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THE IN VITRO UPTAKE OF CATECHOLAMINES INTO RAT BRAIN PREPARATIONS

by

Neil Thomas Brammer B.Sc.

This thesis is submitted to the Council for National Academic Awards in partial fulfilment of the requirements for the degree of Ph.D. The research work having been conducted at Trent Polytechnic, Nottingham.

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Whilst registered as a candidate for the degree of Ph.D. I was not in receipt of another award for a research programme. None of the work contained within this thesis has been used in any other submission for an academic award.

Results contained within this thesis were presented as a poster communication at the January, 1984 meeting of the British Pharmacological Society, held at the University of London. The work entitled Inhibition of dopamine uptake in rat striatal slices by phenylethylamine derivatives was subsequently published in the British Journal of Pharmacology, volume 81, 112P.

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SUMMARY

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The uptake of the catecholamines, dopamine and noradrenaline, was investigated in a number of rat brain preparations. Kinetic analysis of the uptake of dopamine in striatal slices indicated that at least two distinct uptake systems for the compound existed in this region; one with high affinity for dopamine, the other possessing low affinity for dopamine. Distinct high and low affinity uptake systems were also observed for the uptake of dopamine into slices of cerebellum, and the uptake of noradrenaline into both striatal slices and slices of cerebellum.

The uptake of dopamine into striatal slices was further investigated using a number of compounds to inhibit the uptake process. The rank order of decreasing potency of the compounds was shown to be different for the high and low affinity uptake systems in this region.

The uptake of dopamine was also studied in isolated striatal cells, striatal homogenates and striatal synaptosomes. Two uptake systems with differing kinetic parameters were observed in isolated striatal cells, but only a high affinity system was detected in striatal synaptosomes. The results of the experiments using striatal homogenates were inconclusive.

The metabolism of dopamine and noradrenaline in striatal slices following incubation in a high concentration of catecholamine was investigated.

The major metabolites of dopamine and noradrenaline were shown to be 3,4-dihydroxyphenylacetic acid and 3,4-dihydroxyphenylethylglycol, respectively. The accumulation of these metabolites following incubation in their respective precursors at 100 µM was not significantly affected by pretreatment of the rats with 6-hydroxydopamine.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

Dengler et al (1961) demonstrated that slices of cat brain actively accumulated noradrenaline when incubated in a solution of the catecholamine. However the first quantitative study of an uptake mechanism for catecholamines was carried out by Iversen (1963) using the perfused rat heart preparation. The studies revealed an uptake system for catecholamines demonstrating Michaelis-Menten Kinetics. The mechanism involved has further been shown to require the presence of sodium ions in the external medium (Iversen and Kravitz, 1966), to be temperature dependent and to be inhibited by ouabain (Green and Miller, 1966) and by metabolic poisons (Wakade and Furchgott, 1968).

Iversen (1965a) demonstrated a second catecholamine uptake system using the isolated perfused rat heart preparation. This second mechanism appeared only to operate at high catecholamine concentrations and was initially thought to become active only when a threshold concentration of catecholamine had been reached. Subsequently the threshold was shown to be an artefact of the method used. The two uptake systems were termed "Uptake 1" and "Uptake 2" respectively. Further studies showed that hearts from rats treated with antiserum to nerve growth factor showed a decrease in accumulation of noradrenaline via both Uptake 1 and 2 (Iversen, 1965a), and it was concluded that the mechanisms were therefore neuronal. Lightman and Iversen (1969) proposed that when the tissue was perfused with low concentrations of noradrenaline the catecholamine removed by the Uptake 2 system from the surrounding medium was metabolised completely; however at higher perfusion concentrations not all the noradrenaline was metabolised, due to saturation

of the enzymes involved, and some accumulated in the tissue. This situation had led to the earlier conclusion of a threshold value for Uptake 2; Lightman and Iversen concluded that Uptake 2 was in fact active at all concentrations. The system was saturable and there was low affinity between the substrate and its uptake site.

Burgen and Iversen (1965) investigated the structural requirements for the two systems by the use of derivatives of phenylethylamine. It was found that methoxylation, N-substitution or β-hydroxylation of the phenylethylamine base decreased its ability to inhibit Uptake 1, but phenolic hydroxylation at positions 3 or 4, or α-methylation increased the inhibitory action. In the case of Uptake 2 the structural requirements were the virtual reverse of those for Uptake 1. Other differences in the pharmacology of the two mechanisms were reported. In the rat heart Uptake 1 but not Uptake 2, was shown to be stereo-selective for the l-isomer of noradrenaline (Iversen, Jarrot and Simmonds, 1971). Gillespie and Hamilton (1967) and Avakian and Gillespie(1968) had discovered that noradrenaline perfused at high concentrations accumulated in the smooth muscle cells of the spleen and arteries. Investigations into the accumulation of noradrenaline in rat cardiac muscle cells (Farnebo and Malmfors, 1969; Clarke et al, 1969) produced similar results. The characteristics of the accumulation were in all cases similar to those of Uptake 2. It was therefore concluded that Uptake 2 was extraneuronal. Uptake 2 has been found to be reduced by cooling (Gillespie et al, 1970) and to be unaffected by either surgical (Gillespie et al, 1970) or chemical (Clarke and Jones, 1969)

sympathectomy in smooth or cardiac muscle cells. Wakade and Furchgott, (1968), using the isolated left atrium of the guinea-pig found that anoxic conditions or the absence of glucose from the incubation medium reduced the accumulation of noradrenaline from a medium containing a high concentration of the compound, suggesting that the uptake system may be energy dependent. The presence of sodium ion in the medium also appeared to be necessary for Uptake 2 (Gillespie and Towart, 1972 and 1973).

The two mechanisms have been shown to be effectively inhibited by drugs. However, the compounds which inhibit Uptake 1 have little or no inhibitory action on the extraneuronal Uptake 2 mechanism, whilst those affecting Uptake 2 have little or no effect on Uptake 1, i.e. the inhibitory properties of the compounds are relative rather than specific. Cocaine and desipramine (Iversen, 1963) and metaraminol (Iversen, 1965a and 1965b) are all potent inhibitors of Uptake 1, cocaine possibly being specific. The steroids particularly 17-β-oestradiol and corticosterone (Iversen and Salt, 1970; Salt and Iversen, 1972) potently, and possibly specifically, inhibit extraneuronal uptake.

As well as in the peripheral sympathetic nervous system the uptake of catecholamines has been studied in the central nervous system. The use of histochemical methods showed that the catecholamines accumulated in neurones after incubation in low concentrations (Hamberger and Masuoka, 1965; Carlsson et al, 1966; Hamberger, 1967). The uptake of noradrenaline by slices of mouse cerebral cortex was

shown to demonstrate Micahelis-Menten kinetics (Ross and Renyi, 1964). Amphetamine was shown to prevent the accumulation of catecholamines into both noradrenergic and dopaminergic nerve fibres (Coyle and Snyder, 1969) whereas desipramine and chlorpromazine inhibit the uptake of catecholamines into noradrenergic nerves only (Ross and Renyi, 1967; Haggendal and Hamberger, 1967).

The catecholamine uptake mechanism shown to be present in the brain had many other characteristics similar to those of neuronal uptake in the rat heart, namely, temperature dependence, inhibition by ouabain and metabolic poisons and a requirement for sodium ions in the external medium (Colbourn et al, 1968; Keen and White, 1969; Tissari and Bogdanski, 1971; White and Paton, 1972; Harris and Baldessarini, 1973; Holz and Coyle, 1974).

Studies involving different regions of the rat brain were performed. It was shown that the uptake systems varied between the different regions. The striatum accumulated most noradrenaline following incubation in a medium containing this catecholamine, and cerebellum accumulated the least noradrenaline (Snyder et al, 1968a, 1968b). Kinetic analysis of the uptake data gave Km values for the hypothalamus, cerebral cortex, hippocampus and medulla oblongata which were in the range 0.38 to 0.50 μM and Vmax in the range 0.55 to 0.83 nmol.g wet $wt^{-1}.min^{-1}$. Striatal slices, however, gave values of 1.0 μM for Km and 0.33 nmol.g wet $wt^{-1}.min^{-1}$ for Vmax.

Using homogenates of non-striatal areas Snyder and Coyle (1969) found a value for Km of 0.4 μM and for Vmax of 0.56 to 1.0 nmol.g pellet^{-1}.min^{-1} for noradrenaline uptake. The striatal homogenates had a Km of 2.0 μM and Vmax of 20 nmol.g pellet^{-1}.min^{-1}. Snyder and Coyle obtained kinetic parameters of 0.4 μM for Km and 20 nmol.g pellet^{-1}.min^{-1} for Vmax for the uptake of dopamine in striatal homogenates. They also studied dopamine uptake in homogenates of other brain regions. In non-striatal areas the results suggested that two systems existed; the Km values being 0.08 μM and 1.4 μM - high and low affinity systems respectively. Vmax was shown to be in the range 0.34 to 0.66 nmol.g pellet^{-1}.min^{-1} for the high affinity system and 2.5 to 3.4 nmol.g pellet^{-1}. min^{-1} for the low affinity system. In striatal homogenates they obtained values for Km of 0.4 μM and for Vmax 20 nmol.g pellet^{-1}.min^{-1} but were unable to show the presence of a second low affinity uptake system for dopamine. Shaskan and Snyder (1970) successfully demonstrated a striatal low affinity mechanism for dopamine, having a Km in the range 1.0 to 1.3 μM, these values were consistent with those found by Snyder and Coyle (1969) for the uptake of dopamine by low affinity mechanisms in non-striatal brain areas.

Coyle and Snyder (1969) studied the stereoselectivity of the uptake mechanisms in different brain regions. It was demonstrated that noradrenergic neurones appear to possess a mechanism for noradrenaline uptake that is stereoselective at the β-carbon atom for the l-enantiomers. Dopaminergic neurons were not shown to be stereoselective for noradrenaline. It should be noted however, that dopamine itself is not chiral at the β-carbon atom. Structure-activity relationships

of compounds which effect catecholamine uptake in brain tissue were studied by Horn et al (1971). It was found that the requirements for functional groups differed for noradrenergic and dopaminergic neurones. In compounds where the alkylamino side chain had been replaced with a tropine ring there was an increase in their ability to inhibit the uptake of catecholamines into dopaminergic neurones, but a decrease in this effect in noradrenergic fibres. Horn (1973) also studied the structure-activity relationships for the inhibition of uptake into the two types of neurones, using derivatives of phenylethylamine. Their effects were studied in synaptosomes from both the striatal (a dopaminergic) region and the hypothalamic (a noradrenergic) region. It was found that inhibition of neuronal uptake of catecholamines was enhanced by the addition of phenolic hydroxyl groups or by α-methylation of the alkyl side chain. The pattern occurred in both types of neurone but was more pronounced in the noradrenergic synaptosomes. Phenolic methoxylation, N-substitution or ß-hydroxylation of the side chain resulted in a decrease in inhibitory action. The effects of these analogues were the same as those found for the inhibition of noradrenaline uptake by Uptake 1 in the rat heart (Burgen and Iversen, 1965), indicating that the neuronal uptake mechanism is similar in both the central nervous system and its peripheral counterpart. Tuomisto et al (1974) found that the decalin derivative of noradrenaline with the amino group situated gauche to the catechol group of the molecule was a more potent inhibitor of dopamine uptake in the striatum and hypothalamus than the derivative in which the functional groups were in a trans conformation. However, Horn (1974) examined the inhibition of dopamine uptake by two rigid

analogues of dopamine, namely, 2-amino,6,7-hydroxy-1,2,3,4-tetrahydro-naphthalene (ADTN) and 6,7-dihydroxytetrahydroquinoline (norsalsolinol). It was found that of the two analogues the one fixed in the trans (extended) conformation i.e. ADTN, rather than the gauche conformation, was the more potent inhibitor of uptake in both the striatum and hypothalamus; the reverse of Tuomisto's findings. Horn reasoned that the difference in results could be attributed to steric effects produced at the binding site by the large decalin moiety present in the compounds studied by Tuomisto. More recently, in a series of experiments using phenylcyclobutylamines, the trans isomers were found to be slightly more potent inhibitors of uptake than the corresponding cis isomers (Komiskey et al, 1978). Law et al (1982) showed that the exo-isomer of dopamine, in which the catechol and amino groups are in antiperiplaner relationship, possessed more inhibitory activity against dopamine uptake than did the endo-isomer in which the catechol and amino groups are restricted in a gauche relationship.

Rigid analogues of amphetamine, cis and trans-2-phenylcyclopropylamine, were investigated as catecholamine uptake inhibitors (Horn and Snyder, 1972) using synaptosomes from the hypothalamus and striatum of the rat. Trans-2-phenylcyclopropylamine (tranylcypromine) was found to be more potent in this respect in both brain regions investigated. The (-)isomer of tranylcypromine was also shown to be a more potent inhibitor of both noradrenaline and dopamine uptake in the hypothalamus and striatum. In the same study 2-aminoindane was shown to have a higher potency for inhibition than 1-aminoindane in both regions. It was concluded that the results indicated that at the site of uptake

amphetamine is in a conformation where the side chain is fully extended and the amino group above the plane of the ring, i.e. the anticonformation.

The structural requirements for the uptake of noradrenaline in the hypothalamus have also been examined (de Paulis et al, 1978). It was concluded from their work that the noradrenaline transport carrier system is equipped with two active sites. Tricyclic antidepressants have an affinity with one of these sites and phenylethylamines including noradrenaline bind to the other site. Several possible explanations for this apparent dual site are given by the authors. One possibility is that the recognition site for the noradrenaline carrier system may exist in two different conformational states. It is likely that conformational change is part of the functional activity of a transport carrier when binding and releasing its target molecule. Hence it is possible that the tricyclic antidepressants bind to the carriers receptor site in the conformation normally associated with the ejection of the noradrenaline molecule. By occupying the carrier in this conformational state the tricyclic antidepressant agents prevent noradrenaline molecules from being transported across the membrane giving apparent competitive antagonism. Other theoretical explanations for the apparent dual site include the attachment of the tricyclic compounds at a binding site different from that which binds noradrenaline inducing allosteric changes which render the carrier incapable of binding noradrenaline. Thirdly it may be that although the binding site for the amino group is the same for both the tricyclic agents and phenylalkylamines the site which binds the aromatic group may well be different.

An argument against this last explanation is that the structure-activity relationship for amino substitution is different for the two groups of inhibitors. The phenylalkylamine series showed similar potency for inhibition when either primary or secondary amine derivatives were used. Whereas secondary or tertiary amine derivatives are considerably more potent than primary amines of the tricyclic antidepressant group.

Salama et al (1971) had previously shown that the secondary methyl-amines of dihydrobenzazepine, dibenzcycloheptadene and diphenylmethylidene showed greater potency than their primary or tertiary derivatives when used to inhibit noradrenaline uptake in rat cortical tissue. However in these studies all three types of amine derivatives were equipotent in the dibenzocycloheptatriene series. Salama postulated that the hydrophobic area of the "amine pump" receptor which receives the N-methyl groups could distinguish minor differences in the presentation of these groups. It was also found that compounds in which the phenyl rings were co-planar, e.g. carboxazole, were much less potent inhibitors than those in which the phenyl rings were fixed at right angles to each other, e.g. imipramine, amitriptyline and protriptyline. Compounds such as the diphenylmethylidenes which possess unbridged phenyl rings showed intermediate activity. Freeman and Sulser (1972) suggested from work with iprindole, that the tricyclic antidepressants are not required to block the neuronal membrane in order to have a therapeutic effect. Iprindole has been shown not to inhibit the uptake of noradrenaline in rat brain (Crews and Smith, 1981), but it does inhibit dopamine uptake in striatal synaptosomes (Friedman et al, 1977).

The effect of α-alkyl substitution of phenylethylamines on the inhibition of noradrenaline uptake in rat cortical synaptosomes was investigated by de Jong et al (1982) and it was found that the molecule must be in a planar conformation in order to bind to the uptake site, otherwise steric hindrance prevents uptake from taking place.

Tuomisto and Voutilainen (1982) examined catecholamine uptake inhibition using an analogue of nomifensine, Ro-8-4650. Nomifensine is a potent inhibitor of dopamine uptake in brain synaptosomes (Hunt et al, 1974) and is very similar in structure to the tricyclic antidepressants but with two important differences: it does not possess a dimethylene bridge between the phenyl rings and the side chain nitrogen atom is rigidly bound to a ring structure. In the novel analogue the 8-amino group is absent. This compound was shown to be a potent inhibitor of dopamine uptake, hence demonstrating that the 8-amino group of nomifensine is not necessary for its activity as an uptake blocker. The results therefore support the theory that nomifensine binds to the uptake site through its ring nitrogen and the two phenyl rings (Tuomisto, 1977).

The possibility that glycoproteins in the cell membrane of axonal terminals are involved in high affinity uptake was explored by Wang et al (1975). Binding of the lectin, Concanavalin A was shown to have no effect on catecholamine uptake by cortical synaptosomes, neither did trypsin digestion of synaptosomal surface proteins.

The pharmacology of another uptake system in brain tissue was investigated by Hendley et al (1970). Similarities between the uptake of normetanephrine in slices of cerebral cortex and the Uptake 2 system of the rat heart were demonstrated. These included the failure of pretreatment with the neurotoxin 6-hydroxydopamine to influence normetanephrine uptake in brain and the ease with which normetanephrine could be washed out of the brain tissue, an indication that the substance was not firmly bound. The uptake system for normetanephrine was not stereoselective. The rank order of potency of drugs inhibiting the uptake of normetanephrine in untreated rat brain was different from that for the inhibition of noradrenaline uptake. In the case of the 6-hydroxydopamine treated rats the rank order of potency for the inhibition of uptake of normetanephrine closely resembled that of Uptake 2. However, Hendley also showed that phenoxybenzamine a potent inhibitor of noradrenaline Uptake 2, poorly inhibited normetanephrine uptake. It should also be noted that the affinity for normetanephrine in the rat heart (Burgen and Iversen, 1965) is much higher than the affinity in cortical slices, suggesting that it is not Uptake 2 which is involved with the uptake of normetanephrine.

The accumulation of noradrenaline by various preparations of cerebral cortex in the presence and absence of Uptake 1 blockers such as cocaine or desipramine was studied by Burrows et al (1981). It was shown that when neuronal Uptake 1 is blocked a rapid initial uptake of noradrenaline still occurred. This uptake could not be inhibited by normetanephrine but was inhibited by metaraminol, suggesting a novel uptake system was present. Omission of calcium ion from the incubation medium also

reduced the uptake via this novel system which was found predominantly in the synaptosomes. Burrows termed this system calcium sensitive accumulation or CSA.

Uptake inhibitors have also been shown to promote the release of catecholamines from brain tissue (Heikkila et al, 1975). This led to Bauman and Maitre, (1976) challenging the existence of dopamine uptake inhibitors. They proposed that drugs such as benztropine and nomifensine are purely releasers of dopamine. Amphetamine, on the other hand, was proposed to be a pure uptake inhibitor (de Belleroche and Bradford, 1976) and not a direct releaser of dopamine. In experiments involving striatal synaptosomes superfused in conditions which prevented drug effects on re-uptake nomifensine (up to 10^{-5}M) was shown to be unable to stimulate the release of pre-loaded ^{3}H-dopamine from the synaptosomes. However, benztropine possessed a modest stimulatory effect on the release of ^{3}H-dopamine taken up only at high concentrations, 10^{-5}M. Amphetamine was shown to be a potent releaser of ^{3}H-dopamine previously accumulated by brain tissue (Raiteri et al, 1978). This has been shown to occur in vivo (Goodale and Moore, 1975).

Opioid peptides have well documented modulatory effects on the synaptic transmission of neurotransmitters and there is dense innervation of both the striatum and hypothalamus by the β-endorphin and enkephalin neuronal system.

Intracerebral administration of β-endorphin has been shown to increase dopamine uptake into hypothalamic synaptosomes. However in

vitro incubation of hypothalamic and striatal synaptosomes in a wide range of concentrations of β-endorphin failed to increase the uptake of dopamine (George and van Loon, 1982).

Following uptake, catecholamines are metabolised by a series of enzymes prior to release into the blood stream and eventual excretion. There are thought to be two main enzymes involved in catecholamine metabolism. The first is monoamine oxidase (MAO; E.C.1.4.3.4.) which catalyses the oxidative deamination of the catecholamines. The aldehydes produced are immediately converted to acid metabolites by aldehyde dehydrogenase (E.C.1.2.1.3.). Further conversions can occur, alcohol dehydrogenase forms phenylglycols from noradrenaline and phenylethanols from dopamine (Duncan and Sourkes, 1974).

Monoamine oxidase is widely distributed throughout the various tissues of the body, intracellularly it is localised on the outer membrane of the mitochondria. The existence of multiple forms or isoenzymes of MAO was postulated by Johnston (1968). This was based on experiments which demonstrated that clorgyline irreversibly inhibited the activity of rat brain MAO towards serotonin at a concentration considerably lower than those required to inhibit the oxidation of benzylamine. Activity towards tyramine was also shown to be inhibited in a biphasic manner, again suggesting more than one form of MAO. The two sub-types of MAO were termed MAO type A (active toward serotonin) and MAO type B (active toward benzylamine). Dopamine has been shown to be a substrate for both of the subtypes (Yang and

Neff, 1974; Braestrup et al, 1975).

The MAO contained within the dopaminergic neurons of the striatum has been shown to be MAO type A (Demarest et al, 1980) whilst that contained in the glial cells is MAO type B (Strolin Benedetti et al, 1980). Inhibition of MAO A with the selective type A inhibitor clorgyline leads to a build up of dopamine. Deprenyl, a specific type B inhibitor has no such effect (Schoepp and Azzaro, 1981) suggesting that the type A isoenzyme is responsible for the majority of the deamination of dopamine.

The second enzyme involved in catecholamine metabolism is catechol O-methyl transferase (COMT; E.C. 2.1.1.6.). This enzyme catalyses the transfer of methyl groups to substrates containing the catechol grouping. The compound S-adenosylmethionine (SAM) is required as the methyl group donor and magnesium ions are necessary as an activator. Catechol O-methyl transferase can methylate the catecholamine itself or the acid or alcohol metabolites possessing a catechol group.

Catechol O-methyl transferase like MAO is widely distributed throughout the body. Within cells it is freely dispersed in the cytoplasm or loosely bound to membranes within the cellular cytoplasm. In the brain the enzyme is responsible for the O-methylation of the catecholamines at the 3 or meta position (Kopin and Gordon, 1963; Sharman, 1973; Duncan and Sourkes, 1974). This O-methylated compound can also act as a substrate for MAO leading to the production of another aldehyde which is further metabolised by dehydrogenases.

The activity of COMT in the striatum has been shown not to be significantly altered by chemical or electrolytic lesioning of the dopaminergic pathways to the striatum (Uretsky and Iversen, 1970; Marsden et al, 1972) unlike MAO which is significantly reduced by lesions of the pathway with 6-hydroxydopamine (Agid et al, 1973). Evidence for the extraneuronal localisation of COMT was first put forward by Carlsson and Hillarp (1962), this theory has been supported by the work of Kaplan et al, (1979), using immunohistochemical techniques it was demonstrated that the enzyme was present in glial cells but not in the accompanying neuronal elements.

There is no clear evidence of isoenzymes of COMT (Rock et al, 1970). The best known inhibitor of the enzyme is tropolone (Belleau and Burba, 1963). The tropolones are isosteric with catechols and have been shown to be linear non-competitive inhibitors with respect to various common substrates of the enzyme (Borchardt, 1973).

The metabolic pathways for dopamine and noradrenaline in brain tissue were outlined by Rutledge and Jonason (1967) using slices of rabbit cortex incubated in labelled catecholamine. Noradrenaline was shown to be mainly deaminated to form phenolic glycols and dopamine deaminated to form phenolic acids.

The major products of the catabolism of newly formed noradrenaline were the glycols, 3,4-dihydroxyphenylglycol (DOPEG) and 3-methoxy,-4-hydroxyphenylglycol (MOPEG). The formation of these compounds was markedly reduced following pretreatment with the MAO inhibitor

nialamide. The acids dihydroxymandelic acid (DHMA) and vanillyl-mandelic acid (VMA) as well as the amine normetanephrine (NMN) were formed in small quantities with respect to their control values. Relatively more NMN was formed (20% of the total metabolites) from exogenously administered noradrenaline.

The major products of dopamine catabolism were shown to be 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA). 3-methoxytyramine (3-MT) was produced in much smaller quantities, negligible amounts of 3,4-dihydroxyphenylethanol (DOPET) and 3-methoxy-4-hydroxyphenylethanol (MOPET) were also detected. After pretreatment with nialamide the acid metabolites (DOPAC and HVA) were reduced to less than 10% of their normal values. 3-MT was increased 6-fold by this same treatment.

The three main metabolites of dopamine found in the brain are DOPAC, produced by the action of MAO and aldehyde dehydrogenase, 3-MT produced by the action of COMT and HVA which may be produced from either of the preceding metabolites and involves the actions of both the enzymes mentioned above. In addition to these compounds sulphate conjugates of dopamine (Buu et al, 1981), DOPAC and HVA (Gordon et al, 1976; Elchisak et al, 1977; Dedek et al, 1979) have been shown to be present in rat brain. These compounds are believed to be produced by the action of phenolsulphotransferase (PST, E.C. 2.8.2.1.) (Tyce and Rorie, 1982), an enzyme shown to be present in the brain, primarily in neurones (Foldes and Meek, 1974).

It was postulated by Roffler-Tarlov et al (1971) that in the mouse brain DOPAC and HVA levels reflected the intra- and extra-neuronal metabolism of dopamine respectively, and that most if not all the HVA is formed from 3-MT. It was later demonstrated that dopamine is predominantly metabolised to DOPAC and partly removed from the rat brain as HVA after O-methylation (Westerink and Korf, 1976). The turnover rates of DOPAC and HVA were studied in the striatal and mesolimbic areas of the brain of rats sacrificed by decapitation. The DOPAC turnover was found to be 23.3 $nmol.g^{-1}.hr^{-1}$ in the striatum and 22.6 $nmol.g^{-1}.hr^{-1}$ in the mesolimbic structures. The turnover of HVA was 11.2 $nmol.g^{-1}.hr^{-1}$ in the striatum and 6.7 $nmol.g^{-1}.hr^{-1}$ in the mesolimbic structures (Westerink and Korf 1976). The authors concluded that DOPAC turnover probably approximates dopamine turnover.

Westerink and Spaan (1982) studied the turnover of 3-MT and HVA in the rat brain. Using microwave killed rats they obtained values for turnover of 1.9 $nmol.g^{-1}.hr^{-1}$ for 3-MT and 9.1 $nmol.g^{-1}.hr^{-1}$ for HVA in the striatum. It was concluded that 80% of the HVA formed in this region comes from DOPAC. The remaining 20% originating from 3-MT. The authors speculated that there was substantial evidence for there being little, if any COMT activity in dopaminergic terminals and that DOPAC is formed in the nerve endings, transported out from the terminals and then partly methylated to HVA. A portion of this total HVA, designated HVA-I might therefore represent the intra-neuronal metabolism of dopamine. The remaining HVA, HVA-II which

is formed from 3-MT outside the terminal may be derived from dopamine which is not accumulated by the neuronal uptake system.

Pargyline (100 $mg.kg^{-1}$) was shown to totally inhibit MAO activity in the brain and led to the linear accumulation of 3-MT for the first 90 minutes post injection (Kehr, 1976). Assuming that COMT is located extraneuronally then dopamine must be released from the nerve ending into the extraneuronal space prior to O-methylation to 3-MT. Low levels of 3-MT in the control animals indicated that 3-MT was either formed in insufficient amounts to be detectable or was rapidly converted to HVA. Less than 20 $ng.g^{-1}$ tissue of 3-MT (the limit of detectability) were found to be present in the rat brain. This study provided evidence to support the theory that 3-MT is produced from 'released' dopamine in vivo.

Gropetti et al (1977) used combined fluorimetric, mass fragmentographic and radiometric methods to study the levels of labelled dopamine, DOPAC and 3-MT in the striata of rats previously given intracerebroventricular ^{3}H-tyrosine. It was found that the specific activities of 3-MT and DOPAC were much higher than that of dopamine, suggesting that there are two separate compartments for dopamine, one of which is poorly labelled by the isotope. In decapitated rats, rather than those sacrificed by microwave radiation, the level of tritiated 3-MT showed an increase by half, whilst the endogenous 3-MT was 35 times higher than the levels measured in the microwave killed rats. This indicates that following decapitation of the

animal 3-MT formation is mainly due to the conversion of unlabelled dopamine, again suggesting the presence of more than one dopamine compartment.

In conclusion the majority of the evidence suggests the presence of more than a single system being present in the mammalian brain for the removal of catecholamines from the extracellular space prior to their metabolism and eventual removal from the brain. Therefore the existence of these mechanisms was investigated by studying the uptake of catecholamines over a wide range of concentrations and in a number of different preparations. A variety of biochemical and pharmacological methods were used to study the uptake systems in vitro. Attempts were made to provide evidence as to the location of the low affinity systems by the use of chemical lesioning techniques.

MATERIALS

Acetic acid (glacial)	-	Fisons plc
Ascorbic acid	10^{-1}M solution in distilled water	BDH Chemicals Ltd.
Atropine sulphate	10^{-1}M stock solution in distilled water, stored at 4°C	Sigma Chemical Co.Ltd.
Benztropine mesylate	10^{-1}M stock solution in distilled water, stored at 4°C	Merck, Sharp & Dohme Ltd.
Bovine serum albumin	-	Sigma Chemical Co. Ltd.
n-Butanol	-	Sigma Chemical Co. Ltd.
Calcium chloride hexahydrate	-	BDH Chemicals Ltd.
Catechol	10^{-2}M solution in distilled water	BDH Chemicals Ltd.
Copper (11) sulphate pentahydrate	-	BDH Chemicals Ltd.
Desipramine hydrochloride	10^{-1}M stock solution in distilled water, stored at 4°C	Ciba Laboratories Ltd.
3,4-dihydroxybenzylamine hydrobromide	10 mg.ml^{-1} stock solution 0.1M HCl, stored at 4°C	Sigma Chemical Co.Ltd.
D,L 3,4-dihydroxymandelic acid	10 mg.ml^{-1} stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
3,4-dihydroxyphenylacetic acid	10 mg.ml^{-1} stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
D,L 3,4-dihydroxyphenylglycol	10 mg.ml^{-1} stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
3,4-dimethoxyphenylethylamine	10^{-2}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Dimilume	-	United Technologies/ Packard Instrument Co.Ltd
2,4-dinitrophenol GPR	10^{-2}M stock solution in distilled water stored at R.T.	Sigma Chemical Co. Ltd.

Dopamine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
(7,8-^{3}H)-dopamine	40 - 60 Ci.mmol^{-1} stored in 0.02M acetic acid/ ethanol (1 : 1) solution at 4°C	Amersham Int. plc.
(-) ephedrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
(-)- ψ-ephedrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Ethanol (absolute)	-	Fisons plc.
Ethylenediaminetetra-acetic acid (EDTA) disodium salt, 'Analar'	-	BDH Chemicals Ltd.
Ficol type 400	-	Sigma Chemicals Co. Ltd.
Fisofluor '2'	-	Fisons plc.
Fisofluor '3'	-	Fisons plc.
Folin & Ciocalteu's Phenol reagent	2N, stored at 4°C	BDH Chemicals Ltd.
D (-) Fructose 'Analar'	-	BDH Chemicals Ltd.
D-glucose 'Analar'	-	BDH Chemicals Ltd.
Homovanillic acid	10 mg.ml^{-1} stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Hordenine hemisulphate	10^{-1}M stock solution in distilled water, stored at 4°C	Sigma Chemical Co. Ltd.
Hydrochloric acid (conc.) sp.gr 1.16 'Analar'	-	BDH Chemicals Ltd.
6-hydroxydopamine hydrobromide	Freshly prepared in 0.1M HCl or saline containing 0.2 mg.ml^{-1} ascorbic acid	Sigma Chemical Co. Ltd.
Intraval sodium (Thiopentone sodium)	-	May & Baker Ltd.
(-) isoprenaline hydro-chloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Magnesium sulphate heptahydrate 'Analar'	-	BDH Chemicals Ltd.

DL-metanephrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Metaraminol tartrate	10^{-1}M stock solution in distilled water, stored at 4°C	Merck, Sharp & Dohme Ltd.
Methanol HPLC grade	-	Fisons plc.
Methoxyamine hydro chloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
3-methoxy,4-hydroxy phenol glycol; hemi-piperazine salt	10 $mg.ml^{-1}$ stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
3-methoxyisoprenaline	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	United works of Pharmaceutical & Dietetic Products, Budapest, Hungary.
3-methoxytyramine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Methylamine hydrochloride	10^{-1}M stock solution in distilled water, stored at R.T.	BDH Chemical Co. Ltd.
(-) noradrenaline bitartrate	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
1-(7,8-^{3}H)-noradrenaline	30 - 50 $Ci.nmol^{-1}$ stored in 0.02M acetic acid/ethanol (9 : 1) solution at -20°C	Amersham Int. plc.
(-) norephedrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
(-) norpseudoephedrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
DL-normetanephrine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
1-octane sulphonic acid sodium salt. HPLC grade	-	Fisons plc.
DL-octopamine hydrochloride	10^{-1}M stock solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
17-β-oestradiol	10^{-2}M solution in distilled water and Tween 80	Sigma Chemical Co. Ltd.

DL-oxedrine tartrate (Synephrine)	10^{-1}M solution in distilled water, stored at 4°C	Lewis Laboratories Ltd.
95% O_2: 5% CO_2 gas	-	BOC Ltd.
Perchloric acid sp gr 1.54 60% 'Aristar'	0.4M in distilled deionized water	BDH Chemicals Ltd.
β-phenylethylamine hydrochloride	10^{-1}M solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Potassium chloride	-	BDH Chemicals Ltd.
Potassium dihydrogen orthophosphate	-	BDH Chemicals Ltd.
Potassium (+) tartrate	-	BDH Chemicals Ltd.
Sodium carbonate (anhydrous)	-	BDH Chemicals Ltd.
di-sodium hydrogen orthophosphate (anhydrous) A.R.	-	Fisons plc.
Sodium dihydrogen orthophosphate (anhydrous) A.R.	-	Fisons plc.
Sodium metabisulphate 'Analar'	-	BDH Chemicals Ltd.
Soluene - 350	-	United Technologies/ Packard Instrument Co.Inc.
D-(U-^{14}-C)-Sorbitol	Aqueous solution containing 3% ethanol, 150-250 mCi.mmol^{-1} stored at 20°C	Amersham Int. plc.
Sucrose	-	BDH Chemicals Ltd.
Tris-(hydroxymethyl)-methylamine A.R.	-	Fisons plc.
Trypsin (Bovine pancreatic)	-	BDH Chemicals Ltd.
Tyramine hydrochloride	10^{-1}M solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.
Vanillyl mandelic acid	10 mg.ml^{-1} solution in 0.1M HCl, stored at 4°C	Sigma Chemical Co. Ltd.

Krebs' solution

Compound	$mmol.l^{-1}$
NaCl	118
KCl	4.75
KH_2PO_4	1.19
$CaCl_2.6H_2O$	2.55
$Mg\ SO_4.7H_2O$	1.2
$NaHCO_3$	25
D-Glucose	5.56

The Krebs' solution was made up to 1 l with distilled water and initially gassed for 30 min. with 5% carbon dioxide : 95% oxygen, prior to adding 20 $mg.l^{-1}$ ascorbic acid. Gassing brought the pH of the solution to 7.4. Fresh Krebs' solution was prepared each day.

Radiochemical purity

Radiochemical purity was assessed at monthly intervals in order to check that the labelled compounds had not decomposed. The tritiated catecholamines were applied to a 1 cm strip of Whatman No. 1 paper as 5 µl spots of the stored solution. The chromatography paper was then placed in a tank previously equilibrated for 2 hours with an eluent system consisting of n-butanol : glacial acetic acid : water (12 : 3 : 5). The spots were then eluted until the solvent front had crossed the chromatography paper.

