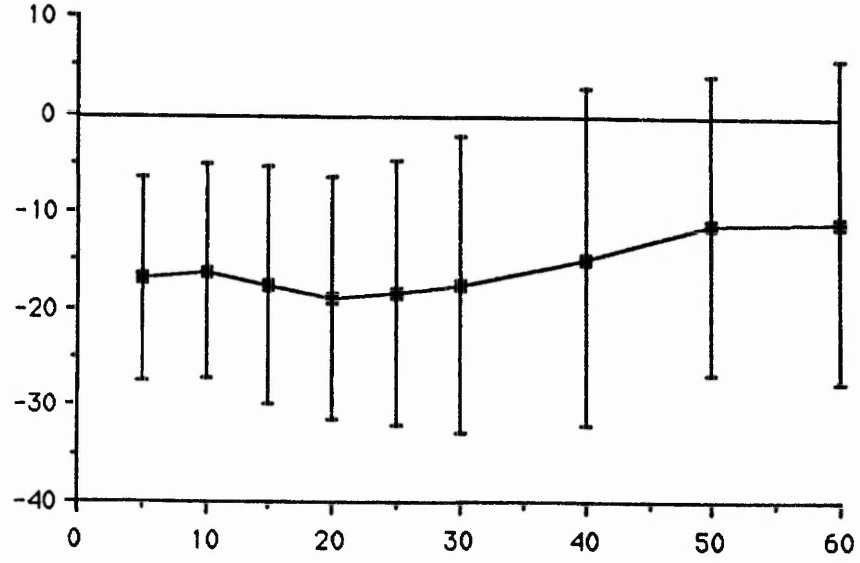
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	MAP (mmHg):	69 (5)	82 (5)
	Mean difference (95% CI): -13 (-28, 1) NS		

MAP = mean (n)

NS = not statistically significant

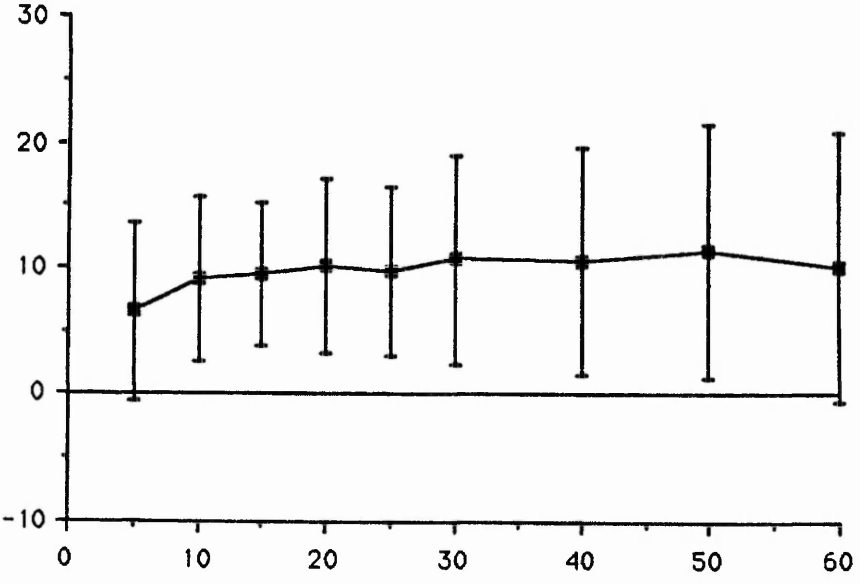
Graphs show mean difference (95% CI); see 2.7

a) AUC = -889 (-1671, -107) mmHg.min ($p < 0.05$)



5 $U.kg^{-1}$ sCT administered at t=0 min

b) AUC = 579 (119, 1039) mmHg.min ($p < 0.05$)

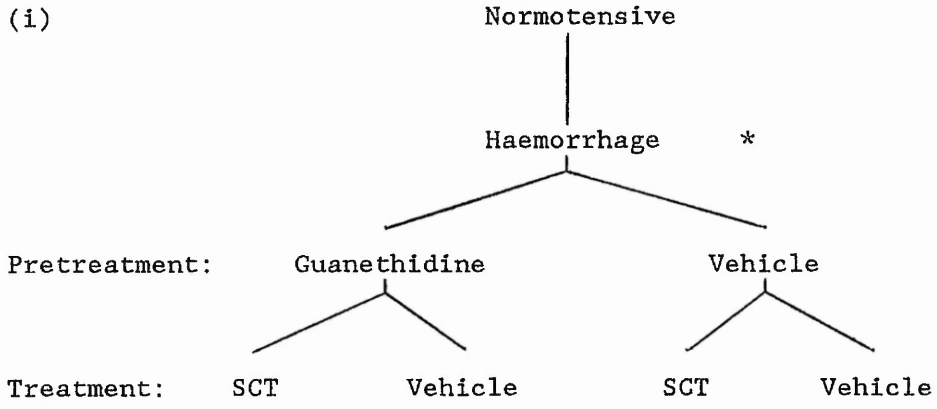


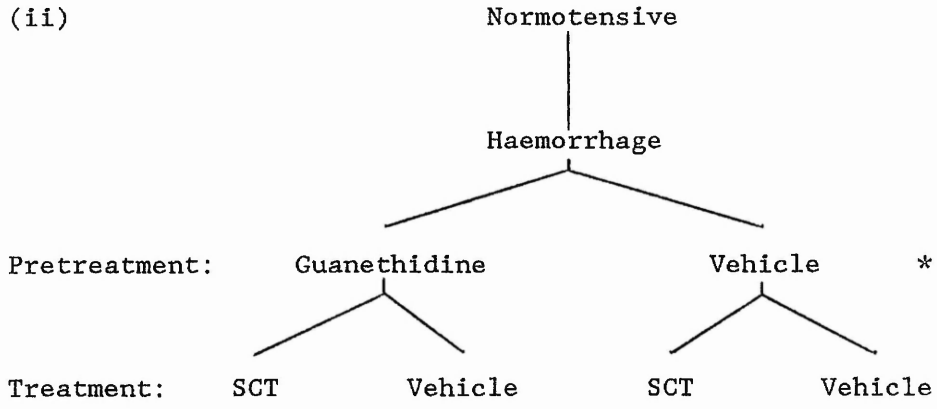
Change in M.A.P. (mmHg) (treated-control)

Time (min)

5 $U.kg^{-1}$ sCT/vehicle administered at t=0 min

Fig.R.32. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 5 $U.kg^{-1}$ SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.32(i) and (ii) overleaf were determined.

Fig.R.32. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 5 U.kg^{-1} SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Haemorrhaged rats were pre-treated with 10 mg.kg^{-1} guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 U.kg^{-1} icv) was administered.

b) 5 U.kg^{-1} sCT (treated) or appropriate vehicle (control) was administered icv, 10 min after pre-treatment with guanethidine (10 mg.kg^{-1} iv), to rats rendered hypotensive by haemorrhage.

Table R.32(i) Post-haemorrhage, pre-pretreatment heart rates

Post-haemorrhage (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	336 (5)	317 (5)
	Mean difference (95% CI): 19 (-40, 79) NS		

Table R.32(ii) Post-haemorrhage, post-pretreatment heart rates

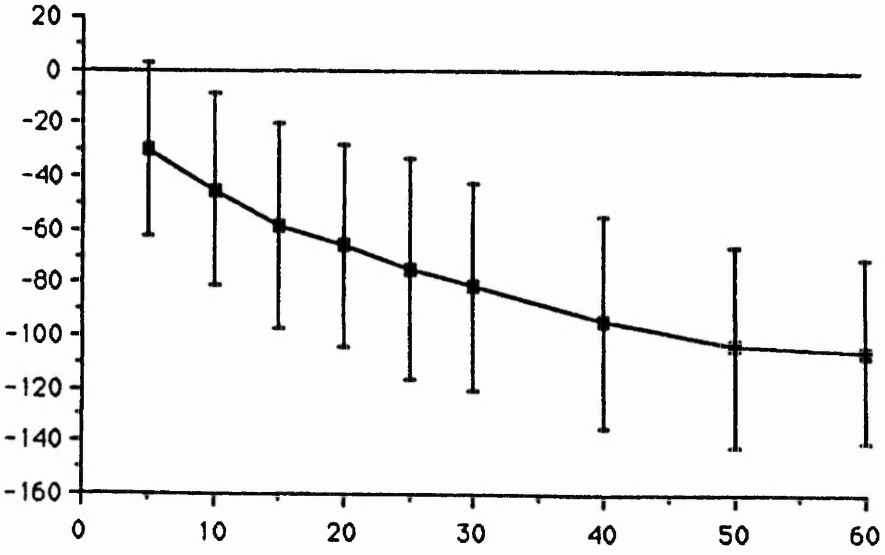
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	383 (5)	323 (5)
	Mean difference (95% CI): 60 (11, 108) $p < 0.05$		

Heart rate = mean (n)

NS = not statistically significant

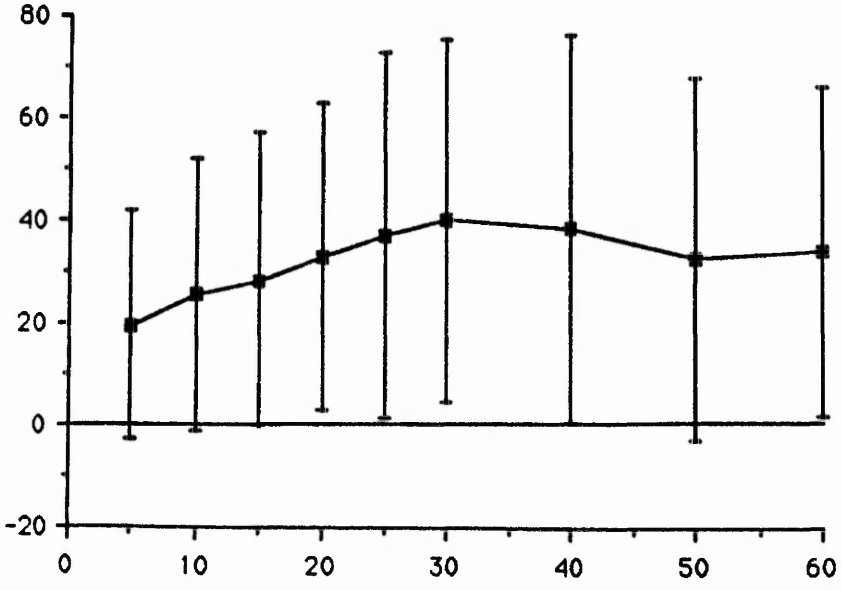
Graphs show mean difference (95% CI); see 2.7

a) AUC = -4473 (-6584, -2363) beats ($p< 0.001$)



5 U.kg^{-1} sCT administered at t=0 min

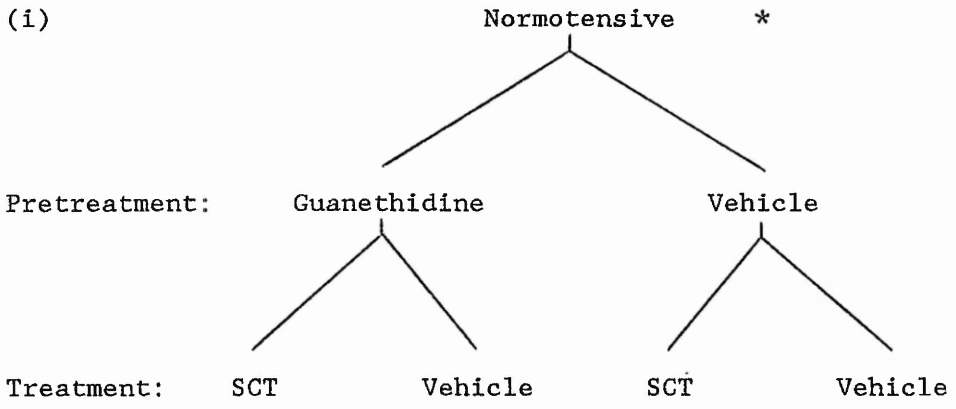
b) AUC = 1917 (101, 3732) beats ($p< 0.05$)

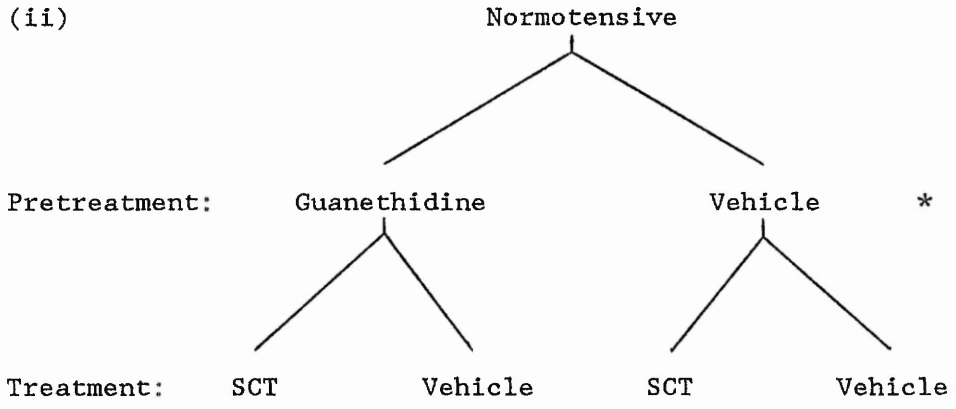


Change in Heart Rate (bpm) (treated-control)

5 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.33. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 5 $U.kg^{-1}$ SCT icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the MAP values depicted in tables R.33(i) and (ii) overleaf were determined.

Fig.R.33. Effect of Pre-treatment with Guanethidine (iv) on the Blood Pressure Response to 5 U.kg^{-1} SCT icv) in Urethane Anaesthetised Normotensive Rats.

a) Normotensive rats were pre-treated with 10 mg.kg^{-1} guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 U.kg^{-1} icv) was administered.

b) 5 U.kg^{-1} sCT (treated) or appropriate vehicle (control) was administered icv, 10 min after pre-treatment with guanethidine (10 mg.kg^{-1} iv), to normotensive rats.

Table R.33(i) Normotensive, pre-pretreatment MAP's

Normotensive (t = -10 min)	Pre-treatment: MAP (mmHg):	Guanethidine 110 (5)	Vehicle 106 (5)
	Mean difference (95% CI): 4 (-17, 21) NS		

Table R.33(ii) Normotensive, post-pretreatment MAP's

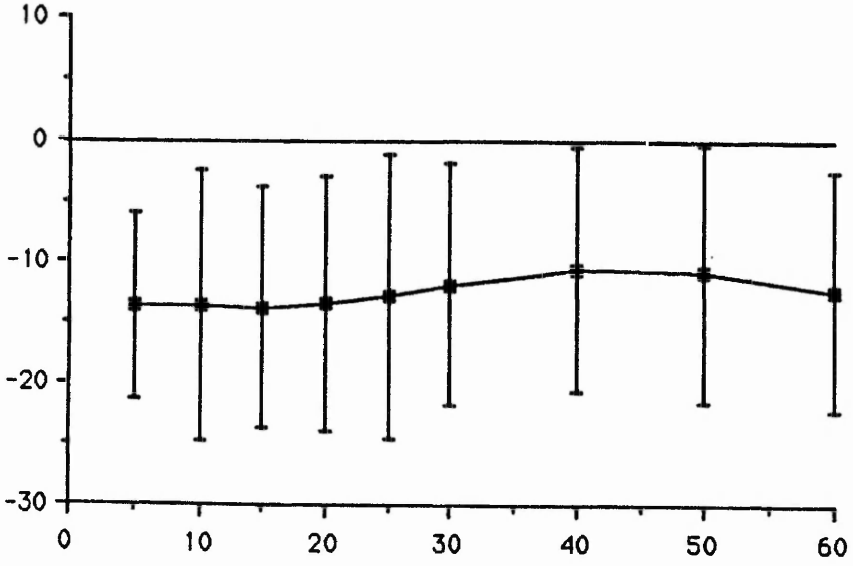
Normotensive + pre-treatment (t = 0 min)	Pre-treatment: MAP (mmHg):	Guanethidine 83 (5)	Vehicle 95 (5)
	Mean difference (95% CI): -12 (-21, -4) $p< 0.05$		

MAP = mean (n)

NS = not statistically significant

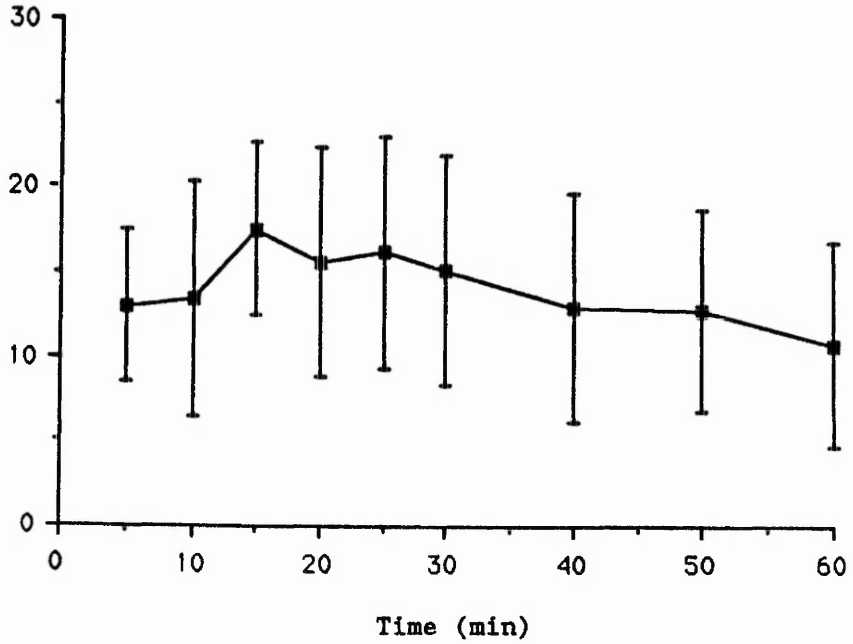
Graphs show mean difference (95 % CI); see 2.7

a) AUC = -701 (-1260, -141) mmHg.min ($p < 0.05$)



5 U.kg^{-1} sCT administered at t=0 min

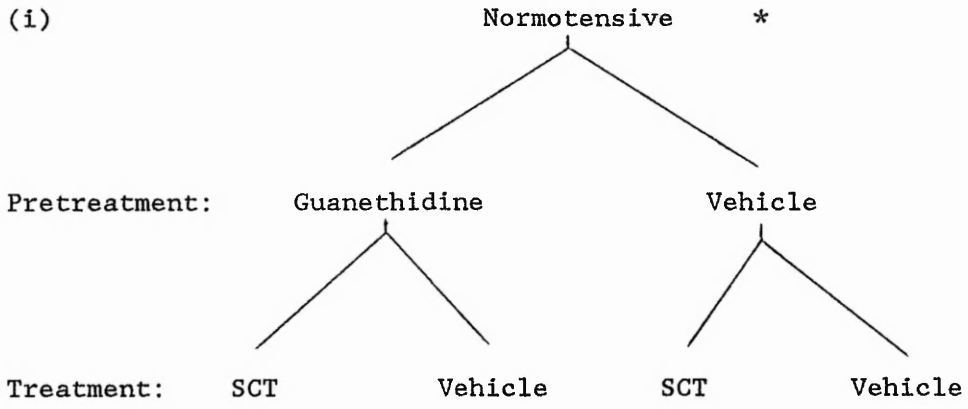
b) AUC = 805 (469, 1140) mmHg.min ($p < 0.001$)

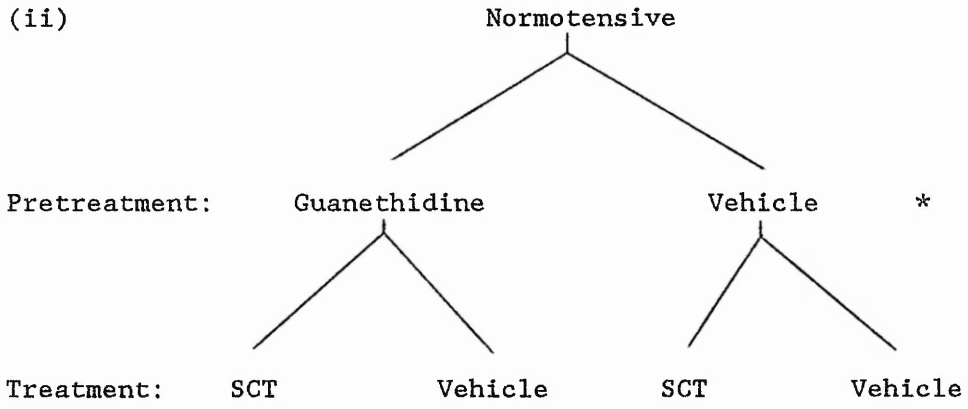


5 U.kg^{-1} sCT/vehicle administered at t=0 min

Change in M.A.P. (mmHg) (treated-control)

Fig.R.34. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 5 $U.kg^{-1}$ SCT (icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.34(i) and (ii) were determined.

Fig.R.34. Effect of Pre-treatment with Guanethidine (iv) on the Heart Rate Response to 5 U.kg^{-1} SCT (icv) in Urethane Anaesthetised Normotensive Rats.

a) Normotensive rats were pre-treated with 10 mg.kg^{-1} guanethidine (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 U.kg^{-1} icv) was administered.

b) 5 U.kg^{-1} sCT (treated) or appropriate vehicle (control) was administered icv, 10 min after pre-treatment with guanethidine (10 mg.kg^{-1} iv), to normotensive rats.