The chromatogram was then dried and sprayed with 5% aqueous ferric chloride in order to locate the catecholamine. The chromatogram was then cut into 1 cm squares from the origin to the solvent front. The squares were placed into individual scintillation vials containing 10 ml of Fisofluor 2 or Dimilume and the radioactivity measured in a scintillation counter.

The positions of the colouration and the peak of radioactivity were compared to check that they coincided. See Fig. 1 and 2.

Fig 1.

Chromatogram of ^{3}H-dopamine stock solution following elution in n-butanol: glacial acetic acid: ethanol (12:3:5).

A. Distribution of radioactivity.

B. Location of dopamine as revealed by spraying with 5% aqueous ferric chloride.

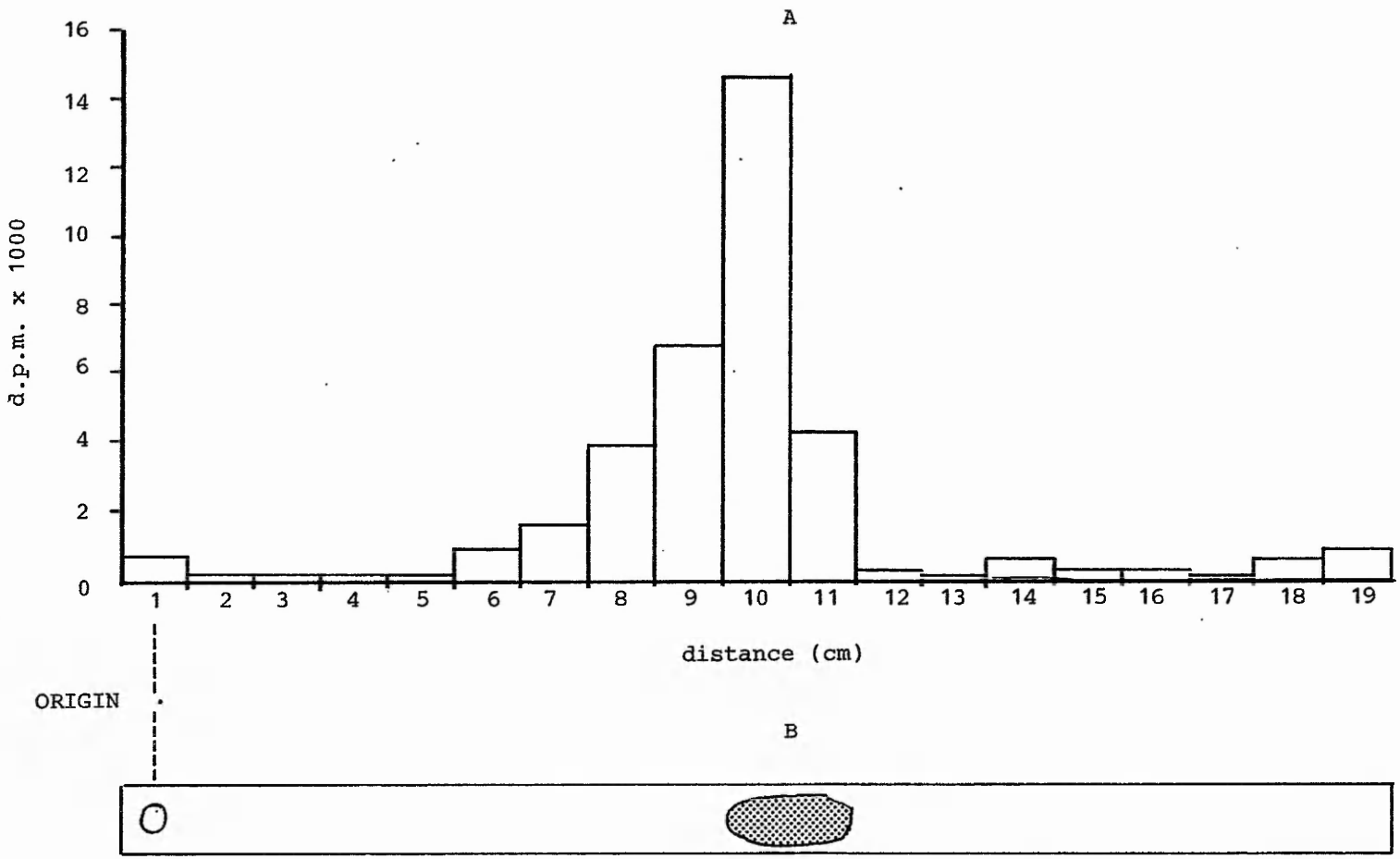
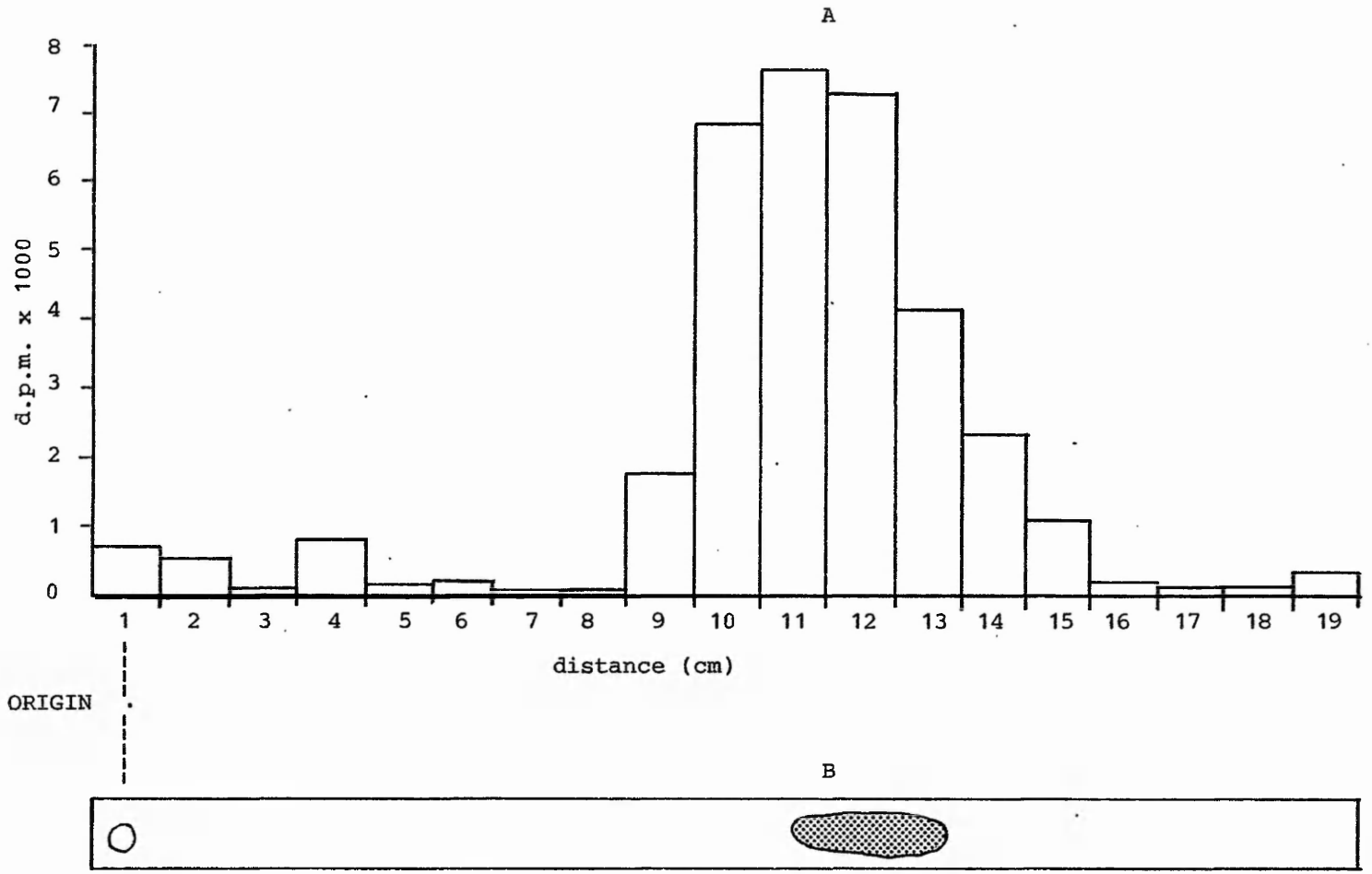

A
16
14
12
10
8
6
4
2
0
d.p.m. x 1000
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
distance (cm)
ORIGIN
B

Fig 2.

Chromatogram of ^{3}H-noradrenaline stock solution following elution in n-butanol: glacial acetic acid: ethanol (12:3:5).

A. Distibution of radioactivity.

B. Location of noradrenaline as revealed by spraying with 5% aqueous ferric chloride.


A
8
7
6
5
4
3
2
1
0
d.p.m. x 1000
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
distance (cm)
ORIGIN
B

METHODS

RADIOLABELLED CATECHOLAMINE UPTAKE EXPERIMENTS

Preparation of radioactive solutions

The required specific activities and concentrations for the ^{3}H-catecholamines were obtained by the addition of a known volume of unlabelled catecholamine solution, diluted with a solution of the salts necessary to produce Krebs' solution to an aliquot of the ^{3}H-catecholamine. An aliquot of ^{14}C-sorbitol of known specific activity was added to the catecholamine solution which was then adjusted to the required final volume by the addition of distilled water. This resulted in a Krebs' solution of the correct concentration, which contained the required concentration of materials.

Preparation of brain slices

Male Sprague-Dawley rats (175 - 250 g) were lightly stunned and sacrificed by decapitation. The skull was exposed and rapidly opened by cutting forwards along the midline. The two halves of the skull were prised apart to reveal the brain and the stump of the spinal cord. After the dura had been cut away the brain was 'scooped out' with a spatula and immediately plunged into ice-cold, oxygenated Krebs' solution. This procedure was carried out as quickly as possible. The brain was removed from the Krebs' solution after two minutes and placed on an ice filled glass Petri dish, the required region of the brain was then dissected out. The brain regions used were as defined by Glowinski and Iversen (1966).

1) Striatum

The striatum was dissected by separating the two cerebral hemispheres and parting the cortex from the mid-brain. By opening up the lateral ventricle this exposed the dorsal surface of the striatum. The cortex and mid-brain structures were then removed.

2) Cerebellum

The cerebellum was dissected from the pons by cutting the cerebellar peduncles.

The dissected brain areas were placed on the platform of a McIlwain Tissue Chopper (Mickel Laboratory Engineering Co.) and 0.5 cm thick sections cut at right angles to the long axis. Tissues which had been sliced were then placed in a test tube containing 5 ml of ice-cold Krebs' solution. The tube and its contents were agitated on a vortex mixer. This separated the tissue into individual slices. The slices were then placed into a Petri dish containing ice-cold Krebs' solution, which was kept continuously oxygenated.

Incubation of brain slices

Six slices were placed in each 25 ml Erlenmeyer flask, two from each of three different rats. The flasks contained 9 ml of ice-cold, oxygenated Krebs' solution, which included a test drug if required. The flask and its contents were then pre-incubated for 5 min at 37°C

in a thermostatically controlled water bath. The medium in the flask was bubbled with 5% carbon dioxide in oxygen which had previously been passed through a Wolfe bottle containing distilled water in order to moisten the gas and thus minimise evaporation of the solution caused by the passage of the gas. The flasks were shaken throughout the experimental period at 120 strokes per minute in order to ensure that the brain slices were kept continuously moving within the incubation medium. At the end of the pre-incubation period 1 ml of Krebs' solution containing ^{3}H-catecholamine and ^{14}C-sorbitol of known concentration and specific activity was added to each flask. The incubation was continued at 37°C for a further 2 minutes. Incubation was terminated by tipping the flask contents onto a nylon mesh. The slices were blotted and individual slices placed in pre-weighed scintillation vials. The vials were weighed again and the weight of the tissue calculated. The slices were digested with 0.5 ml Soluene-350, either overnight at room temperature or by heating for 2 hours in a dry oven at a temperature of 60°C. After the digestion had been completed 0.5 ml 1M HCl was added to the vials in order to neutralise the alkalinity of the Soluene-350, thereby reducing any chemiluminescence to a minimum. Scintillation fluid (10 ml) was added and the samples assayed for radioactivity in a Packard Tri-Carb 300C liquid scintillation counter. The scintillation fluid routinely used for non-aqueous samples was Fisofluor 3; however when this was not available Dimilume was used instead and this did not require the addition of acid for neutralisation. Samples (100 µl) of the incubation media were taken and placed into scintillation

vials containing 10 ml Fisofluor 2 (used for aqueous samples) or Dimilume and the radioactivity present in them measured by liquid scintillation.

Preparation of isolated cells

The required area of tissue was dissected as described previously and sliced into 0.1 mm square sections by the use of a McIlwain tissue chopper. The tissue sections were collected with a microspatula and added to 5 ml of a hypertonic, hexose acid phosphate (HAP) medium containing 5% glucose, 5% fructose, 0.1M phosphate buffer pH 6.0, 1% bovine serum albumin and 1% trypsin (percentages given are weight to volume). After incubating for 5 minutes at 37°C, 5 ml of ice-cold oxygenated Krebs' solution was added (Norton and Poduslo, 1970). The softened tissue was passed with slight suction through a nylon mesh of 200 µm openings placed over the filter funnel of a Buchner filter flask. The procedure was then twice repeated with the use of a new nylon mesh of 60 µm openings. The resultant cell suspension was centrifuged for 2 minutes at 3000 r.p.m. in a Chilspin 2 (MSE scientific instruments) at 0 - 4°C and the supernatant discarded. The pellet of cells was resuspended in 1 ml of ice-cold Krebs' solution.

Incubation of isolated cells

An aliquot (200 µl) of the cell suspension was added to 1.6 ml of oxygenated, ice-cold Krebs' solution, which included a test drug if required, and pre-incubated for 5 minutes at 37°C. A further 200 µl of Krebs' solution, containing ^{3}H-catecholamine and ^{14}C-sorbitol,

was added and the incubation continued for a further 5 minutes. The incubation was terminated when 1 ml of the incubation mixture was removed with a plastic syringe and rapidly passed through a millipore filter holder containing a nitrocellulose filter, pore size 8.0 µm. Filters of this size were used as trial experiments had shown that they retained cells but not cell debris or synaptosomes. The filters and retained cells were washed by passing 2 ml of ice-cold Krebs' solution through the filter, followed by 20 ml of air. The filter was removed from the holder and placed in a scintillation vial containing 10 ml scintillation fluid (Fisofluor-3). After the filter had dissolved (3 to 4 hours at room temperature) the vial was placed in the scintillation counter and the total radioactivity measured. Samples (100 µl) of the incubation media were also assayed for total radioactivity. A further 500 µl of the incubated cell suspension was assayed for protein content (Lowry et al, 1951).

Preparation of homogenates

The required area of brain tissue was dissected out as previously described, weighed and placed in a glass, hand held homogeniser with a ground glass pestle. The tissue was then homogenised until uniform in 1 ml of 0.32M oxygenated sucrose (ice-cold).

Incubation of homogenates

An aliquot (200 µl) of the homogenate was added to 1.6 ml oxygenated Krebs' solution, which included a test drug if required, and pre-incubated for 5 minutes at 37°C. A further 200 µl of Krebs'

solution, containing ^{3}H-catecholamine and ^{14}C-sorbitol, was added and the incubation continued for a further 5 minutes. The incubation was terminated when 1 ml of the incubation mixture was removed with a plastic syringe and passed through a nitrocellulose filter, pore size 0.45 µm. The filters were treated as in the isolated cell experiments prior to determination of the radioactive content. Samples (100 µl) of the incubation media were also assayed for total radioactive content.

Preparation of synaptosomes

The required area of tissue was dissected out as previously described and homogenised until uniform in 0.32M sucrose containing 10 mM tris-HCl (pH 7.4). The homogenate was centrifuged at 900 g for 10 minutes, 0 - 4°C. The pellet of cells was discarded and the supernatant recentrifuged under the same conditions of speed, time and temperature. The resulting supernatant was again centrifuged at 0 - 4°C, but this time at 10,000 g for 20 minutes. The crude synaptosomal fraction yielded thus was resuspended and placed over layers of 13% and 7.5% Ficoll before being centrifuged for 45 minutes at 55,000 g, 0 - 4°C. The synaptosomal band was obtained at the 13%/7.5% Ficoll interphase (Cotman and Matthews, 1971).

The synaptosomes were resuspended in 1 ml of ice-cold Krebs' solution.

Incubation of synaptosomes

A 200 µl aliquot of the synaptosomal suspension was taken and treated in the same manner as for the homogenate experiments. Total radioactivity was measured in both synaptosomes and incubation media samples. The total protein content of the synaptosomes was determined using the method of Lowry et al (1951) as in the isolated cell experiments.

Radioassay of radiochemicals

Labelled compounds which were to be assayed for radioactivity were present either in Krebs' solution, a digest formed by the action of the tissue solubilizer, Soluene-350, or as an homogenate, isolated cell preparations or synaptosomes present on a nitrocellulose filter. The samples were dispersed in 10ml of a scintillant phosphor. Routinely the scintillant phosphor Fisofluor-2 was used for aqueous samples and Fisofluor-3 for non-aqueous samples. However when these phosphors were unavailable Dimilume was used instead. In all cases the samples contained two radioisotopes, tritium and carbon-14. The samples were counted in a Packard Tri-carb model 300C scintillation counter.

In a dual-labelled sample the scintillations are the result of the beta activity of both radionucleides. It is possible to quantify the two separate isotopes provided that their energy spectra are sufficiently different. In the region of the spectrum above the probable maximum pulse height of the lower energy nucleide, in this

case tritium, only disintegrations of the higher energy nucleide (carbon-14) are observed. Regions below the probable maximum pulse height of tritium contain events due to both isotopes (Klein and Eisler, 1966).

The scintillation counter was programmed to set the discriminators to count only carbon-14 disintegrations in one channel, whilst setting a second channel at the maximum counting efficiency for tritium. This channel also counts a certain proportion of the carbon-14 disintegrations. The tritium count was calculated after substracting the counts due to carbon-14.

In order to obtain the number of disintegrations per minute (d.p.m.) due to carbon-14 and tritium, quench curves were constructed for the two isotopes. The efficiency of counting was plotted against the quench indicating parameter, which is based on the external standard channels ratio. The values were programmed into the scintillation counter which then provided data in the form of d.p.m. s for the two separate isotopes. In order to maintain the accuracy of results the quench curves were plotted at monthly intervals and checked against quenched standards of known d.p.m. (Fig.3).

Kinetic analysis of data

After correction for the effects of quenching had been made, the data from the scintillation counter was fed either directly or indirectly to an Apple II computer programmed to calculate values of the mean and standard error for S/v of a given group of samples.

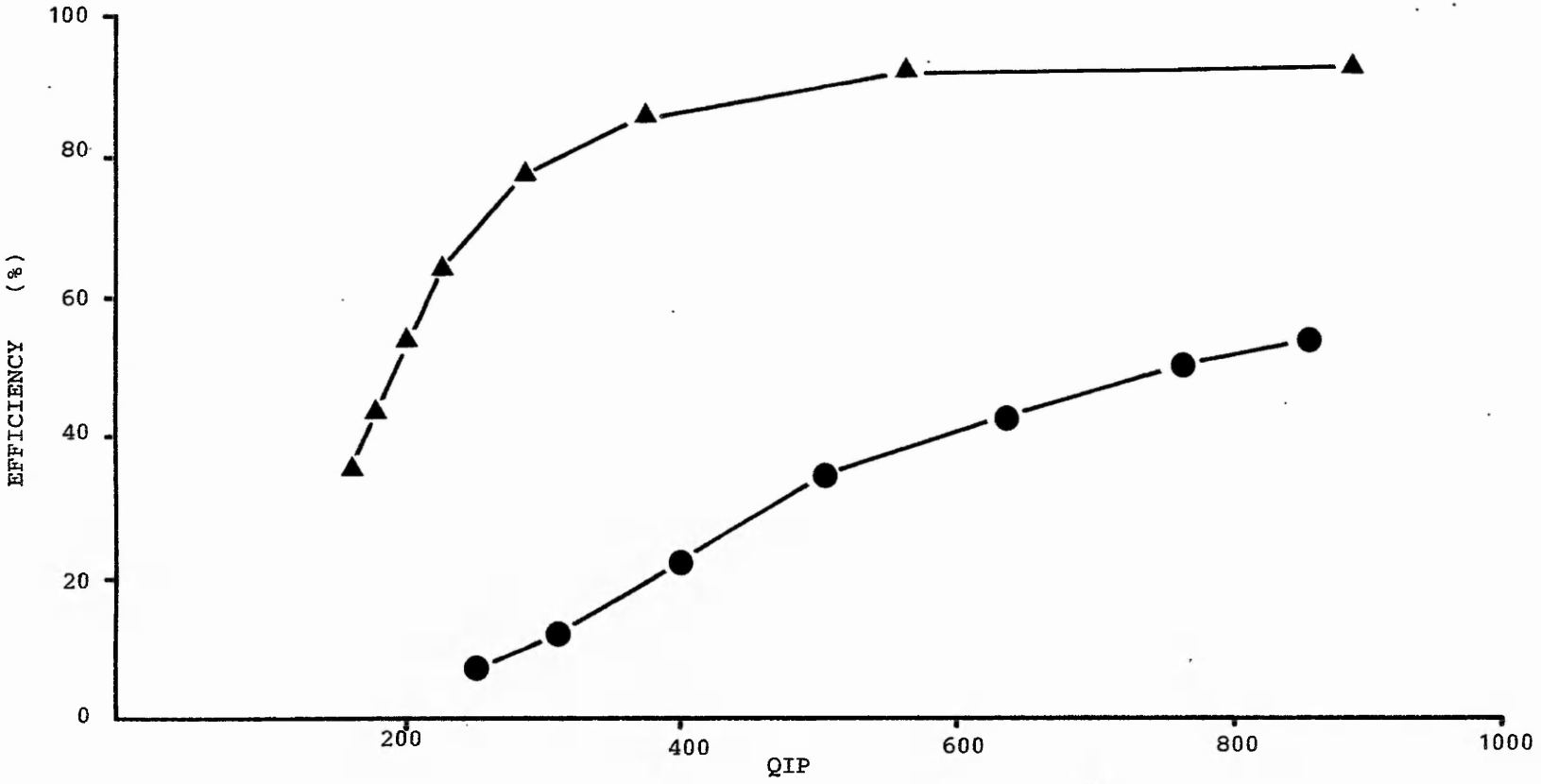
Fig 3.

Standard curves for double isotope experiments involving tritium and carbon-14. Counting efficiency against the quench indicating parameter (QIP).

 tritium.

 carbon-14.

Each point is the mean of 3 determinations.


100
80
60
40
20
0
EFFICIENCY (%)
200
400
600
800
1000
QIP

Where S is the substrate concentration and v is the rate of uptake.

In all the systems investigated the analyses were based on a direct transformation of single substrate saturation kinetics, as described by Dixon and Webb (1964):

$$v = (Vmax.\ S) / (Km + S)$$

where Vmax is the maximum rate of uptake theoretically attained at infinitely high concentrations; Km is the Michaelis constant and S and v are as described above.

Re-arranging gives:

$$S/v = (Km / Vmax) + (S/Vmax)$$

therefore if S/v is plotted against S the result is a straight line.

If $S/v = 0$ then

$$-Km/Vmax = S/Vmax \text{ i.e. } S = -Km$$

The slope of the line is 1/Vmax.

In cases where a graph of S/v against S was not a straight line the above model was not applicable and results were analysed by two other models for uptake.

1) A model based on one saturable uptake system plus diffusion which was directly proportional to the substrate concentration, i.e. according to Fick's first law of diffusion (Mahler and Cordes, 1966).

This can be represented by:

$$v = \left[(Vmax.S) \;/\; (Km + S)\right] + D.\ S$$

where D is the diffusion constant. All other symbols as before.

Rearranging gives:

$$S/v = (Km + S) \;/\; \left[(Vmax) + (Km + S).\ D\right] \quad ....(1)$$

This was called the three parameter model in analyses:

the parameters being Km, Vmax and D.

2) A model based on two saturable uptake mechanisms plus diffusion which was directly proportional to the substrate concentration. The two saturable uptake mechanisms have two different Km and Vmax values. The whole system may be represented by the equation:

$$v = \left[(Vmax.S) \;/\; (Km + S)\right] + \left[(Vmax'.S) \;/\; (Km' + S)\right] + D.\ S$$

where Vmax'and Km'are the kinetic parameters of a second uptake mechanism. The other symbols are as previously defined.

Rearranging gives:

$$S/v = \frac{(Km + S).(Km' + S)}{Vmax.(Km' + S) + Vmax'.(Km + S) + (Km + S).(Km' + S).D}$$

.....(2)

This was called the five parameter model in analyses. The parameters are Km, Vmax, Km', Vmax' and D. Equations (1) and (2) are not linear and so data relevant to them cannot be analysed by linear regression. The data were analysed by a DEC 20 computer using iterative curve fitting programmes which contained equations (1) and (2) respectively. The results of an anaysis were the values for the various kinetic parameters.

Curve fitting analyses

The method used to analyse the kinetic data compared the experimental results with those of a hypothetical model. Two models were used in curve fitting analyses. One represented a system combining a single saturable uptake mechanism with diffusion, i.e. the three parameter model. The other model was a combination of two saturable uptake systems with different kinetic parameters and diffusion i.e. the five parameter model. The models were the simplest involving diffusion and saturable uptake mechanisms. It was possible for models to be formulated which include any number of saturable uptake systems. However as the models become increasingly more complex the reliability of the analyses diminishes due to the increasing ease of the fitting process.

The experimental results were entered into the computer programme in the form of S/v values and their respective standard errors for each value of S. The kinetic parameters to be calculated were given arbitrary 'starting' values. These kinetic parameter values were used to calculate theoretical S/v values for the different values of S. The sum of the squares of the deviations of the calculated S/v values from those of their respective experimental values of S/v were calculated. Each kinetic parameter was altered by the addition or substraction of a fraction of its current value. The fraction by which it was altered was 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128 of the current value. The sum of the squares of the deviation from the experimental values was calculated after each alteration

and compared with the sum of squares of the deviations prior to the alteration. If the sum of squares of the deviations after the alteration in the kinetic parameter was less than that before, then the new value of the kinetic parameter became the current value, otherwise the original value was retained. The sequence of alteration was initially by addition, if this failed to reduce the sum of squares of the deviations the fraction was then substracted from the original value. The process was repeated until altering the values of all the kinetic parameters did not give a reduced sum of the deviations. When this happened the fraction by which the kinetic parameters were altered was decreased. If the fraction by which the current values of the kinetic parameters were altered was 1/128, then on completion of the cycle of altering the kinetic parameters the final values of these parameters were taken to be the best fit obtainable. The fit of the values of S/v predicted from the kinetic parameters compared with the experimental values was computed in terms of the difference between the two values divided by the standard error of the experimental data at that point. The largest difference was taken as the extent of the fit in terms of the standard error. If while cycling through the process of fitting a fit of less than 1/6 of the standard error was achieved the process stopped. This was the limit of the extent of the fit and there would be no point in continuing the process.

If the programme had completed all its cycles of alterations and the fit was greater than one standard error then the fit was considered 'not good'. The term 'good' did not mean that the model was definitely the correct one, it only provided an indication that the model and the experimental data were compatible. The term 'not good' however

suggests that the model and experimental data were not compatible and the model was therefore rejected.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (H.P.L.C.) ANALYSIS OF CATECHOLAMINES AND THEIR METABOLITES

Tissue preparation and incubation

Tissue preparation was carried out in the manner described for the radiolabelled catecholamine uptake experiments using striatal slices. The incubation procedure was also similar, but with the exception that only unlabelled catecholamine compounds (100 µM)were added after the preincubation step instead of tritiated catecholamines and ^{14}C-sorbitol. Incubation was continued for 2, 10 or 30 minutes. After this period the tissue slices were removed by filtration through a nylon mesh.

The catecholamines and their metabolites were assayed by the following method. The slices were transferred to a 1 ml glass homogeniser containing 0.5 ml of 0.4M ice-cold perchloric acid. Dissolved in the perchloric acid solution was 0.15% (wt/vol) sodium metabisulphate, 0.05% (wt/vol) EDTA (disodium salt), (Wagner et al, 1982) and an internal standard of 10 ng/ml dihydroxybenzylamine (DHBA). The homogenate was then centrifuged for 1 minute in a Beckman Microfuge. The supernatant was removed and stored at -20°C for up to one week prior to analysis by HPLC. A sample (500 µl) of the medium was also taken, this was added to 100 µl of the perchloric acid solution and analysed by HPLC.

Reverse phase high performance liquid chromatography analyses

The liquid chromatographic system consisted of a Waters model 6000A or M45 solvent delivery system, with a Waters (200 µl loop) injection port or a Dupont pump and controller with a Rheodyne (50 µl loop) injector. The columns (25 cm x 4.6 mm) used were Spherisorb 5 µ octadecyl silane (ODS) 2. A guard column (5 cm x 4.6 mm) of similar material was used to protect the main analytical column. The detector used was an Environmental Science Associates (ESA) model 5100A electrochemical detector set at + 0.9V. The signal was recorded on a Tarkan W+W 600 recorder which was connected to a Kemtronix Supergrater 3A signal integrator.

The mobile phase consisted of an 84 : 16 (vol/vol) mixture of 0.1M sodium hydrogen orthophosphate and methanol containing 2.6 x 10^{-3}M octane sulphonic acid and 1.0 x 10^{-4}M EDTA. The pH was adjusted to 3.35 units with orthophosphoric acid (3M), (Wagner et al, 1982). All solutions were filtered through a 0.45 µm pore size membrane in a Whatman filtration unit and degassed for at least 30 minutes before use. Tissue and media samples (50 µl) were injected directly on to the column without any further preparation.

Retention times of catecholamines and their metabolites

Samples of noradrenaline, dopamine and their metabolites dissolved in 0.1M perchloric acid containing 10 ng/ml DHBA as internal standard were analysed by reverse phase high performance liquid chromatography (HPLC-RP) using electrochemical detection (ECD). The retention

times, i.e. the time between injection and maximum signal response, of both the various compounds and the internal standard were recorded (see Table 1).

The detector response (peak area) was measured for samples at various known concentrations. The response was shown to be linear in the range 0.2 - 100 ng/50 µl injection, Fig. 4.

Percentage recovery of catecholamines and their metabolites

Known amounts of catecholamines and their metabolites (500 - 1500 ng) were dissolved in 1 ml of 0.4M $HClO_4$. After the addition of 20 ng of fresh striatal tissue the samples were homogenised, centrifuged in a Beckman microfuge and aliquots (50 µl) of supernatant analysed by HPLC under normal experimental conditions. The percentage recovery of each of the compounds was calculated from the peak area and is given in Table 1.

STATISTICS

The Student's t-test was used to test for significantly different sets of data. The probability level used was $p<0.05$, unless otherwise stated in the text.

Fig 4.

HPLC standard curve for dopamine.

Relative peak area against concentration of pure compound.

DHBA (20ng) assigned the arbitrary value of 1.

A 50 µl sample was analysed. Values are the mean ± s.e.m..

Lines were fitted by eye.

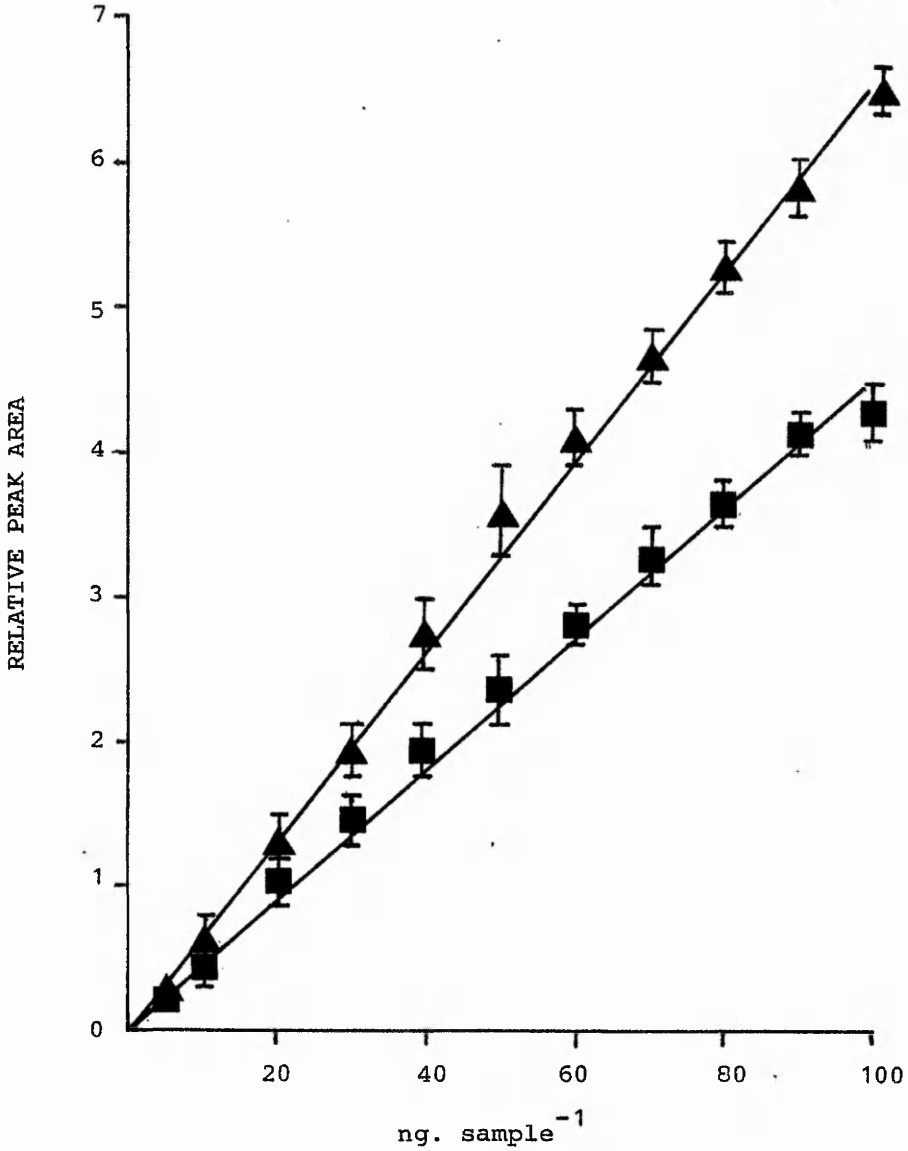

RELATIVE PEAK AREA
0
1
2
3
4
5
6
7
20
40
60
80
100
ng. sample^{-1}

Table 1. Retention times and percentage recovery values for the catecholamines, dopamine and noradrenaline and their major metabolites. The ratio of the percentage recovery to that of the internal standard dihydroxybenzylamine (DHBA) is given.

Compound	Retention Time	% recovery≠	Ratio
Dopamine	25.05	80.3 ± 1.2	0.99
DOPAC	13.68	76.0 ± 1.6	1.05
DOPEG	4.46	64.3 ± 1.8	1.24
DHBA	18.23	79.5 ± 1.5	1.00
DHMA	4.44	74.1 ± 1.5	1.07
HVA	31.33	69.3 ± 2.4	1.15
Noradrenaline	9.79	78.4 ± 0.9	1.01
NMN	19.38	65.7 ± 2.1	1.21
VMA	5.72	72.0 ± 1.4	1.10
3MT	45.67	54.0 ± 1.2	1.47

≠ Each value represents the mean and standard error of 4 separate determinations.