Table R.34(i) Normotensive, pre-pretreatment heart rates

Normotensive (t = -10 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	356 (5)	347 (5)
	Mean difference (95% CI): 9 (-57, 75) NS		

Table R.34(ii) Normotensive, post-pretreatment heart rates

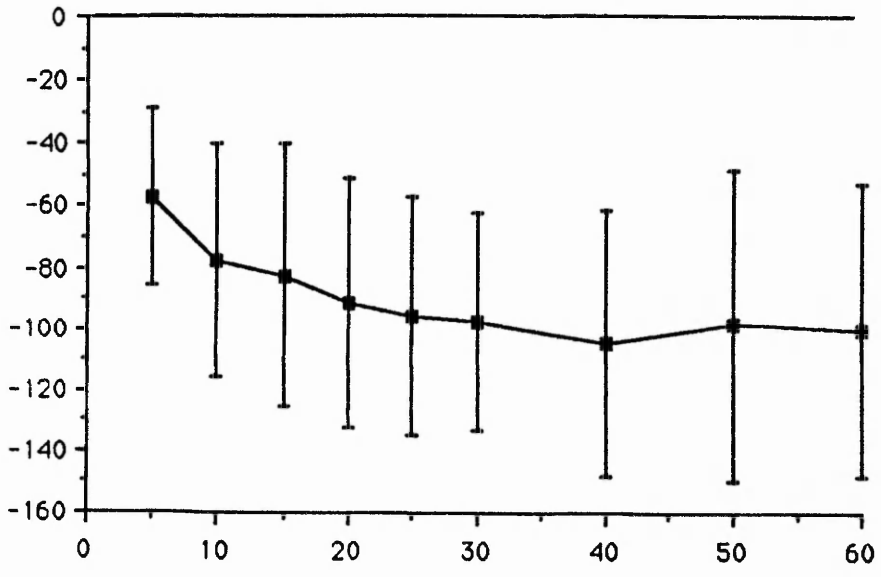
Normotensive + pre-treatment (t = 0 min)	Pre-treatment:	Guanethidine	Vehicle
	Heart rate (bpm):	386 (5)	344 (5)
	Mean difference (95% CI): 42 (-17, 101) NS		

Heart rate = mean (n)

NS = not statistically significant

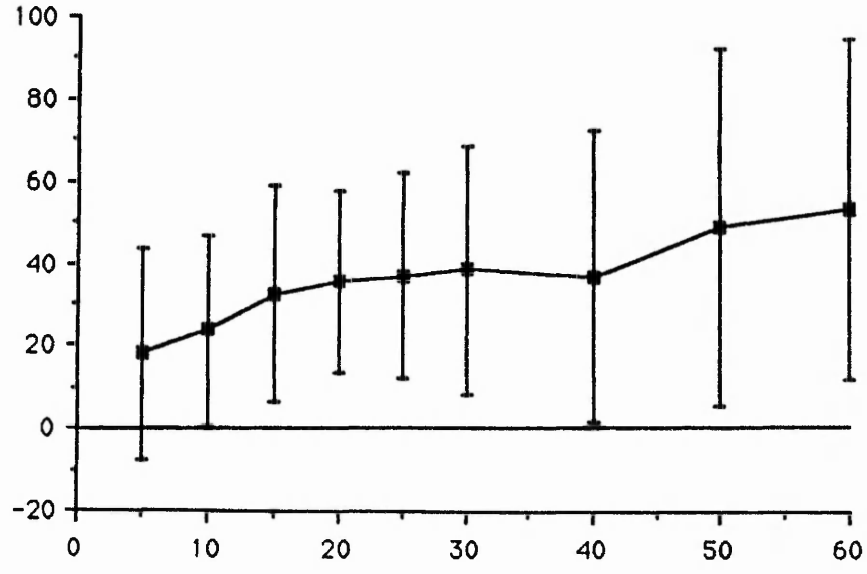
Graphs show mean difference (95% CI); see 2.7

a) AUC = -5295 (-7514, -3075) beats ($p < 0.001$)



5 U.kg^{-1} sCT administered at t=0 min

b) AUC = 2169 (442, 3895) beats ($p < 0.05$)



Change in Heart Rate (bpm) (treated-control)

5 U.kg^{-1} sCT/vehicle administered at t=0 min

6. THE ROLE OF THE ADRENAL GLANDS IN THE CARDIOVASCULAR RESPONSE TO CALCITONIN

6.1 INTRODUCTION

It was hypothesised in the previous chapter that release of catecholamines from the adrenal medulla may be responsible for the excess heart rate and increased MAP observed after central administration of sCT to sympathetically blocked, urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. Thus, the present chapter investigates the role of the adrenal glands in the cardiovascular response to sCT (icv), both with and without pre-treatment with guanethidine, in normotensive rats and haemorrhaged rats.

Stimulation of the sympathetic nervous system can, in addition to exerting a direct effect on blood vessels and the heart, cause release of catecholamines from the adrenal medulla. These hormones then circulate to all parts of the body and cause essentially the same effects on the circulatory system as direct sympathomimetic stimulation (Guyton, 1981). In normotensive animals there exists a functional balance between the activity of each of these components of the sympathetic system; either of them has the ability to compensate for a deficiency of the other. This has been demonstrated by de Champlain and van Ameringen (1972) in experiments with anaesthetised rats. It was observed that bilateral adrenalectomy had no significant effects on blood pressure or heart rate, but, after chemical sympathectomy with 6-OHDA (100 $mg.kg^{-1}$ iv), adrenalectomy resulted in a marked decrease in blood pressure and heart rate. It was suggested

that the latter response was due to the interruption of adrenal medullary secretion of catecholamines since corticosterone acetate (10 $mg.kg^{-1}$) led to a similar hypotensive response. Furthermore,in previously sympathectomised rats, administration of an α-adrenoceptor antagonist (dibenamine at 5 $mg.kg^{-1}$) could mimic the effcts of adrenalectomy. It was also shown that clamping of adrenal vessels caused a rapid fall in blood pressure, whereas unclamping rapidly restored the blood pressure to initial values.

Mueller et al. (1969) investigated the activity of tyrosine hydroxylase, in the heart and adrenal medulla, after 6-OHDA (100 $mg.kg^{-1}$ iv, 2 injections - 8 h apart) in rats. It was observed that cardiac tyrosine hydroxylase activity was markedly reduced whereas adrenal tyrosine hydroxylase activity was significantly increased, thus suggesting an increased catecholamine synthesis rate in the adrenals. It has also been demonstrated that 6-OHDA (200 $mg.kg^{-1}$ iv, 48 h and 24 h before sacrifice) results in an increased firing of the splanchnic nerves of the adrenal glands (Thoenen et al. 1969). These workers transected the nerve fibres leading from the main splanchnic trunk to the left adrenal gland in rats. Administration of 6-OHDA resulted in an increase in tyrosine hydroxylase activity of the innervated adrenal gland, but not the denervated gland.

The sympathoadrenal system is activated by haemorrhage leading to increased plasma levels of catecholamines (Chien and Simchon, 1983). Fredholm et al. (1979) demonstrated that the increase in plasma adrenaline after haemorrhage was abolished by adrenal demedullation,

whereas plasma levels of noradrenaline and dopamine were significantly reduced. Conversely, after treatment with 6-OHDA (50 $mg.kg^{-1}$ iv, 3 and 2 days before experimentation) the increased plasma levels of adrenaline after haemorrhage were unaltered, but the levels of noradrenaline and dopamine were reduced. It was thus concluded that the adrenal medulla is responsible for the increased adrenaline levels seen after haemorrhage and that both the adrenal medulla and sympathetic nerves are responsible for the increased noradrenaline and dopamine levels. Cubedda et al. (1977) suggested that the adrenal glands contribute almost exclusively to the increase in plasma catecholamines induced by haemorrhagic hypotension in anaesthetised dogs. This conclusion was reached after the observation that bilateral adrenalectomy abolished the increase in plasma catecholamine levels.

The adrenal medulla has been implicated in the pressor response to naloxone during cardiovascular shock. Patton et al. (1982) demonstrated that bilateral adrenalectomy attenuated the pressor response to naloxone (2 $mg.kg^{-1}$ + 2 $mg.kg^{-1}.h^{-1}$ iv) during haemorrhage in dogs; this effect was reversed by hydrocortisone. Manugian et al. (1981) measured pre-ganglionic splanchnic nerve (PSN) activity in anaesthetised cats after naloxone (2 $mg.kg^{-1}$ iv or 0.2 $mg.kg^{-1}$ ic). An increase in PSN activity was observed associated with an increase in blood pressure. The role of the adrenal medulla in the pressor response to naloxone (30 $\mu g.rat^{-1}$ icv + 3 $mg.kg^{-1}$ iv) has also been investigated in conscious rats subjected to endotoxin shock. The pressor response was completely prevented by adrenalectomy and selective adrenal demedullation (Holaday et al. 1983).

The following experiments were designed to investigate the effect of centrally administered sCT on MAP and heart rate after bilateral adrenalectomy in urethane anaesthetised normotensive rats and those subjected to haemorrhage. The effect of combination treatment, bilateral adrenalectomy and acute sympathetic blockade, was also investigated.

6.2 RESULTS

NB. Results are expressed as mean difference, ie treated - control (95% CI)

1) Effect of bilateral adrenalectomy and acute sympathetic blockade on the cardiovascular response to icv sCT in urethane anaesthetised rats subjected to haemorrhage

The pressor effect of centrally administered sCT (5 $U.kg^{-1}$) in bilaterally adrenalectomised haemorrhaged rats was not found to be significantly different to the response observed in sham operated haemorrhaged rats (see fig.R.35a). However, bilateral adrenalectomy led to a significant reduction in the pressor response to sCT (icv) in haemorrhaged rats subjected to acute sympathetic blockade. This is demonstrated by the statistically significant decrease in AUC, relative to control, of -559 (-1041, -77) mmHg.min ($p< 0.05$) (see fig.R.35b). In fact, as can be seen in fig.R.35c, the blood pressure response to centrally administered sCT was abolished by the combination treatment in haemorrhaged rats, since the MAP response to sCT was not statistically significantly different from that of the appropriate control for sCT. Pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv) was more effective than bilateral adrenalectomy in reducing the pressor response to centrally administered sCT in rats

rendered hypotensive by haemorrhage. This can be seen in the greater reduction in AUC (-1744 (-2737, -752) mmHg.min; $p< 0.01$) when looking at the effect of guanethidine in adrenalectomised haemorrhaged rats (see fig.R.35d) compared with the effect of adrenalectomy in guanethidine pretreated haemorrhaged rats (AUC, relative to control, = -559 (-1041, -77) mmHg.min; $p< 0.05$; see fig.R.35b).

Bilateral adrenalectomy had no significant effect on the increased heart rate response to sCT (icv) in haemorrhaged rats, as illustrated in fig.R.36a. Furthermore, unlike the blood pressure response, bilateral adrenalectomy had no significant effect on the heart rate response to sCT (icv) in haemorrhaged rats subjected to acute sympathetic blockade (see fig.R.36b). The heart rate response to centrally administered sCT is, however, abolished after the combination treatment, as shown in fig.R.36c, in which the response to sCT is not significantly different from the response to the appropriate vehicle for sCT. Fig.R.36d demonstrates the effect of subjecting bilaterally adrenalectomised haemorrhaged rats to acute sympathetic blockade. It can be seen that pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv) leads to a statistically significant reduction in the pressor response to sCT (icv), as shown by a statistically significant decrease in AUC, relative to control, of -3344 (-5305, -1385) beats ($p< 0.01$). These results would suggest that the peripheral sympathetic nervous system is responsible for the increased-heart rate response to icv sCT in rats subjected to haemorrhage.

2) Effect of bilateral adrenalectomy and acute sympathetic blockade on the cardiovascular response to icv sCT in urethane anaesthetised normotensive rats

The effect of bilateral adrenalectomy in normotensive rats is very similar to that observed in haemorrhaged rats in that no significant effect was observed on the pressor response to icv sCT (see fig.R.37a). Bilateral adrenalectomy did, however, result in a statistically significant reduction in the pressor response to centrally administered sCT in normotensive rats subjected to acute sympathetic blockade. This is demonstrated by the statistically significant reduction in AUC, relative to control, of -675 (-1078, -272) mmHg.min ($p< 0.01$) (see fig.R.37b). Furthermore, this attenuation of the pressor response observed in normotensive rats is greater than that observed in rats rendered hypotensive by haemorrhage. Unlike the response in haemorrhaged rats, the pressor response to sCT (icv) in bilaterally adrenalectomised normotensive rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv) was, however, not abolished, but attenuated, as shown by the statistically significant increase in AUC of 484 (91, 876) mmHg.min ($p< 0.05$) after sCT compared with the appropriate vehicle for sCT (see fig.R.37c). It can be seen in fig.R.37d that pretreatment with guanethidine (10 $mg.kg^{-1}$ iv), unlike the effect in haemorrhaged rats, is as effective in reducing the pressor response to sCT in normotensive rats as is bilateral adrenalectomy. This is reflected in the similarity of the reduction in AUC when comparing the effect of adrenalectomy in guanethidine pretreated normotensive rats (AUC, relative to control, = -675 (-1078, -272) mmHg.min; $p< 0.01$; see fig.R.37b) with the effect of guanethidine in adrenalectomised rats (AUC, relative to control, = -622 (-990, -255) mmHg.min; $p< 0.01$; see fig.R.37d).

As is the case in rats rendered hypotensive by haemorrhage, bilateral adrenalectomy alone had no significant effect on the increased-heart rate response to sCT (icv) in normotensive rats (see fig.R.38a). However, unlike the response observed in haemorrhaged rats, bilateral adrenalectomy led to a statistically significant reduction in the excess heart rate response to centrally administered sCT in normotensive rats subjected to acute sympathetic blockade. This is demonstrated in the statistically significant decrease in AUC, relative to control, of -3102 (-4612, -1592) beats ($p< 0.001$) (see fig.R.38b). Moreover, the heart rate response to sCT (icv) in normotensive rats was actually abolished after the combination treatment, as shown in fig.R.38c, in which the response to sCT is not significantly different from the response to the appropriate vehicle for sCT. Fig.R.38d shows that pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv) led to a greater reduction in the increased heart rate response to sCT (icv) in normotensive rats than did bilateral adrenalectomy. This is demonstrated in the greater reduction in AUC when comparing the effect of guanethidine pretreatment in adrenalectomised normotensive rats (AUC, relative to control, = -8083 (-10858, -5308) beats; $p< 0.001$; see fig.R.38d) with the effect of adrenalectomy in guanethidine pretreated normotensive rats (AUC, relative to control, = -3102 (-4612, -1592) beats; $p< 0.001$; see fig.R.38b).

6.3 DISCUSSION

Bilateral adrenalectomy appears to exert no significant effect on MAP in normotensive rats (see tables R.35(i) and R.37(i)). This would indicate that the adrenal glands do not play a major role in the

maintenance of blood pressure. Furthermore, in normotensive rats subjected to acute sympathetic blockade, bilateral adrenalectomy has no significant effect on MAP compared with sham operated animals (see table R.38(ii)). This suggests that, even in the absence of the peripheral sympathetic nervous system, the adrenal glands do not significantly participate in blood pressure regulation. This is also the case in rats subjected to haemorrhage, ie, the adrenal glands do not exert a significant effect in blood pressure regulation even in the absence of a peripheral sympathetic nervous system (see table R.35(ii)).

The heart rate results indicate that bilateral adrenalectomy alone exerts no significant effects in both normotensive and haemorrhaged rats (see tables R.36(i) and R.38(i)). In normotensive rats this is also true in animals subjected to acute sympathetic blockade (see table R.38(ii)), suggesting that the adrenal glands do not significantly participate in the maintenance of heart rate even in the absence of a peripheral sympathetic nervous system. In haemorrhaged rats subjected to acute sympathetic blockade, however, bilateral adrenalectomy leads to a significant reduction in heart rate compared with sham operated controls (-60 (-11, -20); $p< 0.01$; see table R.36(iii)). Thus, the adrenal glands appear to play a significant role in the regulation of heart rate in the absence of a peripheral sympathetic nervous system. This supports the findings of Fredholm et al.(1979) and Cubedda et al. (1977) who observed a decrease in plasma adrenaline levels after bilateral adrenalectomy in anaesthetised haemorrhaged rats and dogs respectively.

Bilateral adrenalectomy exerts no significant effects on the pressor response to centrally administered sCT (5 $U.kg^{-1}$) in both urethane anaesthetised normotensive rats and those subjected to haemorrhage. However, in normotensive rats subjected to acute sympathetic blockade, bilateral adrenalectomy leads to a significant attenuation of the pressor response to sCT. It would appear that blocking the peripheral sympathetic nervous system is equally as effective as bilateral adrenalectomy in the absence of a peripheral sympathetic nervous system in reducing this pressor response to sCT in normotensive animals. On the other hand, in haemorrhaged rats the combination treatment abolishes the pressor response to centrally administered sCT. Moreover, acute sympathetic blockade is more effective than bilateral adrenalectomy in the absence of a peripheral SNS in this respect.

Conway et al. (1983) investigated the effect of chronic bilateral adrenalectomy on the pressor response to icv morphine (30 μg) in normotensive rats. It was suggested that the opiate-induced increase in plasma catecholamines reflects stimulation of both sympathetic nervous activity and catecholamine release from the adrenal medulla. However, since the opiate-induced increase in blood pressure remained after adrenalectomy it was suggested that noradrenaline release from sympathetic nerve endings was more important than the release of adrenaline from the adrenal medulla, in this pressor effect. This appears to differ from the situation observed after centrally administered sCT in the present study.

Bilateral adrenalectomy has no significant effects on the heart rate response to sCT (icv) in urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. The increased heart rate response observed after sCT in normotensive rats is, however, significantly reduced after bilateral adrenalectomy in rats subjected to acute sympathetic blockade. Furthermore, the combination treatment actually results in abolition of the heart rate response in normotensive animals. The reduction in the excess heart rate response to sCT (icv) is more marked after acute sympathetic blockade than after bilateral adrenalectomy. Haemorrhaged rats again differ from the normotensive rats in that bilateral adrenalectomy, whether performed in the presence or absence of a peripheral sympathetic nervous system, does not affect the increased heart rate response to centrally administered sCT. The heart rate response can be seen to be abolished after the combination treatment, thus suggesting that the peripheral sympathetic nervous system is solely responsible for this effect. These results are in direct conflict with the results obtained in the previous chapter in which an attenuation, and not abolition, of the heart rate response was observed. This would suggest that the presence of the adrenal glands modifies the heart rate response to sCT (icv).

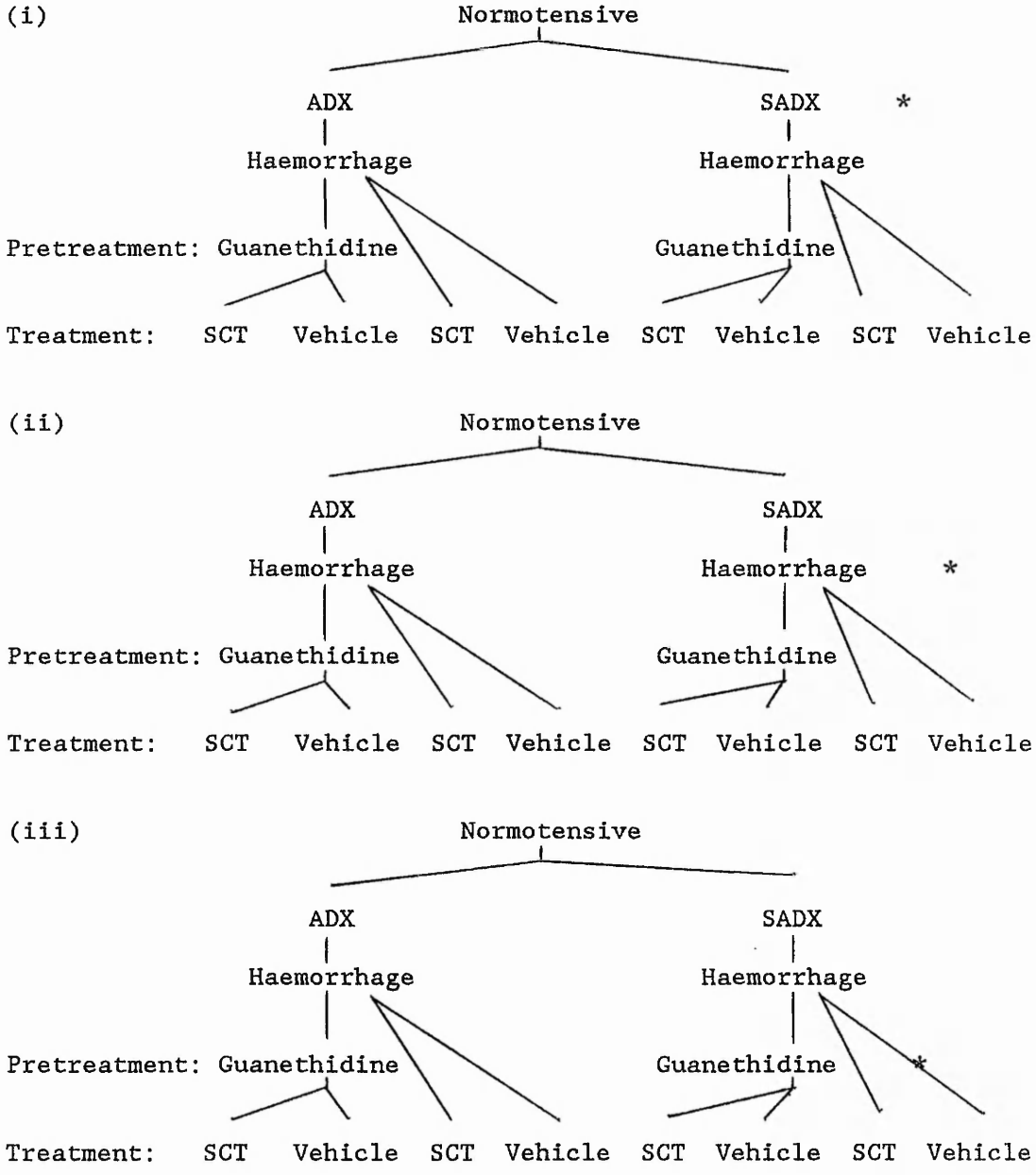
It can be concluded from the above results that the release of catecholamines from the adrenal medulla and the activity of the peripheral sympathetic nervous system are equally effective in mediating the pressor response to centrally administered sCT in urethane anaesthetised normotensive rats. However, some other mechanism(s) must also be involved since removal of both of the above

systems only results in an attenuation of the response. It is possible that this pressor effect of centrally administered sCT may be mediated partly by the action of the renin-angiotensin system. The heart rate response to sCT (icv) in normotensive rats appears to be mediated both by the release of catecholamines from the adrenal medulla and the activity of the peripheral sympathetic nervous system, although the latter system seems to be of greater importance.

The mechanisms responsible for the pressor response and increased heart rate response after icv sCT in urethane anaesthetised rats rendered hypotensive by haemorrhage would appear to differ from those in normotensive rats. The pressor response appears to be mediated by the release of catecholamines from the adrenal medulla and by the activity of the peripheral sympathetic nervous system, but the latter mechanism seems to be of greater importance. The peripheral sympathetic nervous system appears to be solely responsible for the increased heart rate response to sCT (icv) in haemorrhaged rats, but it is unclear why the presence of intact adrenal medullae is important since they do not appear to participate themselves in the response.

It would be of interest to determine the effect of bilateral adrenalectomy, both with and without acute sympathetic blockade, on the plasma levels of catecholamines in urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. The plasma levels of catecholamines could then possibly be correlated with the changes in the cardiovascular parameters observed.

Fig.R.35. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.



* denotes the stages of the experiment at which the MAP values depicted in tables R.35(i), (ii) and (iii) overleaf were determined.

Fig.R.35. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Bilateral adrenalectomised rats (treated) or sham bilateral adrenalectomised rats (control) were rendered hypotensive by haemorrhage. Salmon CT (5 $U.kg^{-1}$) was then administered by icv injection.

b) Bilateral adrenalectomised rats (treated) or sham bilateral adrenalectomised rats (control) were rendered hypotensive by haemorrhage. Ten min after pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv), sCT (5 $U.kg^{-1}$) was administered by icv injection.

Table R.35(i) Pre-haemorrhage, pre-pretreatment MAP's

Normotensive		ADX	SADX
	MAP (mmHg):	109 (5)	104 (5)
	Mean difference (95% CI): 5 (-27, 37) NS		

Table R.35(ii) Post-haemorrhage, pre-pretreatment MAP's

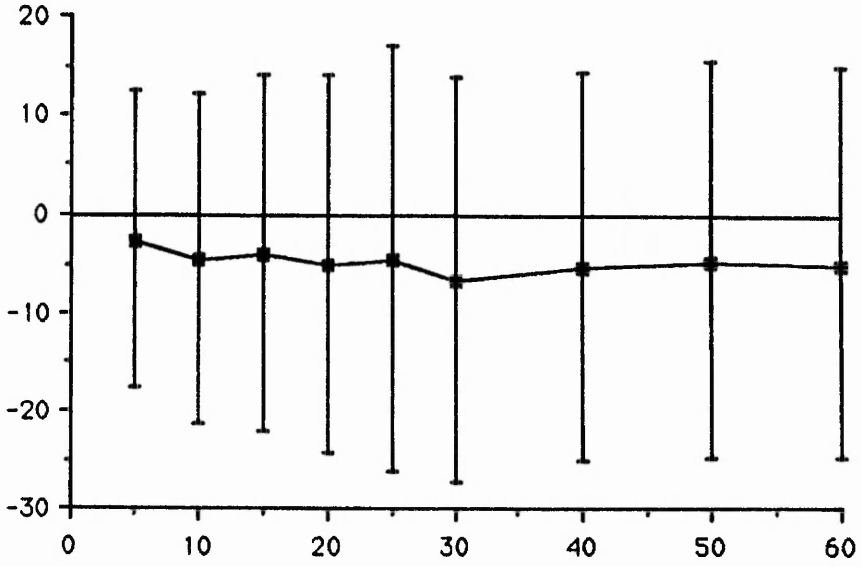
Post-haemorrhage (t = -10 min)		ADX	SADX
	MAP (mmHg):	84 (5)	81 (5)
	Mean difference (95% CI): 3 (-23, 29) NS		

Table R.35(iii) Post-haemorrhage, post-pretreatment MAP's

Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	ADX + Guanethidine	SADX + Guanethidine
	MAP (mmHg):	73 (5)	79 (5)
	Mean difference (95% CI): -6 (-18, 6) NS		

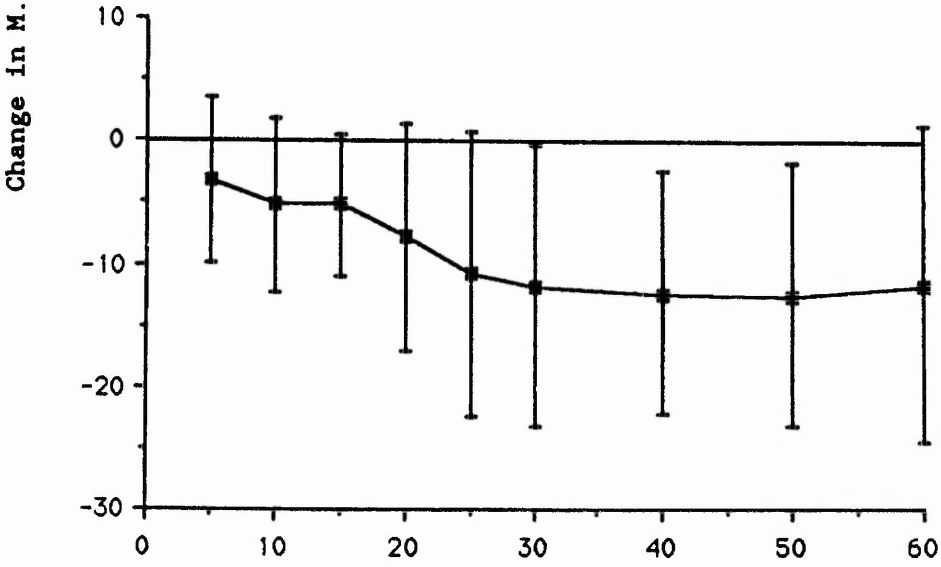
ADX = adrenalectomy
SADX = sham adrenalectomy
MAP = mean (n)
NS = not statistically significant
Graphs show mean difference (95% CI); see 2.7

a) AUC = -283 (-1366, 800) mmHg.min (NS)



5 U.kg^{-1} sCT administered at t=0 min

b) AUC = -559 (-1041, -77) mmHg.min ($p< 0.05$)

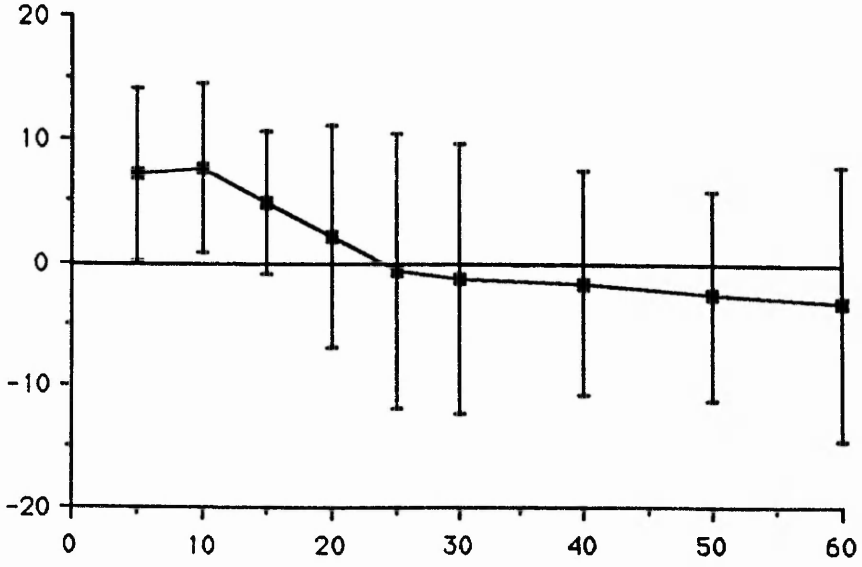


5 U.kg^{-1} sCT administered at t=0 min

Fig.R.35. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

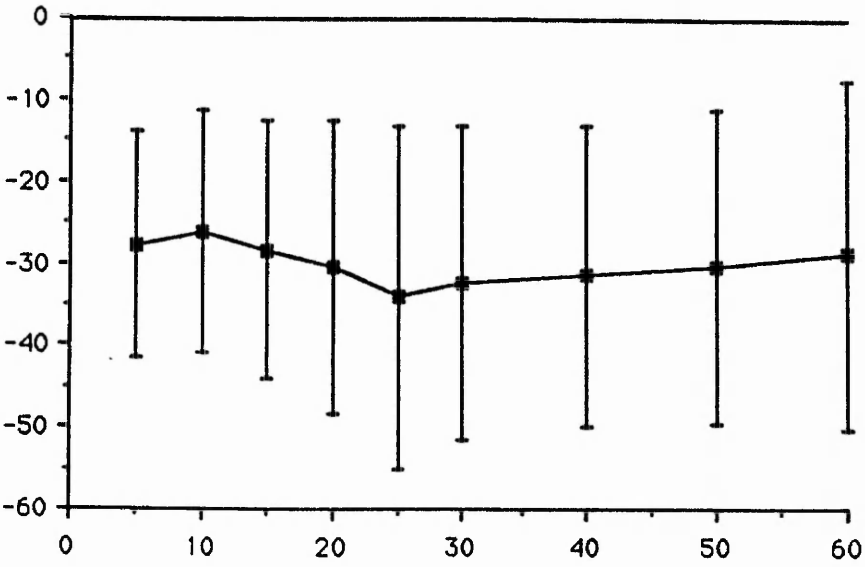
c) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered by icv injection to bilateral adrenalectomised haemorrhaged rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv).
d) 5 $U.kg^{-1}$ sCT (icv) was administered to bilateral adrenalectomised haemorrhaged rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv) (treated) or to bilateral adrenalectomised rats only subjected to haemorrhage (control).

c) AUC = 30 (-411, 472) mmHg.min (NS)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

d) AUC = -1744 (-2737, -752) mmHg.min (p< 0.01)

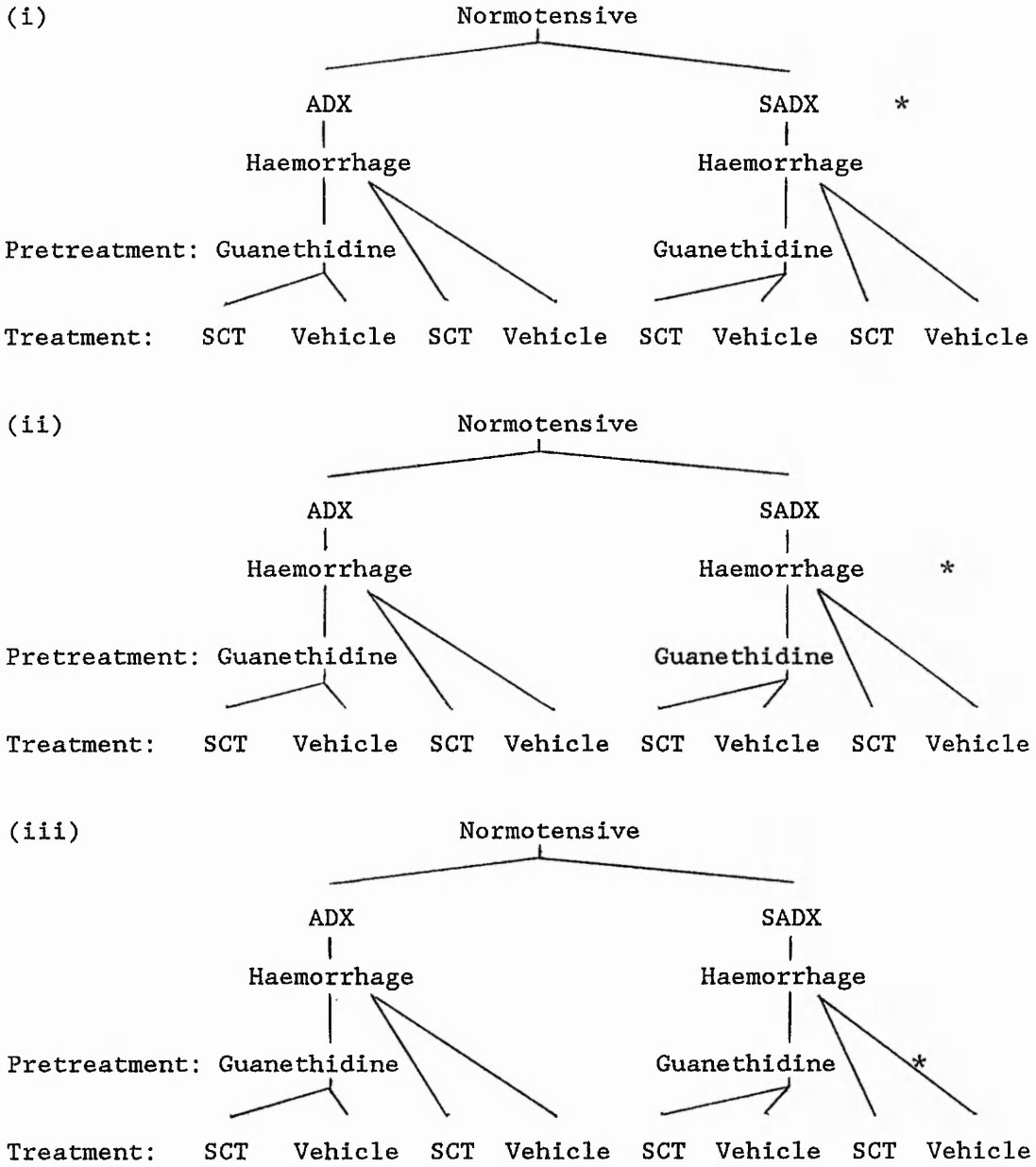


Change in M.A.P. (mmHg) (treated-control)

Time (min)

5 U.kg^{-1} sCT administered at t=0 min

Fig.R.36. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.



* denotes the stages of the experiment at which the heart rate values depicted in tables R.36(i), (ii) and (iii) overleaf were determined.

Fig.R.36. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Bilateral adrenalectomised rats (treated) or sham bilateral adrenalectomised rats (control) were rendered hypotensive by haemorrhage. Salmon CT (5 $U.kg^{-1}$) was then administered by icv injection.

b) Bilateral adrenalectomised rats (treated) or sham bilateral adrenalectomised rats (control) were rendered hypotensive by haemorrhage. Ten min after pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv), sCT (5 $U.kg^{-1}$) was administered by icv injection.

Table R.36(i) Pre-haemorrhage, pre-pretreatment heart rates

Normotensive		ADX	SADX
	Heart rate (bpm):	350 (5)	375 (5)
	Mean difference (95% CI): -25 (-72, 22) NS		

Table R.36(ii) Post-haemorrhage, pre-pretreatment heart rates

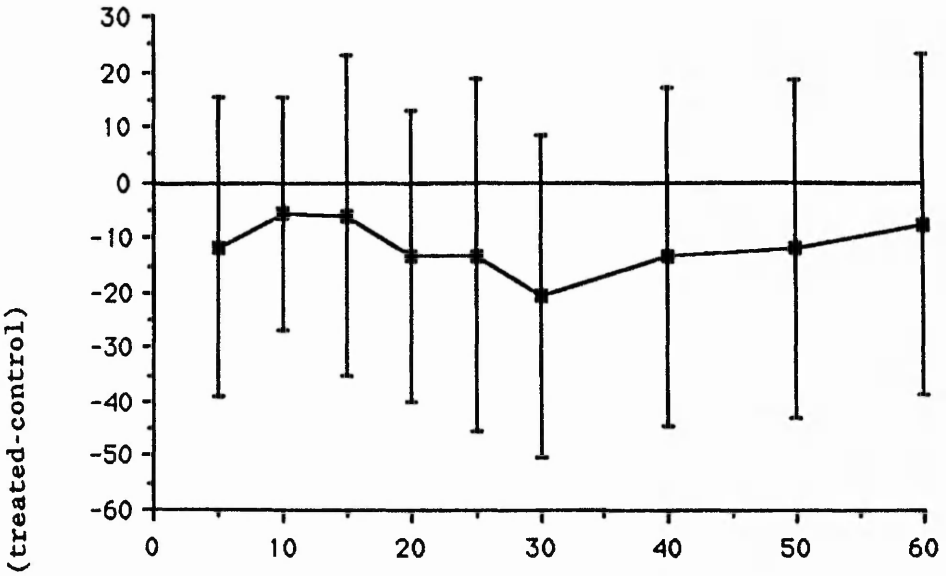
Post-haemorrhage (t = -10 min)		ADX	SADX
	Heart rate (bpm):	348 (5)	354 (5)
	Mean difference (95% CI): -6 (-67, 55) NS		

Table R.36(iii) Post-haemorrhage, post-pretreatment heart rates

Post -haem + pre-treatment (t = 0 min)	Pre-treatment:	ADX + Guanethidine	SADX + Guanethidine
	Heart rate (bpm):	366 (5)	426 (5)
	Mean difference (95% CI): -60 (-100, -20) $p< 0.01$		

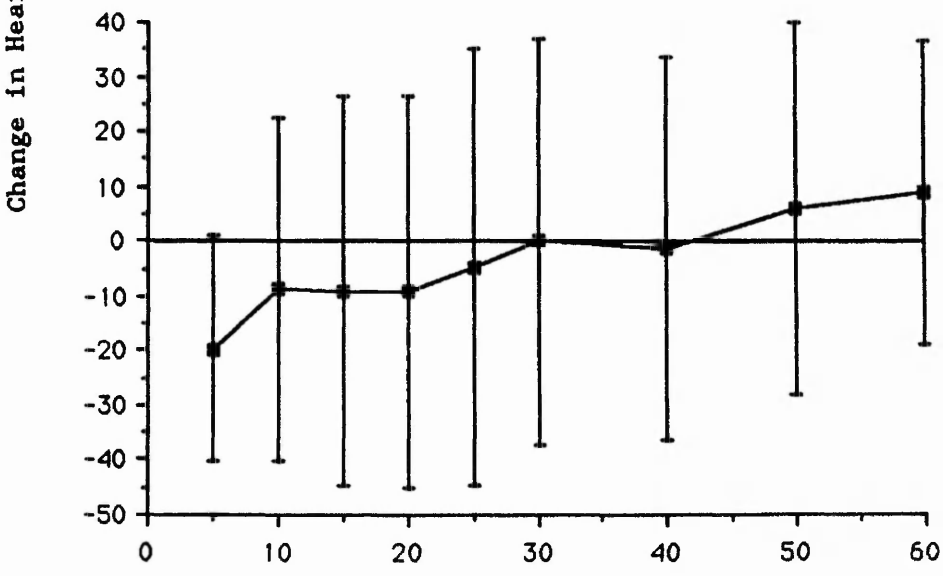
ADX = adrenalectomy
SADX = sham adrenalectomy
Heart rate = mean (n)
NS = not statistically significant
Graphs show mean difference (95% CI); see 2.7

a) AUC = -697 (-2257, 864) beats (NS)



5 U.kg^{-1} sCT administered at t=0 min

b) AUC = -167 (-2012, 1678) beats (NS)



Change in Heart Rate (bpm) (treated-control)

Time (min)

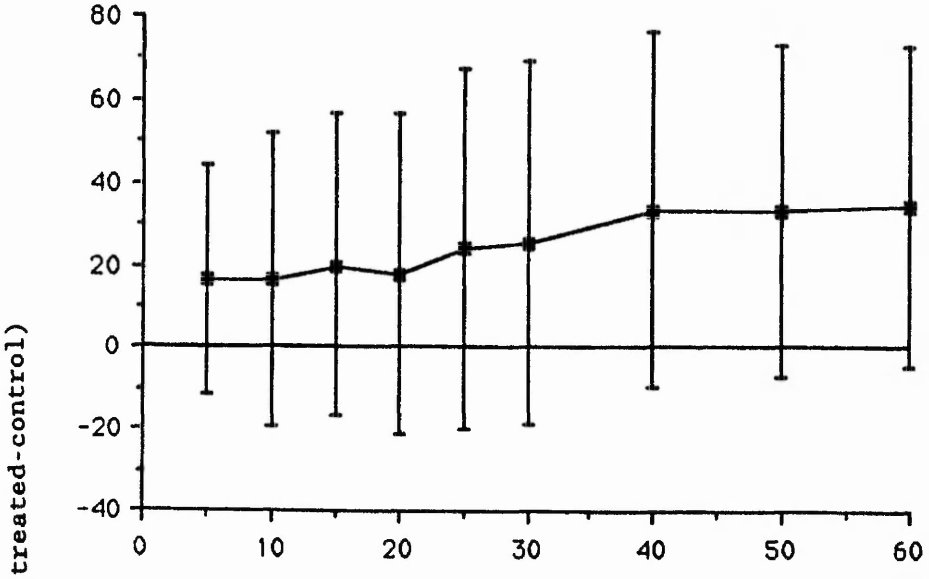
5 U.kg^{-1} sCT administered at t=0 min

Fig.R.36. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

c) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered by icv injection to bilateral adrenalectomised haemorrhaged rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv).