RESULTS

RADIOLABELLED CATECHOLAMINE UPTAKE EXPERIMENTS

Accumulation of ^{14}C-sorbitol by slices

After 5 min preincubation at 37°C in Krebs' solution slices of rat striatum or cerebellum were incubated in Krebs' solution containing catecholamine (0.1 or 500 µM) and ^{14}C-sorbitol (100 nCi.ml^{-1}). Incubation in the radioactive solution was continued for times in the range 0.5 - 6.0 min. The incubation was terminated and the radioactivity determined. Media samples (100 µl) were also assayed for radioactivity. The accumulation of ^{14}C-sorbitol was expressed in terms of the tissue to medium ratio (T/M) :

$$\frac{\text{nCi } ^{14}\text{C-sorbitol.g wet weight tissue}^{-1}}{\text{nCi } ^{14}\text{C-sorbitol.ml medium}^{-1}}$$

The time course for the accumulation of ^{14}C-sorbitol in striatal slices is presented in Fig. 5. Accumulation was not linear with time at either of the catecholamine concentrations studied. The accumulation does not appear to have reached a plateau by the end of the incubation period i.e. 6 min. The sorbitol in the tissue is taken as an indication of the size of the extracellular space and hence used to correct for only ^{3}H-catecholamine that is in the slice but not taken up by the cells.

Time course for the accumulation of $^{3}H_2O$ by slices

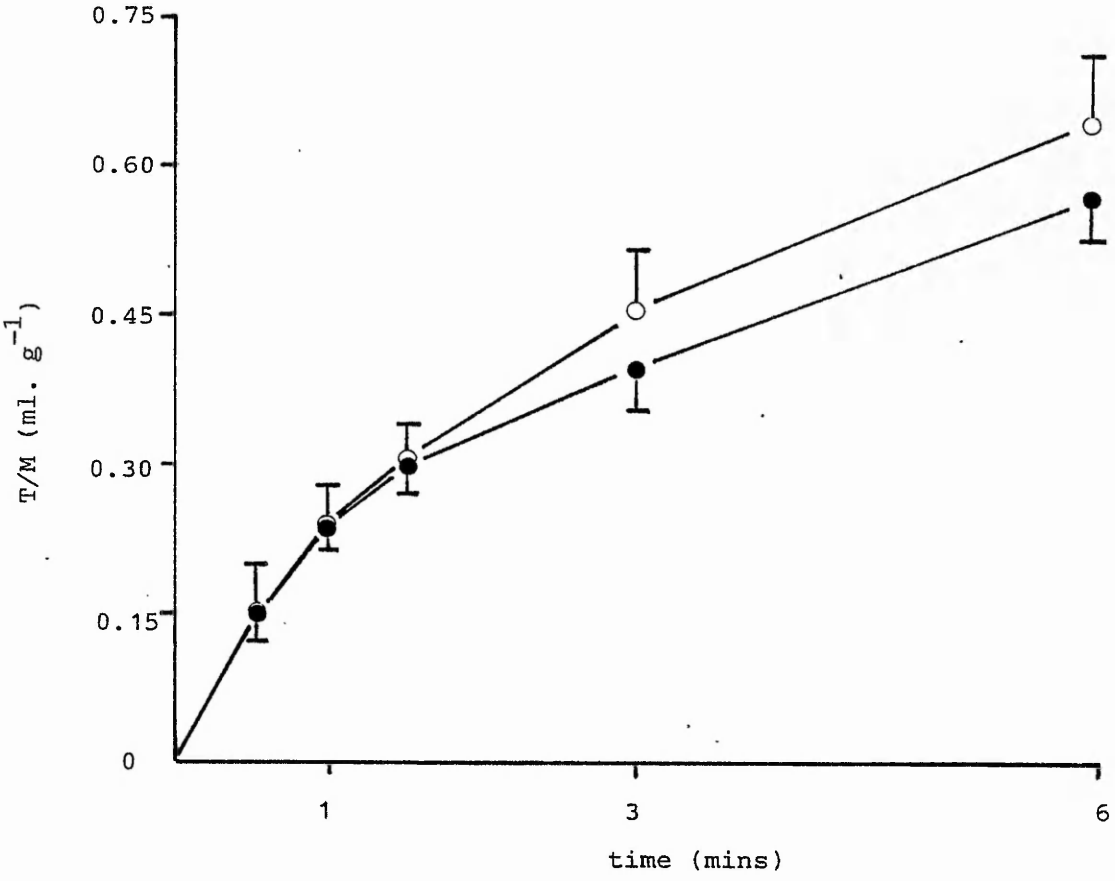
After 5 min preincubation in Krebs' solution at 37°C slices of rat striatum or cerebellum were incubated in Krebs' solution containing tritiated water ($^{3}H_2O$ - final concentration 500 nCi.ml^{-1} for times in the range 0.5 - 6.0 min. At the end of the incubation

Fig 5.

Time course for the accumulation of ^{14}C-sorbitol, in terms of the T/M ratio, in slices of rat striatum. Each point is the mean and standard error of at least six values.

○——○ 0.1 μ M dopamine

●——● 500 μM dopamine


0.75
0.60
0.45
0.30
0.15
0
T/M (ml. g^{-1})
1
3
6
time (mins)

period the radioactivity in each slice was determined as previously described. Aliquots (100 µl) of the incubation media were also taken and their radioactive content determined. The $^{3}H_2O$ accumulated in the slices was calculated after correction for the extracellular space. The results for the accumulation of $^{3}H_2O$ by striatal slices are shown graphically in Fig 6. The time course for accumulation of $^{3}H_2O$ was shown to be linear for both striatal slices and slices of cerebellum.

Time course for the accumulation of ^{3}H-dopamine by striatal slices

After 5 min preincubation in Krebs' solution at 37°C slices of striatum were incubated in Krebs' solution but with the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 500 µM or 0.1 µM). The time course for the incubations was in the range 0.5 - 6.0 min. The radioactivity present in both the individual slices and the media were determined. The accumulation of tritium was taken as being equivalent to the uptake of dopamine. No correction was applied for the metabolism of the labelled dopamine as this is assumed to occur after the molecule is taken up by the tissue. The dopamine content of each slice was expressed in terms of nmol.g wet wt^{-1} after correction for the extracellular space by the use of ^{14}C-sorbitol. The time course for the uptake of ^{3}H-dopamine at the two concentrations used is given in Fig. 7.

Fig 6.

Time course for the accumulation of $^{3}H_{2}O$ by striatal slices. Each point is the mean and standard error of at least six determinations. The line was fitted by eye.

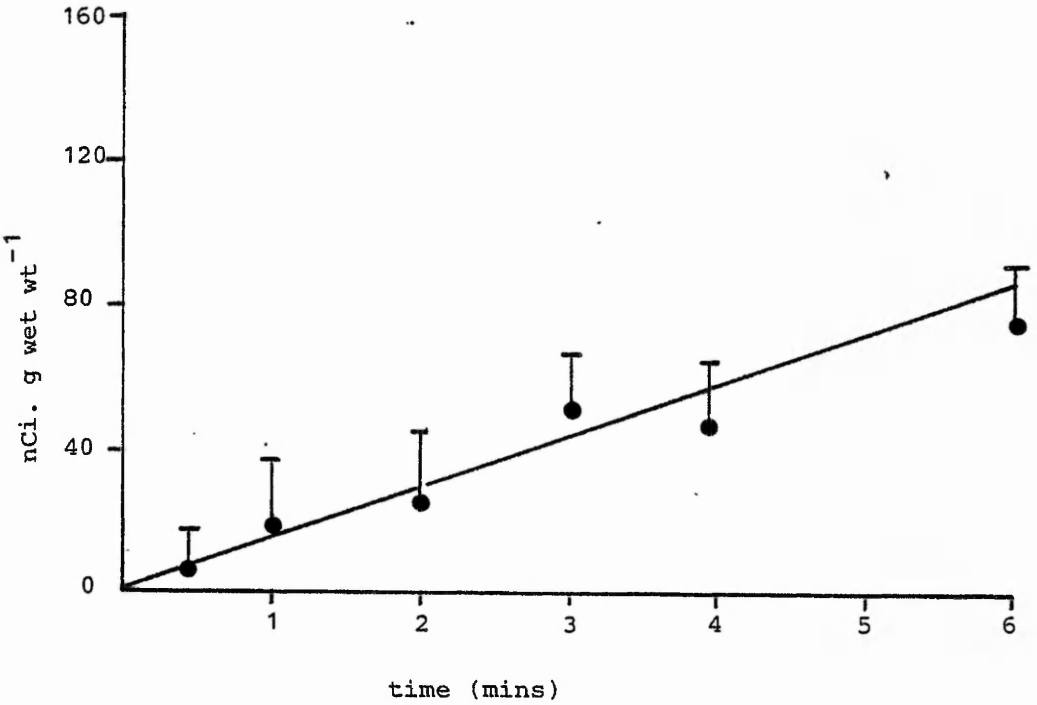
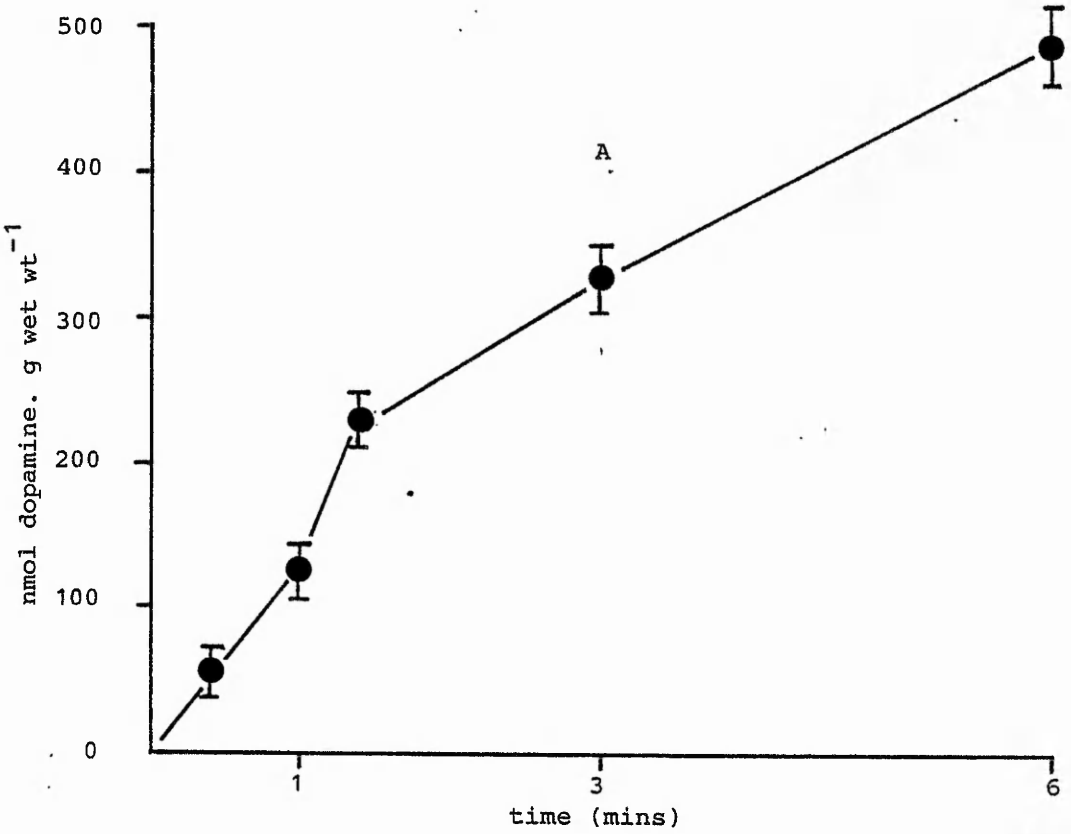


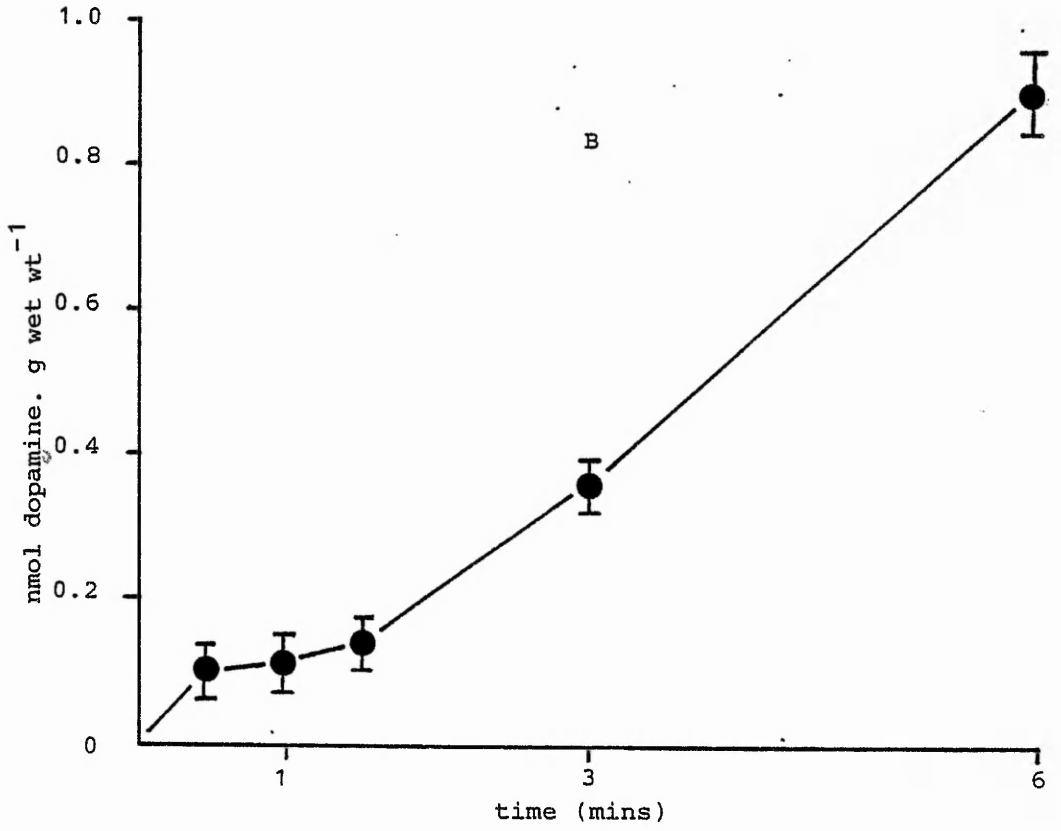
Fig 7.

Time course for the cellular accumulation of ^{3}H-dopamine by striatal slices. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 µM.

B. Accumulation at 0.1 µM.


500
400
300
200
100
0
A
nmol dopamine. g wet wt^{-1}
1
3
6
time (mins)


1.0
0.8
0.6
0.4
0.2
0
B
nmol dopamine. g wet wt^{-1}
1
3
6
time (mins)

There is an initial linear accumulation of dopamine for a period of 2 to 3 min at the 500 µM concentration and for 6 min at the 0.1 µM concentration. At 3 min the tissue incubated at 500 µM dopamine had taken up 329 nmol.g wet wt^{-1} (T/M = 0.66) and that incubated at 0.1 µM dopamine 0.36 nmol.g wet wt^{-1} (T/M = 3.6).

From these results a two min incubation period was chosen for measuring the initial rate of accumulation in the kinetic experiments.

Kinetics of the uptake of ^{3}H-dopamine by striatal slices

After a 5 min preincubation in Krebs' solution at 37°C, slices of striatum were incubated for 2 min following the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM). Enough tissue was taken from each rat for it to provide 2 slices for incubation in each of the concentrations of dopamine studied. Samples of the incubation media (100 µl) were also taken and the radioactive content measured. Using the rate of uptake (nmol.g wet wt^{-1}.min^{-1}) during the initial 2 min the S/v value was calculated for each of the dopamine concentrations studied. The values of S/v at the various concentrations of dopamine (S) were given in Table 2. Plotting S/v against S gave Fig. 8. After a relatively rapid initial rise in the value of S/v the graph begins to level out. However over the range of concentrations used the graph does not appear to be reaching an asymptote parallel to the x axis. The three parameter model i.e. one saturable uptake system plus diffusion, gave kinetic parameters considered not to be a 'good

Fig 8.

S/v against S graph for the accumulation of tritium (^{3}H-dopamine) by slices of rat striatum. Each point is the mean and standard error of at least thirty-six determinations.

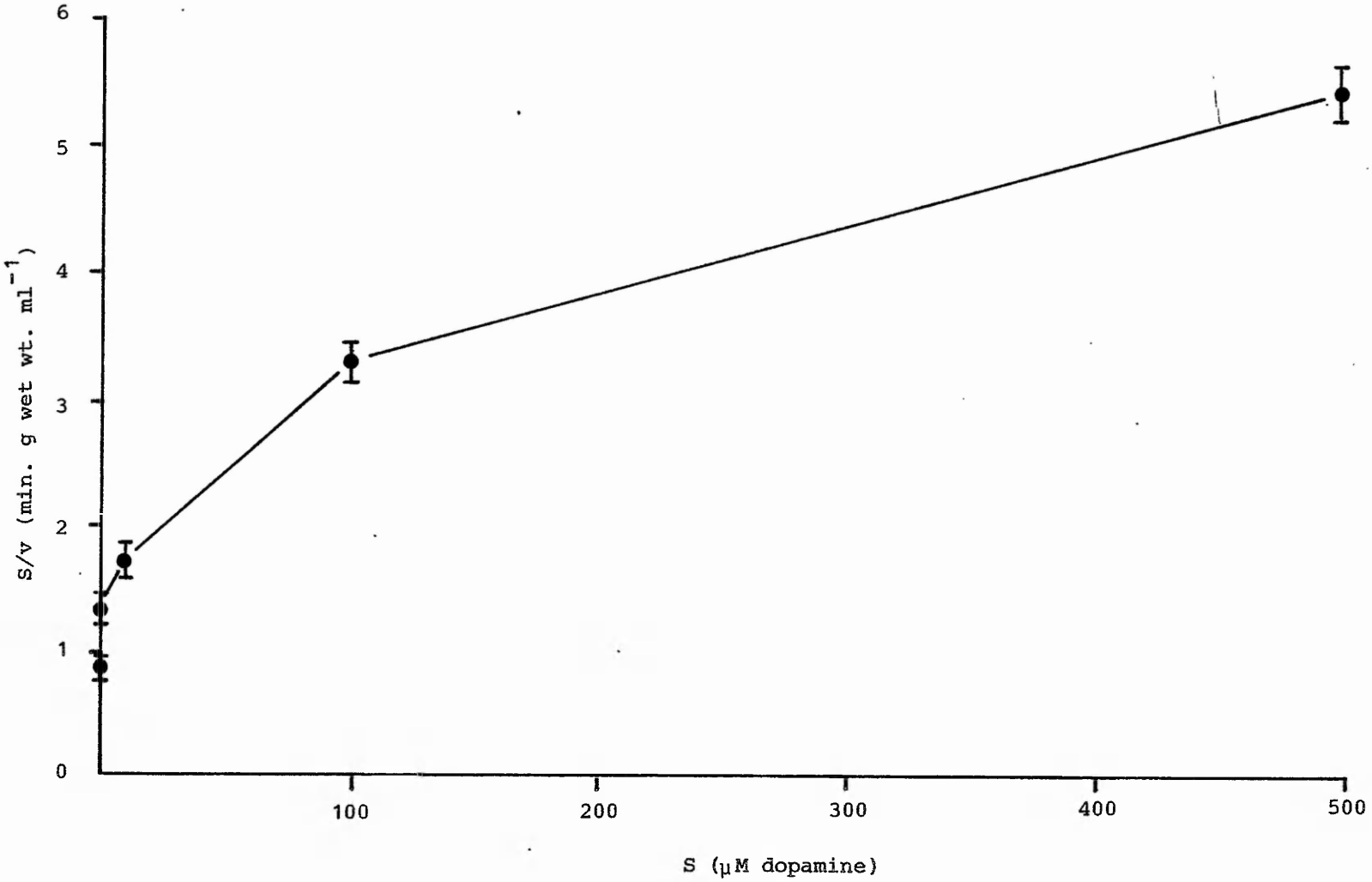

6
5
4
3
2
1
0
S/v (min. g wet wt. ml^{-1})
100
200
300
400
500
S (μM dopamine)

Table 2. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in slices of striatum. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g wet wt.ml^{-1})	Calc S/v No. of parameters	
		3	5
0.1	0.842 ± 0.071	1.029	0.844
1.0	1.301 ± 0.098	1.066	1.303
10.0	1.667 ± 0.126	1.408	1.676
100.0	3.281 ± 0.092	3.362	3.301
500.0	5.366 ± 0.228	5.300	5.419
(n = 36)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		17.254	22.425
Km (μM)		21.048	40.346
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.088
Km' (μM)			0.081
D (ml.g wet wt.$^{-1}$.min^{-1})		0.157	0.144
Fit (s.e.)		>1	<0.25

Table 3. Values for the percentage contribution of the two uptake systems plus diffusion for ^{3}H-dopamine uptake in striatal slices. The values are determined from the kinetic data presented in Table 2.

Dopamine conc.	% contribution		
(μM)	high affinity	low affinity	diffusion
0.1	41.0	46.8	12.2
1.0	10.6	70.6	18.8
10.0	1.5	74.5	24.0
100.0	0.3	52.4	47.3
500.0	0.1	22.4	77.5

fit' of the experimental results. The five parameter model i.e. two saturable uptake systems plus diffusion, when used produced values for the kinetic parameters which were less than 0.25 standard error different from the experimental results for all concentrations of dopamine studied. See Table 2. This was considered a 'good fit'. The kinetic parameters calculated from the two types of model are included in Table 2. The percentage contribution, to total uptake, of the two uptake systems plus diffusion is presented in Table 3.

Time course for the uptake of ^{3}H-noradrenaline by striatal slices

After 5 min preincubation in Krebs' solution at 37°C slices of striatum were incubated in Krebs' solution with the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1} and ^{3}H-noradrenaline (final concentration 300 nCi.ml^{-1}; 500 µM or 0.1 µM). The time course for the incubation was in the range 0.5 - 6.0 min. See Fig. 9.

An initial linear phase of the graph was evident for both concentrations of noradrenaline investigated. This linear phase persisted for at least two min before the rate of uptake began to decrease. The tissue incubated in 500 µM noradrenaline accumulated 297 nmol.g wet wt^{-1} by 3 min (T/M = 0.59), at 0.1 µM noradrenaline concentration 0.322 nmol.g wet wt^{-1} were accumulated in 3 min (T/M = 3.22).

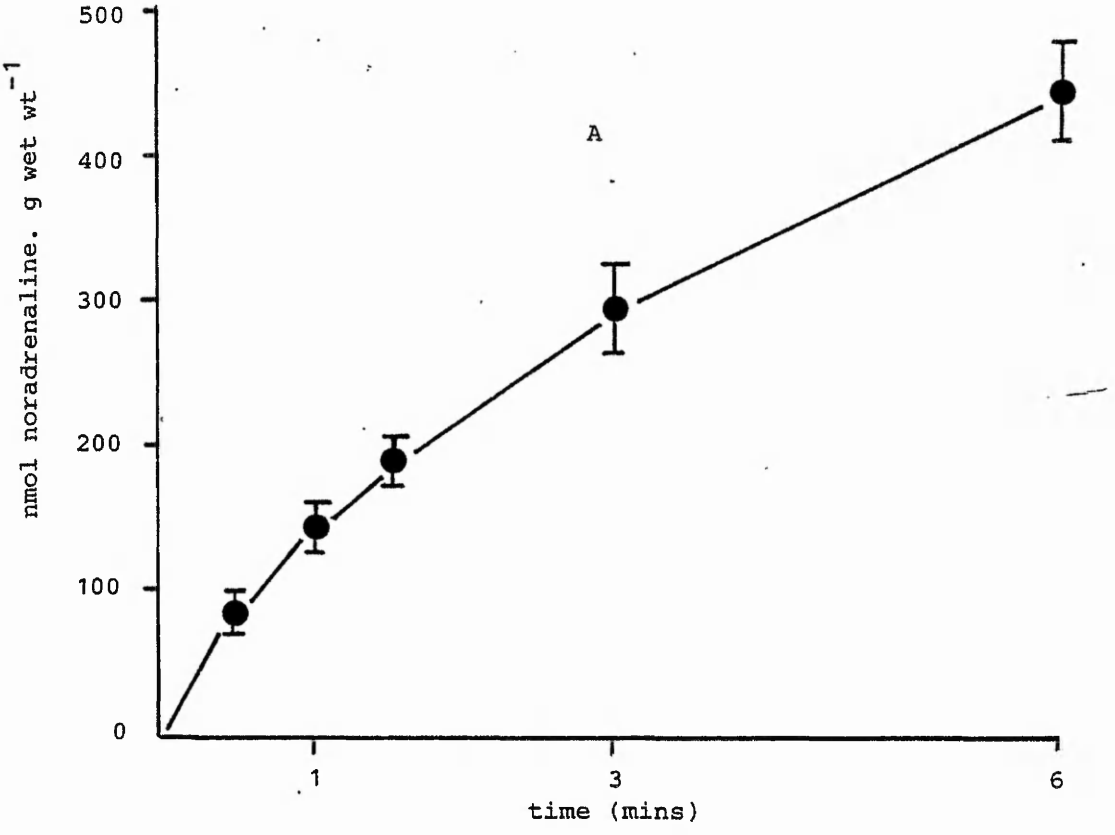
As uptake was linear for at least the first 2 min of the time course this period was selected for the study of the initial rate of accumulation.

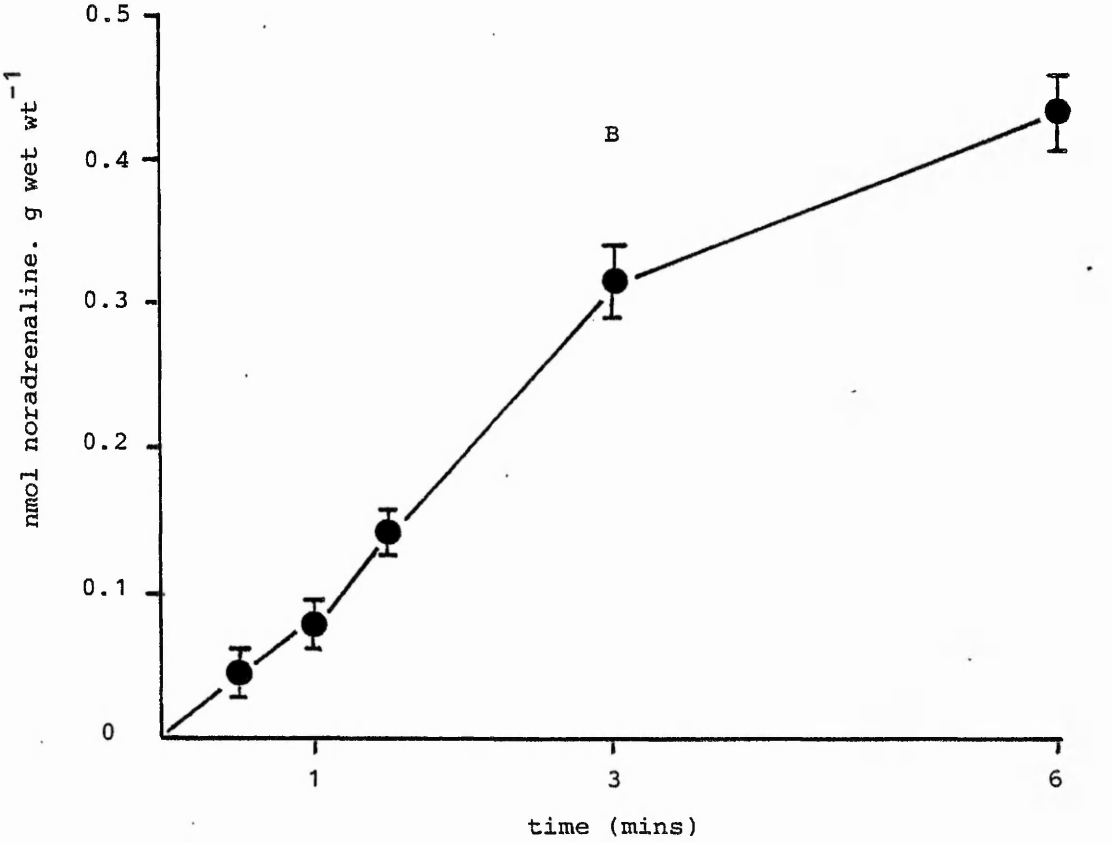
Fig 9.

Time course for the cellular accumulation of ^{3}H-noradrenaline by striatal slices. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 µM.

B. Accumulation at 0.1 µM.


500
400
300
200
100
0
nmol noradrenaline. g wet wt^{-1}
A
1
3
6
time (mins)


0.5
0.4
0.3
0.2
0.1
0
nmol noradrenaline. g wet wt^{-1}
B
1
3
6
time (mins)

Kinetics of the uptake of ^{3}H-noradrenaline by striatal slices

After 5 min preincubation in Krebs' solution at 37°C, striatal slices were incubated for 2 min following the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-noradrenaline (final concentration 300 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM). Enough tissue was taken from each rat to provide 2 slices for incubation at each of the concentrations of noradrenaline studied. The values of S/v calculated from the initial rate of accumulation, nmol.g wet wt^{-1}.min^{-1}, are summarised in Table 4. Plotting S/v against S gave the graph shown in Fig. 10. As with the dopamine uptake experiments using striatal slices (Fig. 8) the graph appears to be levelling out; although not reaching a parallel with the x axis. This follows on from an initial relatively rapid rise in S/v when the values of S are in the range 0.1 to 10.0 µM. The three parameter model gave values with a fit of greater than one standard error, not a 'good fit'. The five parameter model produced values with a fit of less than 0.2 standard errors, considered a 'good fit' to the experimental data.

Time course for the uptake of ^{3}H-dopamine by cerebellar slices

The methods used were the same as those described for the study of dopamine uptake in striatal slices except that cerebellar slices were used instead. The time course was shown to be linear for at least the first two min at both of the dopamine concentrations studied (i.e. 500 µM or 0.1 µM), see Fig. 11. After 3 min the cerebellar slices incubated in 0.1 µM dopamine had accumulated 0.091 nmol.g wt^{-1} (T/M = 0.91) dopamine and those incubated in 500 µM dopamine had

Fig 10.

S/v against S graph for the accumulation of tritium (^{3}H-noradrenaline) by slices of rat striatum. Each point is the mean and standard error of at least twelve determinations.

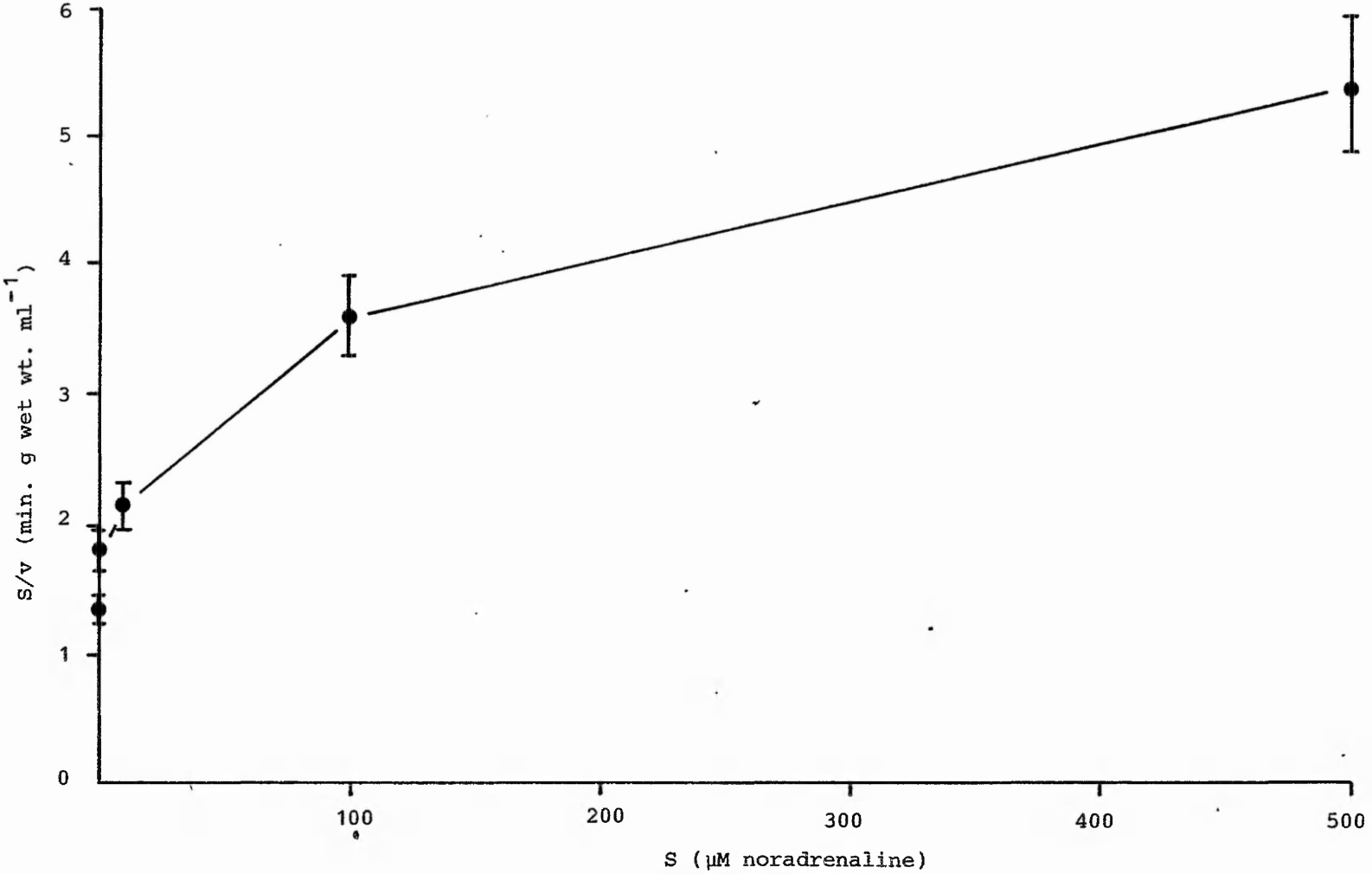

6
5
4
3
2
1
0
S/v (min. g wet wt. ml^{-1})
100
200
300
400
500
S (µM noradrenaline)

Table 4. Values of S/v for the accumulation of tritium, from ^{3}H-noradrenaline, in slices of striatum. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

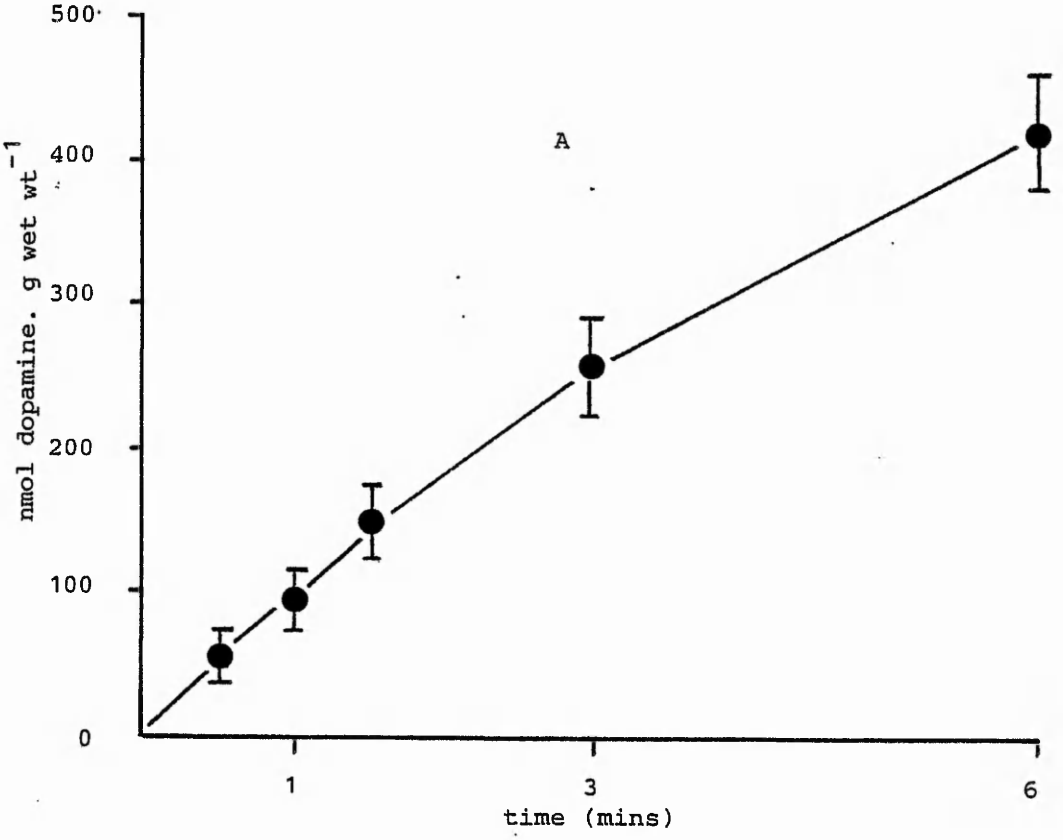
Conc. (μM)	Exp.S/v (min.g wet wt.ml^{-1})	Calc.S/v No. of parameters	
		3	5
0.1	1.597 ± 0.106	1.676	1.603
1.0	1.812 ± 0.160	1.711	1.821
10.0	2.170 ± 0.183	2.037	2.185
100.0	3.566 ± 0.328	3.767	3.603
500.0	5.385 ± 0.536	5.287	5.464
(n = 12)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		13.293	21.053
Km (μM)		30.645	59.131
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.086
Km' (μM)			0.604
D (ml.g wet wt^{-1}.min^{-1})		0.166	0.148
Fit (s.e.)		>1	<0.2

Fig 11.

Time course for the cellular accumulation of ^{3}H-dopamine by slices of cerebellum. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 µM.

B. Accumulation at 0.1 µM.


A
500
400
300
200
100
0
nmol dopamine. g wet wt^{-1}
1
3
6
time (mins)

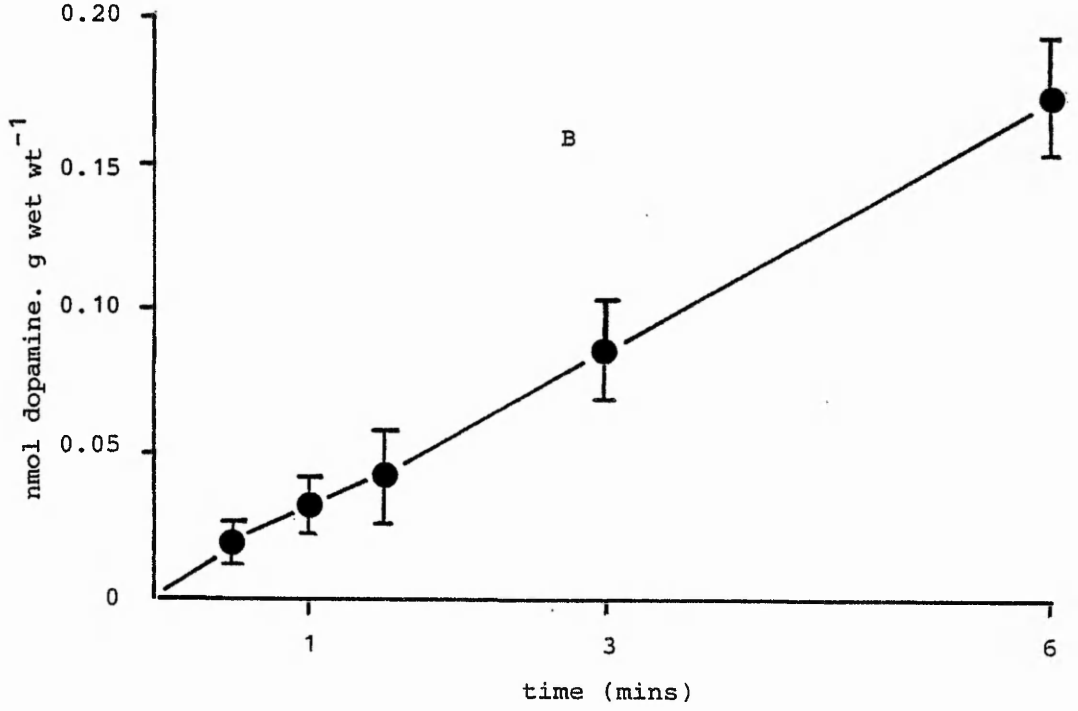

B
0.20
0.15
0.10
0.05
0
nmol dopamine. g wet wt^{-1}
1
3
6
time (mins)

Fig 12.

S/v against S graph for the accumulation of tritium (^{3}H-dopamine) by slices of rat cerebellum. Each point is the mean and standard error of at least twelve determinations.

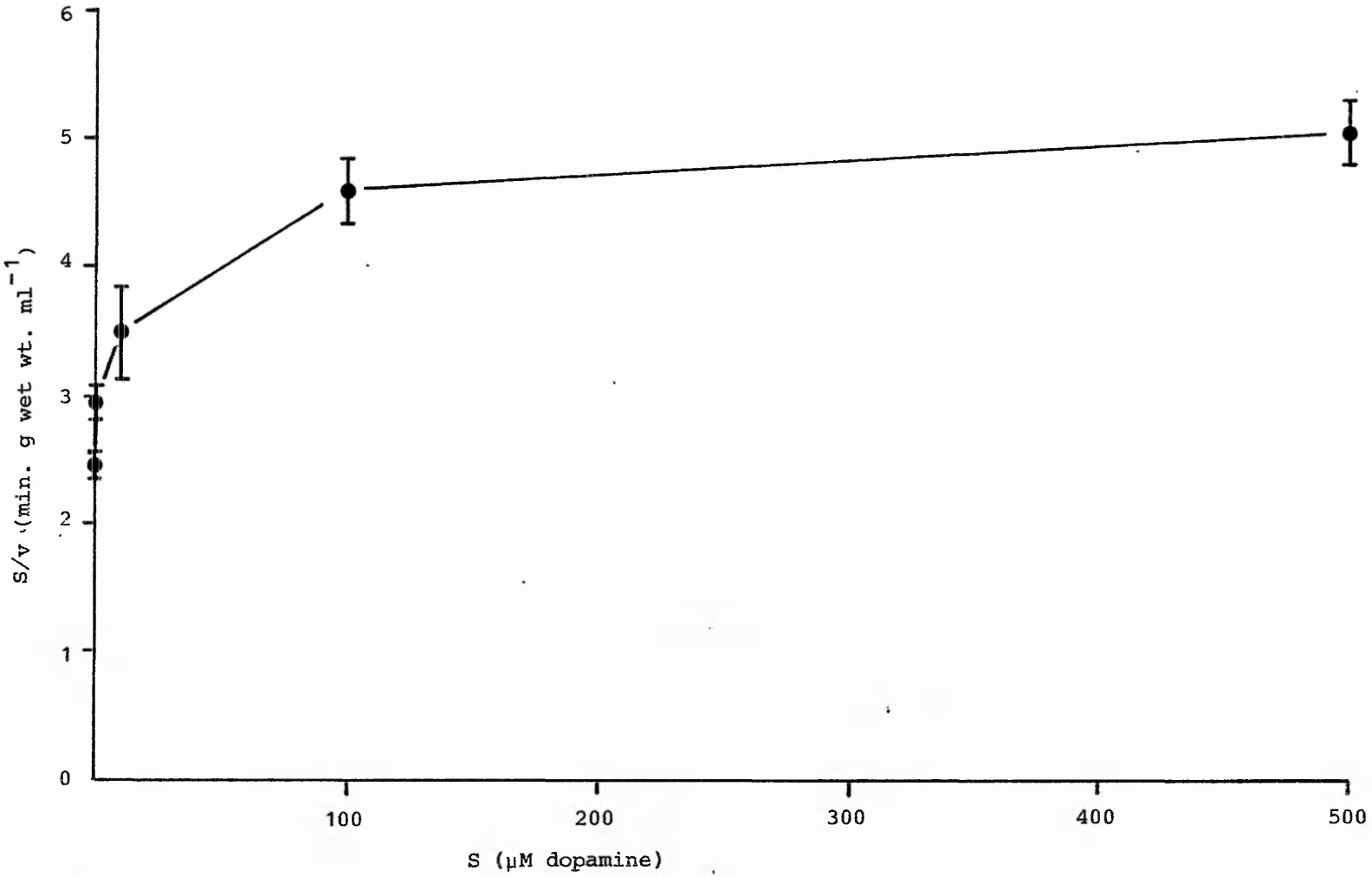

6
5
4
3
2
1
0
S/v (min. g wet wt. ml^{-1})
100
200
300
400
500
S (μM dopamine)

Table 5. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in slices of cerebellum. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp.S/v (min.g wet wt.ml^{-1})	Calc.S/v No. of parameters	
		3	5
0.1	2.447 ± 0.096	2.513	2.459
1.0	2.950 ± 0.137	2.807	2.965
10.0	3.510 ± 0.354	3.966	3.495
100.0	4.590 ± 0.244	4.769	4.658
500.0	5.068 ± 0.247	4.884	5.061
(n = 12)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		0.647	2.628
Km (μM)		3.230	18.240
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.008
Km' (μM)			0.016
D (ml.g wet wt^{-1}.min^{-1})		0.205	0.194
Fit (s.e.)		>1	<0.3

accumulated 256 nmol.g wet wt^{-1} (T/M = 0.512) dopamine.

Kinetics of the uptake of ^{3}H-dopamine by cerebellar slices.

The methods used were as in the kinetic studies using striatal slices except that cerebellar slices were used. A 2 min incubation period was chosen from the data provided by the time course experiments. Plotting S/v against S gave Fig. 12. The graph shows an initial relatively rapid rise in the value of S/v with increasing values of S in the range 0.1 to 10 µM dopamine. The graph then begins to level out, but never reaches a point where it becomes parallel to the x axis.

The values of S/v calculated using the two curve fitting models are given in Table 5. along with their relevant kinetic parameters. Values for the fit were greater than one standard error for the three parameter model, and hence it was not considered a 'good fit'. The five parameter model gave values for the fit which were less than 0.3 standard errors different from the experimental values; a 'good fit'.

Time course for the uptake of ^{3}H-noradrenaline by cerebellar slices

The methods used were the same as those described for the study of noradrenaline uptake in striatal slices except that cerebellar slices were used.

The time course was shown to be linear for at least the first 2 min at both of the noradrenaline concentrations studied (Fig. 13). After 3 min the cerebellar slices incubated at 0.1 μM noradrenaline had accumulated 0.112 nmol.g wet wt^{-1} (T/M = 1.12) noradrenaline and those incubated in 500 μM noradrenaline 145 nmol.g wet wt^{-1} (T/M = 0.29) noradrenaline.

Kinetics of the uptake of ^{3}H-noradrenaline by cerebellar slices

The methods used were the same as those described for the study of the uptake kinetics of noradrenaline striatal slices except that cerebellar slices were used. A 2 min incubation period was chosen using the data from the previous time course experiments. Plotting S/v against S gave Fig. 14. The graph shows an initial relatively rapid rise in the value of S/v in the concentration (S) range 0.1 μM to 10 μM noradrenaline. The graph then begins to level out but never becomes parallel to the x axis.

The values of S/v calculated using the two curve fitting models are given in Table 6. along with their relevant kinetic parameters. Values for the fit were greater than one standard error for the three parameter model, and hence not considered a 'good fit', but less than 0.2 standard errors for the five parameter model; a 'good fit'.

Inhibition studies

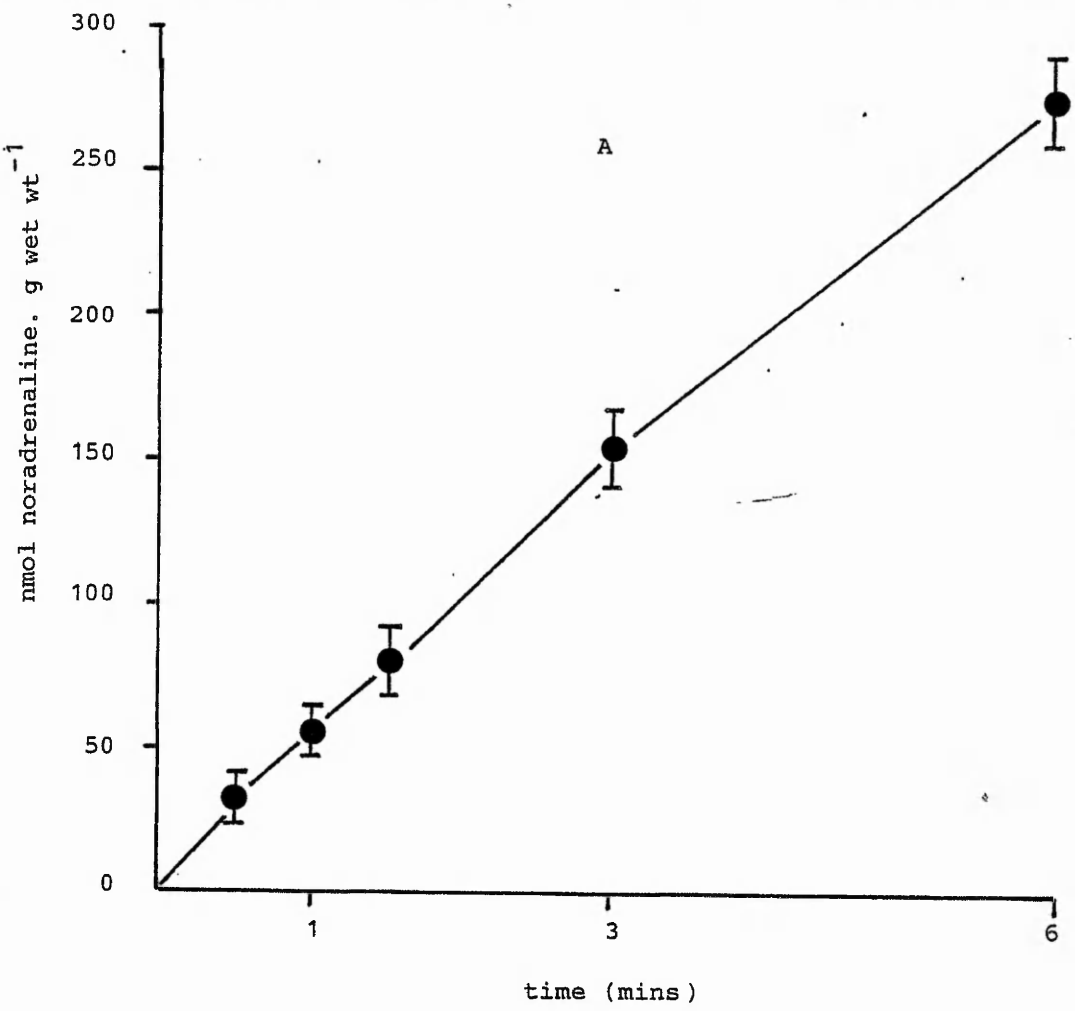
A number of phenylethylamine derivatives were screened as possible inhibitors of the second uptake system for dopamine demonstrated in the kinetic experiments. The concentration of dopamine selected

Fig 13.

Time course for the cellular accumulation of ^{3}H-noradrenaline by slices of rat cerebellum. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 µM.

B. Accumulation at 0.1 µM.


A
nmol noradrenaline. g wet wt^{-1}
300
250
200
150
100
50
0
1
3
6
time (mins)

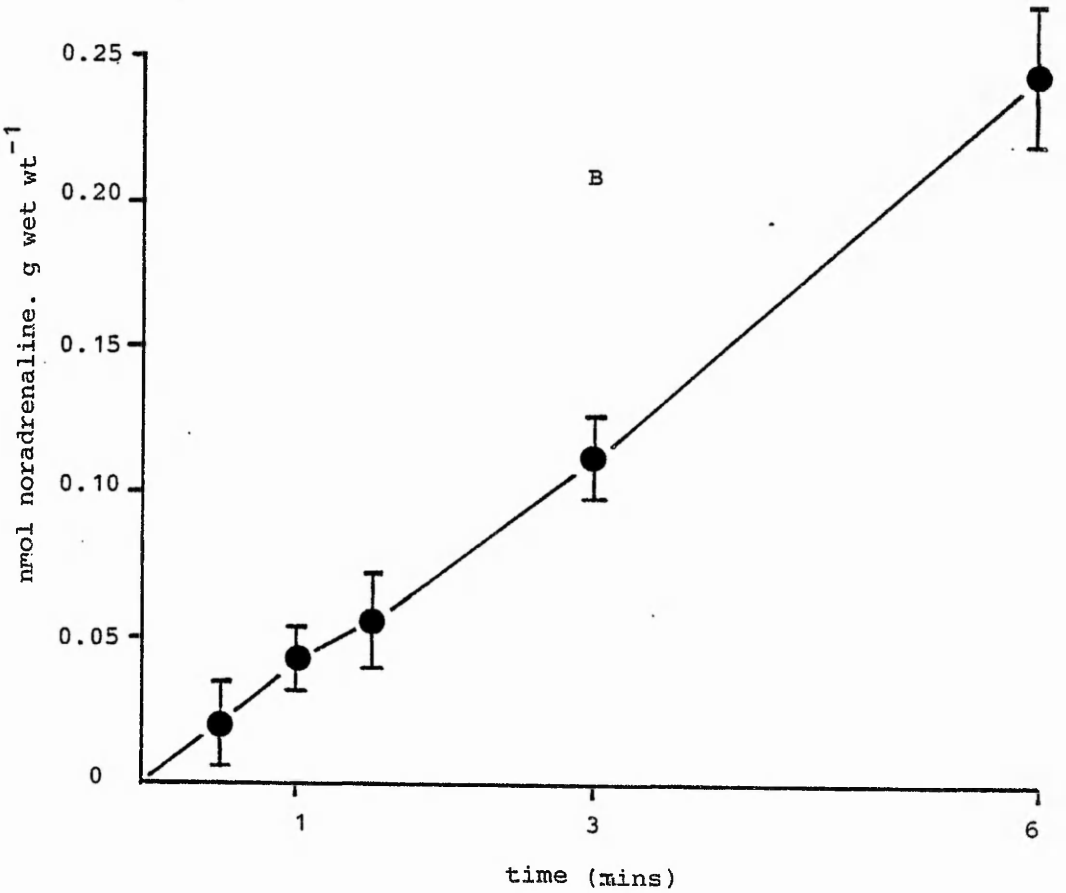

B
nmol noradrenaline. g wet wt^{-1}
0.25
0.20
0.15
0.10
0.05
0
1
3
6
time (mins)

Fig 14.

S/v against S graph for the accumulation of tritium (^{3}H-noradrenaline) by slices of rat cerebellum. Each point is the mean and standard error of at least twelve determinations.

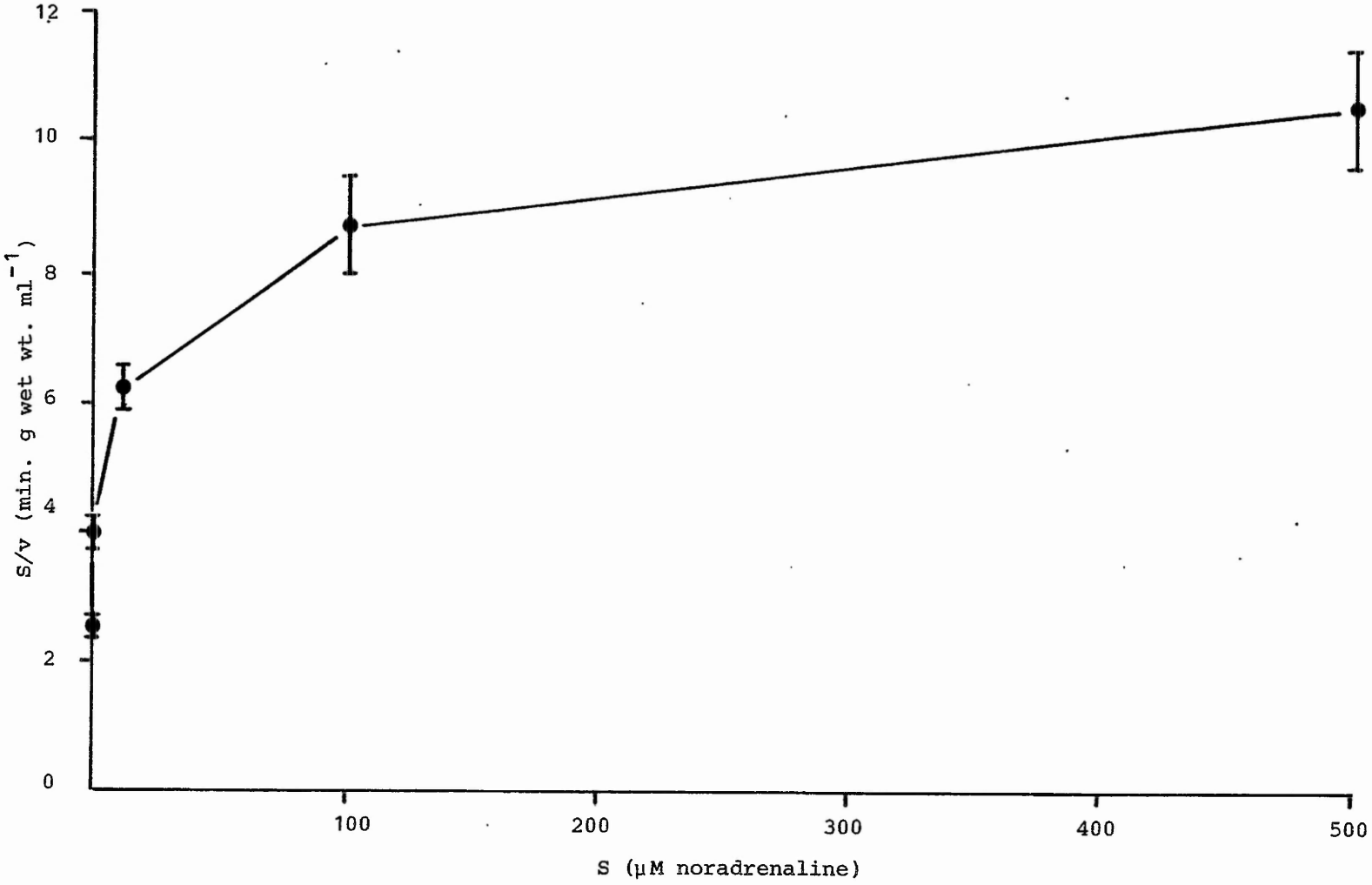

12
10
8
6
4
2
0
S/v (min. g wet wt. ml^{-1})
100
200
300
400
500
S (µM noradrenaline)

Table 6. Values of S/v for the accumulation of tritium, from ^{3}H-noradrenaline, in slices of cerebellum. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp.S/v (min.g wet wt.ml^{-1})	Calc.S/v No. of parameters	
		3	5
0.1	2.586 ± 0.182	2.781	2.593
1.0	4.067 ± 0.283	3.461	4.084
10.0	6.255 ± 0.301	6.544	6.264
100.0	8.738 ± 0.713	9.141	8.885
500.0	10.626 ± 0.915	9.552	10.597
(n = 12)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		0.609	3.526
Km (μM)		2.278	48.832
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.127
Km' (μM)			0.461
D (ml.g wet wt^{-1}.min^{-1})		0.104	0.088
Fit (s.e.)		>1	<0.2

for these studies was 100 μM. At this concentration the second (low affinity) uptake system is theoretically responsible for approximately 53% of the total uptake observed (see Table 3). The uptake system for dopamine that is present in the striatum was studied as it accumulates a larger proportion of the total uptake than do the systems shown to be present in the cerebellum for dopamine or for noradrenaline in either region.

After 5 min preincubation in Krebs' solution at 37°C, which may be modified by the presence of a test drug at a concentration of 10^{-3}M, ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 100 μM) were added. Incubation was continued for a further 2 min in the presence of the radiolabel. Slices from each individual rat brain were incubated in drug free medium at the same time, these provided control data against which the effects of the test drugs were compared. The percentage inhibition of the ^{3}H-dopamine uptake was calculated from the equation:

$$\% \text{ inhibition} = \left(\frac{\text{(1-uptake in the presence of the test drug)}}{\text{uptake in control slices}} \right) \times 100\%$$

The variability of the uptake within the group of slices studied in the inhibition experiments was estimated by arbitrarily assigning one of the slices as 'X' and comparing the remaining values to X as 'tests'. The percentage inhibition of the tests compared to the control value X was calculated for all the slices and the standard error estimated. This standard error was used in the Students 't' test on unpaired data so as to determine if the uptake of ^{3}H-dopamine

Table 7. Compounds screened for uptake inhibition at a concentration of 10^{-3}M. Each value is the mean and standard error of at least 6 determinations. Dopamine concentration 100 µM.

Compound	% inhibition	Compound	% inhibition
Adrenaline	39.16 ± 7.4	Metanephrine*	-5.86 ± 4.7
Atropine	25.56 ± 1.9	Methoxamine*	11.68 ± 4.2
Benztropine	52.21 ± 3.0	Methoxy-isoprenaline*	-9.83 ± 3.6
Catechol*	0.19 ± 2.4	Methylamine*	6.09 ± 2.4
Desipramine	48.07 ± 5.1	Noradrenaline	42.94 ± 5.8
Dimethoxyphenyl-ethylamine*	13.33 ± 6.3	Norephedrine*	7.06 ± 8.5
Dopamine	55.15 ± 5.1	Norψephedrine	37.00 ± 5.7
Ephedrine*	5.32 ± 3.2	Normetanephrine*	16.06 ± 6.6
ψ-ephedrine	21.02 ± 8.0	Octopamine	26.32 ± 2.5
Isoprenaline	25.00 ± 2.6	17β-oestradiol*	16.46 ± 5.3
Hordenine	18.86 ± 1.2	Oxedrine*	10.14 ± 4.7
6-hydroxydopamine	41.48 ± 3.7	Phenylethylamine*	9.89 ± 3.9
Metaraminol	40.95 ± 2.5	Tyramine	35.64 ± 2.7

*The compounds marked were not significantly different from the control values at the $p<0.05$ level using the two tailed Student's 't' test.

in the presence of a test drug was significantly different from the control.

The standard error of the control values from the ^{3}H-dopamine uptake inhibition experiments (n = 20) was 1.52 nmol.g wet wt^{-1}.min^{-1} i.e. 4.7% inhibition. The variability was checked in all experiments involving inhibition of ^{3}H-dopamine uptake into striatal slices, it was shown not to vary significantly.

The percentage inhibition of uptake (dopamine concentration (100 µM) for the compounds screened are given in Table 7.

Inhibition of ^{3}H-dopamine uptake in striatal slices

Drugs which had a significant effect on the uptake of ^{3}H-dopamine in the striatum under the conditions of the screening experiments were investigated further. Dose inhibition curves were constructed for these compounds following their incubation with striatal slices in the presence of dopamine at a concentration of either 100 µM or 0.1 µM.

After a 5 min preincubation in Krebs' solution at 37°C containing the drug under investigation (present in the concentration range 10^{-2}M to 10^{-8}M) ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1})and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 100 µM or 0.1 µM) were added. Incubation was continued for a further 2 min. The slices of striatum were arranged so that some were incubated in a drug free medium i.e. these acted as control slices, at the same time other slices were

incubated in varying concentrations of the selected test compound. The standard error for the control group was ± 5.3% (incubations in 100 µM dopamine) and ± 3.0% (incubation in 0.1 µM dopamine) and is displayed on the graphs as a shaded area. The percentage inhibition of the uptake of ^{3}H-dopamine was determined as in the previous screening experiments. The results are presented graphically in Figs. 15 to 24. and as Table 8.

In order to calculate the IC_{50} value it is normally assumed that 100% inhibition of uptake can be achieved. However as can be seen from the results of the kinetic experiments it appears that at least two separate uptake systems plus diffusion are in operation and the data indicates that because of diffusion complete inhibition of ^{3}H-dopamine uptake is not possible. The maximum amount of possible inhibition is approximately 53% (52.4% low affinity, 0.3% high affinity) in the case of dopamine uptake by striatal slices incubated in 100 µM dopamine and approximately 88% (low affinity 46.8%, high affinity 41%) for striatal slices incubated in 0.1 µM dopamine. These figures are based upon the assumption that the saturable uptake systems can be inhibited completely by a test drug while the fraction of the total uptake due to diffusion cannot be inhibited by the test drug.

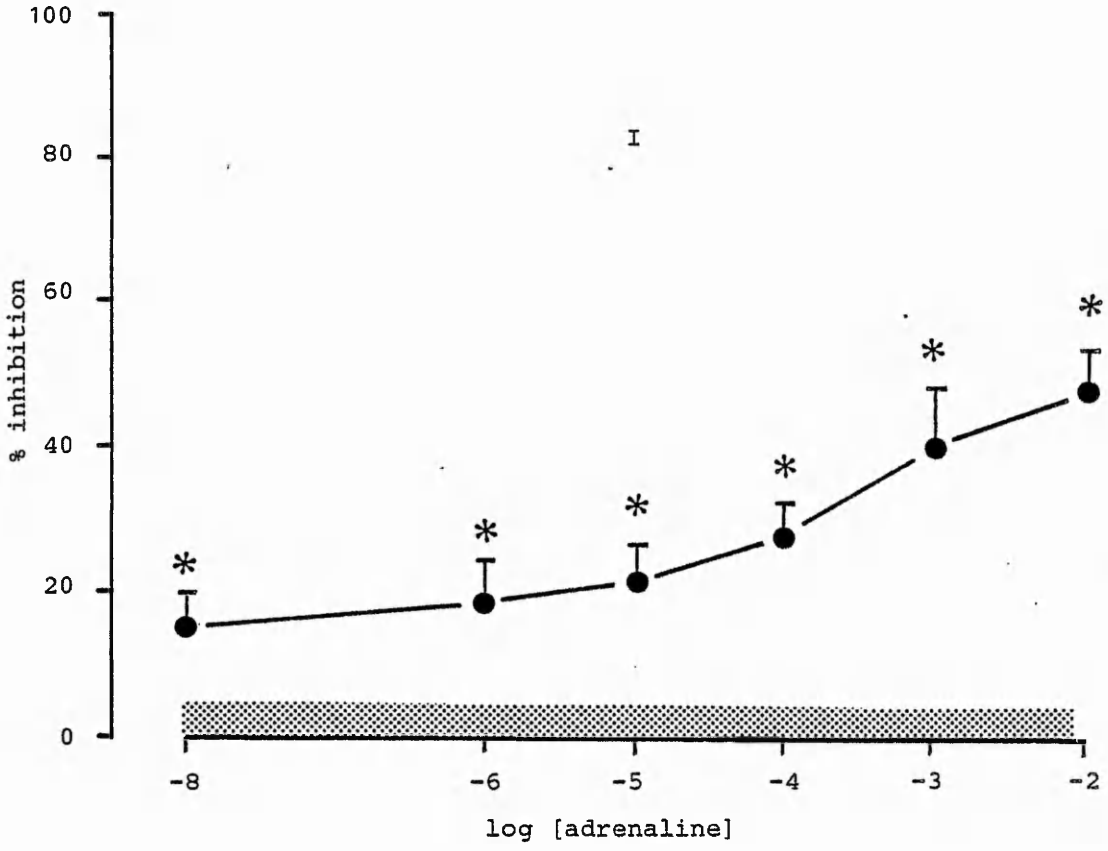
This 'theoretical' maximum value of inhibition was obtained experimentally. The value representing half this maximum has been taken as the IC_{50} value. That is, the maximum amount of inhibition is 60%, therefore the level of the IC_{50} is taken as the value producing 30% inhibition of TOTAL uptake, called the $E_{30}IC_{50}$. The maximum

Fig 15.

Inhibition of the accumulation of ^{3}H-dopamine (100 µM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Adrenaline

II. Benztropine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [adrenaline]

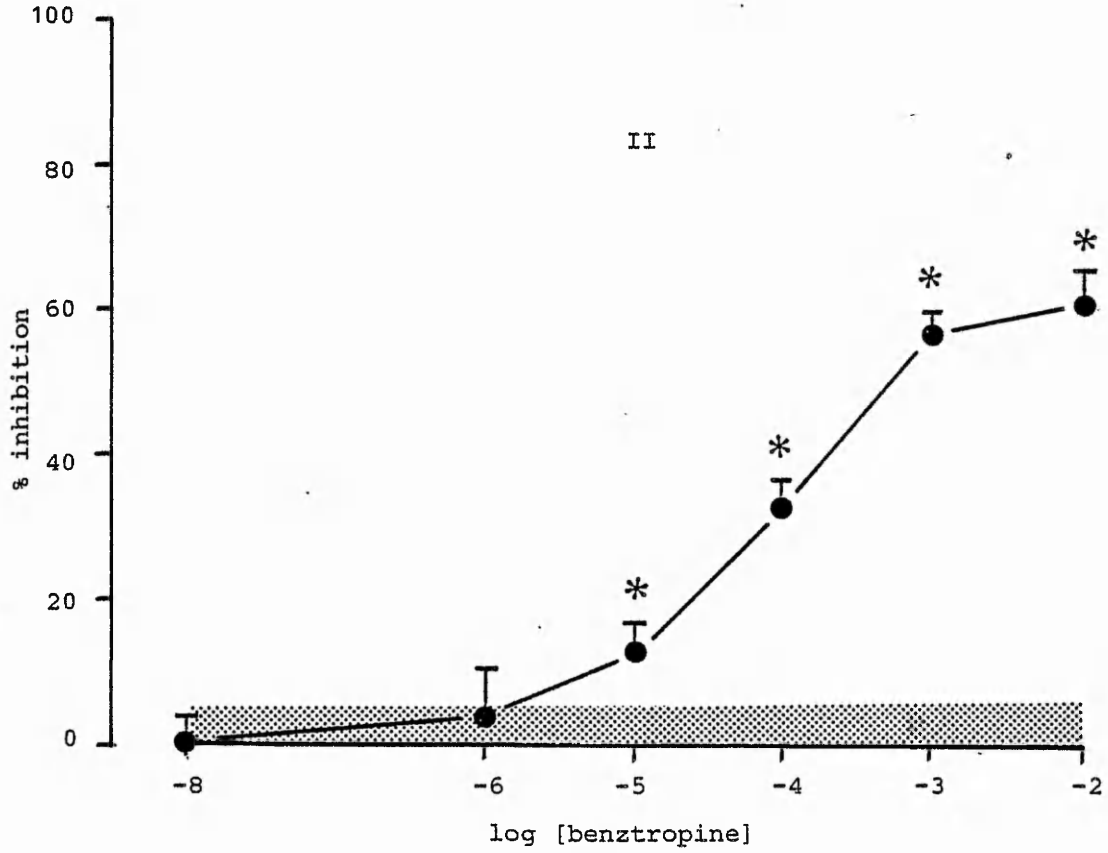
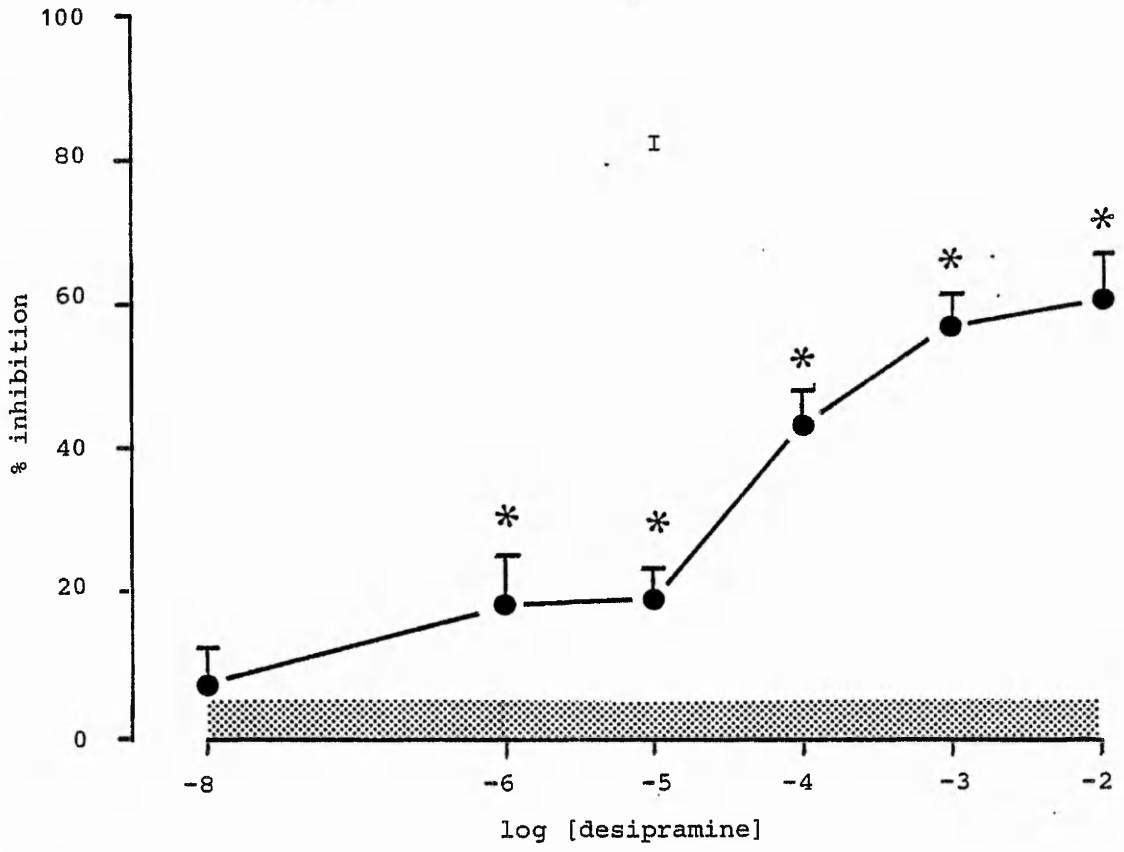

100
80
60
40
20
0
% inhibition
II
*
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [benztropine]

Fig 16.