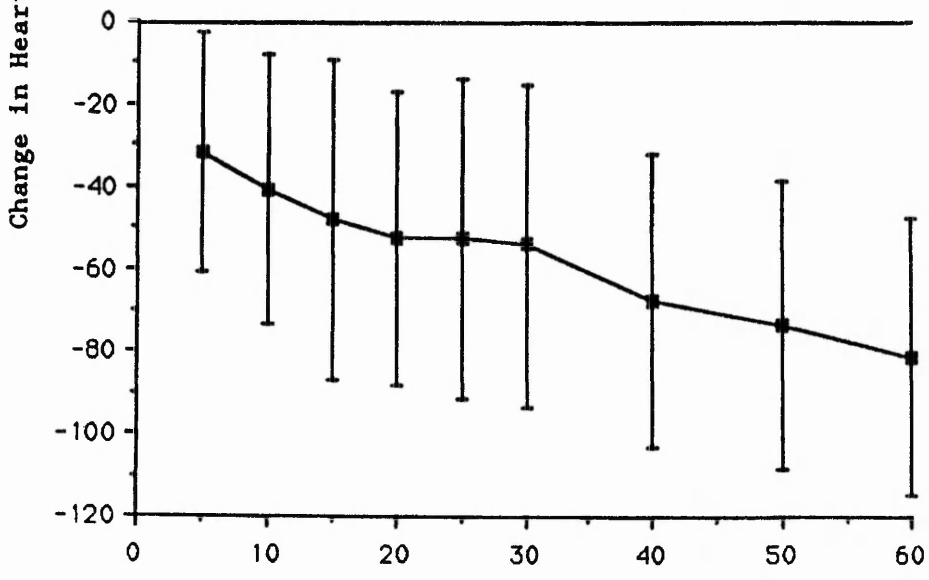
d) 5 $U.kg^{-1}$ sCT (icv) was administered to bilateral adrenalectomised haemorrhaged rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv) (treated) or to bilateral adrenalectomised rats only subjected to haemorrhage (control).

c) AUC = 1507 (-707, 3722) beats (NS)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

d) AUC = -3344 (-5305, -1383) beats ($p< 0.01$)

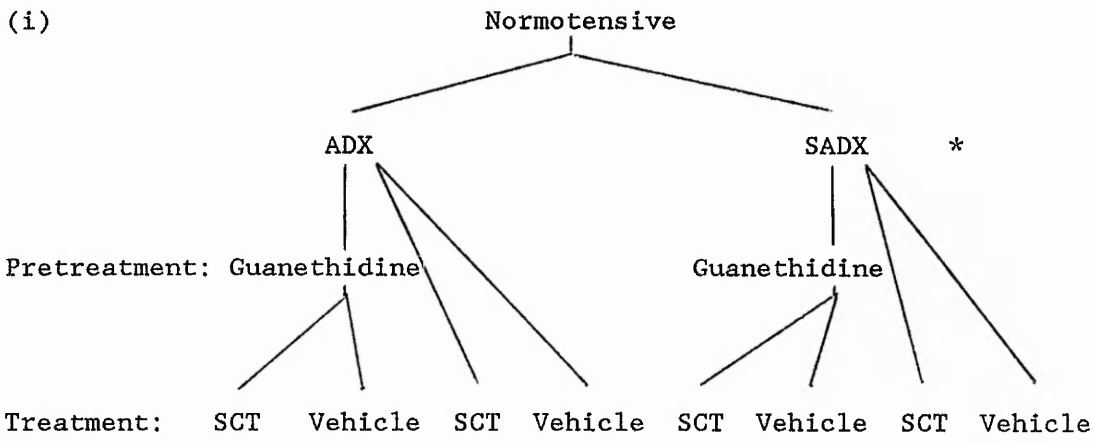


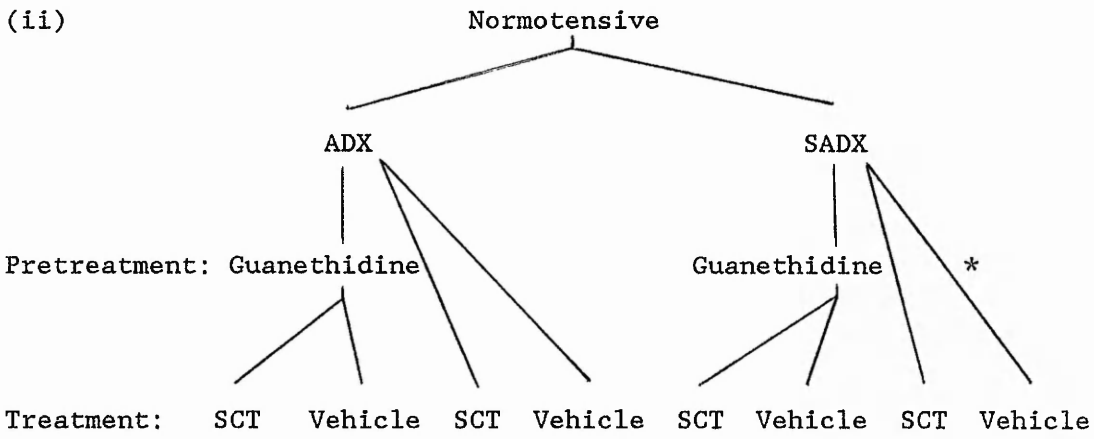
Change in Heart Rate (bpm) (treated-control)

Time (min)

5 U.kg^{-1} sCT administered at t=0 min

Fig.R.37. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the MAP values depicted in tables R.37(i) and (ii) overleaf were determined.

Fig.R.37. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

a) Bilateral adrenalectomised normotensive rats (treated) or sham bilateral adrenalectomised normotensive rats (control) were administered sCT (5 $U.kg^{-1}$) by icv injection.

b) Bilateral adrenalectomised normotensive rats (treated) or sham bilateral adrenalectomised normotensive rats (control) were given sCT (5 $U.kg^{-1}$) by icv injection 10 min after pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv).

Table R.37(i) Normotensive, pre-pretreatment MAP's

Normotensive		ADX	SADX
	MAP (mmHg):	112 (5)	108 (5)
	Mean difference (95% CI): 4 (-15, 23) NS		

Table R.37(ii) Normotensive, post-pretreatment MAP's

Normotensive + pre-treatment (t = 0 min)	Pre-treatment:	ADX + Guanethidine	SADX + Guanethidine
	MAP (mmHg):	76 (5)	83 (5)
	Mean difference (95% CI): -7 (-20, 4) NS		

ADX = adrenalectomy

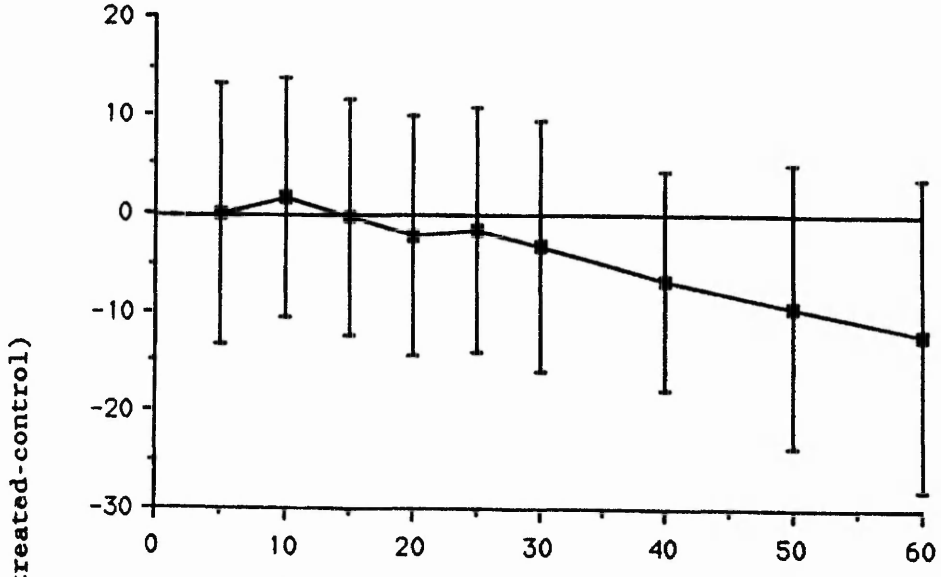
SADX = sham adrenalectomy

MAP = mean (n)

NS = not statistically significant

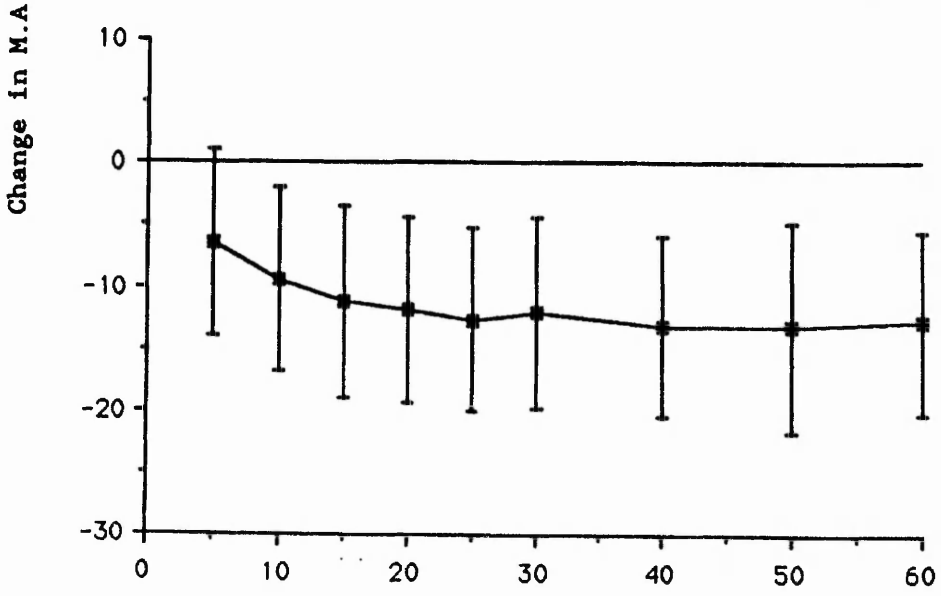
Graphs show mean difference (95% CI); see 2.7

a) AUC = -260 (-923, 403) mmHg.min (NS)



5 U.kg^{-1} sCT administered at t=0 min

b) AUC = -675 (-1078, -272) mmHg.min ($p < 0.01$)



Change in M.A.P. (mmHg) (treated-control)

Time (min)

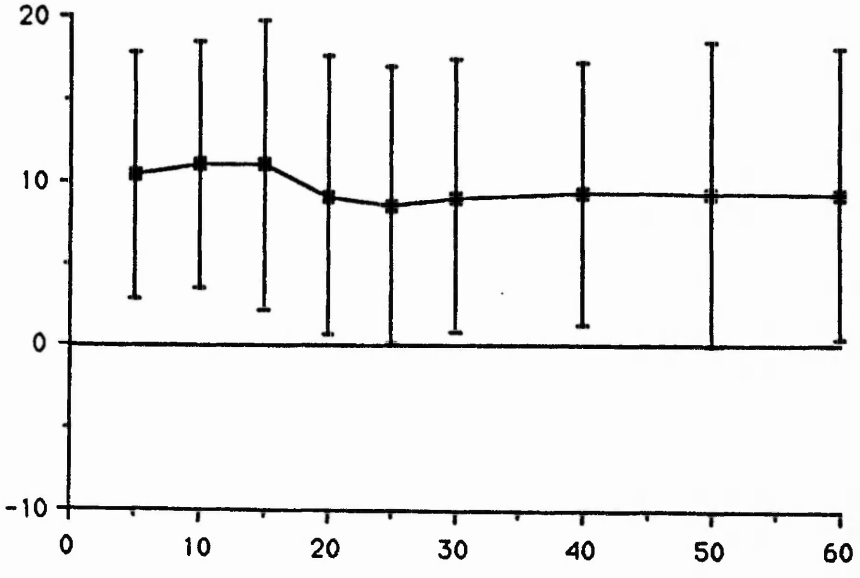
5 U.kg^{-1} sCT administered at t=0 min

Fig.R.37. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the MAP Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

c) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered by icv injection to bilateral adrenalectomised normotensive rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv).

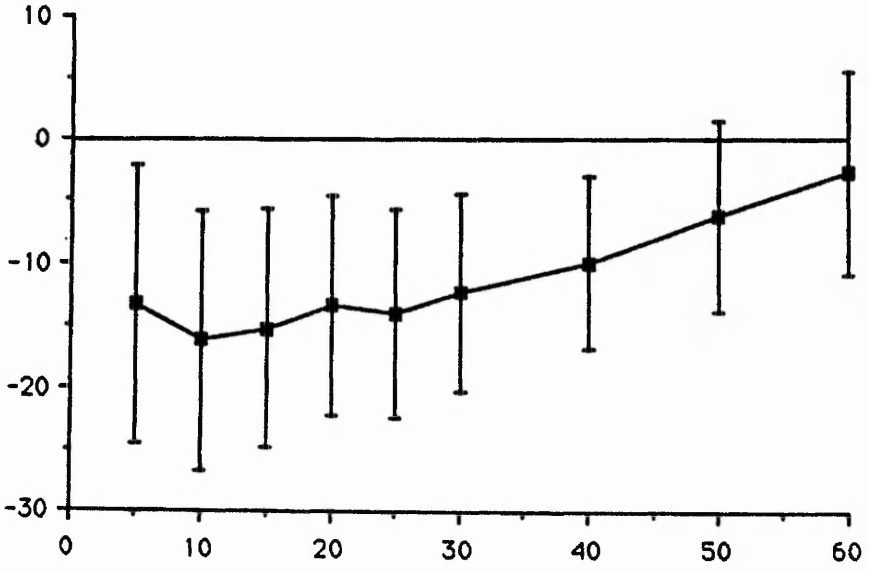
d) 5 $U.kg^{-1}$ sCT (icv) was administered to bilateral adrenalectomised normotensive rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv) (treated) or to bilateral adrenalectomised normotensive rats only (control).

c) AUC = 484 (91, 876) mmHg.min ($p < 0.05$)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

d) AUC = -622 (-990, -255) mmHg.min ($p < 0.01$)

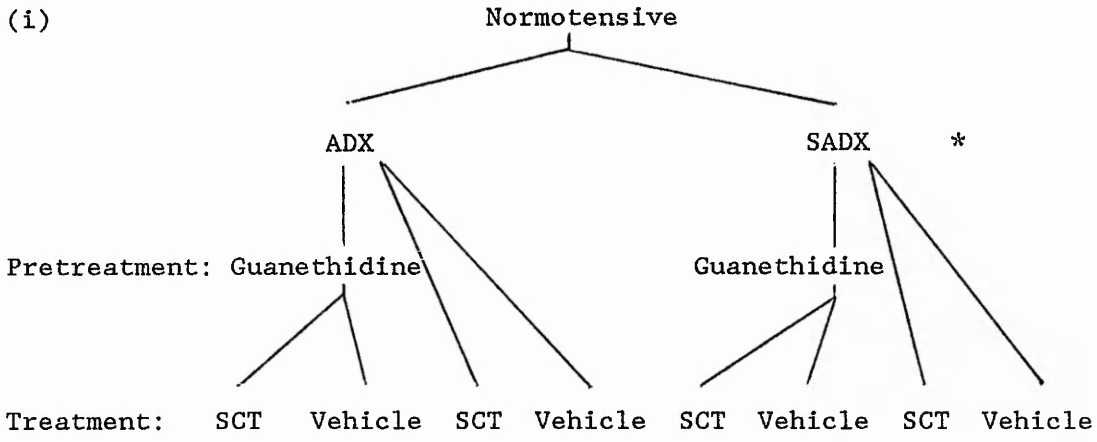


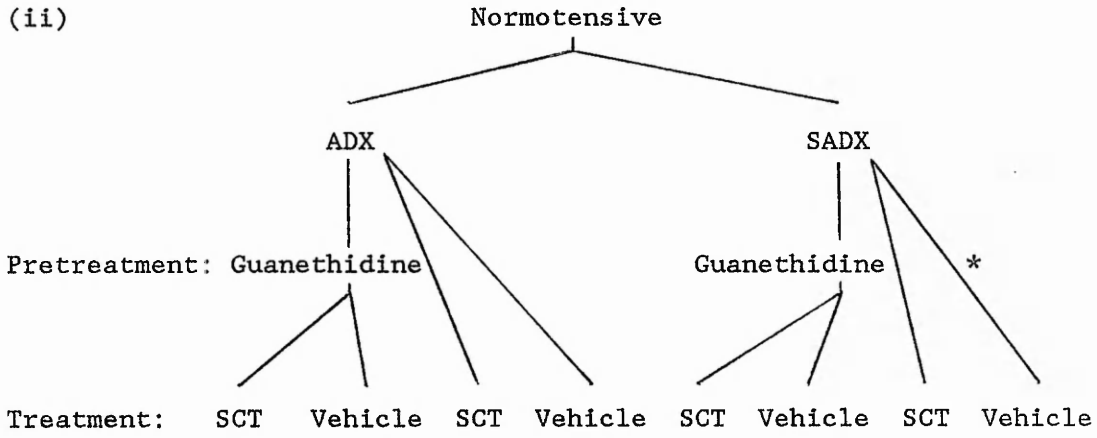
Change in M.A.P. (mmHg) (treated-control)

Time (min)

5 U.kg^{-1} sCT administered at t=0 min

Fig.R.38. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.38(i) and (ii) overleaf were determined.

Fig.R.38. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

a) Bilateral adrenalectomised normotensive rats (treated) or sham bilateral adrenalectomised normotensive rats (control) were administered sCT (5 $U.kg^{-1}$) by icv injection.

b) Bilateral adrenalectomised normotensive rats (treated) or sham bilateral adrenalectomised normotensive rats (control) were given sCT (5 $U.kg^{-1}$) by icv injection 10 min after pre-treatment with guanethidine (10 $mg.kg^{-1}$ iv).

Table R.38(i) Normotensive, pre-pretreatment heart rates

Normotensive		ADX	SADX
	Heart rate (bpm):	344 (5)	371 (5)
	Mean difference (95% CI): -27 (-72, 18) NS		

Table R.38(ii) Normotensive, post-pretreatment heart rates

Normotensive + pre-treatment (t = 0 min)	Pre-treatment:	ADX + Guanethidine	SADX + Guanethidine
	Heart rate (bpm):	404 (5)	432 (5)
	Mean difference (95% CI): -28 (-67, 10) NS		

ADX = adrenalectomy

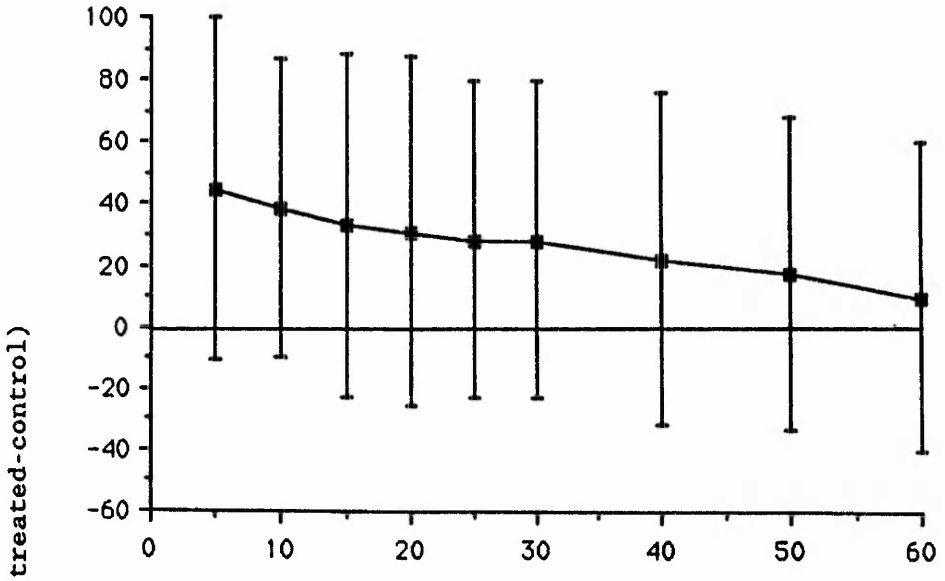
SADX = sham adrenalectomy

Heart rate = mean (n)

NS = not statistically significant

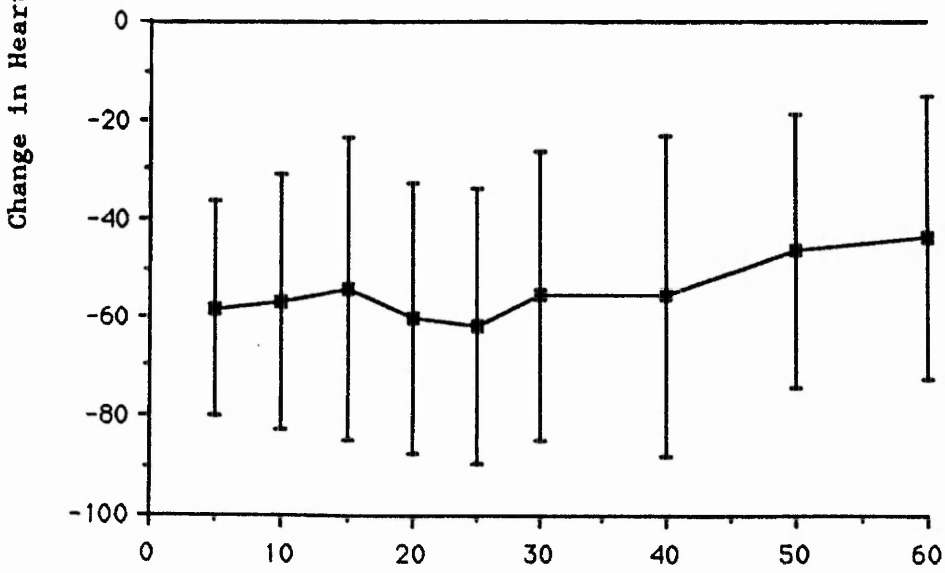
Graphs show mean difference (95% CI); see 2.7

a) AUC = 1551 (-1406, 4509) beats (NS)



5 U.kg^{-1} sCT administered at t=0 min

b) AUC = -3102 (-4612, -1592) beats ($p< 0.001$)



Change in Heart Rate (bpm) (treated-control)

Time (min)

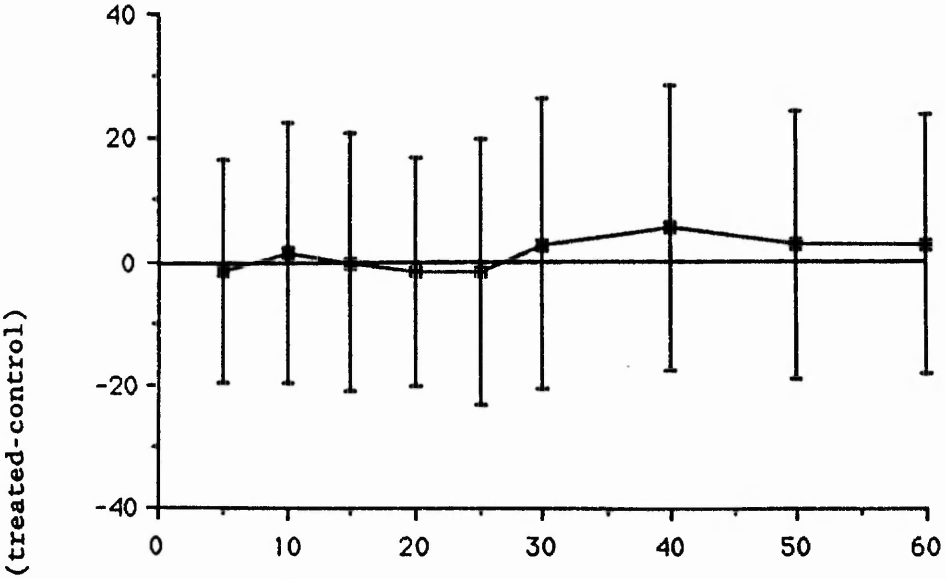
5 U.kg^{-1} sCT administered at t=0 min

Fig.R.38. Effect of Bilateral Adrenalectomy and Pre-treatment with Guanethidine (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

c) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered by icv injection to bilateral adrenalectomised normotensive rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv).

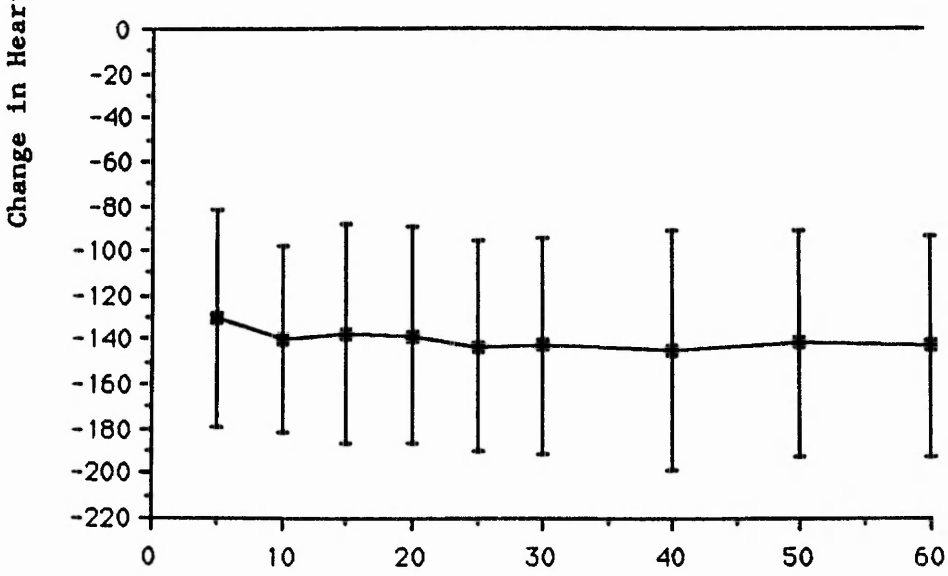
d) 5 $U.kg^{-1}$ sCT (icv) was administered to bilateral adrenalectomised normotensive rats pre-treated with guanethidine (10 $mg.kg^{-1}$ iv) (treated) or to bilateral adrenalectomised normotensive rats only (control).

c) AUC = 113 (-990, 1216) beats (NS)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

d) AUC = -8083 (-10858, -5308) beats ($p < 0.001$)



5 U.kg^{-1} sCT administered at t=0 min

7. CALCITONIN AND THE RENIN - ANGIOTENSIN SYSTEM

7.1 INTRODUCTION

It was suggested in the previous chapter that the pressor response to centrally administered sCT in urethane anaesthetised normotensive rats subjected to acute bilateral adrenalectomy and acute peripheral sympathetic blockade may be due to the increased activity of the renin - angiotensin system. The results presented below were obtained after central administration of sCT to urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage after acute blockade of the renin - angiotensin system using the angiotensin converting enzyme inhibitor, enalapril.

When arterial pressure is decreased, as in haemorrhage, the juxtaglomerular cells of the kidney secrete an enzyme, renin, into the blood. Adrenal insufficiency, upright posture and a low sodium diet are also stimuli which increase renin secretion (Obika, 1986; Sweet, 1977). This enzyme then cleaves the Leu-Leu bond on angiotensinogen to release a decapeptide, angiotensin I (AgI). Within a few seconds after formation of AgI, two amino acids are split from it to form the octapeptide, angiotensin II (AgII). This reaction is catalysed by angiotensin converting enzyme (ACE) and occurs almost entirely in the small blood vessels of the lungs. Angiotensin II persists in the blood for approximately one minute, but is rapidly inactivated by a number of blood and tissue enzymes, collectively called angiotensinase. Nearly all of the biological effects of AgI <u>in vivo</u> can be ascribed to its conversion to AgII. (Sweet, 1977; Severs and Daniels-Severs, 1973).

AgII causes vasoconstriction, especially of the arterioles, which leads to an increased peripheral resistance thus raising the arterial pressure back toward normal. Mild constriction of the veins is also observed which increases the mean circulatory filling pressure and thus promotes increased tendency for venous return of blood to the heart. AgII also causes constriction of the renal arterioles, thereby causing the kidneys to retain both water and salt, thus increasing the body fluid volume which helps to raise arterial pressure (Guyton, 1981).

AgII can produce a detectable response in animals and man at about 5 $ng.kg^{-1}$ (iv). Blood pressure increases within 15 - 30 sec and reaches a peak within 1 - 2 min. The arterial blood pressure response to AgII in the intact animal depends on the initial smooth muscle tone, the degree of baroreceptor inhibitory activity and direct effects on the heart. The cardiovascular response to AgII is also modified by change in the state of sodium balance, the level of sympathetic nervous system activity, nephrectomy and the type and level of anaesthesia (Sweet, 1977).

AgII stimulates the release of catecholamines from the adrenal medulla and facilitates ganglionic transmission (Ferrario et al. 1972). In turn, the sympathetic nervous system exerts an important influence on many of the haemodynamic and hormonal variables that determine the net release of renin by the kidneys (Ferrario et al. 1985). Direct electrical stimulation of the sympathetic nerve supply to the kidney and iv infusions of adrenaline or noradrenaline will increase renin secretion. A number of drugs which alter sympathetic nerve activity

or deplete catecholamine storage granules in nerve terminals will blunt but not completely abolish the increase in renin secretion which follows haemorrhage (Sweet, 1977)

Anaesthesia has been demonstrated to have an important effect on the renin - angiotensin system. Volicier and Loew (1971) studied the effect of AgII in conscious rats and in those anaesthetised with urethane (1.3 $g.kg^{-1}$ iv), pentobarbitone (35 $mg.kg^{-1}$ iv) or chloralose(55 $mg.kg^{-1}$ iv). Urethane anaesthesia was shown to diminish the pressor response to single doses of AgII (0.04 - 0.08 $\mu g.kg^{-1}$ iv) and to iv infusion of AgII (1 $\mu g.h^{-1}$). The authors suggested that urethane partially blocks the sympathetic nervous system and so prevents the indirect component of AgII action which is mediated through the sympathetic nervous system.

Clementi et al. (1986) administered sCT to conscious rats and observed an increase in plasma renin activity after 1.5 $U.kg^{-1}$ icv, 4 $U.kg^{-1}$ iv and 20 $U.kg^{-1}$ im. Centrally administered sCT was also associated with an increase in systolic blood pressure. The authors suggested that sCT (icv) may increase plasma renin activity through an interference with the tryptaminergic pathway and, in turn, enhance blood pressure. This does not explain, however, the increase in plasma renin activity after iv or im sCT which the authors attributed to the natriuretic and diuretic effects of the peptide when injected peripherally. Kurtz et al. (1988) observed a significant increase in renin release from isolated rat renal juxtaglomerular epitheloid cells after addition of sCT (10^{-6} M).

The ACE inhibitor, enalapril, is used in the following experiments to acutely block the renin - angiotensin system. This pro - drug is converted to active enalaprilic acid. The dose of enalapril used was assessed by the AgI pressor assay, in which the precursor peptide AgI is injected iv and the pressor response recorded. The response is identical to the same dose of AgII since AgI is rapidly converted by ACE. In the presence of an ACE inhibitor, the pressor response to AgI is markedly reduced (Unger et al. 1983). Administration of ACE inhibitors leads to vascular dilatation and hence a reduction in total peripheral resistance. Reflex tachycardia is absent and there is no direct cardiac stimulatory effect. The arterial dilatation may be attributed to various mechanisms including the lowering of AgII levels, enhanced sensitivity of vascular α-adrenoceptors, stimulation of pre-synaptic α_2-adrenoceptors causing diminished release of noradrenaline, and central effects (Sweet and Blaine, 1984).

The present chapter investigates the role of the renin - angiotensin system in the pressor response to sCT (icv) in urethane anaesthetised normotensive rats and those subjected to haemorrhage.

7.2 RESULTS

NB. Results are expressed as mean difference, ie treated - control (95% CI)

1) Effect of iv enalapril on the pressor response to iv AgI in urethane anaesthetised normotensive rats and those subjected to haemorrhage

Pretreatment of urethane anaesthetised haemorrhaged rats with enalapril (2 $mg.kg^{-1}$ iv) resulted in a statistically significant

reduction in the pressor response to 30 $ng.rat^{-1}$ AgI (iv), administered 10 min and 90 min after enalapril ($p< 0.01$) (see Fig 39). The pressor response to 300 $ng.rat^{-1}$ AgI (iv) was significantly reduced 20 min and 100 min after pretreatment with enalapril ($p< 0.001$) (see Fig.R.39). It is therefore concluded that enalapril at a dose of 2 $mg.kg^{-1}$ (iv) is sufficient to inhibit ACE activity for up to 100 min in urethane anaesthetised rats rendered hypotensive by haemorrhage.

Pretreatment of urethane anaesthetised normotensive rats with enalapril (2 $mg.kg^{-1}$ iv) resulted in a statistically significant reduction in the pressor response to 30 $ng.rat^{-1}$ AgI (iv) for up to 90 min ($p< 0.01$) and a significant reduction in the pressor response to 300 $ng.rat^{-1}$ AgI (iv) for up to 100 min ($p< 0.001$) (see Fig.R.40). It would therefore appear that enalapril, at a dose of 2 $mg.kg^{-1}$ (iv), also effectively inhibits ACE activity in urethane anaesthetised normotensive rats.

2) Effect of iv enalapril on the cardiovascular response to icv sCT in urethane anaesthetised rats rendered hypotensive by haemorrhage

Pretreatment of rats with enalapril (2 $mg.kg^{-1}$ iv) resulted in a statistically significant reduction in the pressor response to sCT (5 $U.kg^{-1}$ icv) as shown by the statistically significant reduction in the AUC, relative to control, of -883 (-1308, -459) mmHg.min ($p< 0.001$) (see Fig.R.41a). However, the pressor response to 5 $U.kg^{-1}$ sCT administered by icv injection is not abolished by pretreatment with enalapril but is significantly attenuated, as shown by the

statistically significant increase in MAP after sCT compared with the appropriate control for sCT. The increase in AUC, relative to control, of 798(569,1027) mmHg.min is statistically significant ($p< 0.001$) (see Fig.R.41b).

Pretreatment of urethane anaesthetised haemorrhaged rats with enalapril (2 $mg.kg^{-1}$ iv) does not appear to exert any effect on the heart rate response to icv sCT (see Figs.R.42a and b). It thus appears that the pressor response to centrally administered sCT in urethane anaesthetised rats subjected to haemorrhage is significantly attenuated by acute blockade of the renin - angiotensin system, but that the heart rate response is unaffected.

3) Effect of iv enalapril on the cardiovascular response to icv sCT in urethane anaesthetised normotensive rats

It can be seen from Figs.R.43 and R.44 that inhibition of ACE activity with enalapril (2 $mg.kg^{-1}$ iv) exerts no effect on the pressor response and heart rate response to centrally administered sCT (5 $U.kg^{-1}$) in urethane anaesthetised normotensive rats. A significant pressor response was still obtained 5 min post - sCT administration and although the AUC was not significantly increased there is a trend for an overall increase in MAP. This would indicate that the renin - angiotensin system does not play a role in the cardiovascular response to sCT (icv) in urethane anaesthetised normotensive rats.

7.3 DISCUSSION

Enalapril (2 $mg.kg^{-1}$ iv) effectively blocked the pressor response to both 30 $ng.rat^{-1}$ AgI and 300 $ng.rat^{-1}$ AgI administered iv to urethane

anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. Blockade of the renin - angiotensin system was effective for at least 100 min and so was of an adequate duration for the experiments involving sCT. The pressor response to AgI was maintained for only approximately 1 min in the absence of enalapril (results not shown). Enalapril alone led to a decrease in basal MAP both in normotensive rats and those subjected to haemorrhage, the effect being more marked in normotensive rats (see tables R.39(ii), R.40(ii), R.41(ii) and R.43(ii)). There was, however, no real effect on heart rate (see tables R42(ii) and R.44(ii)).

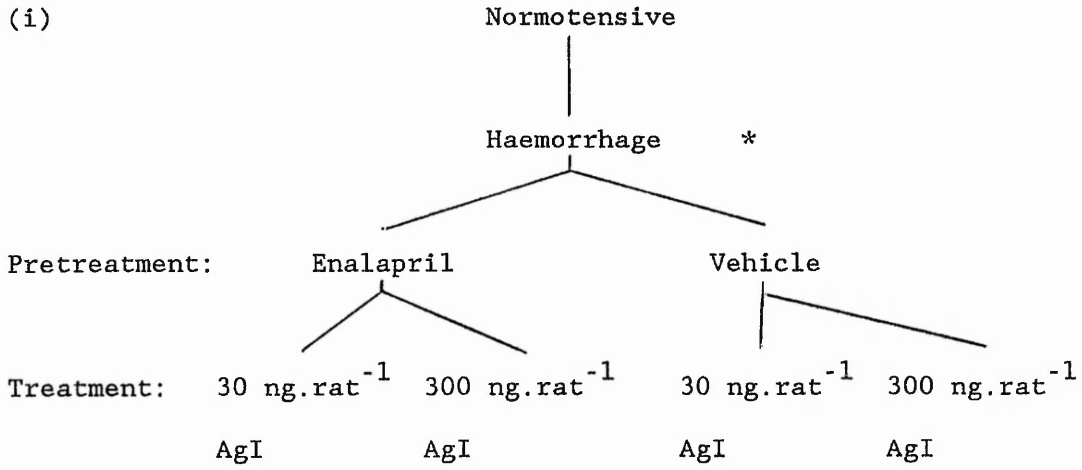
In urethane anaesthetised haemorrhaged rats with an intact peripheral sympathetic nervous system and adrenal glands, blockade of the renin-angiotensin system leads to a significant attenuation of the pressor response to centrally administered sCT, suggesting that sCT (icv) may exert its effect partly through the latter system. This is not an expected result after observing the results presented in chapter 4 in which the pressor response to sCT (icv) is abolished in haemorrhaged rats after acute peripheral sympathectomy and acute bilateral adrenalectomy. It can be concluded that both the peripheral sympathetic nervous system and adrenal glands must be active in order for the renin - angiotensin system to be involved in the pressor response to icv sCT. Since the sympathetic nervous system has effects on and is affected by the renin - angiotensin system (Ferrario et al. 1972; Ferrario et al. 1985; Sweet, 1977) it may be postulated that sCT (icv) leads to a release of catecholamines which then influence the release of renin which leads ultimately to the pressor effect of AgII. Thus, after peripheral sympathectomy and bilateral adrenalectomy, the

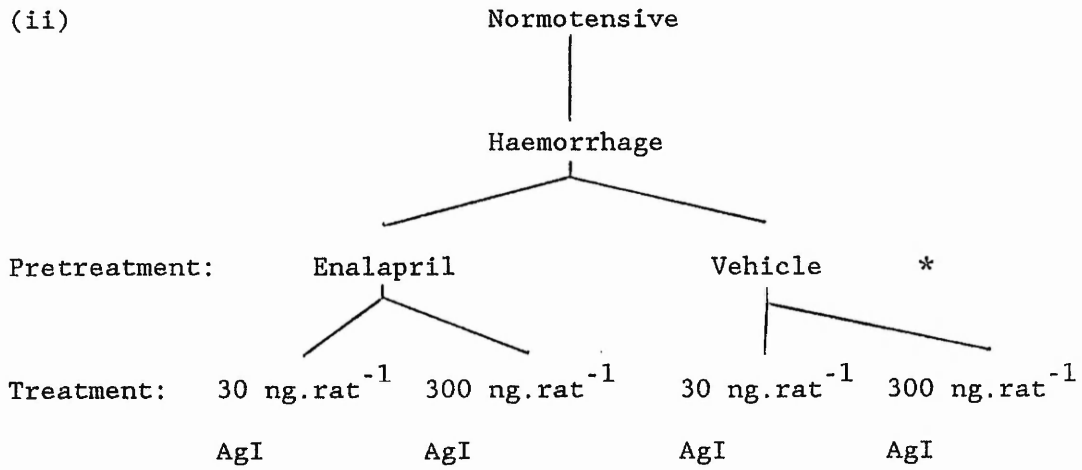
renin - angiotensin system plays no part in the pressor response to sCT (icv). The renin - angiotensin system does not appear to be involved in the increased heart rate response to centrally administered sCT.

The effect of enalapril on the pressor response to sCT (icv) in urethane anaesthetised normotensive rats appears to differ from that detailed above. Blockade of the renin - angiotensin system has no effect on the pressor response to icv sCT. The increased heart rate response also remains unaffected. This conflicts with the results of Clementi et al. (1986) who observed an increase in plasma renin activity after sCT (1 - 5 $U.kg^{-1}$ icv) in normotensive conscious rats. It would be of interest to measure plasma renin activity after treatment with sCT (icv) in order to confirm the observations of Clementi et al. (1986). Further work on conscious animals would eliminate any complicating effects of anaesthetic.

In conclusion, the renin - angiotensin system would appear to be involved in the pressor response to sCT (icv) in urethane anaesthetised haemorrhaged rats, providing that the peripheral sympathetic nervous system and adrenal glands are intact, but would not appear to be involved in the pressor response to sCT (icv) in urethane anaesthetised normotensive rats.

Fig.R.39. Effect of Enalapril (iv) on the Pressor Response to Angiotensin I (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.39(i) and (ii) overleaf were determined.

Fig.R.39. Effect of Enalapril (iv) on the Pressor Response to Angiotensin I (iv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Haemorrhaged rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (0.9% NaCl) (control) by iv administration. Ten min and 90 min after pre-treatment, 30 $ng.rat^{-1}$ AgI, and 20 min and 100 min after pre-treatment, 300 $ng.rat^{-1}$ AgI was administered by iv injection.