Inhibition of the accumulation of ^{3}H-dopamine (100 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Desipramine

II. Hordenine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [desipramine]

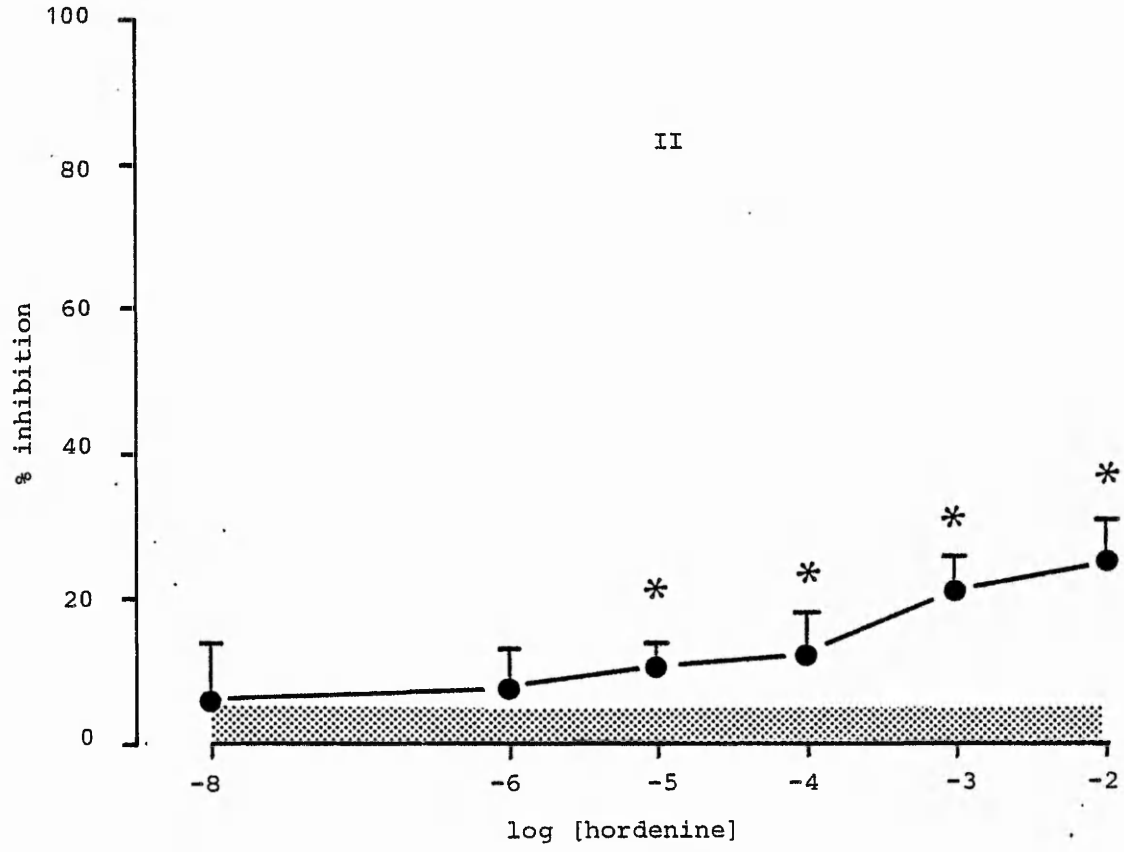
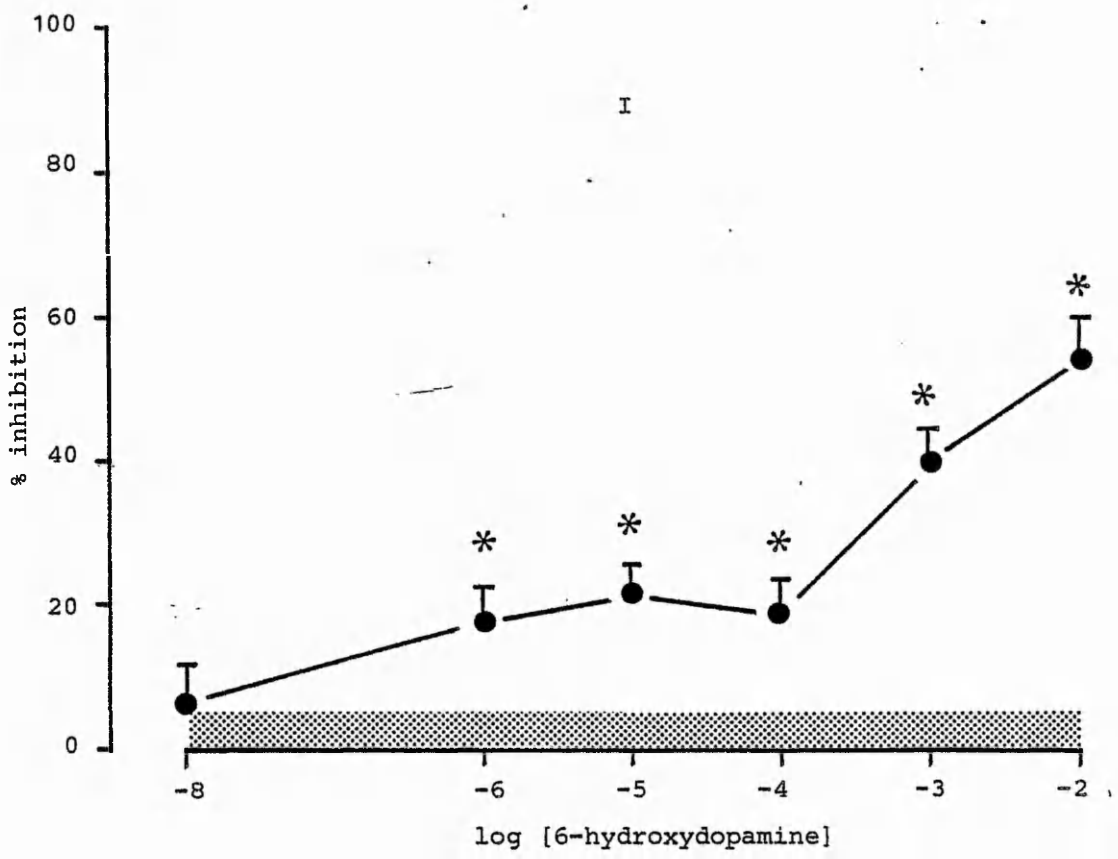

100
80
60
40
20
0
% inhibition
II
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [hordenine]

Fig 17.

Inhibition of the accumulation of ^{3}H-dopamine (100 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. 6-Hydroxydopamine

II. Isoprenaline


I
100
80
60
40
20
0
% inhibition
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [6-hydroxydopamine]

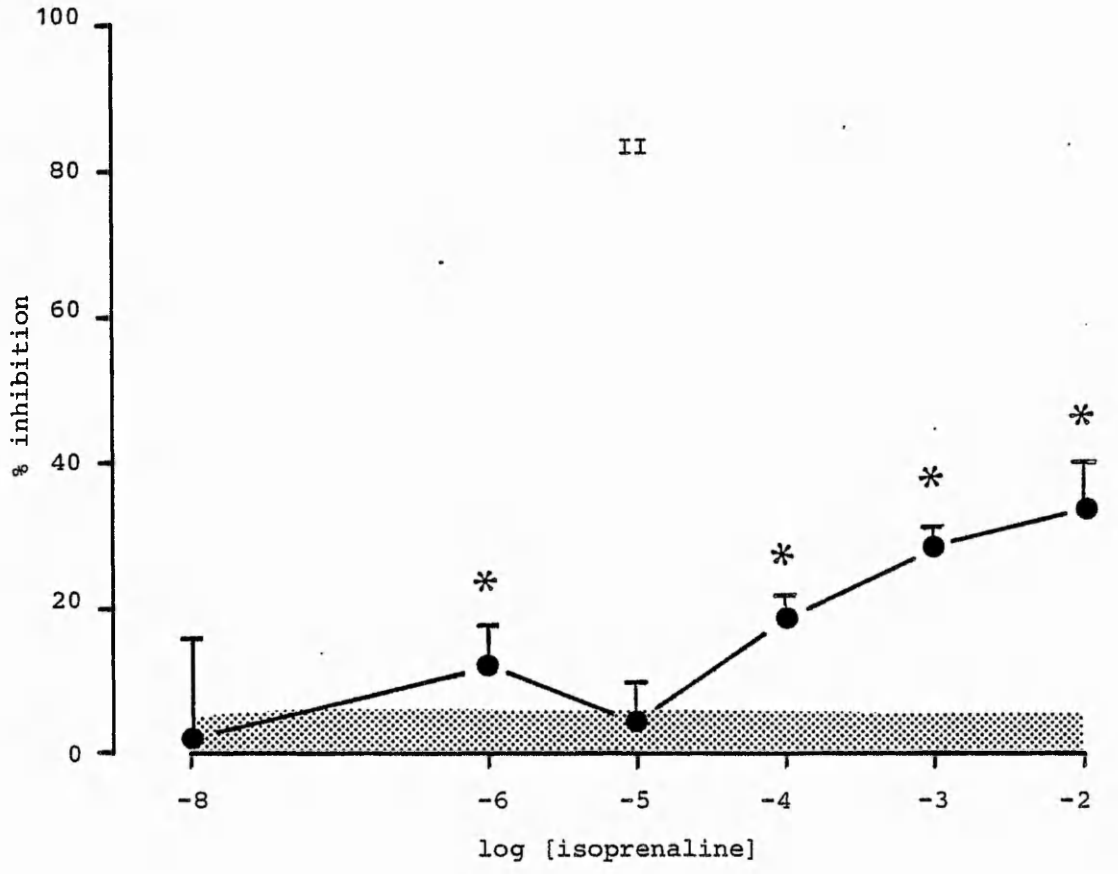
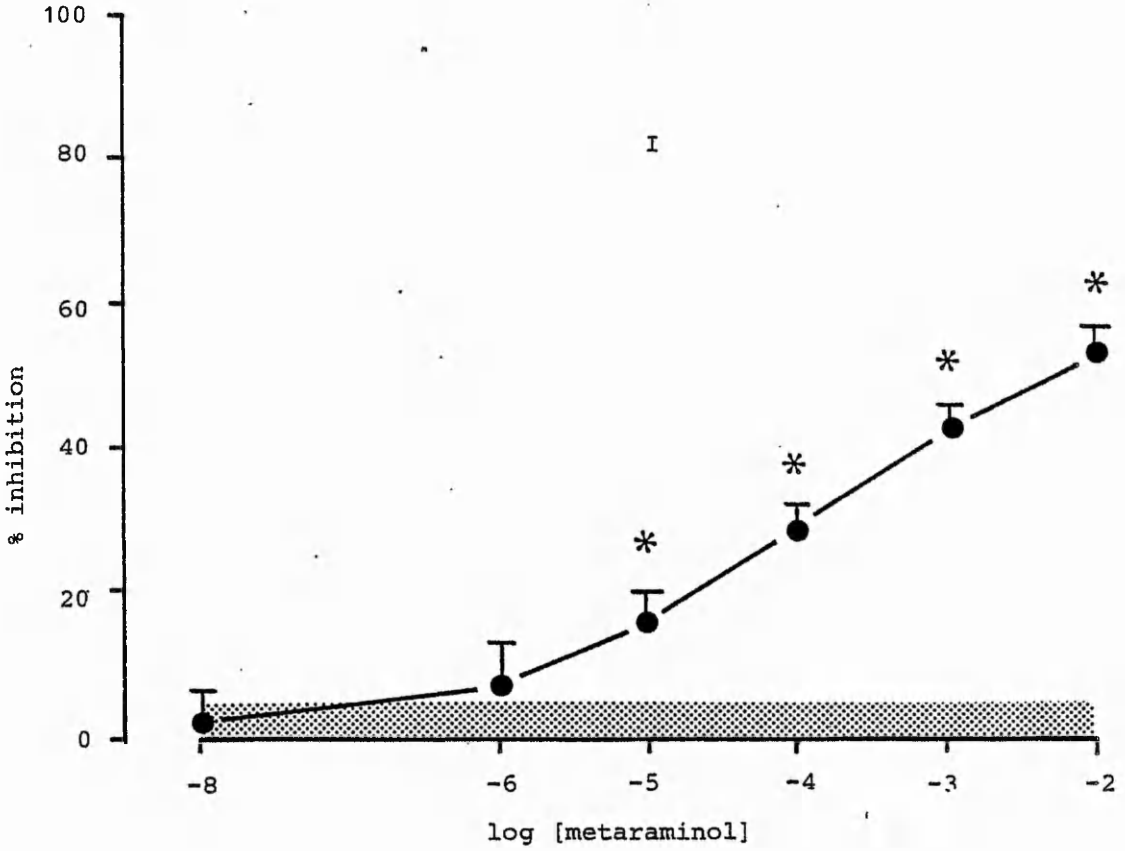

II
100
80
60
40
20
0
% inhibition
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [isoprenaline]

Fig 18.

Inhibition of the accumulation of ^{3}H-dopamine (100 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Metaraminol

II. Noradrenaline


I
100
80
60
40
20
0
% inhibition
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [metaraminol]

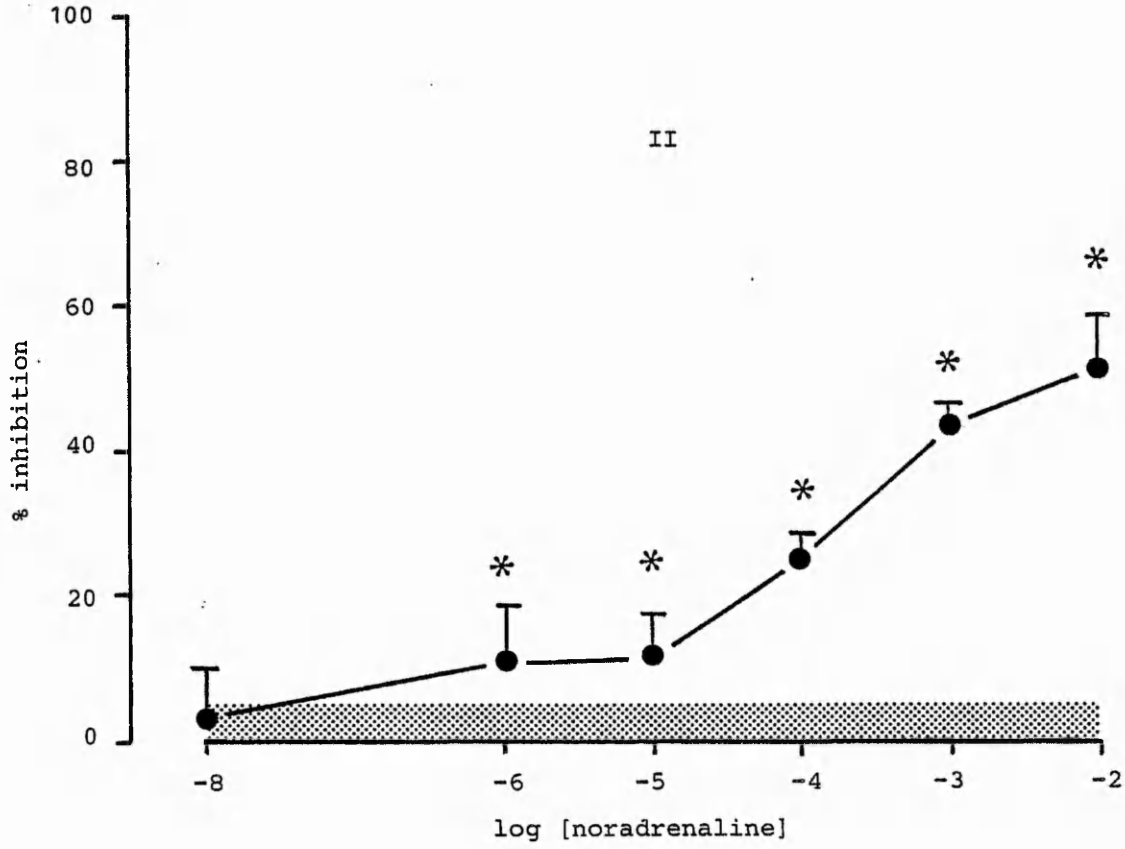
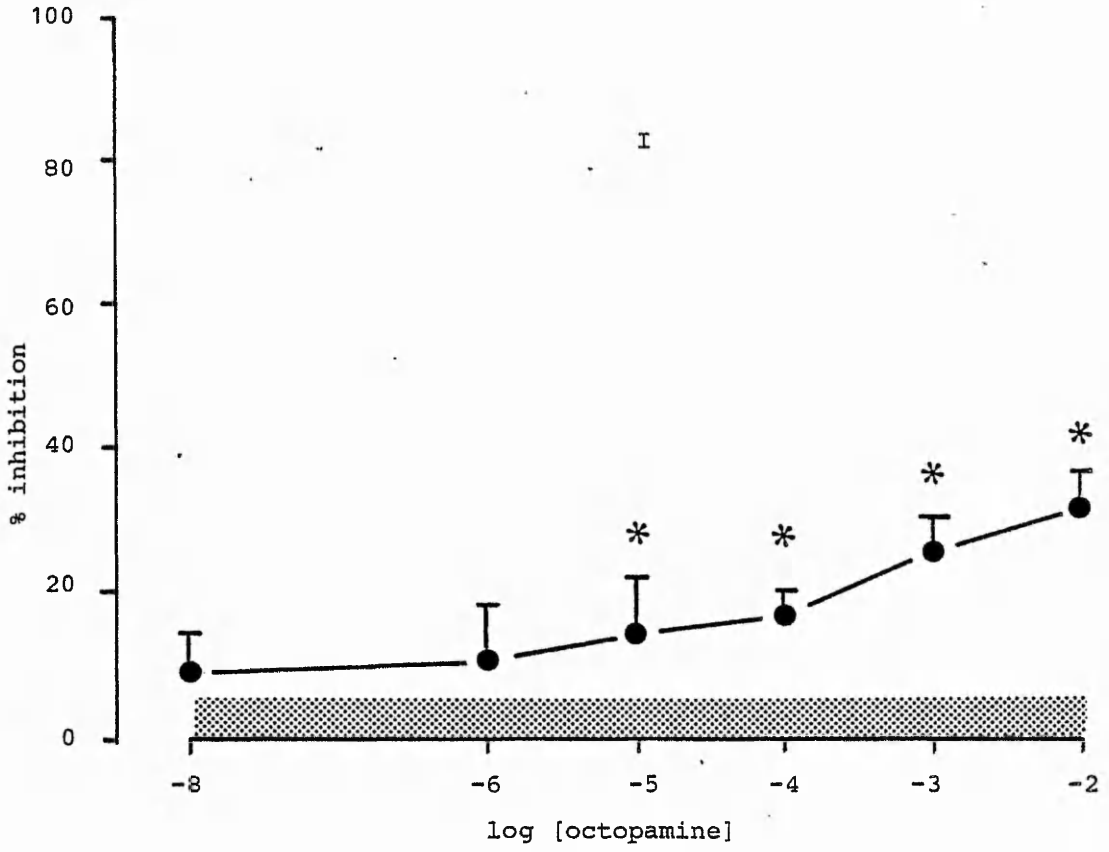

II
100
80
60
40
20
0
% inhibition
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [noradrenaline]

Fig 19.

Inhibition of the accumulation of ^{3}H-dopamine (100 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Octopamine

II. Tyramine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [octopamine]

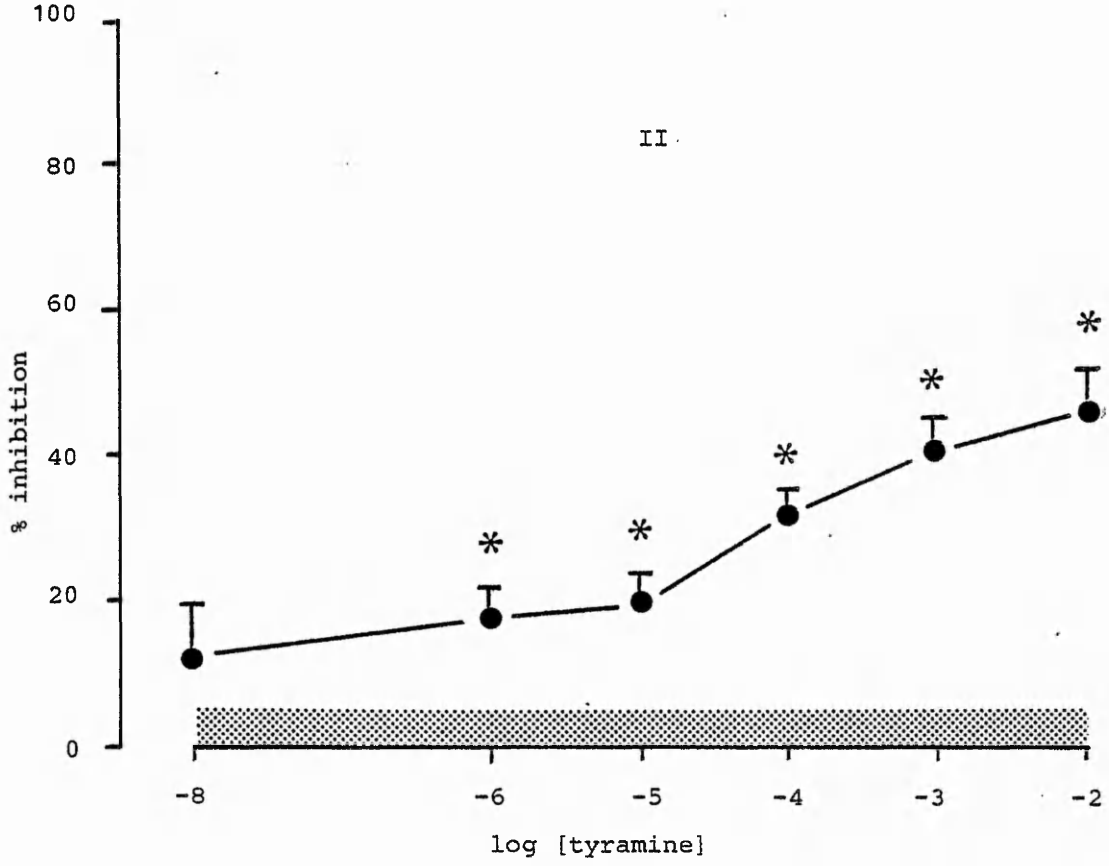
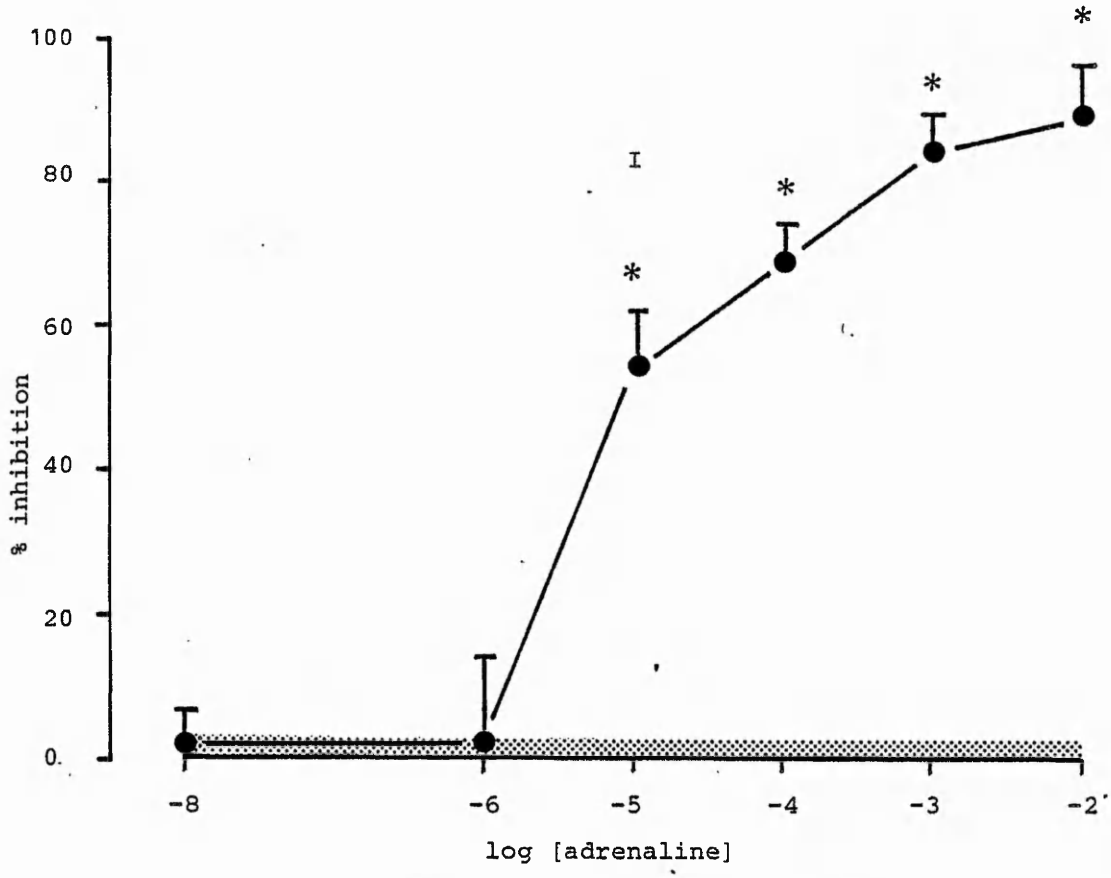

100
80
60
40
20
0
% inhibition
II
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [tyramine]

Fig 20.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μ M) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Adrenaline

II. Benztropine


100
80
60
40
20
0.
% inhibition
I
*
-8
-6
-5
-4
-3
-2
log [adrenaline]

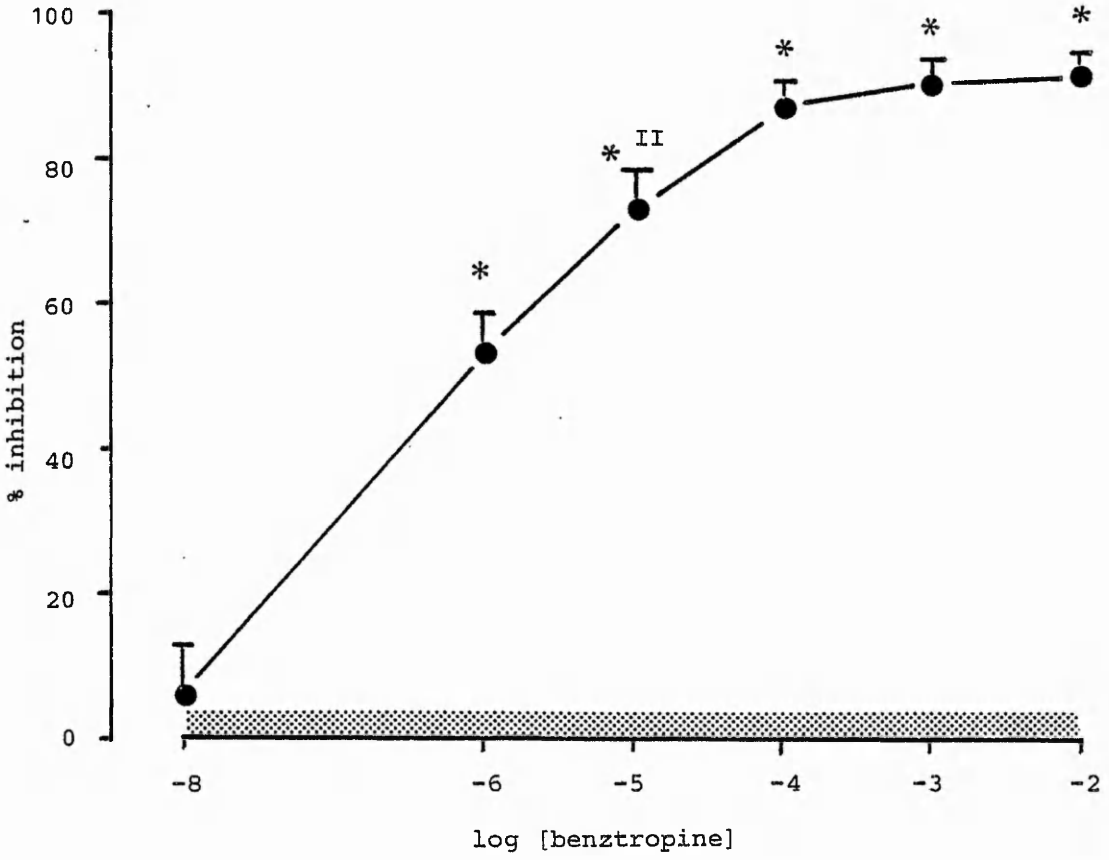
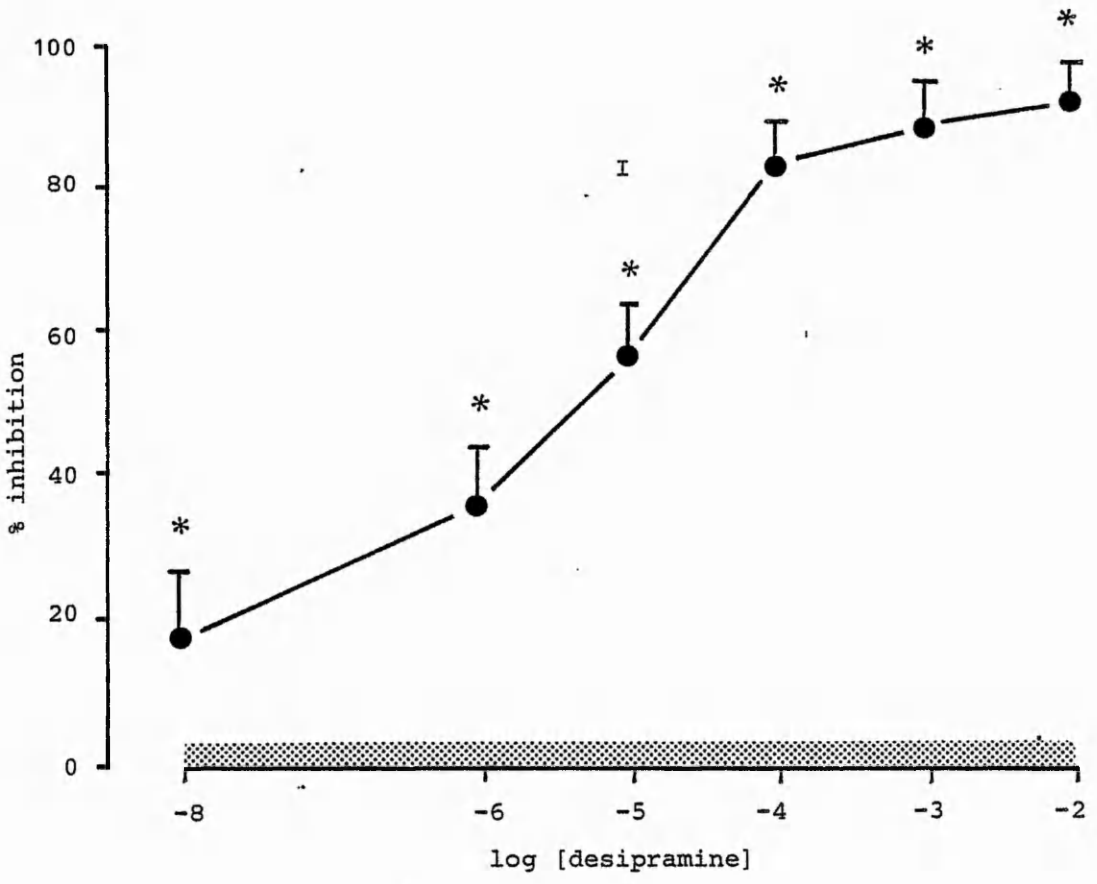

100
80
60
40
20
0
% inhibition
II
*
-8
-6
-5
-4
-3
-2
log [benztropine]

Fig 21.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Desipramine

II. Hordenine


100
80
60
40
20
0
% inhibition
I
-8
-6
-5
-4
-3
-2
log [desipramine]