Table R.39(i) Post-haemorrhage, pre-pretreatment MAP's

Post-haemorrhage (t = -10 min)	Pre-treatment: MAP (mmHg):	Enalapril 81 (4)	Vehicle 67 (4)
	Mean difference (95% CI): 14 (-7, 35) NS		

Table R.39(ii) Post-haemorrhage, post-pretreatment MAP's

Post-haem + pre-treatment (t = 0 min)	Pre-treatment: MAP (mmHg):	Enalapril 64 (4)	Vehicle 69 (4)
	Mean difference (95% CI): -5 (-31, 21) NS		

MAP = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

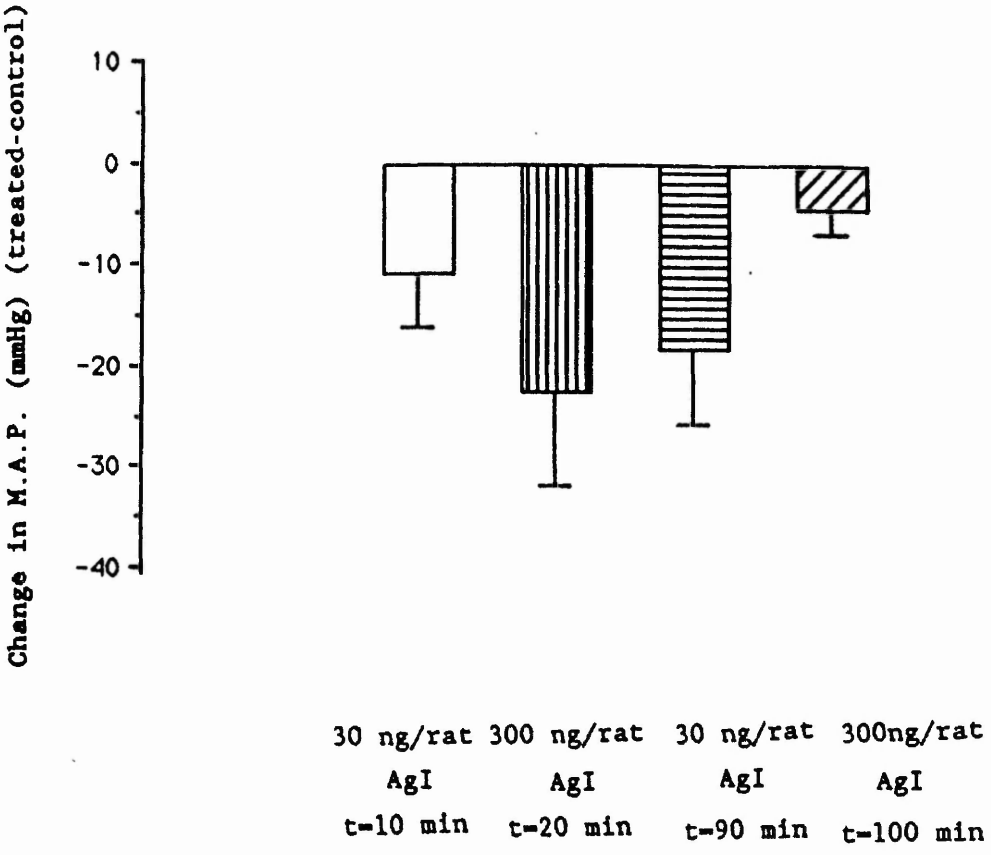
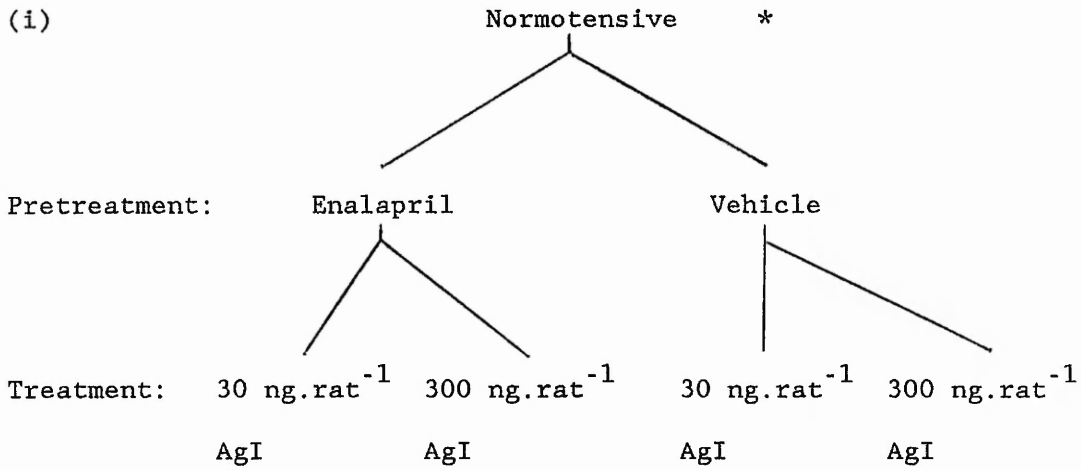
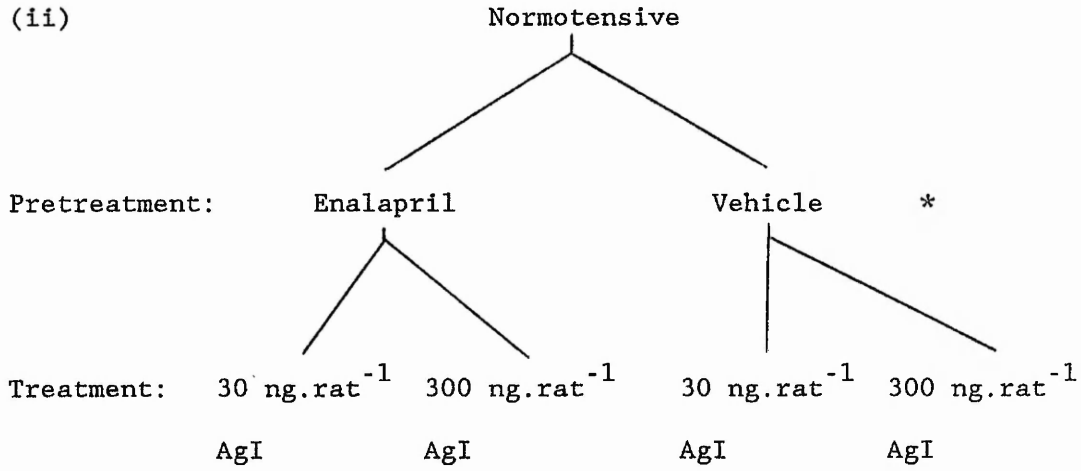

Change in M.A.P. (mmHg) (treated-control)
10
0
-10
-20
-30
-40
30 ng/rat AgI t=10 min
300 ng/rat AgI t=20 min
30 ng/rat AgI t=90 min
300ng/rat AgI t=100 min

Fig.R.40. Effect of Enalapril (iv) on the Pressor Response to Angiotensin I (iv) in Urethane Anaesthetised Normotensive Rats





* denotes the stages of the experiment at which the MAP values depicted in tables R.40(i) and (ii) overleaf were determined.

Fig.R.40. Effect of Enalapril (iv) on the Pressor Response to Angiotensin I (iv) in Urethane Anaesthetised Normotensive Rats

b) Normotensive rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (0.9% NaCl) (control) by iv administration. Ten min and 90 min after pre-treatment, 30 $ng.rat^{-1}$ AgI, and 20 min and 100 min after pre-treatment, 300 $ng.rat^{-1}$ AgI was administered by iv injection.

Table R.40(i) Normotensive, pre-pretreatment MAP's

Normotensive (t = -10 min)	Pre-treatment: MAP (mmHg):	Enalapril 98 (4)	Vehicle 106 (4)
	Mean difference (95% CI): -8 (-29, 13) NS		

Table R.40(ii) Normotensive, post-pretreatment MAP's

Normotensive + pre-treatment (t = 0 min)	Pre-treatment: MAP (mmHg):	Enalapril 66 (4)	Vehicle 105 (4)
	Mean difference (95% CI): -39 (-58, -20) $p< 0.01$		

MAP = mean (n)

NS = not statistically significant

Graphs show mean difference (95% CI); see 2.7

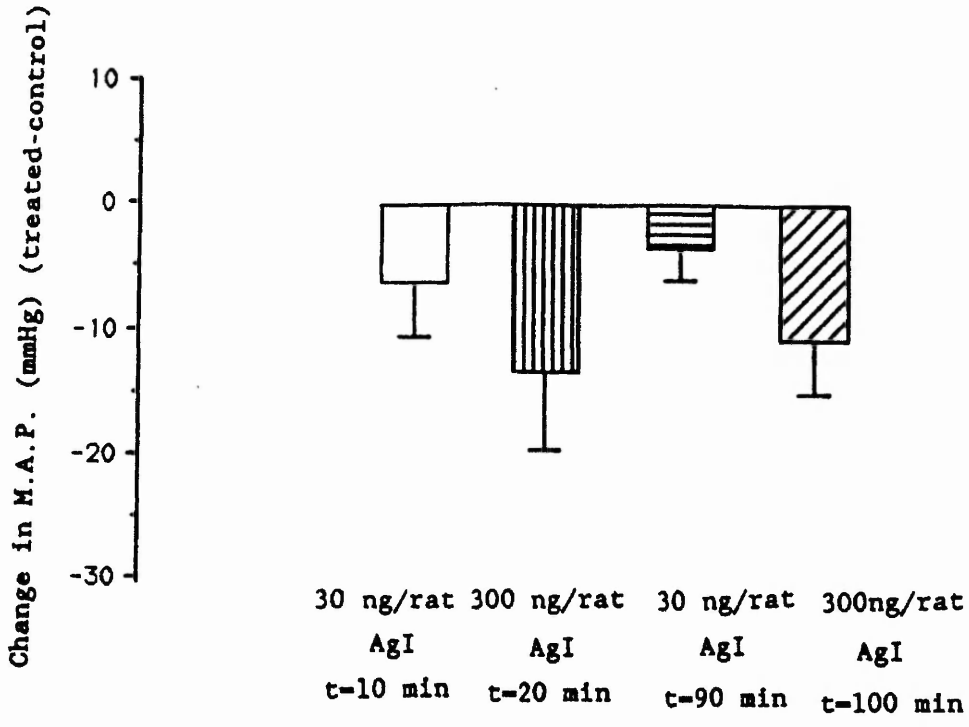
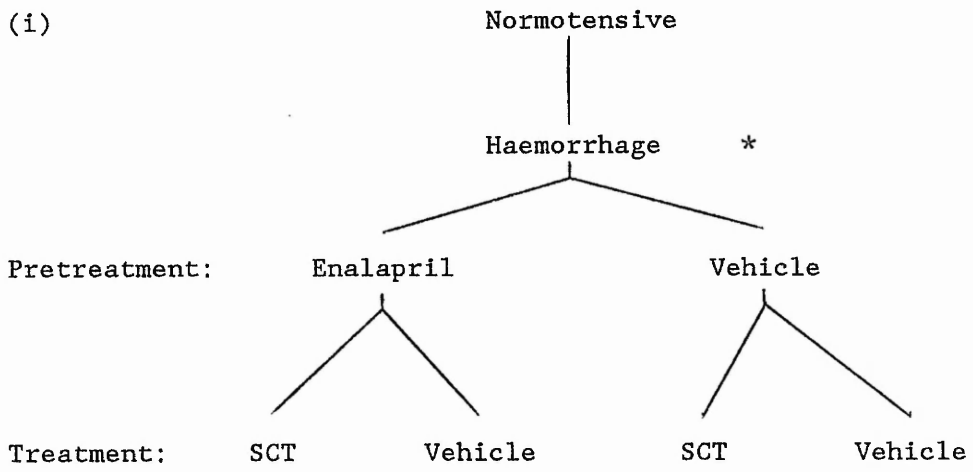
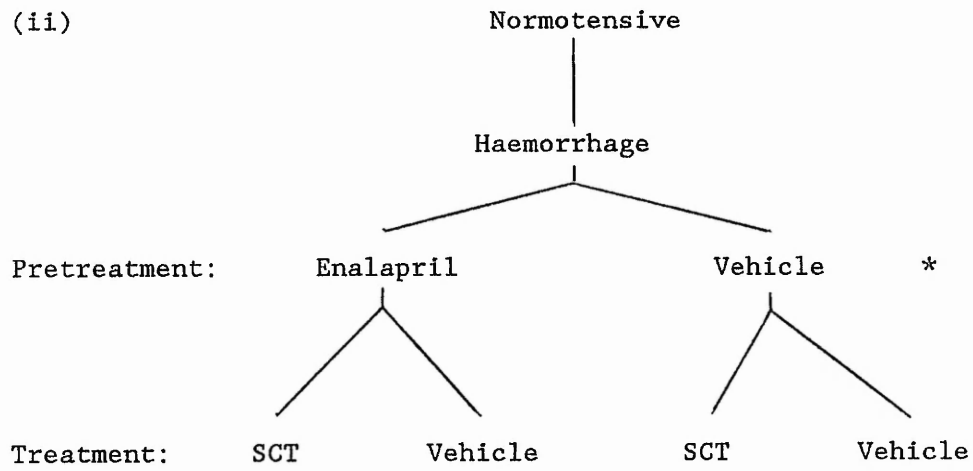

Change in M.A.P. (mmHg) (treated-control)
10
0
-10
-20
-30
30 ng/rat AgI t-10 min
300 ng/rat AgI t-20 min
30 ng/rat AgI t-90 min
300ng/rat AgI t-100 min

Fig.R.41. Effect of Pre-treatment with Enalapril (iv) on the Blood Pressure Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the MAP values depicted in tables R.41(i) and (ii) overleaf were determined.

Fig.R.41. Effect of Pre-treatment with Enalapril (iv) on the Blood Pressure Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Haemorrhaged rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (0.9% NaCl) (control) by iv administration. Ten min later, sCT (5 $U.kg^{-1}$ icv) was administered.

b) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered icv), 10 min after pre-treatment with enalapril (2 $mg.kg^{-1}$ iv), to rats rendered hypotensive by haemorrhage.

Table R.41(i) Post-haemorrhage, pre-pretreatment MAP's

Post-haemorrhage (t = -10 min)	Pre-treatment:	Enalapril	Vehicle
	MAP (mmHg):	81 (5)	84 (5)
	Mean difference (95% CI): -3 (-19, 13) NS		

Table R.41(ii) Post-haemorrhage, post-pretreatment MAP's

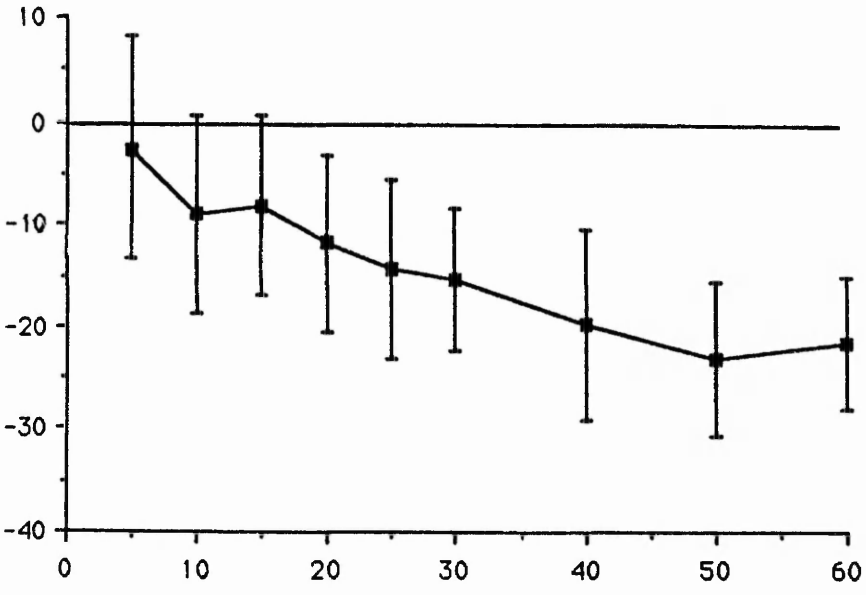
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Enalapril	Vehicle
	MAP (mmHg):	58 (5)	87 (5)
	Mean difference (95% CI): -29 (-45, -13) $p< 0.01$		

MAP = mean (n)

NS = not statistically significant

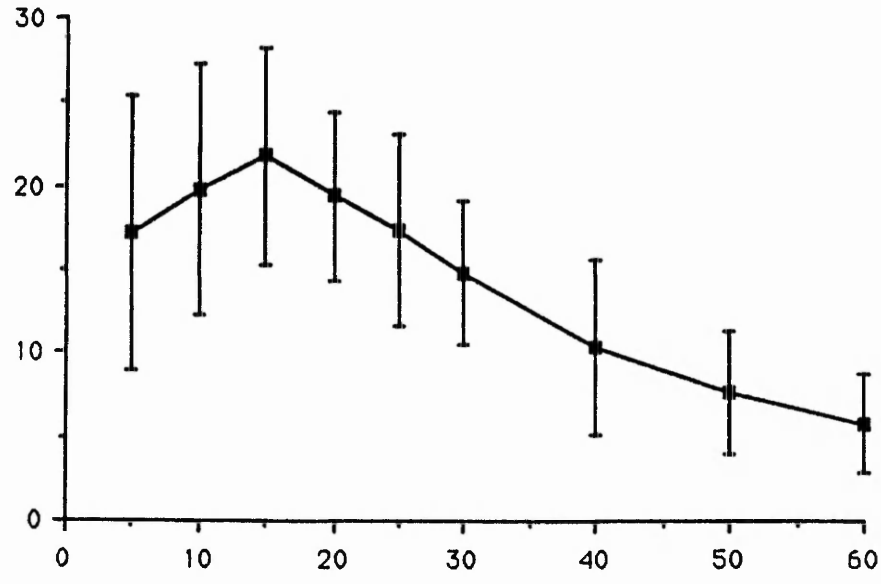
Graphs show mean difference (95% CI); see 2.7

a) AUC = -883 (-1308, -459) mmHg.min (p< 0.001)



5 U.kg^{-1} sCT administered at t=0 min

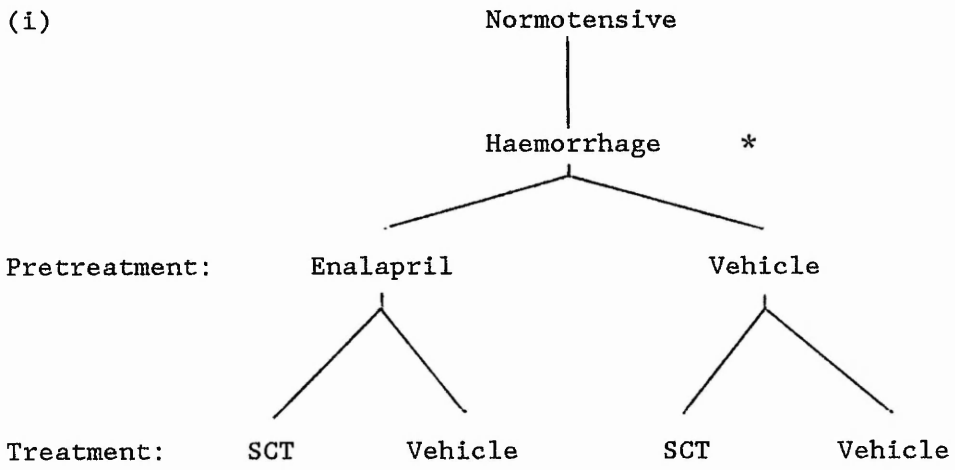
b) AUC = 798 (569, 1027) mmHg.min (p< 0.001)

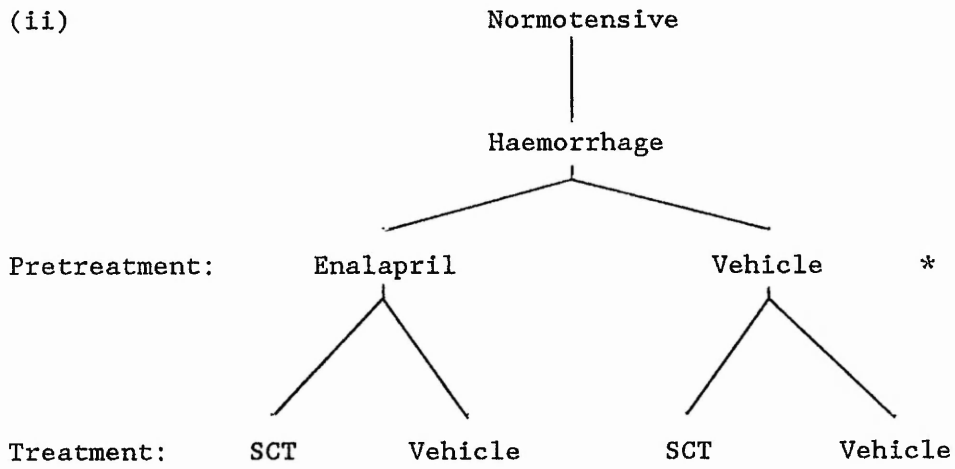


Change in M.A.P. (mmHg) (treated-control)

5 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.42. Effect of Pre-treatment with Enalapril (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.42(i) and (ii) overleaf were determined.

Fig.R.42. Effect of Pre-treatment with Enalapril (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Rats subjected to Haemorrhage.

a) Haemorrhaged rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (0.9% NaCl) (control) by iv administration. Ten min later, sCT (5 $U.kg^{-1}$ icv) was administered.

b) 5 $U.kg^{-1}$ sCT (treated) or appropriate vehicle (control) was administered icv, 10 min after pre-treatment with enalapril (2 $mg.kg^{-1}$ iv), to rats rendered hypotensive by haemorrhage.