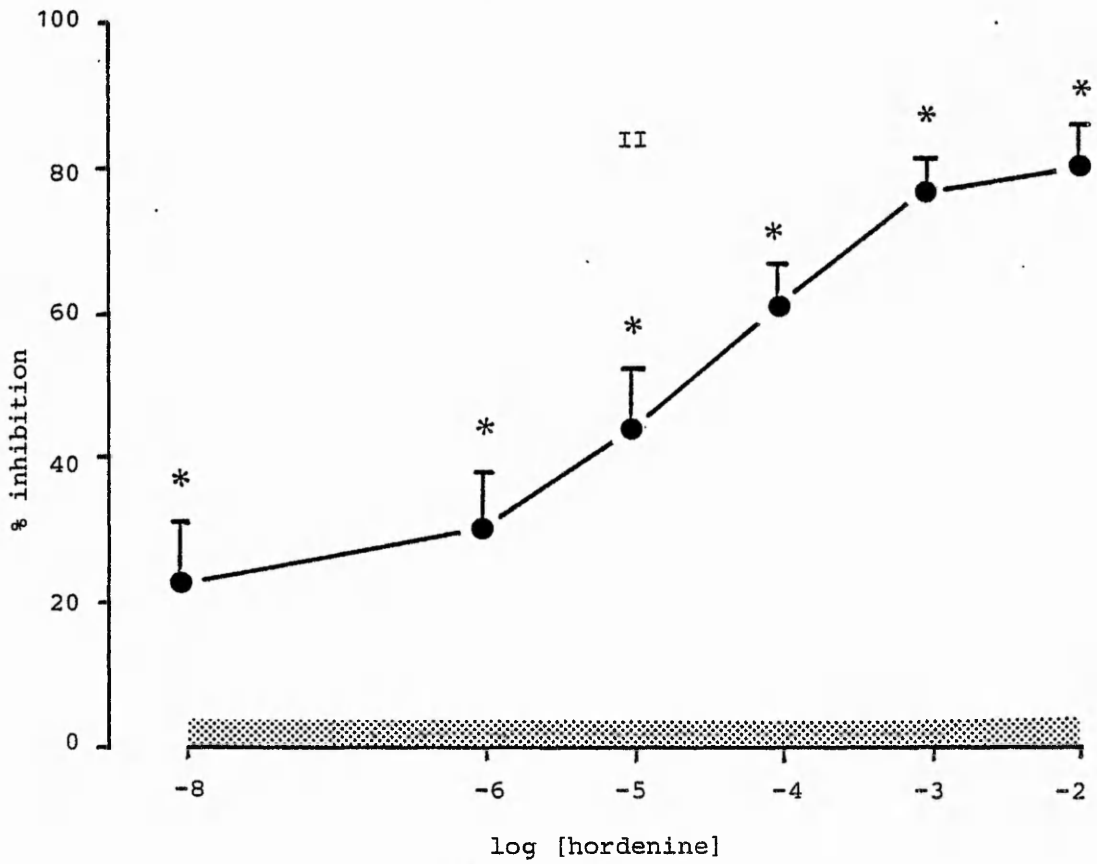
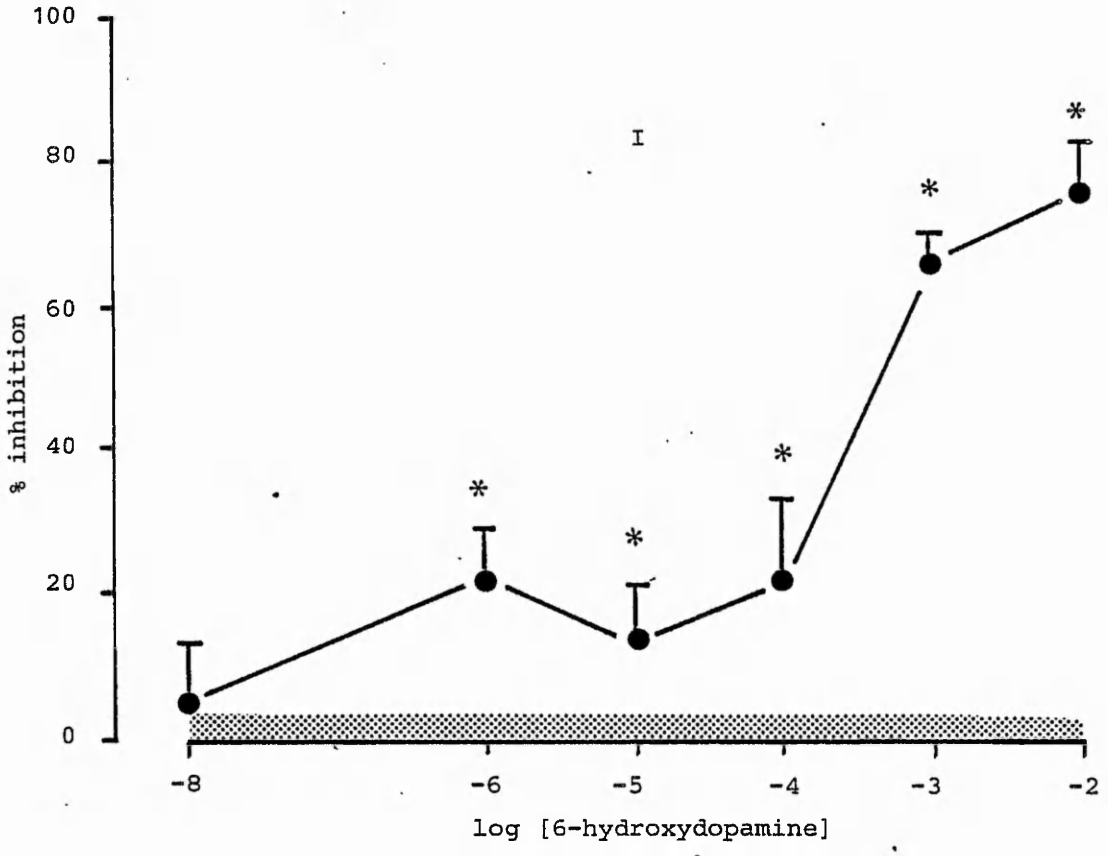

100
80
60
40
20
0
% inhibition
II
-8
-6
-5
-4
-3
-2
log [hordenine]

Fig 22.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. 6-Hydroxydopamine

II. Isoprenaline


100
80
60
40
20
0
% inhibition
I
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [6-hydroxydopamine]

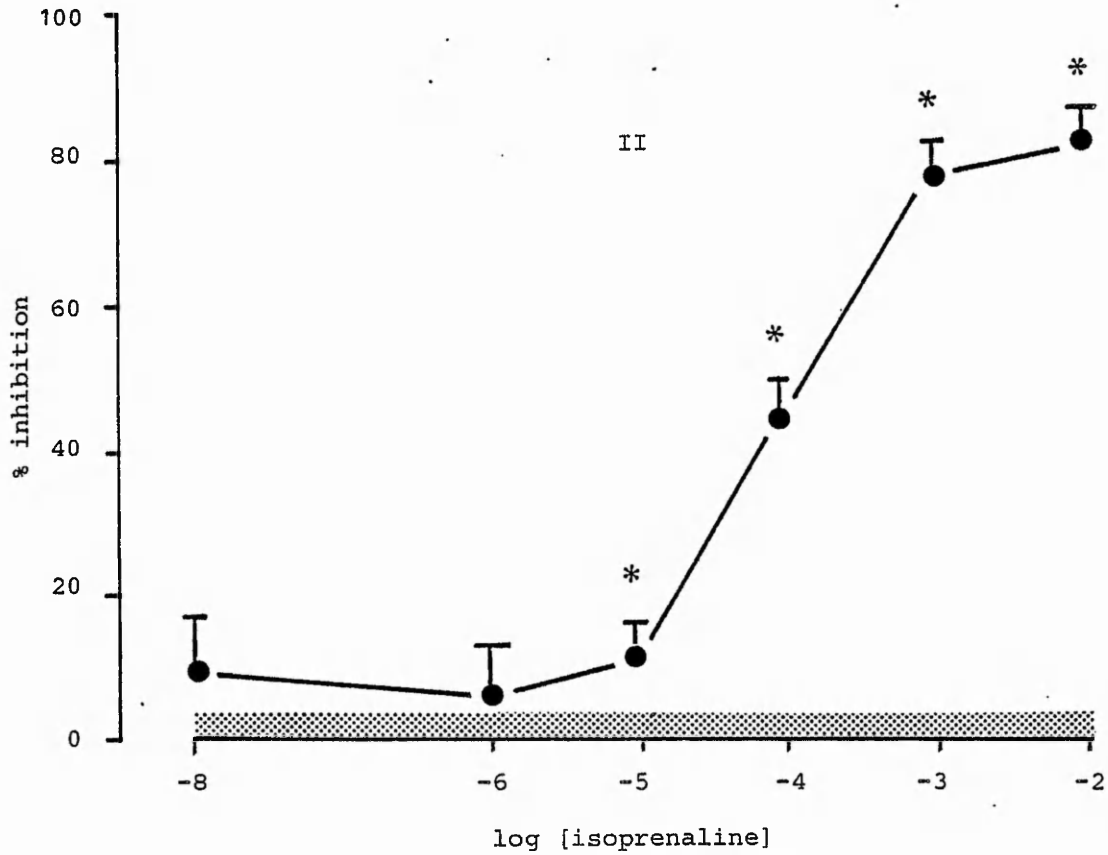
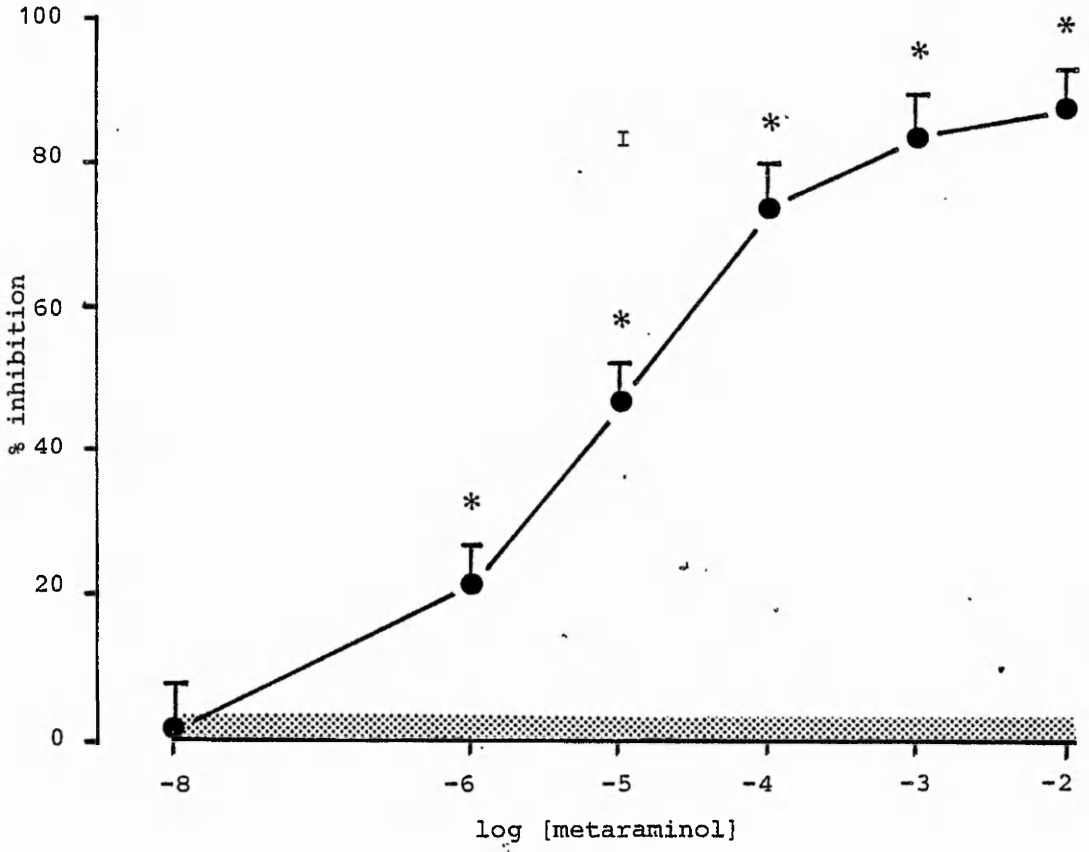

100
80
60
40
20
0
% inhibition
II
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [isoprenaline]

Fig 23.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Metaraminol

II. Noradrenaline


100
80
60
40
20
0
% inhibition
I
*
-8
-6
-5
-4
-3
-2
log [metaraminol]

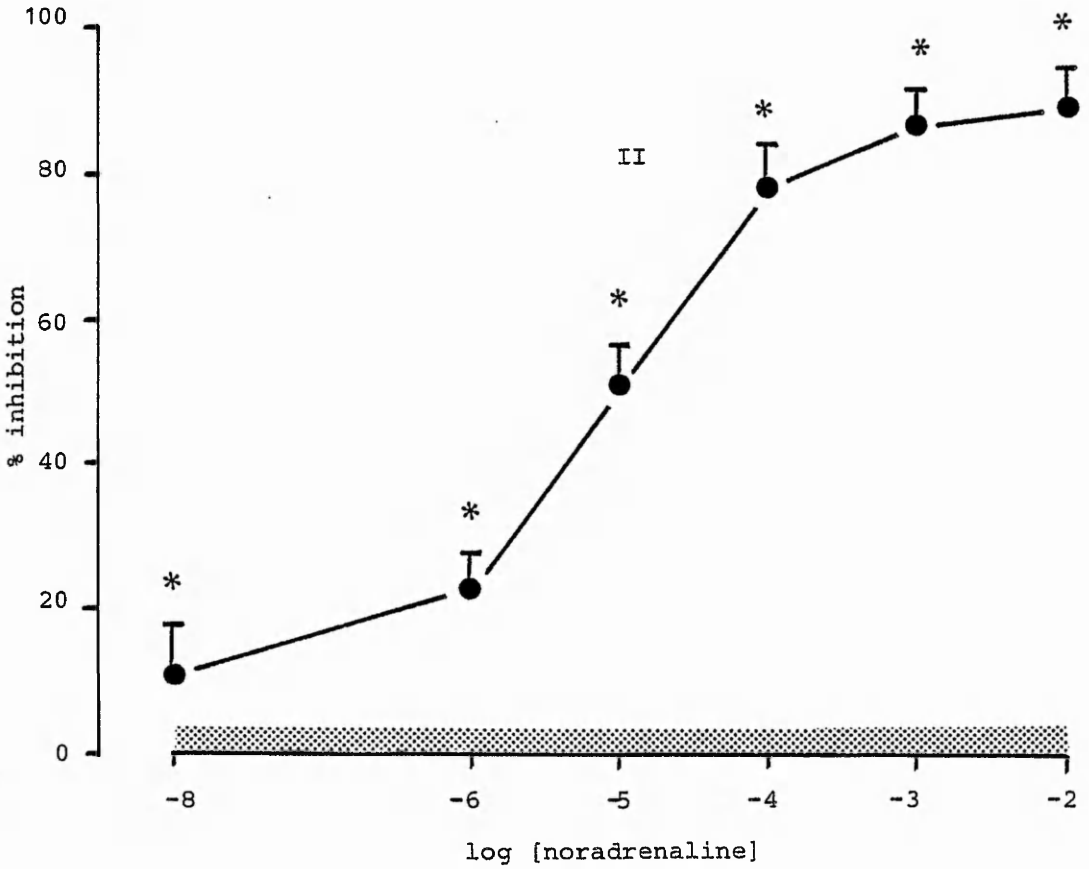
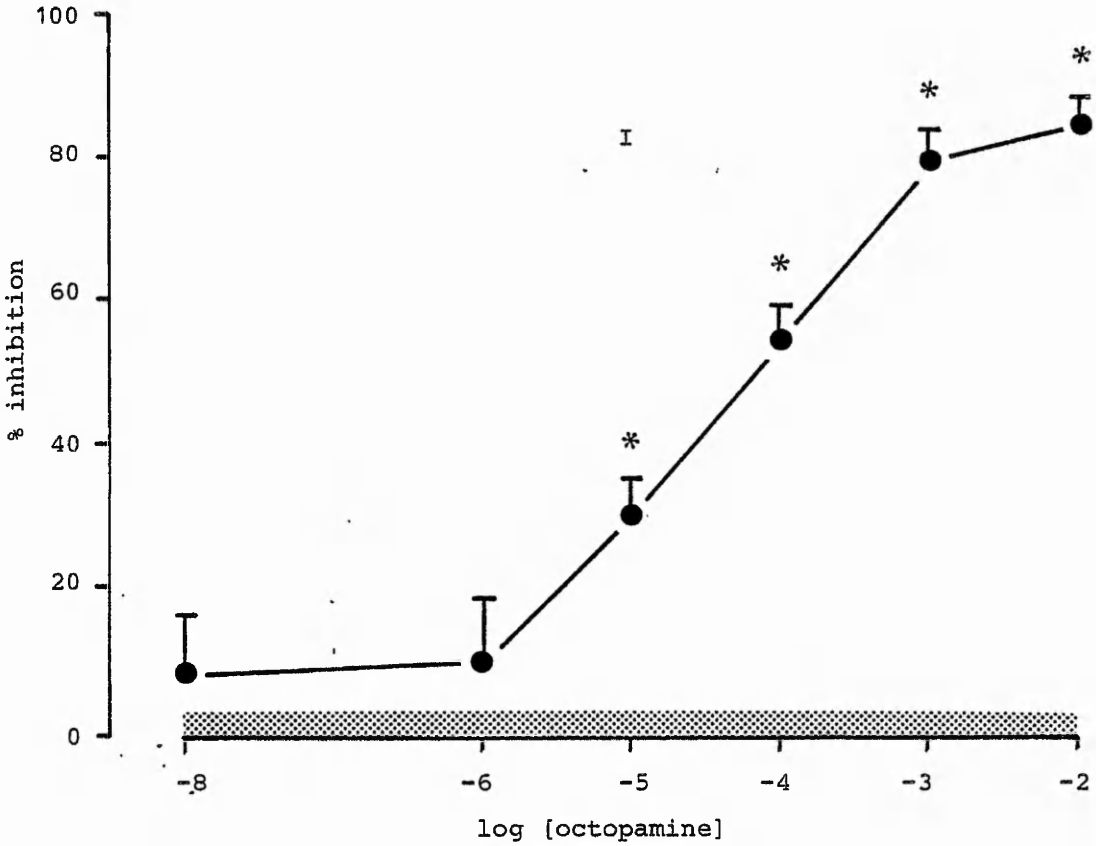

100
80
60
40
20
0
% inhibition
II
*
-8
-6
-5
-4
-3
-2
log [noradrenaline]

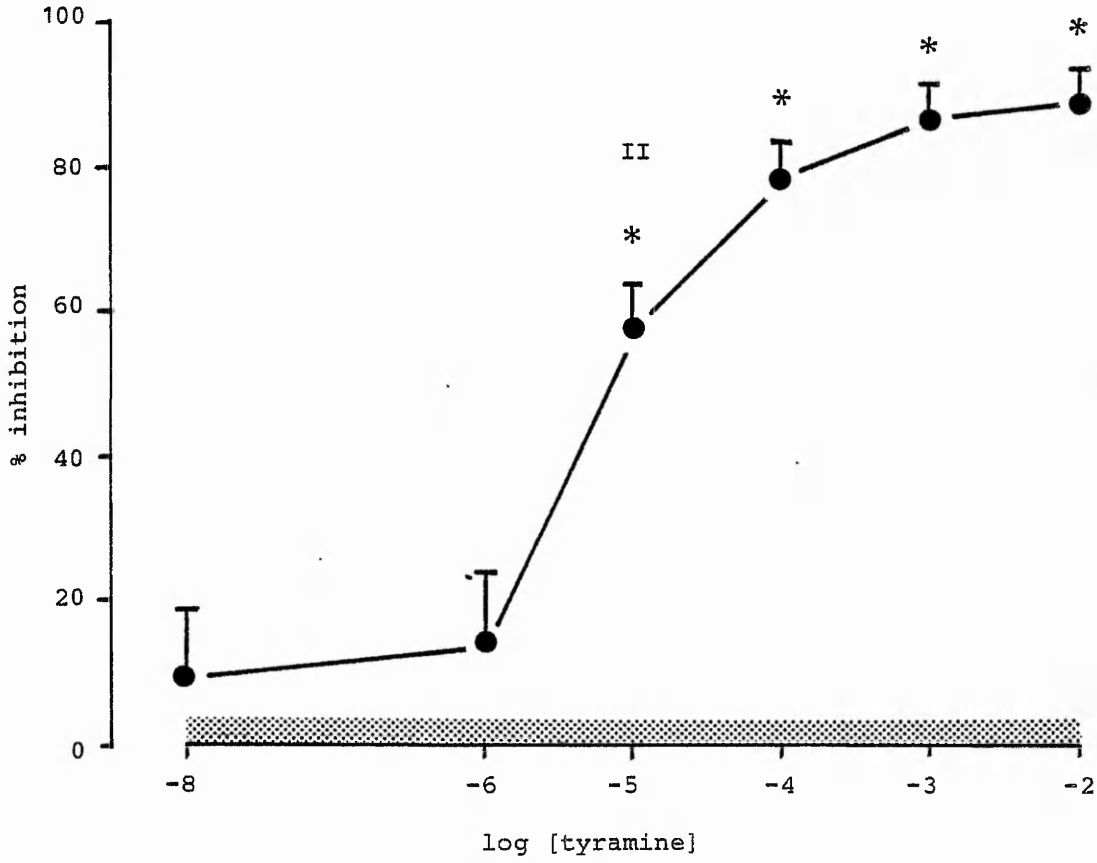
Fig 24.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in slices of rat striatum. Each point is the mean and standard error of at least five determinations. Points marked * are significantly different from the control.

I. Octopamine

II. Tyramine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [octopamine]


100
80
60
40
20
0
% inhibition
II
*
*
*
*
*
-8
-6
-5
-4
-3
-2
log [tyramine]

value of inhibition, obtained experimentally, in striatal slices incubated in 0.1 μM dopamine was 90%. Half this maximum inhibition value was termed the $E_{45}IC_{50}$.

As expected all the drugs investigated produced inhibition of dopamine uptake at 100 μM (c.f. screening experiments). Uptake inhibition was also shown to occur when slices were incubated in 0.1 μM dopamine in the presence of these compounds. The values at which the compounds produced half maximum inhibition are given in Table 8. The decreasing rank order of potency for the inhibition of dopamine uptake in striatal slices (100 μM) was: desipramine > benztropine = tyramine > adrenaline = metaraminol > noradrenaline = 6-hydroxydopamine > isoprenaline > octopamine = hordenine. The decreasing rank order of potency for the inhibition of dopamine uptake in striatal slices at 0.1 μM was: benztropine > noradrenaline = desipramine = metaraminol = tyramine > hordenine > adrenaline = octopamine > isoprenaline > 6-hydroxydopamine.

The values for the $E_{30}IC_{50}$ and $E_{45}IC_{50}$ were calculated using a generalised linear interactive modelling (GLIM) programme on a DEC-20 computer.

The differences in the rank order of potency suggest that two pharmacologically different dopamine uptake systems are present in the striatum.

Table 8. IC_{50} values for the drugs used to inhibit the accumulation of ^{3}H-dopamine (0.1 and 100 µM) in slices of rat striatum. These values were calculated using the GLIM computer programme.

Inhibitor	$E_{30}IC_{50}$ (100 µM)	$E_{45}IC_{50}$ (0.1 µM)
Adrenaline	7.9×10^{-5}M	1.3×10^{-5}M
Benztropine	3.5×10^{-5}M	7.4×10^{-7}M
Desipramine	7.6×10^{-6}M	3.7×10^{-6}M
Hordenine	1.5×10^{-1}M	8.0×10^{-6}M
6-hydroxydopamine	1.5×10^{-4}M	3.2×10^{-4}M
Isoprenaline	2.3×10^{-2}M	9.9×10^{-5}M
Metaraminol	7.9×10^{-5}M	3.9×10^{-6}M
Noradrenaline	1.2×10^{-4}M	3.4×10^{-6}M
Octopamine	1.0×10^{-1}M	2.4×10^{-5}M
Tyramine	2.5×10^{-5}M	4.5×10^{-6}M

Effect of low sodium media on ^{3}H-dopamine uptake by striatal slices

The slices of striatum were preincubated in a modified Krebs' solution at 37°C containing either:

1) a reduced sodium ion content (25 mM) but containing sucrose (220 mM) to maintain tonicity.
2) a reduced sodium ion content (25 mM) but containing choline chloride (118 mM) to maintain tonicity.

After 5 min preincubation ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 100 µM or 0.1 µM) were added. These solutions were prepared in media identical to those used for the respective preincubation stage. Incubation was for 2 min. Control slices were incubated in normal Krebs' medium at the same time as the test systems. The percentage inhibition of uptake was calculated relative to these control values. See Fig. 25.

Incubating in the modified low sodium solution containing sucrose did not inhibit accumulation of ^{3}H-dopamine at an incubation concentration of 100 µM dopamine. However replacement of the sodium ions with choline chloride resulted in significant inhibition of ^{3}H-dopamine uptake at this concentration. Incubation of the striatal slices at 0.1 µM dopamine concentration in either low sodium medium resulted in significant inhibition of ^{3}H-dopamine uptake. The inhibition was significantly greater for the medium containing choline chloride than for the medium containing sucrose.

Fig 25.

Inhibition of the accumulation of ^{3}H-dopamine in slices of rat striatum. Each point is the mean and standard error of at least six determinations.

I. Accumulation at 100 µM dopamine

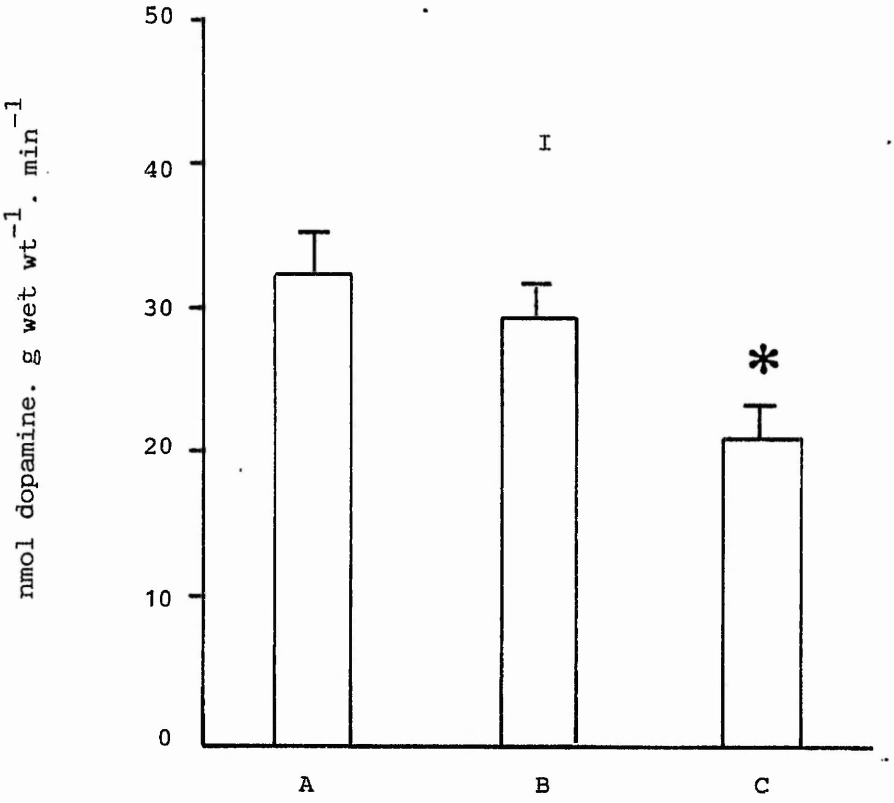
II. Accumulation at 0.1 µM dopamine

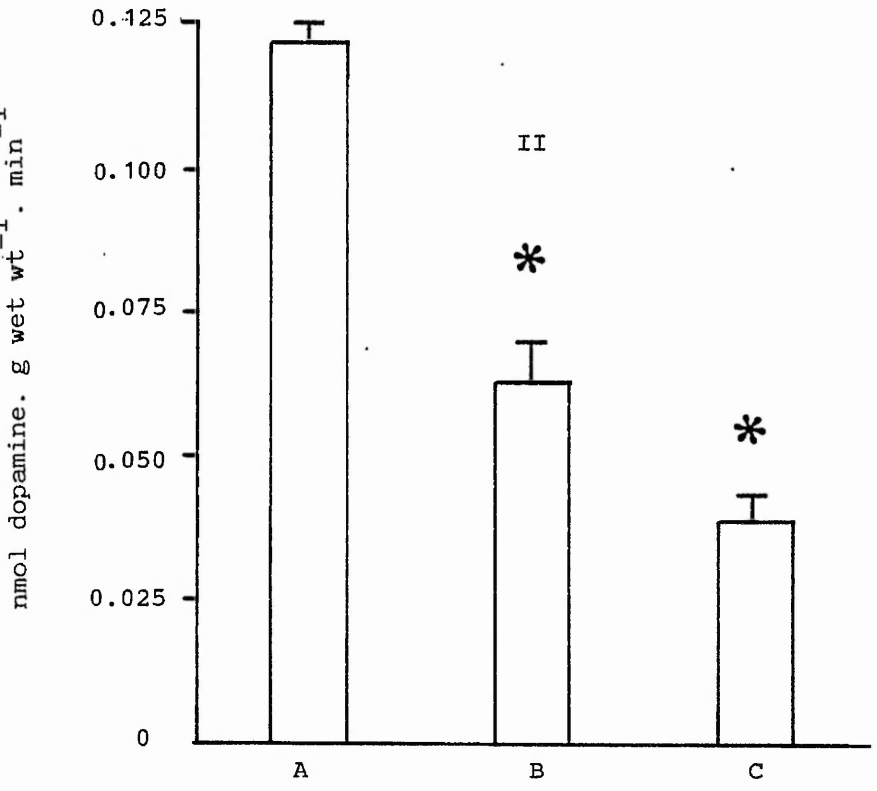
(A) control

(B) low sodium (25mM) medium containing sucrose (220mM)

(C) low sodium (25mM) medium containing choline chloride (118mM)

Points marked * are significantly different from the control.


nmol dopamine. g wet wt^{-1}. min^{-1}
50
40
30
20
10
0
I
*
A
B
C


nmol dopamine. g wet wt^{-1}. min^{-1}
0.125
0.100
0.075
0.050
0.025
0
II
*
*
A
B
C

Effect of 2,4-dinitrophenol on ^{3}H-dopamine uptake by striatal slices

The slices of striatum were preincubated in Krebs' solution at 37°C containing 10^{-3} M 2,4-dinitrophenol (DNP), a metabolic inhibitor. After 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 100 µM or 0.1 µM) were added. Incubation was continued for 2 min. The results are displayed graphically in Fig. 26.

Incubation of the striatal slices with dopamine in the presence of DNP resulted in significant inhibition of dopamine uptake at both of the concentrations investigated.

Effect of temperature on ^{3}H-dopamine uptake by striatal slices

After 5 min preincubation in ice-cold Krebs' solution ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM were added. Incubation was continued for 2 min at 0°C. Control experiments incubated at 37°C were carried out simultaneously. See Table 9.

The reduction of the temperature of incubation from 37°C to 0°C resulted in significant inhibition of dopamine uptake at all the concentrations studied. The percentage inhibition of uptake increased with decreasing dopamine concentration and ranged from 79% inhibition at 500 µM dopamine to 95% inhibition at 0.1 µM dopamine.

Fig 26.

Inhibition of the accumulation of ^{3}H-dopamine in slices of rat striatum. Each point is the mean and standard error of at least six determinations.

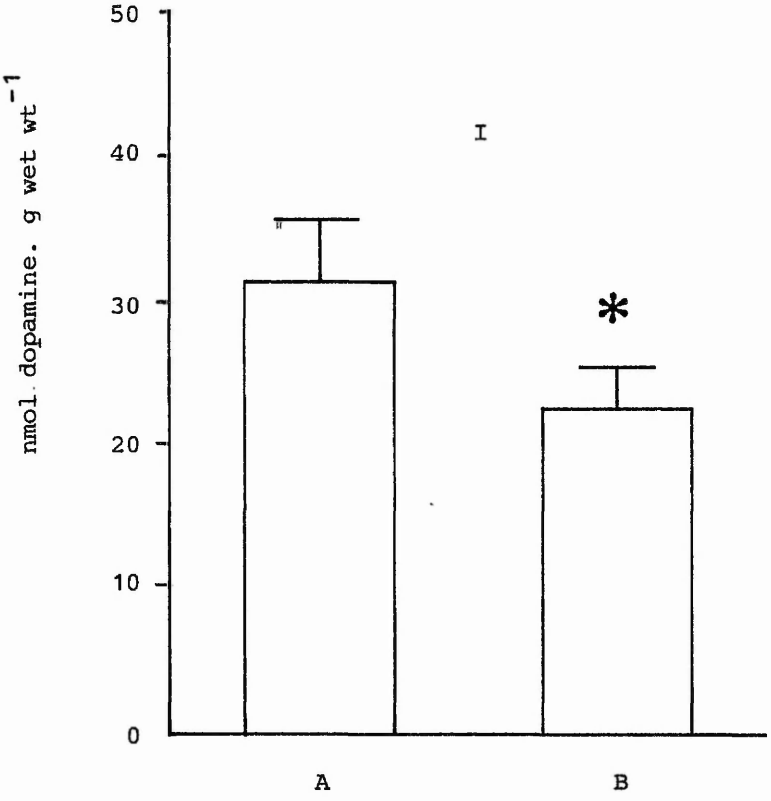
I. Accumulation at 100 μM dopamine

II. Accumulation at 0.1 μM dopamine

(A) control

(B) 2,4-dinitrophenol (10^{-3} M)

Points marked * are significantly different from the control.


50
40
30
20
10
0
nmol dopamine. g wet wt^{-1}
I
*
A
B

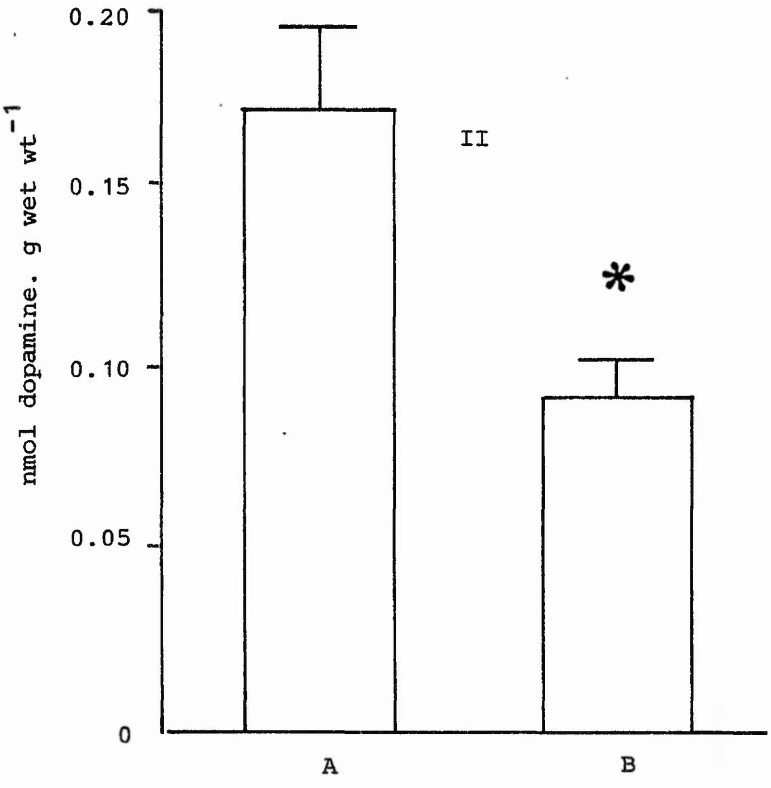

0.20
0.15
0.10
0.05
0
nmol dopamine. g wet wt^{-1}
II
*
A
B

Table 9. Values for the accumulation of tritium, from ^{3}H-dopamine, in slices of striatum incubated at 0°C and 37°C. Experimental data are mean ± standard error. Percentage inhibition as compared to uptake at 37°C is also presented.

Dopamine conc. (μM)	Uptake at 37°C (nmol.g wet $wt^{-1}.min^{-1}$)	Uptake at 0°C (nmol.g wet $wt^{-1}.min^{-1}$)	Inhibition %
0.1	0.12± 0.01	0.006 ± 0.001	95
1.0	0.88 ± 0.06	0.062 ± 0.01	93
10.0	5.82 ± 0.42	0.409 ± 0.09	93
100.0	30.44 ± 1.6	3.653 ± 0.40	88
500.0	93.16 ± 7.1	19.563 ± 5.10	79

Effect of 6-hydroxydopamine pretreatment on ^{3}H-dopamine uptake by striatal slices

Slices of striatum from rats pretreated 5 days before with 6-hydroxydopamine (6-OHDA), 250 µg administered i.c.v. were pre-incubated for 5 min in Krebs' solution at 37°C. Following the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM) incubation was continued for a further 2 min. Enough tissue was taken from each rat to provide 2 slices of tissue for incubation at each of the concentrations of dopamine studied. Control slices of striatum from untreated rats were investigated concomitantly, as were slices of striatum from sham operated rats given an i.c.v. injection of the vehicle (0.9% saline).

Plotting the values of S/v obtained against S gave Fig. 27. The graphs were of the characteristic curved shape which begins to level out with increasing substrate concentration. The graphs for the values of S/v obtained from the control and sham operated animals were very similar. The data derived for the 6-OHDA treated animals produced a curve which was displaced above the other curves, as a result of a reduction in uptake. The S/v values for the 6-OHDA treated rats were significantly different from the control values at dopamine concentrations of 0.1, 1.0 and 10 µM. None of the S/v values for the sham operated rats varied significantly from their respective control values.

Fig 27.

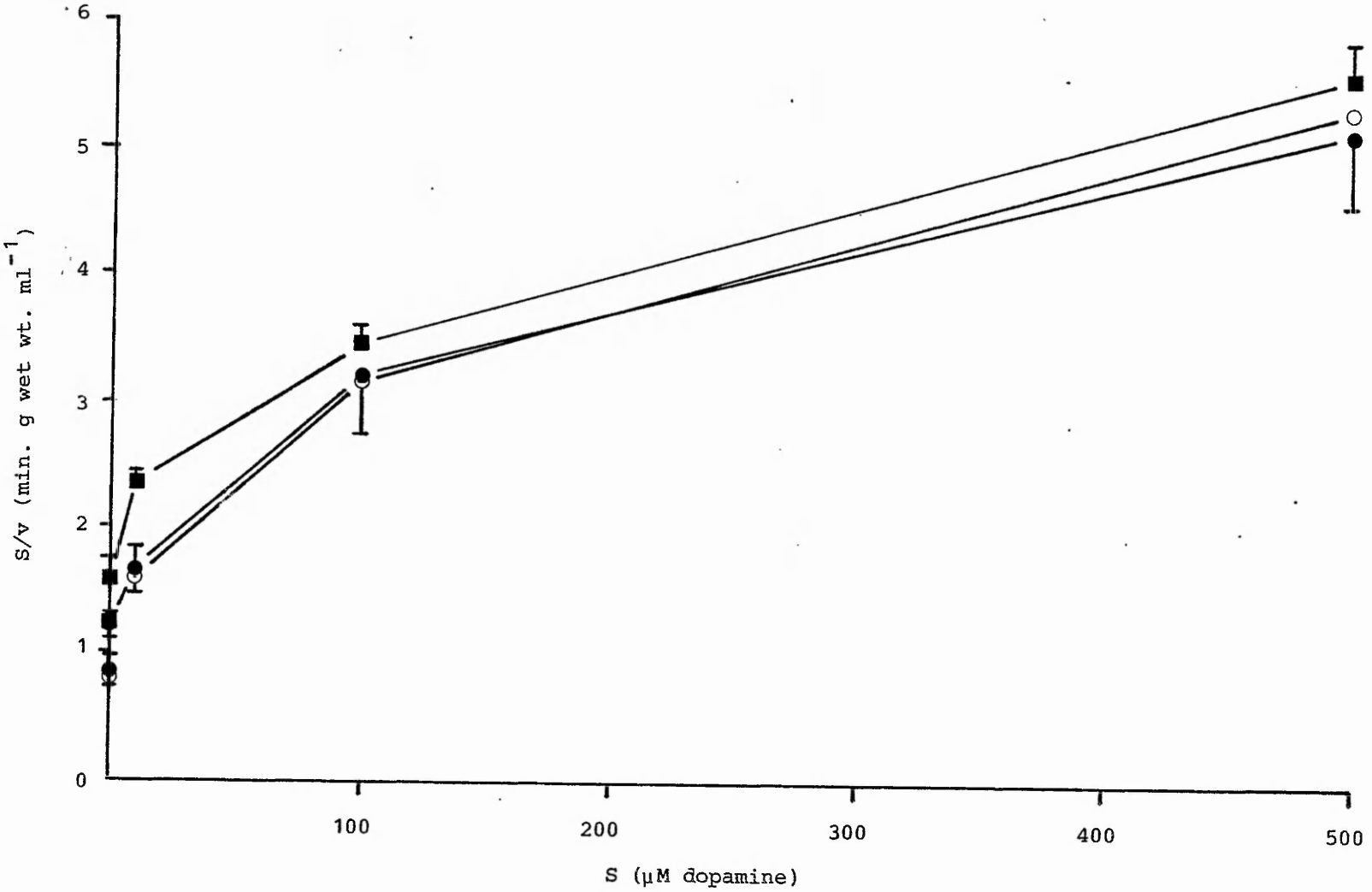
S/v against S graph for the accumulation of tritium (^{3}H-dopamine) by slices of rat striatum, from:

●——● control

○——○ sham operated

■——■ 6-hydroxydopamine pre-treated

groups of animals. Each point is the mean and standard error of at least four determinations.


S/v (min. g wet wt. ml^{-1})
0
1
2
3
4
5
6
100
200
300
400
500
S (µM dopamine)

The data were analysed kinetically. The three parameter model gave values for the fit which were not considered a 'good fit' to the experimental values for all three of the preparations studied. The five parameter model gave a fit of less than 0.2 standard errors for the control data and the data from the sham operated animals. The data from the 6-OHDA treated animals had a fit of less than 0.15 standard errors, hence all three were considered a 'good fit'. The Km and Vmax values for the high affinity system were similar for the control and sham operated animals, but the Km for the 6-OHDA treated animals showed a 10 fold increase and the Vmax more than doubled. The Km and Vmax values for the low affinity system were very similar for the control and sham operated animals. The value of the Km for the low affinity system of the animals given 6-OHDA was three times greater than the control value. The Vmax value was approximately double. The diffusion constant was slightly lower than for the 6-OHDA treated animals compared to the controls and sham operated animals. See Tables 10 to 12.

Effect of 6-OHDA on the uptake of ^{3}H-noradrenaline by striatal slices

Rats were pretreated with 6-OHDA as above. After 5 days the animals were sacrificed. Striatal slices were preincubated in Krebs' solution at 37°C for 5 min. Following the addition of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-noradrenaline (final concentration 300 nCi.ml^{-1}; 0.1 - 500 µM) incubation was continued for a further 2 min. The cellular accumulation of tritium at all concentrations of ^{3}H-noradrenaline was measured using slices from an individual brain. Control slices from untreated rats were studied simultaneously.

Table 10. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in slices of striatum from control rats on the 6-OHDA study. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g wet wt.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.870 ± 0.092	1.019	0.872
1.0	1.250 ± 0.104	1.078	1.261
10.0	1.686 ± 0.177	1.651	1.673
100.0	3.210 ± 0.464	3.733	3.280
500.0	5.170 ± 0.573	4.826	5.242
(n = 6)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		8.636	21.475
Km (μM)		10.709	39.372
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.133
Km' (μM)			0.193
D (ml.g wet wt^{-1}min^{-1})		0.192	0.154
Fit (s.e.)		>1	<0.25

Table 11. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in slices of striatum from sham operated rats on the 6-OHDA study. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit.

Conc. (μM)	Exp. S/v (min.g wet wt.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.785 ± 0.046	1.030	0.886
1.0	1.285 ± 0.055	1.091	1.291
10.0	1.632 ± 0.061	1.610	1.634
100.0	3.182 ± 0.185	3.551	3.210
500.0	5.356 ± 0.409	4.603	5.396
(n = 4)			
Vmax (nmol.g wet wt.$^{-1}$.min^{-1})		9.282	24.39
Km (μM)		11.930	42.60
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.083
Km' (μM)			0.098
D (ml.g wet wt^{-1}.min^{-1})		0.201	0.141
Fit (S.E.)		>1	<0.2

Table 12. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in slices of striatum from 6-OHDA pre-treated rats. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g wet wt.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	1.246 ± 0.152	1.493	1.244
1.0	1.613 ± 0.104	1.580	1.621
10.0	2.378 ± 0.097	2.253	2.382
100.0	3.514 ± 0.171	4.032	3.537
500.0	5.628 ± 0.311	4.692	5.662
(n = 18)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		4.96	38.22
Km (μM)		10.53	134.30
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.43
Km' (μM)			0.97
D (ml.g wet wt^{-1}.min^{-1})		0.205	0.117
Fit (s.e.)		>1	<0.15

Plotting S/v against S gave Fig. 28. The characteristic curved graph levelling out with increasing values of S was produced, however the value of S/v sharply decreased between 0.1 and 1.0 μM (S) prior to the development of the curve. The values of S/v for the 6-OHDA treated animals were higher than the corresponding values for the controls, but only at 0.1 μM (S) were the points significantly different. The results were analysed kinetically and the control data found to be a good fit to the five parameter model, less than 0.2 standard errors. The data from the 6-OHDA treated animals was incompatible with any of the models used for analysis. See Tables 13 and 14.

Effect of Pargyline on the uptake of ^{3}H-dopamine by striatal slices

Slices of rat striatum were preincubated in Krebs' solution at 37°C containing pargyline (an inhibitor of both types of monoamine oxidase) at a concentration in the range 10^{-8} to 10^{-3}M. After 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 or 100 μM) was added. The incubation was continued for a further 2 min. Control slices were incubated at the same time in Krebs' solution containing no drug. Percentage inhibition was calculated relative to these control values. See Fig. 29.

Pargyline had no significant effect on dopamine uptake by striatal slices at either of the concentrations studied.

Fig 28.

S/v against S graph of the accumulation of tritium (^{3}H-noradrenaline) by slices of rat striatum, from:

● ——— ● control

■ ——— ■ 6-hydroxydopamine pre-treated

groups of animals. Each point is the mean and standard error of at least six determinations.

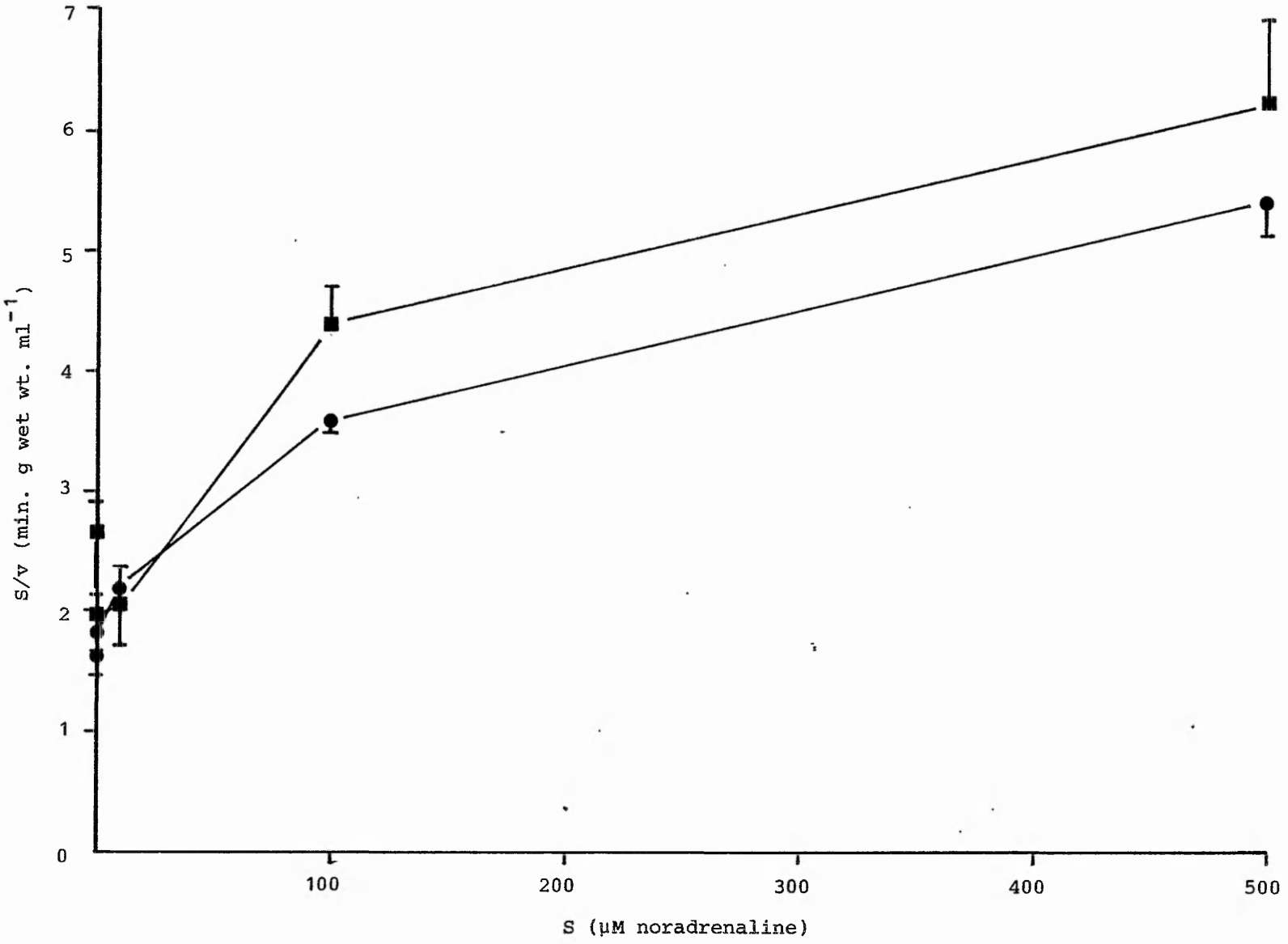

7
6
5
4
3
2
1
0
100
200
300
400
500
S/v (min. g wet wt. ml^{-1})
S (μM noradrenaline)

Table 13. Values of S/v for the accumulation of tritium, from ^{3}H-noradrenaline, in slices of striatum from control rats on the 6-OHDA study. Experimental data are meam ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (µM)	Exp. S/v (min.g wet $wt^{-1}.ml^{-1}$)	Cal S/v No. of parameters	
		3	5
0.1	1.612 ± 0.132	1.758	1.616
1.0	1.822 ± 0.144	1.785	1.821
10.0	2.203 ± 0.150	2.039	2.233
100.0	3.576 ± 0.078	3.610	3.592
500.0	5.389 ± 0.245	5.404	5.435
(n = 6)			
Vmax (nmol.g wet $wt^{-1}.min^{-1}$)		17.922	21.705
Km (µM)		42.936	64.170
Vmax' (nmol.g wet $wt^{-1}.min^{-1}$)			0.133
Km' (µM)			0.879
D (ml.g wet $wt^{-1}.min^{-1}$)		0.153	0.147
Fit (s.e.)		>1	<0.2

Table 14. Values of S/v for the accumulation of tritium, from ^{3}H-noradrenaline, in slices of striatum from 6-OHDA pre-treated rats. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g wet $wt^{-1}.ml^{-1}$)	Cal S/v No. of parameters 3
0.1	2.673 ± 0.281	2.081
1.0	1.982 ± 0.163	2.114
10.0	2.039 ± 0.340	2.428
100.0	4.418 ± 0.316	4.332
500	6.258 ± 0.696	6.439
(n = 18)		
Vmax (nmol.g wet $wt^{-1}.min^{-1}$)		14.467
Km (μM)		41.023
D (ml.g wet $wt^{-1}.min^{-1}$)		0.130
Fit (s.e.)		>1

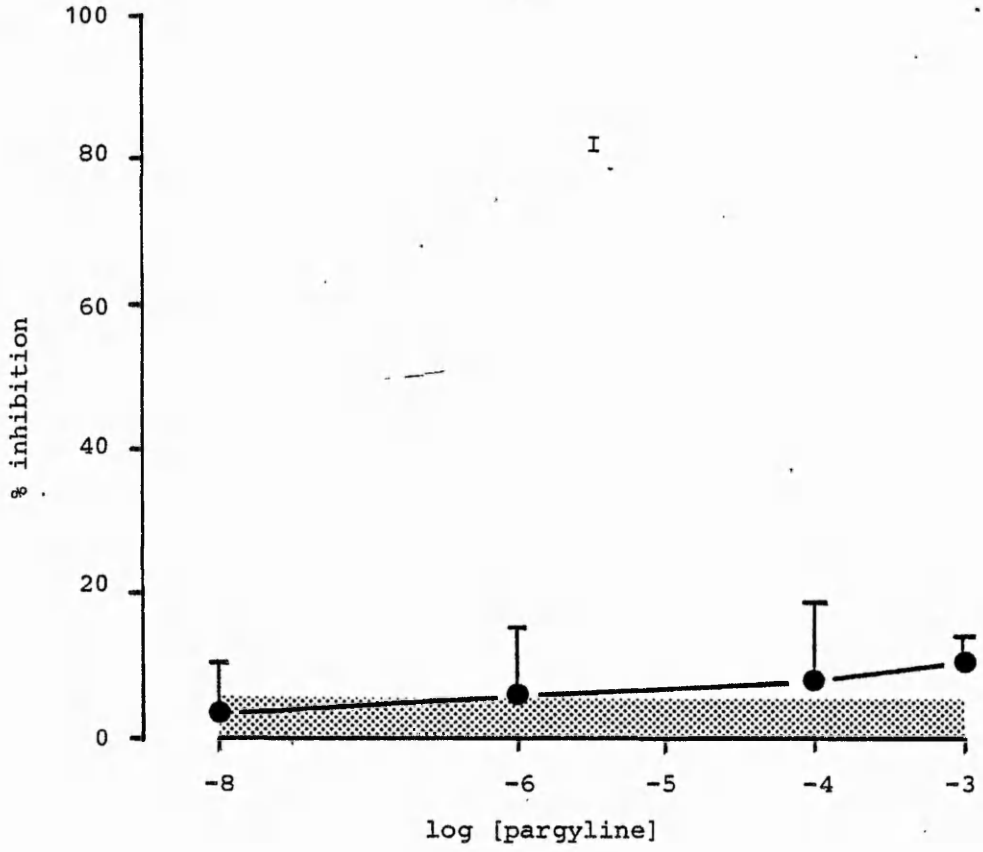
Fig 29.

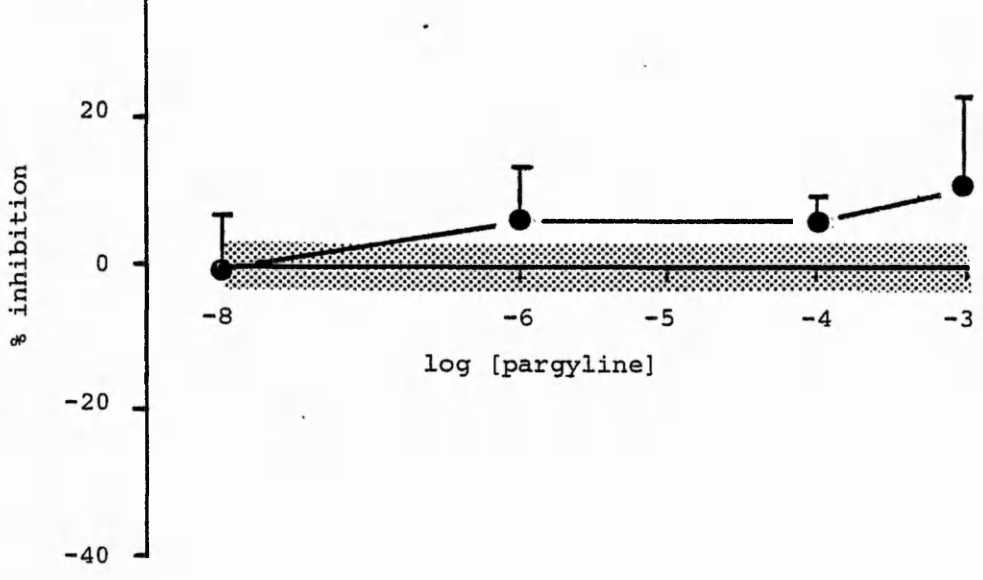
Inhibition of the accumulation of ^{3}H-dopamine in slices of rat striatum, by pargyline. Each point is the mean and standard error of at least six determinations.

I. Accumulation at 100 μM dopamine.

II. Accumulation at 0.1 μM dopamine.

Points marked * are significantly different from the control.


100
80
60
40
20
0
I
% inhibition
-8
-6
-5
-4
-3
log [pargyline]


60
40
20
0
-20
-40
II
% inhibition
-8
-6
-5
-4
-3
log [pargyline]

Effect of DOPAC and HVA on ^{3}H-dopamine uptake by striatal slices

Following preincubation for 5 min in Krebs' solution at 37°C containing either 3,4-dihydroxyphenylacetic acid (DOPAC) or homo vanillic acid (HVA) in the concentration range 10^{-3} to 10^{-8}M ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi^{3} ml^{-1}; 0.1 or 100 µM) were added. The incubation was continued for a further 2 min. Percentage inhibition was calculated using appropriate controls. See Figs. 30 and 31. HVA had no significant effect on dopamine uptake by striatal slices at either of the incubation concentration studied. DOPAC had no significant effect on dopamine uptake at the 0.1 µM dopamine concentration, but significantly inhibited dopamine (100 µM) uptake at 10^{-4} and 10^{-3}M, maximum inhibition was 23%.

Uptake of ^{3}H-dopamine by isolated striatal cells

Isolated cells were prepared by the mechanical disruption of brain tissue through a series of nylon meshes of varying size. The resulting suspension was incubated in Krebs' solution at 37°C for 5 min prior to the addition of ^{14}C-sorbitol and ^{3}H-dopamine. The cells were separated from the incubation medium by passing them in suspension in the media through a nitrocellulose filter, pore size 8.0 µM.

Passive accumulation and binding of ^{14}C-sorbitol and ^{3}H-dopamine by the filter membrane was assessed. The experiment was carried out as above but in the absence of any cells. Five minutes after the addition of the radioactive compounds a sample of the labelled

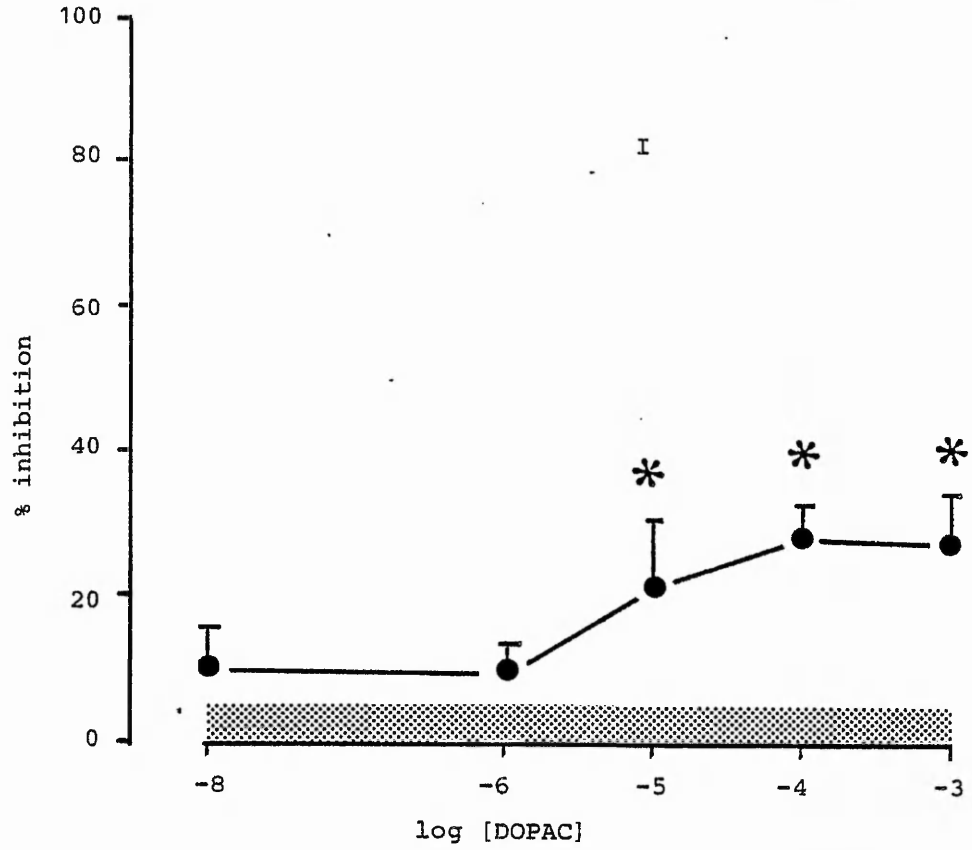
Fig 30.

Inhibition of the accumulation of ^{3}H-dopamine (100 μM) in slices of rat striatum. Each point is the mean and standard error of at least six determinations.

I. DOPAC

II. HVA

Points marked * are significantly different from the control.


100
80
60
40
20
0
% inhibition
I
*
*
*
-8
-6
-5
-4
-3
log [DOPAC]

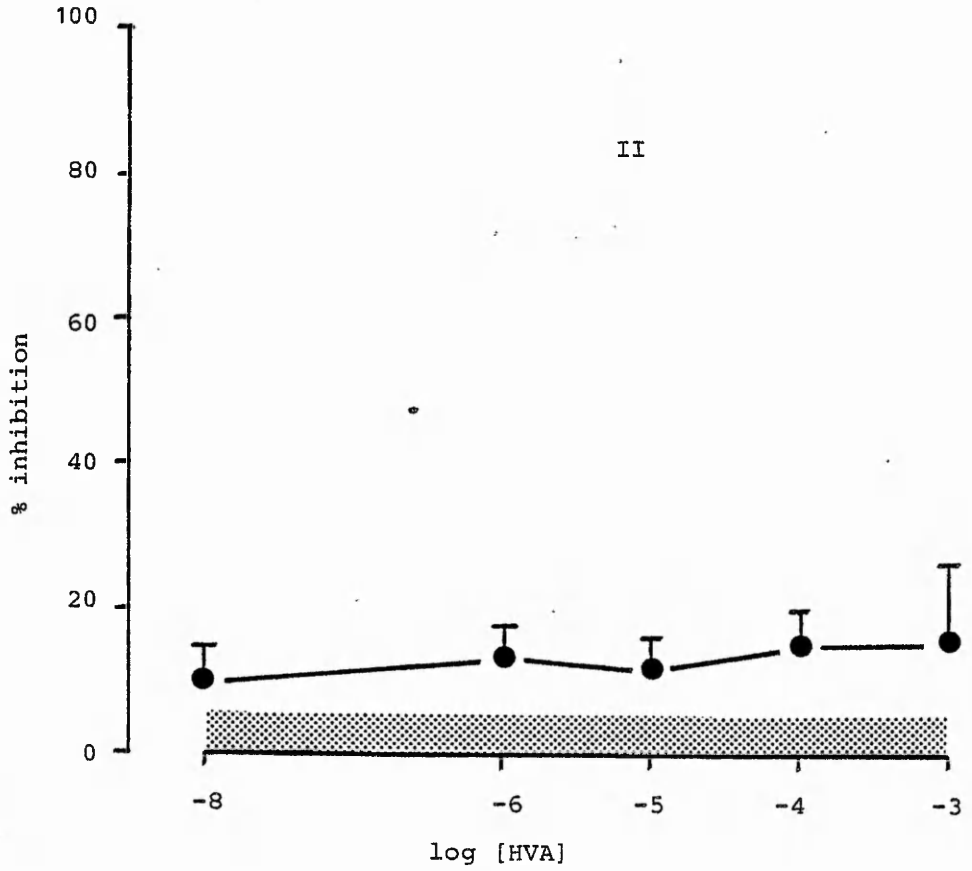

100
80
60
40
20
0
% inhibition
II
-8
-6
-5
-4
-3
log [HVA]

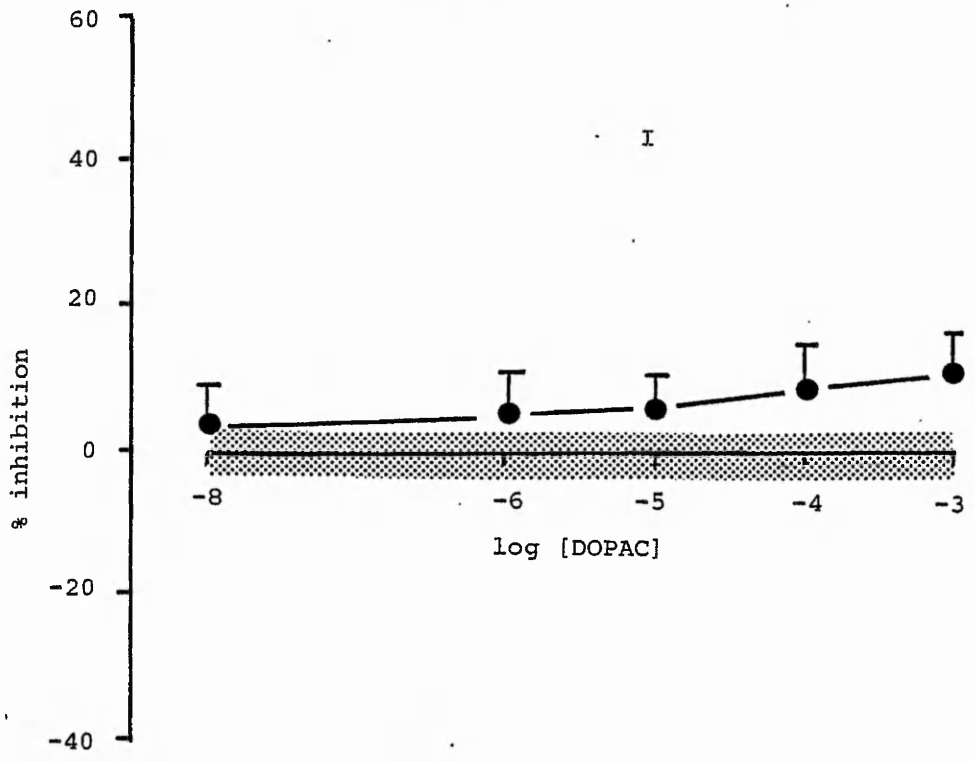
Fig 31.

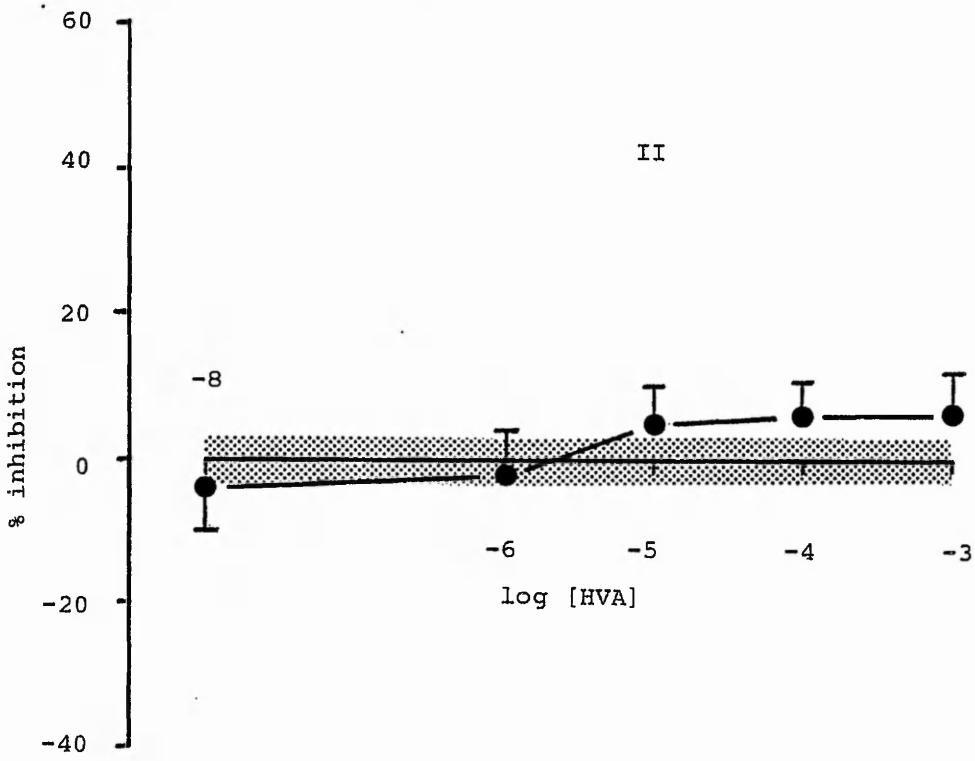
Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in slices of rat striatum. Each point is the mean and standard error of at least six determinations.

I. DOPAC

II. HVA

Points marked * are significantly different from the control.


60
40
20
0
-20
-40
% inhibition
I
-8
-6
-5
-4
-3
log [DOPAC]


60
40
20
0
-20
-40
% inhibition
II
-8
-6
-5
-4
-3
log [HVA]

medium was passed through the filter as in the experiments. The amount of tritium and carbon-14 retained on the filters was measured . See Table 15.

There were no significant differences in the results at $P<0.05$ level using the F-test. Results suggest that account had to be taken of label binding to the filter hence ^{14}C-sorbitol was still used as a basis for calculating the background binding.

Time course for the uptake of ^{3}H-dopamine by isolated striatal cells

Following a 5 min preincubation in Krebs' solution at 37°C, ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 and 500 µM) were added to the striatal cell suspension. The time course for the incubations was in the range 0.5 - 5.0 min. The radioactivity present in the cells and the media were determined as described previously. The protein content of the cells was also determined and the tritium content of the cells expressed in terms of nmol dopamine.g protein^{-1}. The amount of tritium present in the medium retained (both in the extra cellular spaces and on the nitrocellulose filters) was estimated from the amount of carbon-14 retained. This value was then subtracted from the total amount of tritium measured. The time courses for the uptake of ^{3}H-dopamine at the two incubation concentrations studied are given in Fig. 32.

The uptake of dopamine in isolated striatal cells was seen to be linear for at least 5 min at 500 µM and 5 min at 0.1 µM dopamine.

Table 15. Values for the accumulation of tritium from ^{3}H-dopamine and carbon-14 from ^{14}C-sorbitol, by nitrocellulose filters. Experimental data are mean ± standard error.