Table R.42(i) Post-haemorrhage, pre-pretreatment heart rates

Post-haemorrhage (t = -10 min)	Pre-treatment:	Enalapril	Vehicle
	Heart rate (bpm):	347 (5)	335 (5)
	Mean difference (95% CI): 12 (-20, 44) NS		

Table R.42(ii) Post-haemorrhage, post-pretreatment heart rates

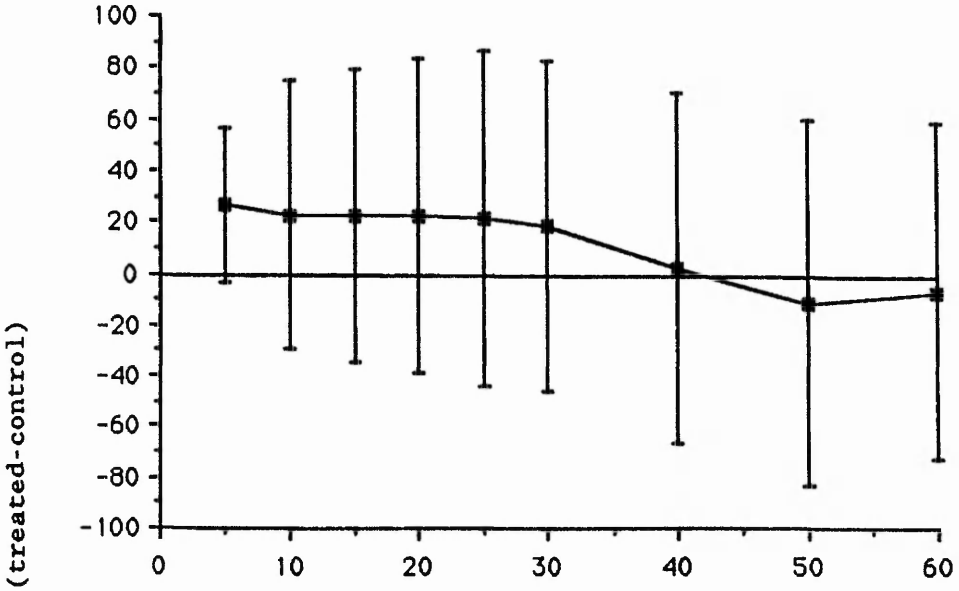
Post-haem + pre-treatment (t = 0 min)	Pre-treatment:	Enalapril	Vehicle
	Heart rate (bpm):	330 (5)	346 (5)
	Mean difference (95% CI): -16 (-59, 26) NS		

Heart rate = mean (n)

NS = not statistically significant

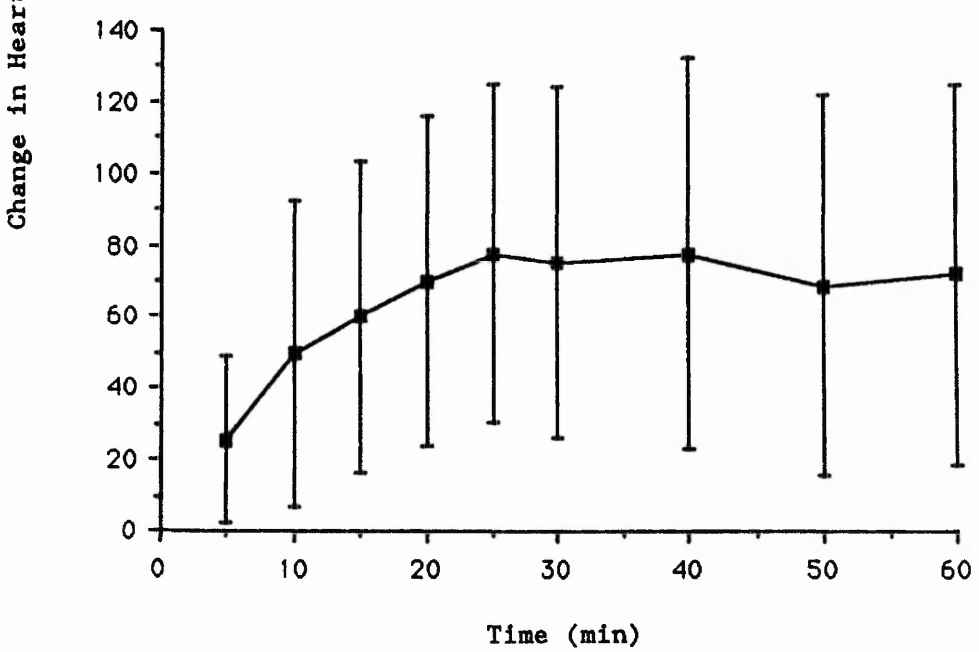
Graphs show mean difference (95% CI); see 2.7

a) AUC = 564 (-2915, 4042) beats (NS)



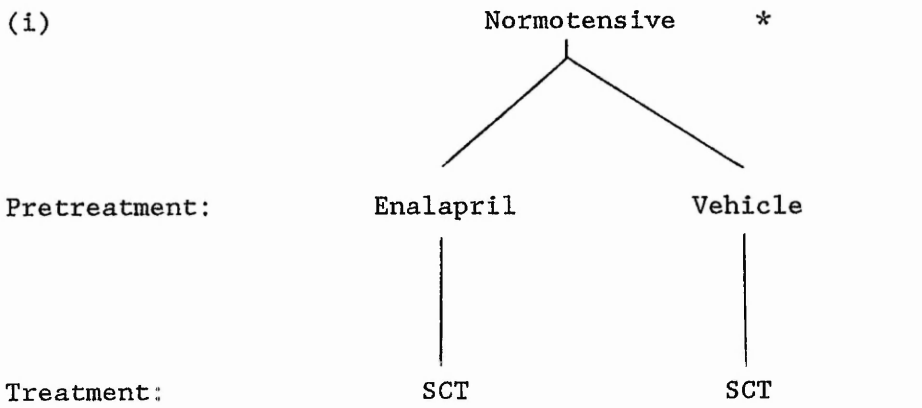
5 U.kg^{-1} sCT administered at t=0 min

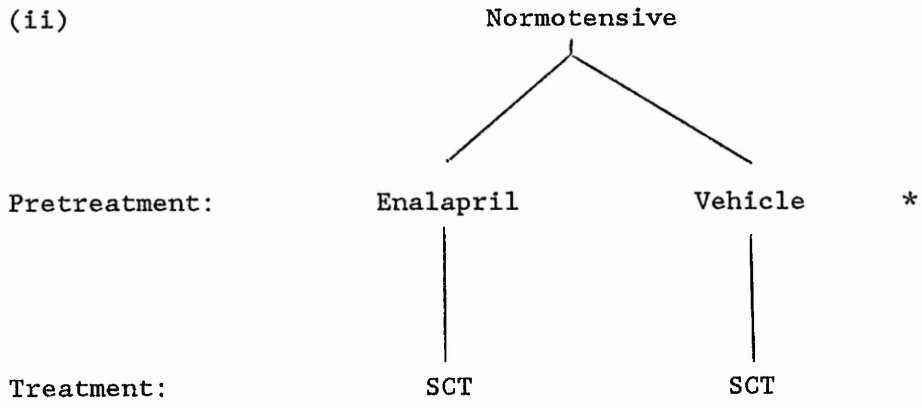
b) AUC = 3804 (1161, 6448) beats ($p< 0.01$)



5 U.kg^{-1} sCT/vehicle administered at t=0 min

Fig.R.43. Effect of Pre-treatment with Enalapril (iv) on the Blood Pressure Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the MAP values depicted in tables R.43(i) and (ii) overleaf were determined

Fig.R.43. Effect of Pre-treatment with Enalapril (iv) on the Blood Pressure Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

Normotensive rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 $U.kg^{-1}$ icv) was administered.

Table R.43(i) Normotensive, pre-pretreatment MAP's

Normotensive (t = -10 min)	Pre-treatment: MAP (mmHg):	Enalapril 106 (5)	Vehicle 109 (5)
	Mean difference (95% CI): -3 (-25, 19) NS		

Table R. 43(ii) Normotensive, post-pretreatment MAP's

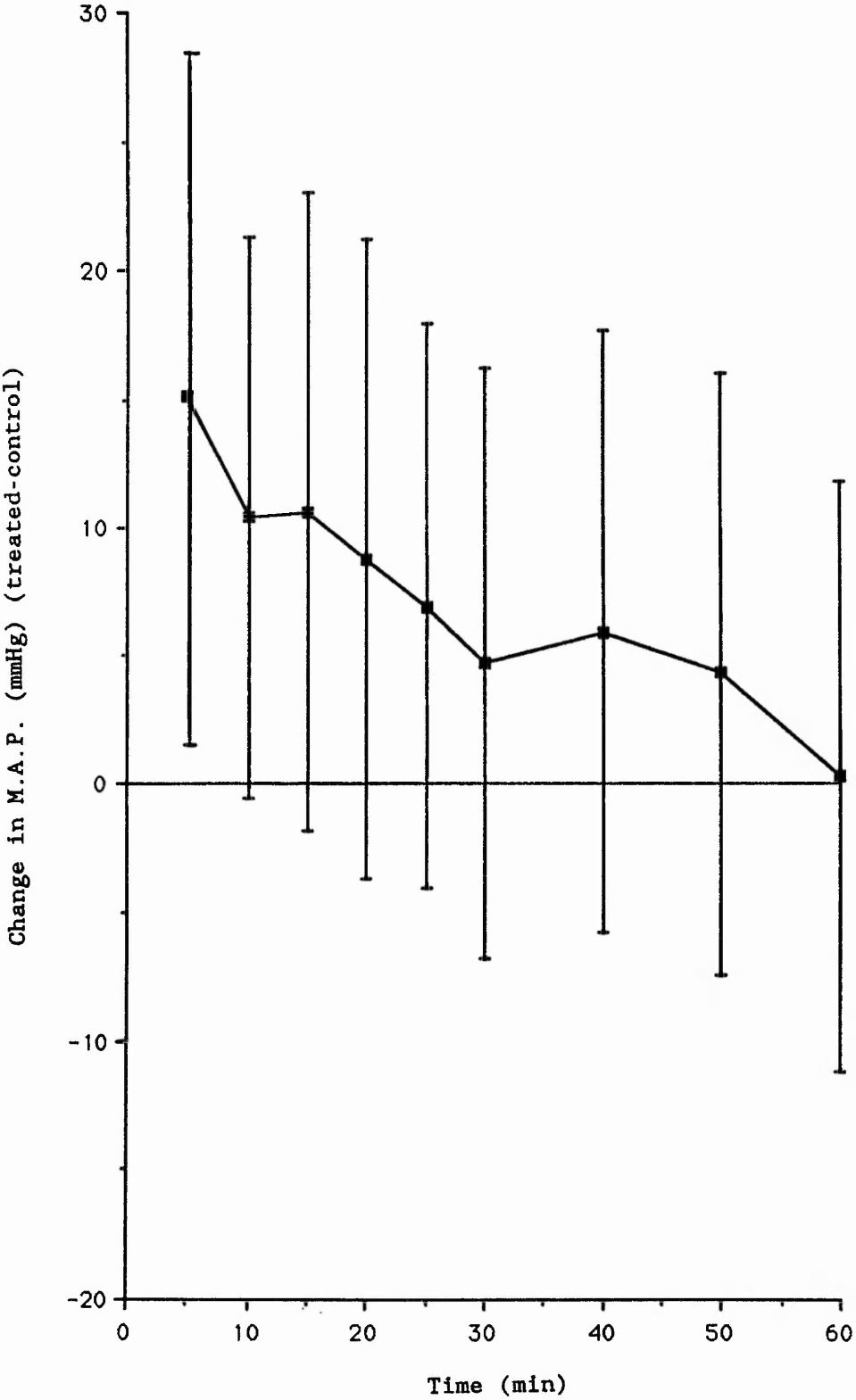
Normotensive + pre-treatment (t = 0 min)	Pre-treatment: MAP (mmHg):	Enalapril 74 (5)	Vehicle 105 (5)
	Mean difference (95% CI): -31 (-54, -9) $p< 0.05$		

MAP = mean (n)

NS = not statistically significant

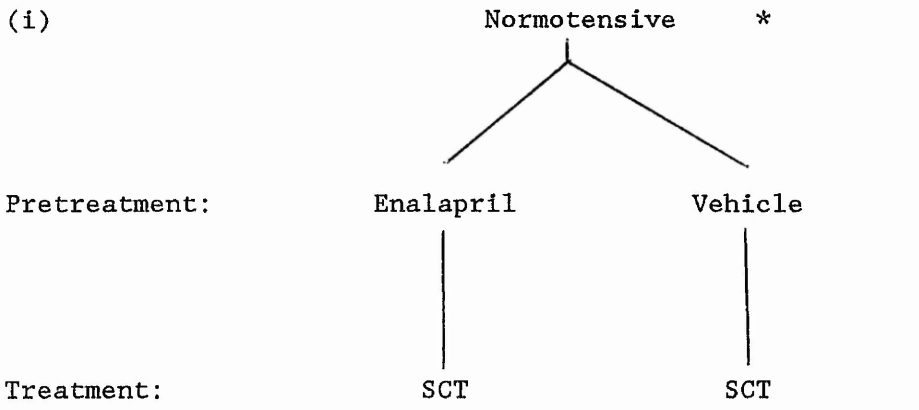
Graph shows mean difference (95% CI); see 2.7

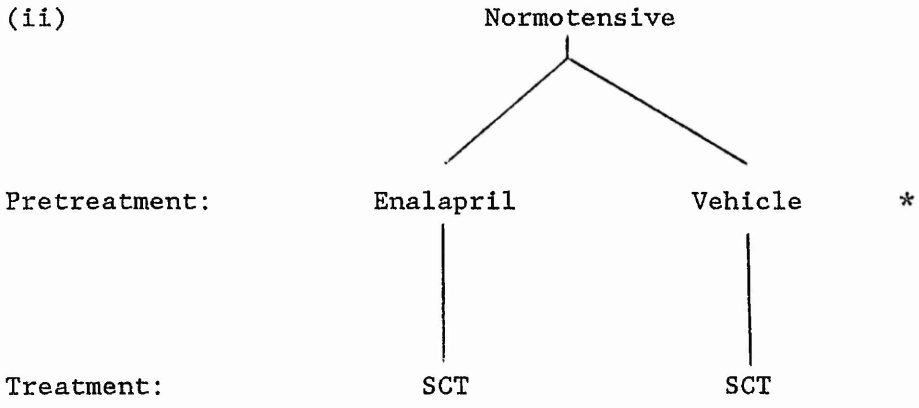
AUC = 402 (-247, 1052) mmHg.min (NS)



5 U.kg^{-1} sCT administered at t=0 min

Fig.R.44. Effect of Pre-treatment with Enalapril (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.





* denotes the stages of the experiment at which the heart rate values depicted in tables R.44(i) and (ii) overleaf were determined.

Fig.R.44. Effect of Pre-treatment with Enalapril (iv) on the Heart Rate Response to SCT (icv) in Urethane Anaesthetised Normotensive Rats.

Normotensive rats were pre-treated with 2 $mg.kg^{-1}$ enalapril (treated) or appropriate vehicle (control) by iv administration. Ten min later, sCT (5 $U.kg^{-1}$ icv) was administered.

Table R.44(i) Normotensive, pre-pretreatment heart rates

Normotensive (t = -10 min)	Pre-treatment:	Enalapril	Vehicle
	Heart rate (bpm):	365 (5)	375 (5)
	Mean difference (95% CI): -10 (-45, 23) NS		

Table R.44(ii) Normotensive, post-pretreatment heart rates

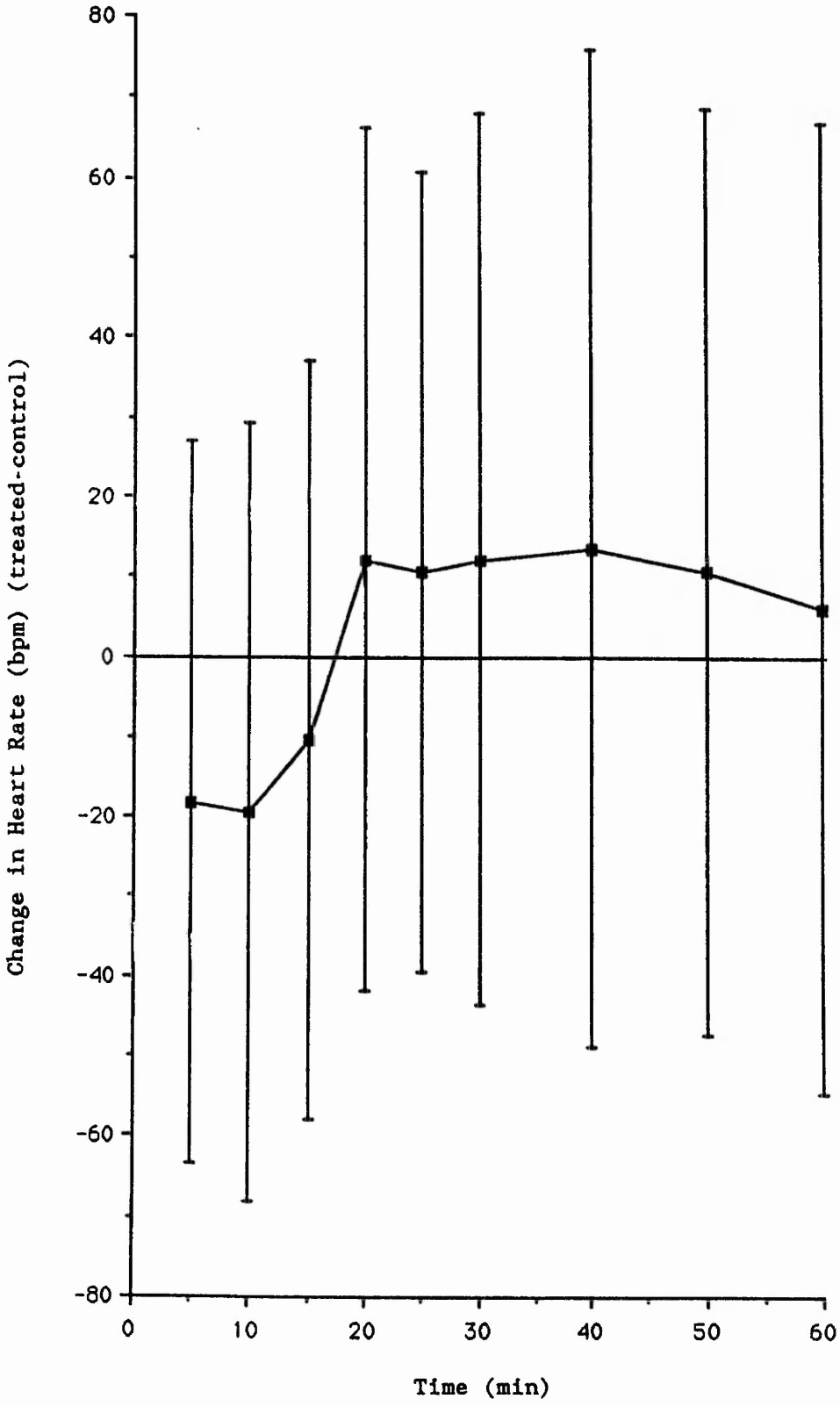
Normotensive + pre-treatment (t = 0 min)	Pre-treatment:	Enalapril	Vehicle
	Heart rate (bpm):	350 (5)	371 (5)
	Mean difference (95% CI): -21 (-67, 25) NS		

Heart rate = mean (n)

NS = not statistically significant

Graph shows mean difference (95% CI); see 2.7

AUC = 238 (-2791, 3267) beats (NS)



5 $U.kg^{-1}$ sCT administered at t=0 min

8. DISCUSSION

8.1 The cardiovascular effects of peripherally administered sCT

The results presented in this thesis demonstrate that peripherally administered sCT exerts a pressor effect in urethane anaesthetised rats rendered hypotensive by haemorrhage, but is devoid of effect in urethane anaesthetised normotensive rats. These results confirm those of Bates et al. (1983b). The pressor response is accompanied by a tendency for a decrease in heart rate. Conversely, when sCT (iv) is administered to pentobarbitone anaesthetised rats subjected to endotoxin shock, there is a trend for a decrease in blood pressure, and a significant decrease in heart rate. Quimby and Resnick (1985) observed a rapid and profound hypotension in baboons given iv sCT followed by toxic shock syndrome toxin-type 1 (TSST-1). This hypotension was not observed in baboons which had undergone bilateral thyroidectomy. The authors, therefore, concluded that immunoreactive CT (iCT) is involved in the pathophysiology of the hypotension in TSST-1-induced toxic shock syndrome. It would be of interest to investigate the effect of iv sCT on blood pressure and heart rate of rats subjected to bilateral thyroidectomy and endotoxin shock in order to investigate whether iCT is involved in the pathophysiology of the hypotension in this particular shock model.

The pressor response to iv sCT in urethane anaesthetised haemorrhaged rats is abolished after pretreatment with the adrenergic neurone blocking agent, guanethidine, thereby indicating that the pressor response is mediated by the peripheral sympathetic nervous system. A similar effect was observed by Bates et al. (1984a) after inducing

chemical sympathectomy with 6-OHDA in urethane anaesthetised rats rendered hypotensive by haemorrhage. Moreover, these workers demonstrated that the pressor response to sCT (iv) is not affected by bilateral vagotomy or vasopressin antagonism suggesting that modification of vagal afferents or secretion of vasopressin is not involved in the response.

An attenuation of the increased heart rate response to iv sCT was seen after guanethidine in haemorrhaged rats. It is, therefore, concluded that the peripheral sympathetic nervous system is partly involved in the heart rate response to iv sCT. Other mechanisms contributing to this effect may include the release of catecholamines from the adrenal medulla or an inhibitory action on the parasympathetic nervous sytem.

It is well documented that during haemorrhage there is an increase in sympathetic discharge with increases in plasma catecholamines (Hess et al. 1983). It may, therefore, be hypothesised that sCT potentiates existing neuronal activity; this would account for the lack of effect of iv sCT in urethane anaesthetised normotensive rats. This may also explain the differences in response to peripheral administration of sCT to urethane anaesthetised rats compared with pentobarbitone anaesthetised rats. When sCT (iv) is given to rats anaesthetised with pentobarbitone and subjected to haemorrhage, the peptide exerts no effects on the cardiovascular parameters investigated. Since the results presented in this thesis indicate that the peripheral sympathetic nervous system is more active during pentobarbitone anaesthesia than during urethane anaesthesia, it may be postulated that the peripheral sympathetic nervous system is stimulated to such an extent that the administration of sCT (iv) can stimulate it no

further. It would be of interest to investigate any changes in plasma catecholamine levels after administration of anaesthetic and/or sCT. It would also be advantageous to investigate the effects of peripherally administered sCT in conscious animals thereby removing the confounding influences of the anaesthetic.

8.2 The site of action of peripherally administered sCT

Calcitonin has been demonstrated to cross the blood-brain barrier albeit in small amounts; approximately 1% of the dose administered (Doepfner, 1983). It is, therefore, feasible that peripherally administered sCT may exert its effects centrally by leakage through the blood-brain barrier into the CNS thereby stimulating the release of catecholamines from the sympathoadrenal axis. This is unlikely, however, since sCT given centrally, at a dose of 1 $U.kg^{-1}$, did not exert any significant effects on blood pressure or heart rate (see chapter 4). If approximately 1% of the iv dose was indeed reaching the CNS then it would be expected that sCT at a dose of at least 0.2 $U.kg^{-1}$ (icv) would exert significant cardiovascular effects. The involvement of the CNS in the cardiovascular responses induced by peripherally administered sCT could be further investigated by the administration of a ganglion blocking agent prior to the peripheral administration of sCT.

The results presented in this thesis would, therefore, indicate that the pressor effect of peripherally administered sCT is mediated by the peripheral sympathetic nervous system. If one discounts the possible involvement of the CNS then this effect could be achieved either by a direct action of sCT on peripheral sympathetic neurones or the release of neurotransmitter secondary to the interaction of sCT with CT

receptors or possibly receptors for the related peptide, CGRP. Calcitonin and CGRP have a similar molecular structure and so it is feasible that each peptide could act on the other's receptor. In fact, CGRP is thought to activate CT receptors in the bone and kidney since it has been shown to inhibit bone resorption by active rat osteoclasts and to lower plasma calcium when injected into young rats (Zaidi et al. 1988), although CGRP is 100 - 1000 times less potent than CT in this respect. To support this, it has been demonstrated that in the rat kidney, which contains high affinity binding sites for sCT (half maximal inhibition of binding of[^{125}I]-sCT by 10^{-9} M sCT), rCGRP inhibits binding of[^{125}I]-sCT approximately as well as hCT (half maximal inhibition of binding by 10^{-6} M) (Goltzman and Mitchell, 1985).

The pressor effect of sCT is unlikely to involve interaction at CGRP receptors since it has been demonstrated that cardiovascular receptors for CGRP show no cross-reactivity with sCT (Breimer et al. 1988). Furthermore, iv CGRP exerts dissimilar effects to iv sCT on the cardiovascular system; iv CGRP leads to sustained reductions in MAP in conscious rats (Fischer et al. 1983a) and in anaesthetised rats (Marshall et al. 1986). These cardiovascular effects are associated specifically with the CGRP receptor on vascular smooth muscle (Siren and Feuerstein, 1988). CT receptors have not so far (May 1989) been demonstrated in the cardiovascular system and so it is most likely that the haemodynamic effects of peripherally administered sCT are due to a direct interaction of sCT with peripheral sympathetic neurones.

8.3 The cardiovascular effects of centrally administered sCT

Centrally administered sCT (5 $U.kg^{-1}$) has been demonstrated to exert a pressor effect in both urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. This response appears to be more marked in rats subjected to haemorrhage. These results are in accord with those of Bates et al. (1984c). The pressor response observed is associated with an increase in heart rate, and again, this is most evident in haemorrhaged rats. This phenomenon of an increase in blood pressure concomitant with an increase in heart rate has also been observed after central administration of other peptides such as leucine-enkephalin (Schaz et al. 1980) and bradykinin (Unger et al. 1981).

Acute blockade of the peripheral sympathetic nervous system using the adrenergic neurone blocking agent guanethidine, leads to a significant attenuation of the pressor response and increased heart rate response to icv sCT in both urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage. These reults are in direct contrast to those of Bates et al. (1984c) who demonstrated that the blood pressure response to icv sCT was unaffected by chemical sympathectomy using 6-OHDA. It is unclear why such a difference in results should occur, but it may be explained by the differences in inducing blockade of the peripheral sympathetic nervous system.

It is apparent from the results presented in this thesis that the peripheral sympathetic nervous system is involved in both the pressor

response and increased heart rate response to centrally administered sCT in urethane anaesthetised normotensive rats and those subjected to haemorrhage. Presumably this is due to the release of catecholamines from sympathetic nerve endings. It would, therefore, be of interest to investigate the effect of icv sCT on plasma catecholamine levels in the presence and absence of guanethidine. It is also apparent that the peripheral sympathetic nervous system is not the only mediator of the cardiovascular response to centrally administered sCT, since the presence of guanethidine only leads to an attenuation, and not abolition, of the response.

Since catecholamines are also released from the adrenal medulla it was of interest to investigate the effect of bilateral adrenalectomy, both with and without acute peripheral sympathetic nervous system blockade, on the response to icv sCT. The results indicate that, in urethane anaesthetised normotensive rats, the adrenal glands and peripheral sympathetic nervous system are equally as effective in mediating the pressor response to icv sCT. It is clear, however, that some other mechanism must be involved since bilateral adrenalectomy in the presence of guanethidine only resulted in a significant attenuation of the pressor response. The increased heart rate response to icv sCT appears to be mediated both by the peripheral sympathetic nervous system and the adrenal glands although the peripheral sympathetic nervous system seems to be of greater importance.

The pressor response to icv sCT in urethane anaesthetised rats subjected to haemorrhage would appear to be mediated by the adrenal glands and peripheral sympathetic nervous system, although the latter

system seems to be more important. Conversely, the increased heart rate response to icv sCT is mediated solely by the peripheral sympathetic nervous system. The presence of intact adrenal glands seems to be necessary in mediating the increase in heart rate, although the reason for this is unclear because the adrenal glands themselves do not appear to participate in the response.

It is apparent that some other mechanism is involved in the pressor response to icv sCT in urethane anaesthetised normotensive rats. The involvement of the renin-angiotensin system was, therefore, examined. After blockade of the renin-angiotensin system with the angiotensin converting enzyme (ACE) inhibitor, enalapril, it became apparent that this system is not involved in the pressor response or, indeed increased heart rate response, to icv sCT in normotensive rats. Conversely, pretreatment with enalapril of urethane anaesthetised rats rendered hypotensive by haemorrhage resulted in a significant attenuation of the pressor response to centrally administered sCT. It may be postulated that icv sCT leads to the release of catecholamines from the peripheral sympathetic neurones and from the adrenal medulla which then stimulate the release of renin ultimately resulting in a pressor effect partly mediated by AgII. It would be of interest to examine the effect of icv sCT on plasma renin activity and plasma catecholamine levels in the presence or absence of enalapril in order to confirm or refute this hypothesis. The renin-angiotensin system does not appear to be involved in the increased heart rate response to icv sCT in urethane anaesthetised haemorrhaged rats.

To summarise, it is hypothesised that the release of catecholamines from peripheral sympathetic neurones and from the adrenal medullae are equally involved in the pressor response to icv sCT in urethane anaesthetised normotensive rats. However, some other, as yet unexplained, mechanism must be involved. The release of catecholamines from peripheral sympathetic neurones and the adrenal medullae are involved in the increased heart rate reponse to icv sCT in these rats, although the peripheral sympathetic nervous system seems to be of greater importance. Conversely, the release of catecholamines from peripheral sympathetic neurones and the adrenal medullae as well as the vasoconstrictor effect of AgII are involved in the pressor effect of icv sCT in urethane anaesthetised rats rendered hypotensive by haemorrhage. The peripheral sympathetic nervous system, however, appears to be most important. The release of catecholamines from peripheral sympathetic neurones and the presence of intact adrenal medullae seem to mediate the increased heart rate response to icv sCT in these haemorrhaged rats.

8.4 The site of action of centrally administered sCT

As has been discussed above, centrally administered sCT is thought to exert its cardiovascular effects primarily through the release of catecholamines. The question now arises as to the site of action of centrally administered sCT. The primary cardiovascular control centre is in the medulla, but other areas of the brain, particularly in the cerebral cortex and the hypothalamus, have an important influence on blood pressure. Nerve impulses pass from these areas to the medulla and, through synaptic connections, alter the discharge of the primary medullary neurones (Vander et al. 1980). The hypothalamus also relays

with sympathetic preganglionic efferents which arise in the intermediolateral cell column in the spinal cord and which supply the noradrenergic neurones of sympathetic ganglia and the adrenal medulla (Struthers and Dollery, 1985).

The hypothalamus has been demonstrated to contain a high density of binding sites for sCT (Goltzman and Mitchell, 1985). Thus, it may be postulated that centrally administered sCT diffuses to the hypothalamus and interacts with CT receptors which then leads to the stimulation of noradrenergic neurones in this area. This finally leads to the release of catecholamines from peripheral sympathetic nerve endings and the adrenal medullae culminating in the observed increases in MAP and heart rate.

The possible correlation of CT binding sites in the hypothalamus with the cardiovascular effects of the peptide can also be extended to include the anorexic and gastrointestinal effects of the peptide. It is assumed that the ventromedial nucleus balances the stimulatory effect of the lateral hypothalamic area. Thus, an inhibitory effect of CT on the lateral hypothalamic area could produce the inhibition of feeding and gastric acid secretion observed after central administration of sCT. Furthermore, binding of sCT to the dorsal horn of the spinal cord and the periaqueductal grey (PAG) correlates with the antinococeptive effects of sCT (Sagar et al. 1984). It is noticeable that the raphe nuclei, which contains sCT binding sites, is rich in the cell bodies of tryptaminergic neurones and so the question of an interaction between CT and central 5-HT neurones must be addressed.

Central administration of sCT was found not to have any significant effect on 5-HT/5-HIAA levels in rat brain but there was a trend for an increase in these monoamines (see chapter.3). However, it should be noted that the anaesthetic may well be masking any response to icv sCT, since the anaesthetic itself was shown to induce increases in 5-HT/5-HIAA levels. These results lend support to those of Guidobono et al. (1984) who observed an increase in rat brain 5-HIAA levels in response to icv sCT administration to conscious rats. Moreover, the central tryptaminergic system has been demonstrated to be implicated in sCT induced antinociception (Bates et al. 1983a; Clementi et al. 1984; 1985). Guidobono et al (1986a) suggested that sCT induced antinociception requires the integrity of the central catecholaminergic system and not the central tryptaminergic system (as discussed in chapter.3). This would support the findings presented in this thesis concerning the importance of catecholaminergic neurones in mediating the cardiovascular effects of both iv and icv sCT.

The involvement of central tryptaminergic systems in the cardiovascular response to sCT (icv) cannot, however, be discounted. Clementi et al. (1986) suggested that sCT (icv) increases blood pressure in conscious rats by an increase in plasma renin activity through an interference with the central tryptaminergic system. Further work is required to clarify the role of the central tryptaminergic system in the cardiovascular response to sCT. More information would be obtained by the use of selective 5-HT receptor agonists, such as 5-carboxamidotryptamine and 2-methyl-5-hydroxytryptamine and antagonists, such as methysergide, cyproheptadine and ketanserin.

8.5 The interaction of CGRP with CT receptors in the CNS

Receptors for CT and CGRP have been mapped extensively in the CNS of the rat. Thus, Goltzman and Mitchell (1985) demonstrated that maximal binding of [^{125}I]-sCT occurred in the hypothalamus with less in the midbrain. Minimal binding was demonstrable in the spinal cord. Conversely, maximal binding of [^{125}I]-CGRP was found in the spinal cord, with less in the pituitary. Significant binding was also observed in the midbrain and cerebellum. Henke et al. (1985) extended these observations by subjecting rat brain and spinal cord to autoradiography with [^{125}I]-rCGRP and [^{125}I]-sCT. Binding sites for rCGRP were demonstrated in the lateral hypothalamus, vestibular nuclei, colliculi, medial geniculate body, corpus mallimare and the molecular layer of the cerebellum which lacked binding sites for sCT. In contrast, no rCGRP labelling was seen over the anterior and dorsomedial hypothalamus which showed high sCT binding.

Salmon CT and CGRP have been shown to cross-react at each others receptor, but only at 1000-fold higher concentrations than is necessary to bind to their own receptors (Breimer et al. 1988). Moreover, similar pharmacological effects have been observed after central administration of sCT and CGRP. Both peptides have been shown to inhibit gastric acid secretion (Morley et al. 1981a; Lenz et al. 1985a), produce anorexic effects (Levine and Morley, 1981; Tannenbaum and Goltzman, 1985) and to result in antinociception (Bates et al. 1981b; 1984b), which raises the possibility of a common site of action. Calcitonin gene-related peptide, however, is approximately 1000-times less potent than sCT in producing the above effects, which suggests that CGRP may be interacting with CT receptors in the CNS.

As has been demonstrated in this thesis, sCT (icv) results in a pressor effect and increased heart rate response in urethane anaesthetised normotensive and haemorrhaged rats. Calcitonin gene-related peptide (icv) has also been demonstrated to exert an increase in blood pressure and heart rate in conscious normotensive rats (Fischer et al. 1983a). However, CGRP is much less potent than sCT in this respect in that 220 pmol - 2200 pmol CGRP result in smaller increases in blood pressure and heart rate than does 290 pmol sCT (5 U icv). It may, therefore, be postulated that centrally administered CGRP exerts its cardiovascular effects by interaction with central CT receptors. This differs from the cardiovascular effects of peripherally administered CGRP which are thought to be associated specifically with peripheral CGRP receptors (see 8.1). It would be advantageous to compare the haemodynamic effects of sCT and CGRP in internally controlled experiments using the same animal model (ie, all animals conscious or all animals anaesthetised; normotensive animal model or hypotensive animal model).

8.6 Therapeutic potential of sCT

Salmon CT (icv) has been demonstrated to increase MAP and heart rate in urethane anaesthetised normotensive rats and those rendered hypotensive by haemorrhage, an effect mediated primarily by the CNS control of sympathetic tone. Conversely, sCT (iv) results in a pressor effect and increased heart rate response only in rats subjected to haemorrhage, an effect which is thought to be mediated by peripheral sympathetic tone. It is, therefore, possible that treatment of hypovolaemic shock with iv sCT may be of therapeutic benefit. Furthermore, the antinociceptive effects of the peptide

would be additive to the putative beneficial effect of sCT compared with treatment with naloxone which may antagonise the antinociceptive effect of endogenous and exogenous opiates.

Administration of sCT (iv) to rats rendered hypotensive by endotoxin shock was found not to exert any effects on MAP, but resulted in a decrease in heart rate. This would suggest that sCT has no place in the treatment of endotoxin shock, and in fact may exacerbate the condition. Indeed, sCT may well be involved in the pathophysiology of the hypotension of endotoxin shock.

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CALCITONIN AND THE SYMPATHETIC NERVOUS SYSTEM IN HAEMORRHAGIC SHOCK

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Peripherally administered salmon calcitonin (sCT) exerts a pressor effect in rats rendered hypotensive by haemorrhage. This may involve an increase in sympathetic tone since chronic pretreatment with 6-hydroxydopamine (6-OHDA) greatly attenuates the response to peripheral (i.v.) sCT (Bates et al, 1984), although not to intracerebroventricular (i.c.v.) sCT (Bates et al, 1984). We have investigated the role of the sympathetic nervous system in the response to sCT during haemorrhagic shock by determining the effect of sCT after acute sympathetic blockade using guanethidine.

Sprague-Dawley rats (♂, 150-300g) were anaesthetised with urethane (1.6g.Kg^{-1} i.p. and s.c.) and the jugular vein and carotid artery cannulated for drug administration and the measurement of blood pressure respectively. Animals which were to receive sCT or vehicle by i.c.v. injection were placed in a stereotaxic frame for the duration of the experiment. Animals were bled by the withdrawal of arterial blood such that mean arterial pressure (M.A.P.) was reduced by 20%.

After a 20 min. stabilisation period, guanethidine (10mg.Kg^{-1} i.v.) or vehicle (0.9% NaCl) was administered. Ten minutes later, sCT (10U.Kg^{-1} i.v. or 20U.Kg^{-1}i.v. in 0.15M sodium phosphate buffer containing 0.1% BSA), sCT (1U.Kg^{-1} i.c.v. or 5U.Kg^{-1} i.c.v. in 50mM tris-buffer containing 0.1% BSA), or appropriate vehicle were administered. M.A.P. was monitered for 60 min. Statistical analysis was by analysis of variance followed by the Students 't' test as appropriate.

Table 1. Change in M.A.P. (mmHg) after drug treatment in haemorrhaged rats.

Pretreatment	Drug	5min.	15min.	30min.	60min.
Vehicle	Vehicle (i.v.)	2±2	0±2	3±2	5±2
(n,6)	sCT (10U.Kg^{-1} i.v.)	9±2*	5±1*	2±2	4±1
	sCT (20U.Kg^{-1} i.v.)	18±2***	11±2**	12±3	14±4
Guanethidine	Vehicle (i.v.)	2±1	1±1	2±1	-2±2
(n,6)	sCT (10U.Kg^{-1} i.v.)	3±1	4±1	3±2	1±1
	sCT (20U.Kg^{-1} i.v.)	3±1	3±1	1±1	2±2
Vehicle	Vehicle (i.c.v.)	16±3	6±3	3±4	-1±3
(n,5)	sCT (1U.Kg^{-1} i.c.v.)	17±3	12±5	8±5	7±5
	sCT (5U.Kg^{-1} i.c.v.)	28±4*	30±5**	29±6**	20±6*
Guanethidine	Vehicle (i.c.v.)	4±2	3±1	1±2	-1±2
(n,5)	sCT (1U.Kg^{-1} i.c.v.)	6±1	5±1	5±1	2±1
	sCT (5U.Kg^{-1} i.c.v.)	11±3	12±3*	12±4*	9±5

mean ±s.e.m. *p<0.05; **p<0.01; ***p<0.001

The pressor response to i.v. sCT was completely abolished after guanethidine, whereas the response to i.c.v. sCT was reduced but not abolished. These results suggest that the central and peripheral mechanisms of the pressor response to sCT in haemorrhaged rats may not be identical. The difference between these results and those using 6-OHDA may be due to differences between acute and chronic sympathectomy.

Bates, R.F.L. et al. (1984) Br. J. Pharmacol. 81, Proc. Suppl., 157P
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786P

THE ROLE OF THE ADRENAL GLANDS IN THE PRESSOR RESPONSE TO CALCITONIN

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The pressor response observed after peripheral administration of salmon calcitonin (sCT) to rats rendered hypotensive by haemorrhage is abolished after acute sympathetic blockade using guanethidine. However, the pressor response to centrally administered sCT in haemorrhaged rats is significantly attenuated, but not abolished, after guanethidine (Bates et al, 1987).

We have investigated the role of the adrenal glands, after acute sympathetic blockade, in the response to intracerebroventricular (i.c.v.) administration of sCT, both in normotensive rats and those subjected to haemorrhagic shock.

Male Sprague-Dawley rats (200-300g) were anaesthetised with urethane (1.6g.Kg^{-1}; 50% i.p. and 50% s.c.) and bilaterally adrenalectomised (ADX), or sham adrenalectomy (SADX) in control animals. Animals were prepared for drug administration as previously reported (Bates et al, 1987). After a 20 min. stabilisation period, guanethidine (10mg.Kg^{-1} i.v.) or vehicle (0.9% NaCl i.v.) was administered. Ten minutes later, sCT (5U.Kg^{-1} i.c.v. in 25μl) or vehicle (50mM tris-buffer containing 0.1% BSA i.c.v. in 25μl) was administered by stereotaxic placement. Mean arterial pressure (M.A.P.) was monitored for 60 min. Statistical analysis was by the Student's 't' test. Results are expressed as mean area under the blood pressure curve (AUC).

Table 1. Mean AUC (mmHg.min.) after 5U.Kg^{-1} sCT (i.c.v.) in normotensive and haemorrhaged rats. (mean ± s.e.m.; n=5; $^{*}p<0.05$, $^{**}p<0.01$.

PRETREATMENT	NORMOTENSIVE 60 min.		POST-HAEMORRHAGE 60 min.	
SADX	+1058 ± 304		+2227 ± 290	
ADX	+ 798 ± 103		+1944 ± 438	
SADX + Guanethidine	+ 850 ± 130	**	+ 758 ± 122	*
ADX + Guanethidine	+ 175 ± 146		+ 200 ± 199	
ADX	+ 798 ± 103	**	+1944 ± 438	**
ADX + Guanethidine	+ 175 ± 146		+ 200 ± 199	

In both the normotensive animals and the haemorrhaged animals, ADX alone has no effect on the response to sCT. In the presence of guanethidine, however, ADX leads to a significant further reduction in this pressor response.

In normotensive animals either the peripheral sympathetic nervous system or the adrenal glands can maintain the pressor response to sCT. In haemorrhaged animals the peripheral sympathetic nervous system is more important than the adrenal glands in the pressor response to sCT.

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CALCITONIN IN ENDOTOXIN SHOCK.

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Naloxone raises blood pressure after endotoxin (Holaday et al, 1978) and after haemorrhage. Salmon calcitonin (sCT) has a pressor effect in rats rendered hypotensive by haemorrhage (Bates et al, 1983), but Quimby et al, 1985 have shown a depressor effect of sCT in baboons during toxic shock syndrome. We have therefore compared the effects of calcitonin with those of naloxone on blood pressure of anaesthetised rats subjected to endotoxin shock.

Sprague-Dawley rats (150-300g) were anaesthetised with i.p. sodium pentobarbital (60mg.kg^{-1}). The trachea, jugular vein, and carotid artery were cannulated for artificial ventilation, drug administration, and the measurement of blood pressure respectively. Rats were maintained on a ventilator (50 strokes/min) throughout the experiment, and anaesthesia was maintained by s.c. sodium pentobarbital as required. After a 20 min stabilisation period an i.v. infusion of E.coli lipopolysaccharide (0127:B8) was initiated (2mg.kg^{-1} over 2 hr at 0.03 ml/min). This produced a rapid fall in mean arterial pressure (M.A.P.), the maximum response (-47 ± 8mm Hg) occurring at 30 min. Twenty-five min after the start of the endotoxin infusion i.v. naloxone in 0.9% NaCl, sCT in 0.15M sodium phosphate buffer containing 0.1% BSA, or appropriate vehicles were administered. M.A.P. was monitored for a further 95 min. Statistical analysis was by analysis of variance followed by the students 't' test as appropriate.

Table 1 Increase in M.A.P. after drug treatment in rats subjected to endotoxin shock.

Treatment	ΔM.A.P. (mmHg) : post drug treatment ($\bar{x}$ ± S.E., *$p < 0.05$) n= 6.		
	15min	35min	75min
Endotoxin alone	7 ± 3	2 ± 10	4 ± 11
Endotoxin + naloxone (10mg.kg^{-1})	27 ± 6	31 ± 6 *	30 ± 7 *
Endotoxin + 0.9% NaCl	18 ± 4	9 ± 5	6 ± 7
Endotoxin + sCT (10U.kg^{-1})	18 ± 6	14 ± 7	11 ± 5
Endotoxin + sodium phosphate	29 ± 6	19 ± 4	16 ± 4

In the rats treated with endotoxin infusion and either vehicle there was a transient increase in M.A.P. during the first 15 min. These increases, however, were not significant. As shown in table 1, naloxone resulted in a significant increase in M.A.P. at 35 min. This pressor response was maintained for the duration of the experiment. In contrast, sCT had no significant effects on M.A.P. It was noted, however, that when compared with the corresponding control sCT tended towards a lower rather than a higher M.A.P.

We have shown that sCT had no significant effect on M.A.P. of rats treated with endotoxin.

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