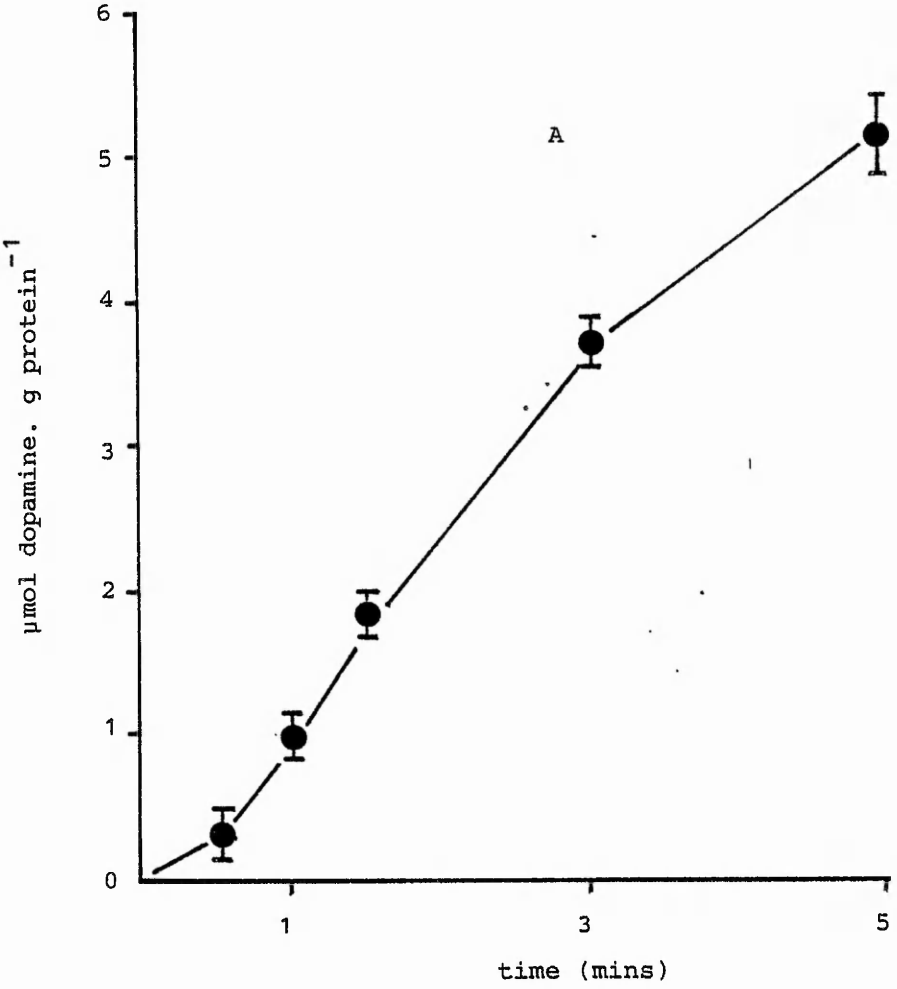
^{3}H-dopamine (μM)	(nmol.g wet wt filter)		
	^{3}H-dopamine	^{14}C-sorbitol	^{3}H/^{14}C
0.1	7.27 ± 0.36	6.52 ± 0.75	1.11
1.0	6.41 ± 0.68	5.69 ± 0.77	1.13
10.0	5.82 ± 0.80	5.59 ± 1.04	1.04
100.0	7.25 ± 0.97	6.60 ± 0.45	1.10
500.0	6.41 ± 0.44	5.55 ± 0.49	1.15

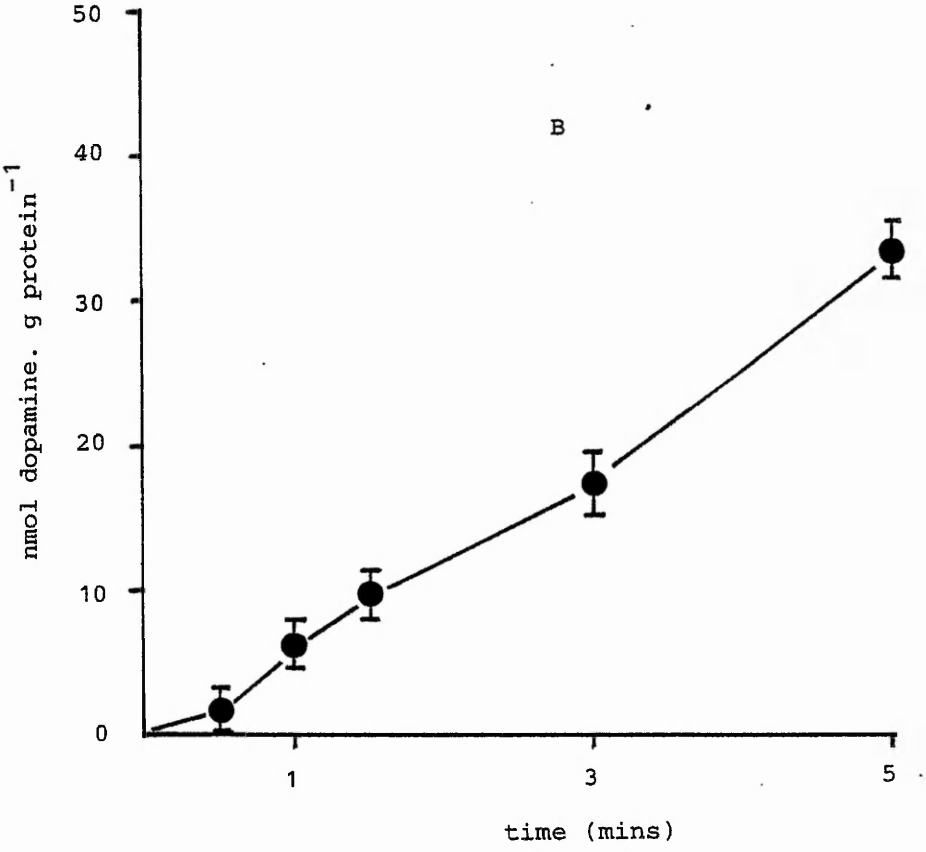
Fig 32.

Time course for the accumulation of ^{3}H-dopamine by isolated striatal cells. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 µM

B. Accumulation at 0.1 µM


A
µmol dopamine. g protein^{-1}
0
1
2
3
4
5
6
1
3
5
time (mins)


B
nmol dopamine. g protein^{-1}
0
10
20
30
40
50
1
3
5
time (mins)

Kinetics of the uptake of ^{3}H-dopamine by isolated striatal cells

Following a 5 min preincubation period in Krebs' solution at 37°C the cells were incubated for a further 5 min in the presence of ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 to 500 µM). Uptake in cells was measured concomitantly for all concentrations of substrate by pooling cells from 3 different animals. The period of incubation (5 min) was determined from the linear portion of the time course. The initial rate of uptake was calculated in terms of nmol.g protein^{-1}. min^{-1}. Plotting the values of S/v against S gave Fig. 33.

The graph showed the characteristic curved shape levelling out with increasing values of S. However the graph never reaches a point where it becomes parallel to the x axis. The experimental data were then analysed kinetically. The three parameter model gave values for the fit which were not considered a 'good fit'. The five parameter model produced a fit of less than 0.5 standard errors, a 'good fit'. See Table 16.

Inhibition studies using isolated striatal cells

After 5 min preincubation in Krebs' solution at 37°C, which contained the drug under test at a concentration in the range 10^{-3}M to 10^{-8}M, ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 µM or 10 µM) were added. A dopamine concentration of 10 µM was selected as at this concentration the theoretical maximum inhibition is 55%, of

Fig 33.

S/v against S graph of the accumulation of tritium (^{3}H-dopamine) by isolated striatal cells. Each point is the mean and standard error of at least nine determinations.

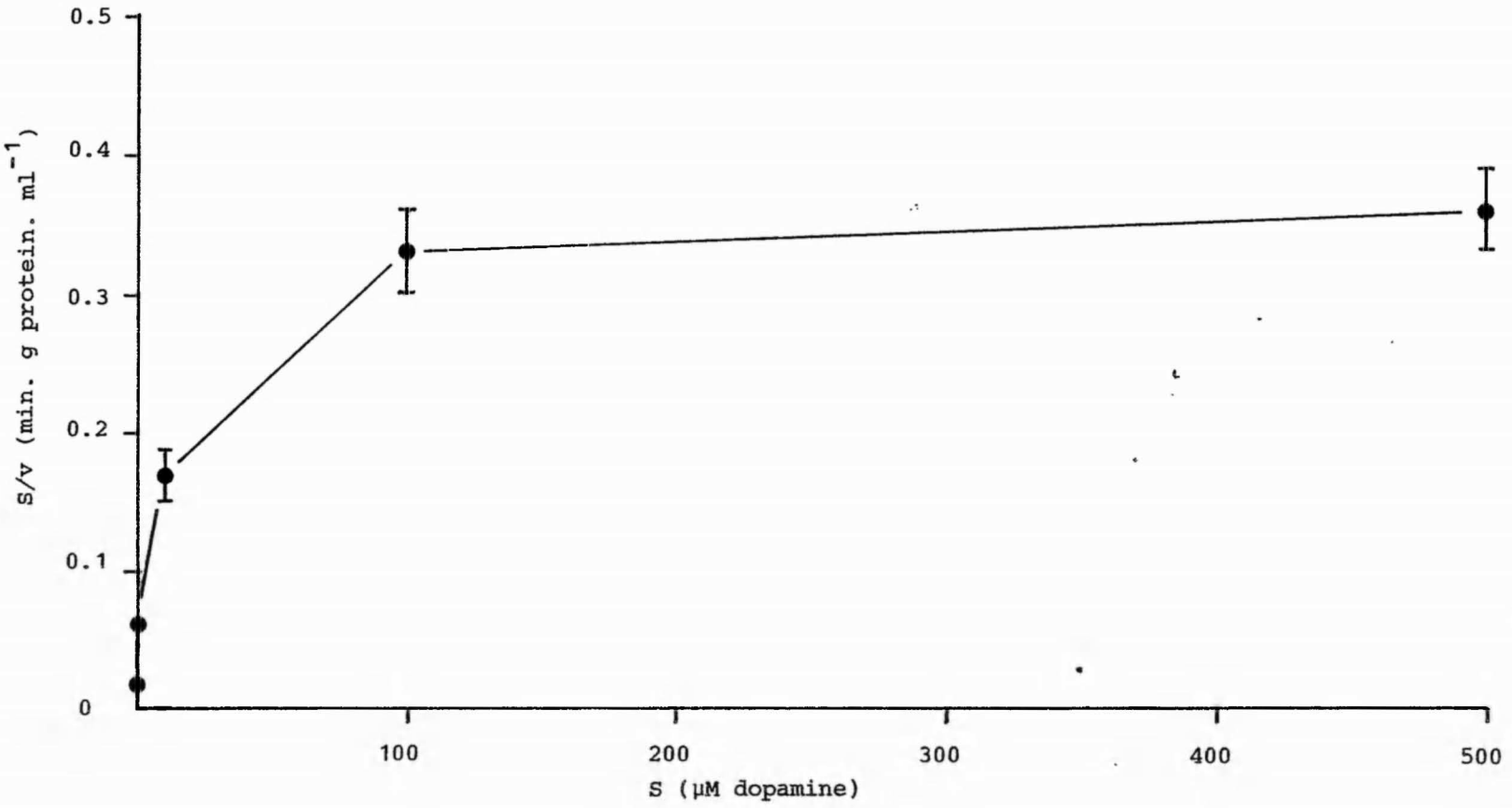

0.5
0.4
0.3
0.2
0.1
0
S/v (min. g protein. ml^{-1})
100
200
300
400
500
S (µM dopamine)

Table 16. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in an isolated cell preparation of striatal tissue. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g protein.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.018 ± 0.002	0.018	0.019
1.0	0.063 ± 0.009	0.048	0.064
10.0	0.170 ± 0.019	0.19	0.17
100.0	0.333 ± 0.030	0.33	0.33
500.0	0.363 ± 0.028	0.36	0.37
(n = 9)			
Vmax (nmol.g protein^{-1}.min^{-1})		25.81	36.33
Km (μM)		0.418	3.29
Vmax' (nmol.g protein^{-1}.min^{-1})			4.746
Km (μM)			0.015
D (ml.g protein^{-1}.min^{-1})		2.78	2.65
Fit (s.e.)		>1	<0.5

which the low affinity system contributes 47% and the high affinity system 8%. At a concentration of 0.1 µM dopamine the contributions are 20% (low affinity) and 76% (high affinity) i.e. a theoretical maximum inhibition of 96%, see Table 17. Incubation was continued for a further 5 min in the presence of the radiolabelled compounds. An aliquot of the suspension was removed and the cells separated by the use of a nitrocellulose filter. Uptake was measured concomitantly for all concentrations of substrate by pooling cells from 3 different animals. Control experiments were carried out simultaneously. The variability of the control values within the isolated striatal cell experiments was estimated by the same technique that was used in the inhibition experiments involving striatal slices, see Page 81. The standard error of the control values from the dopamine uptake experiments using isolated striatal cells was ± 6.0% (0.1 µM dopamine) and ± 3.6% (10 µM dopamine). This value is displayed on the graphs as a shaded area. See Figs. 34 to 37.

The maximum inhibition obtained experimentally was 92% (at 0.1 µM dopamine) and 81% (at 10 µM dopamine). The uptake of dopamine (0.1 µM) in isolated striatal cells was significantly inhibited in a concentration dependant manner by all four compounds studied. However at 10 µM dopamine, benztropine had no significant effect on the uptake of dopamine. The remaining three compounds, desipramine, octopamine and noradrenaline significantly inhibited dopamine uptake.

Table 17. Values for the percentage contributions of the two uptake systems plus diffusion for ^{3}H-dopamine uptake in isolated striatal cells. Values are determined from the kinetic data presented in Table 16.

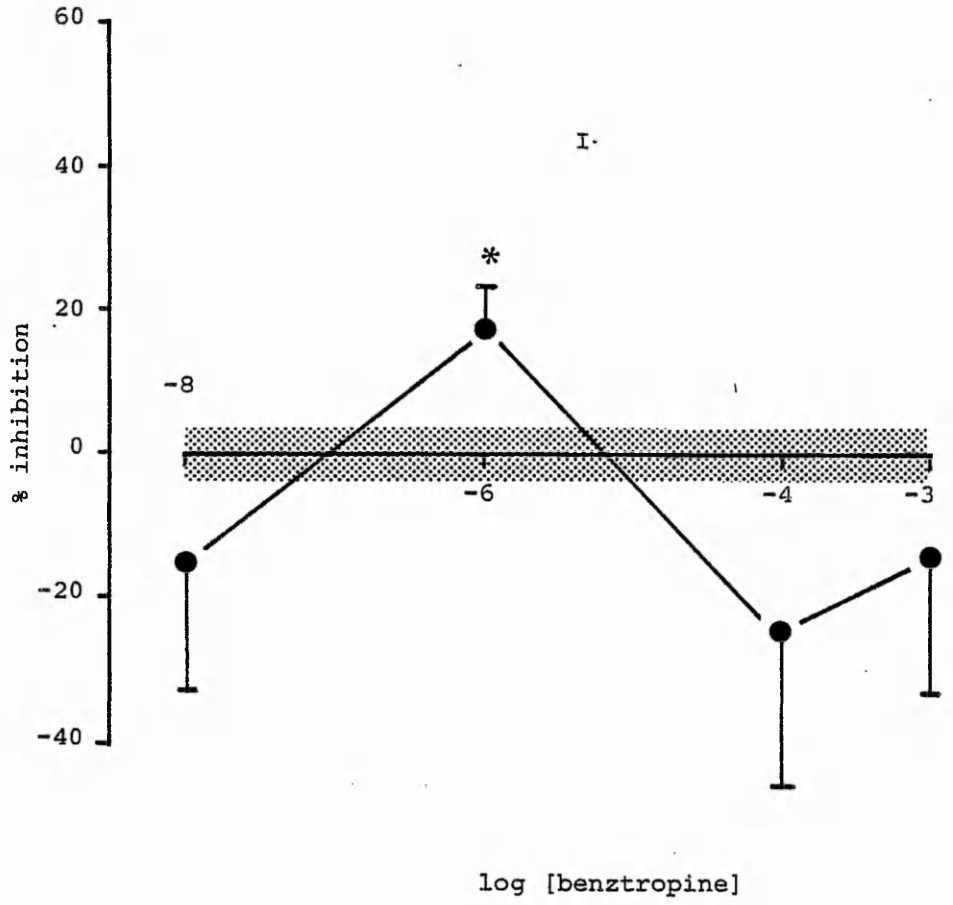
Dopamine conc.	% contribution		
(μM)	high affinity	low affinity	diffusion
0.1	75.73	19.63	4.84
1.0	29.60	53.64	16.76
10.0	8.09	46.70	45.21
100.0	1.56	11.55	86.90
500.0	0.35	2.65	97.00

Fig 34.

Inhibition of the accumulation of ^{3}H-dopamine (10 μM) in isolated striatal cells. Each point is the mean and standard error of at least four determinations. Points marked * are significantly different from the control.

I. Benztropine

II. Desipramine


I
60
40
20
0
-20
-40
% inhibition
-8
-6
-4
-3
*
log [benztropine]

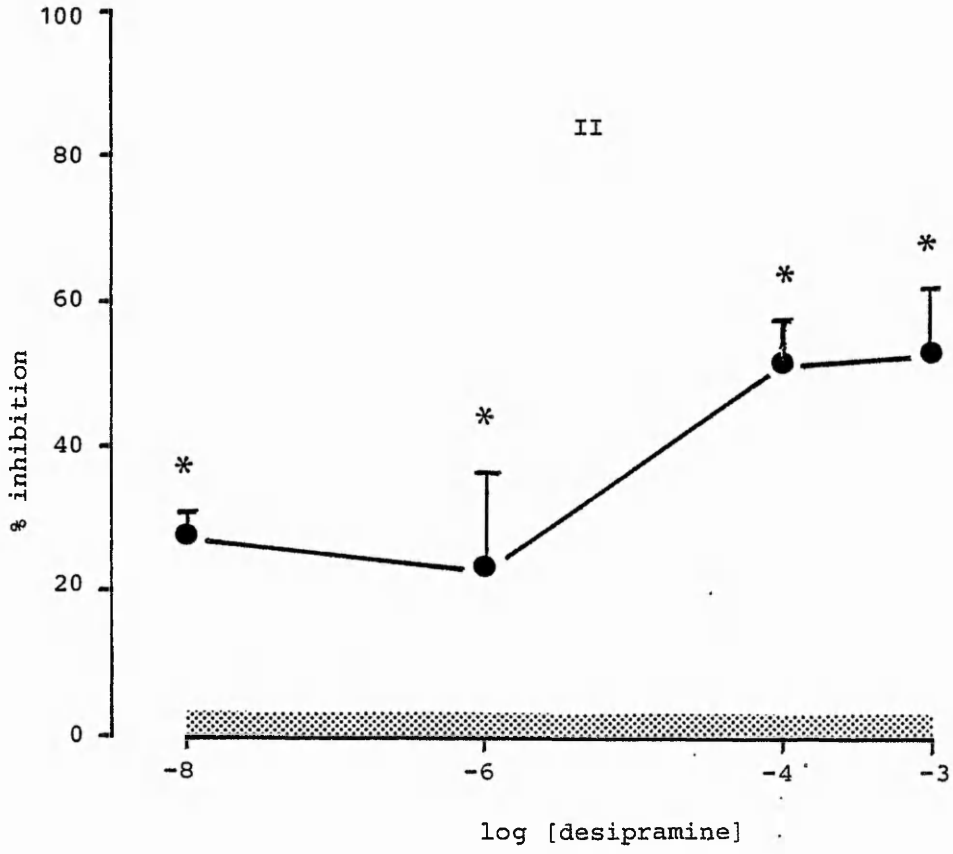
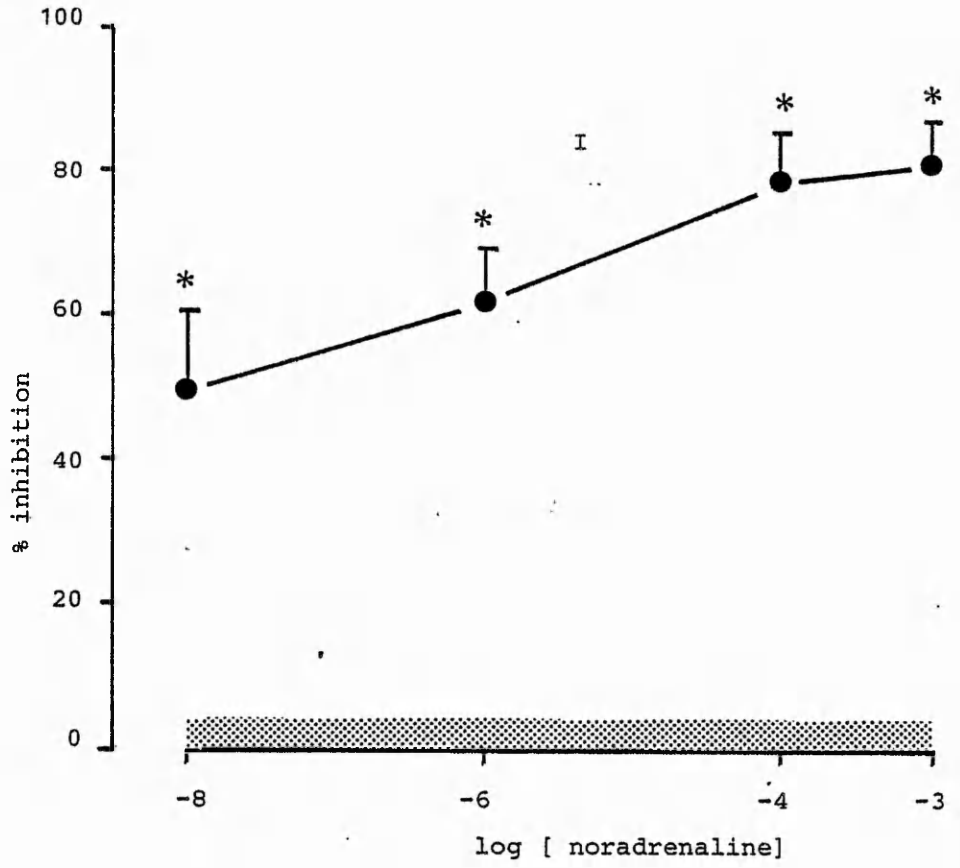

II
100
80
60
40
20
0
% inhibition
-8
-6
-4
-3
*
log [desipramine]

Fig 35.

Inhibition of the accumulation of ^{3}H-dopamine (10 μM) in isolated striatal cells. Each point is the mean and standard error of at least four determinations. Points marked * are significantly different from the control.

I. Noradrenaline

II. Octopamine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
-8
-6
-4
-3
log [noradrenaline]

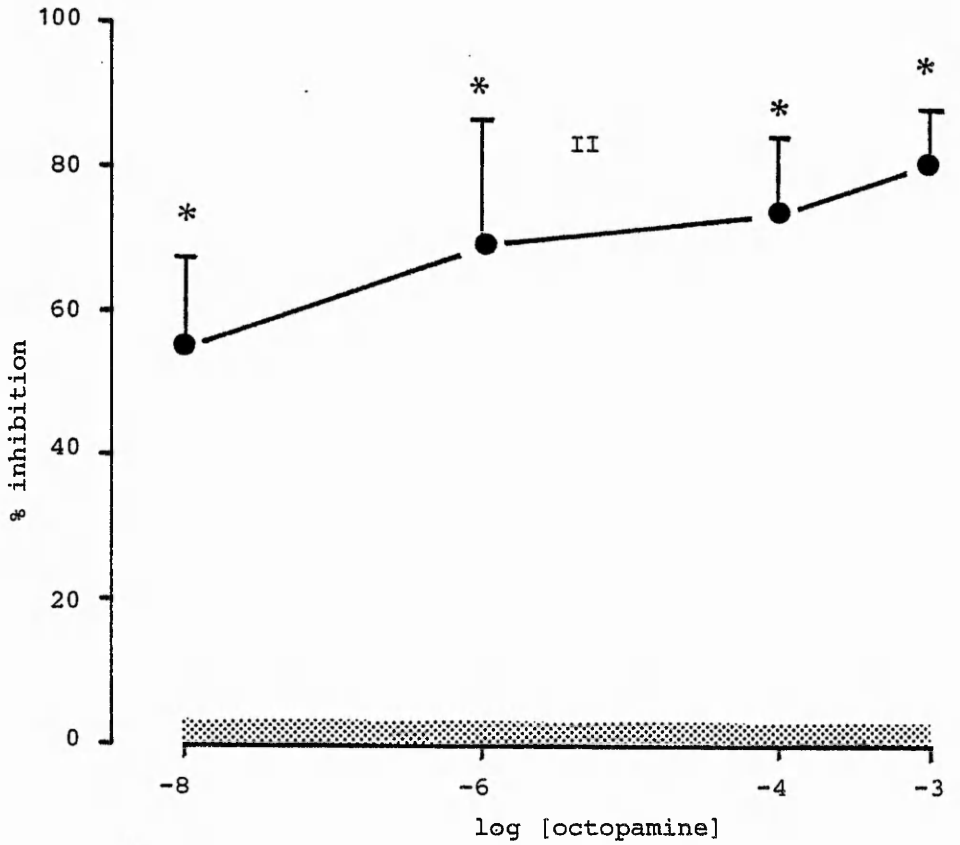
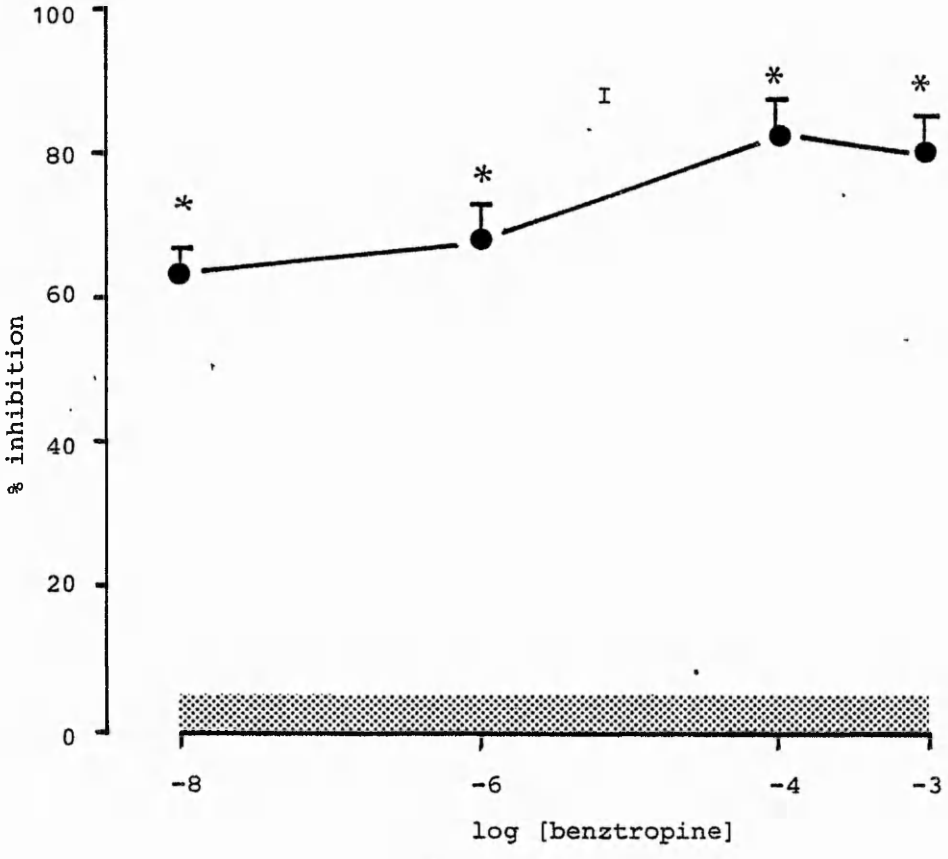

100
80
60
40
20
0
% inhibition
II
*
*
*
*
-8
-6
-4
-3
log [octopamine]

Fig 36.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in isolated striatal cells. Each point is the mean and standard error of at least four determinations. Points marked * are significantly different from the control.

I. Benztropine

II. Desipramine


100
80
60
40
20
0
% inhibition
I
*
*
*
*
-8
-6
-4
-3
log [benztropine]

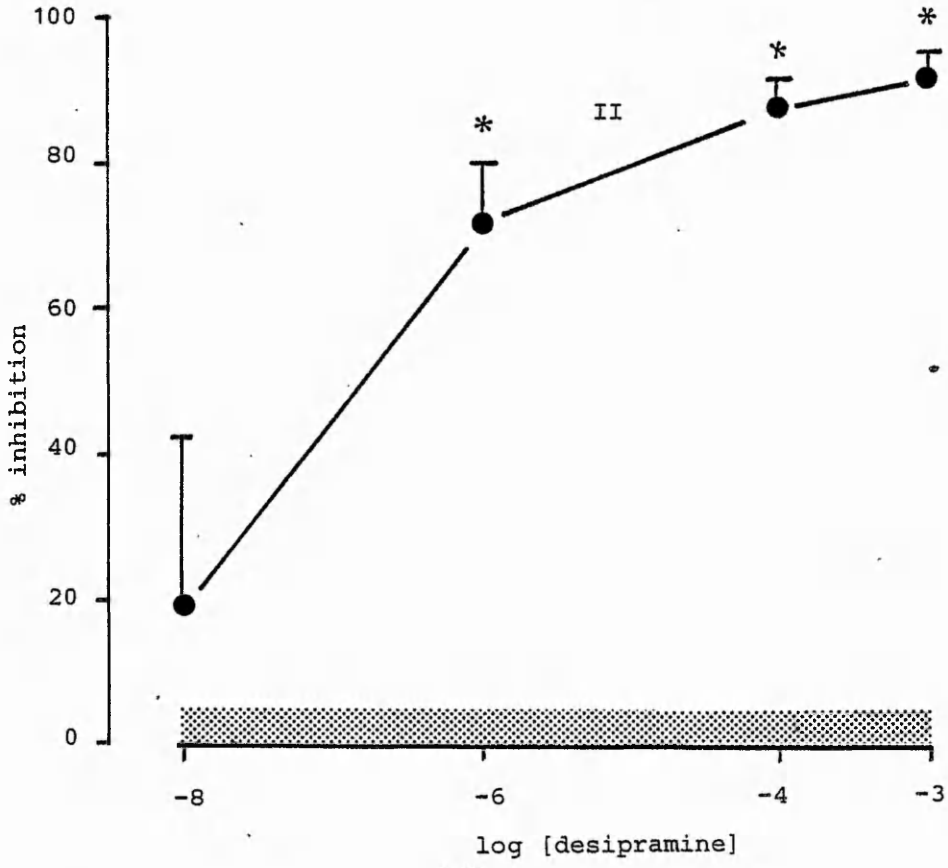
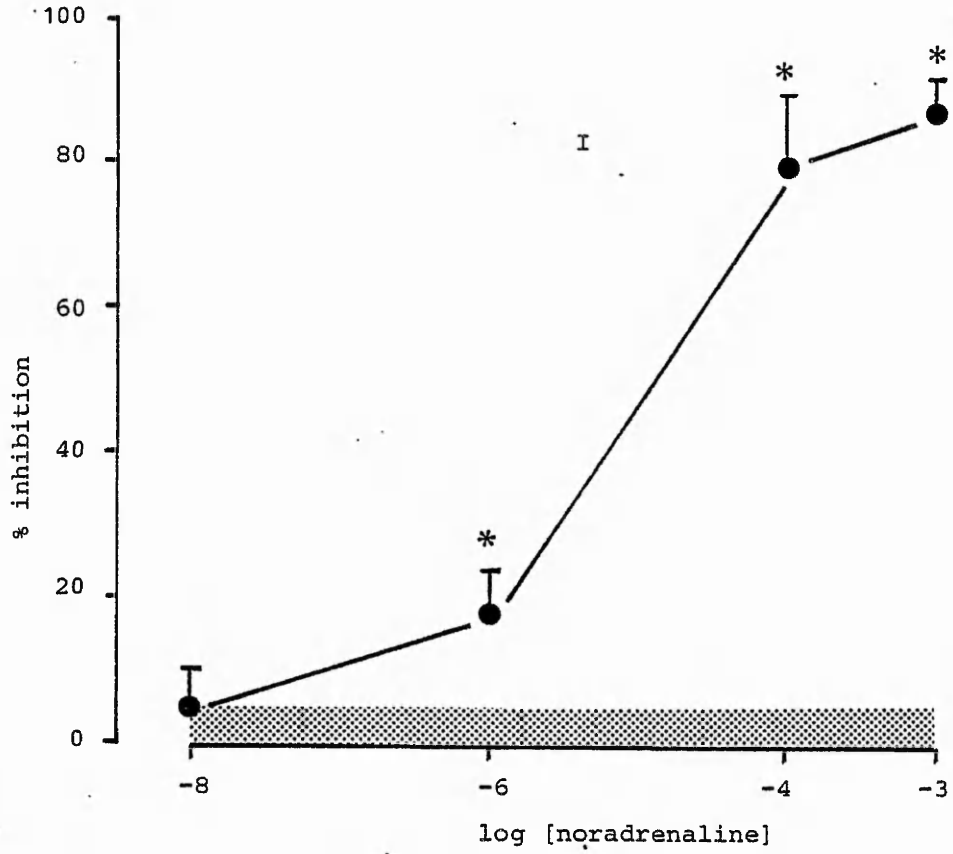

100
80
60
40
20
0
% inhibition
II
*
*
*
*
-8
-6
-4
-3
log [desipramine]

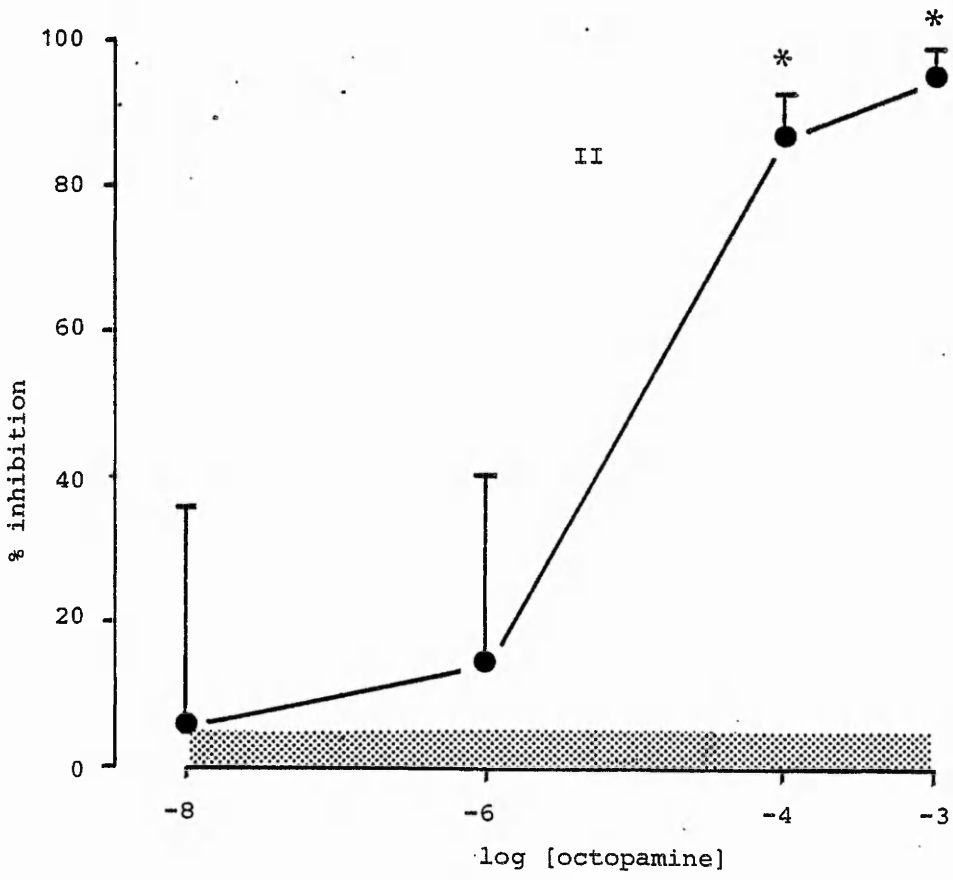
Fig 37.

Inhibition of the accumulation of ^{3}H-dopamine (0.1 μM) in isolated striatal cells. Each point is the mean and standard error of at least four determinations. Points marked * are significantly different from the control.

I. Noradrenaline

II. Octopamine


100
80
60
40
20
0
% inhibition
I
*
*
*
-8
-6
-4
-3
log [noradrenaline]


100
80
60
40
20
0
% inhibition
II
*
*
-8
-6
-4
-3
log [octopamine]

Effect of low sodium media on ^{3}H-dopamine uptake by isolated striatal cells

The isolated cell preparations were preincubated in modified Krebs' solution at 37°C, with a reduced sodium ion content (25 mM) but containing either sucrose (220 mM) or choline chloride (118 mM) to maintain tonicity.

After 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (100 nCi.ml^{-1}; 0.1 µM or 10 µM) were added. Control experiments in normal Krebs' medium were carried out simultaneously. The incubation was continued for 5 min. See Fig. 38.

Incubation in a medium with a reduced sodium ion content but containing sucrose had no significant effect on the uptake of ^{3}H-dopamine at either of the dopamine concentrations investigated. However, replacement of the sodium ions with choline resulted in significant inhibition of the ^{2}H-dopamine uptake, at both 0.1 µM and 10 µM incubation concentrations.

Effect of 2,4-dinitrophenol on ^{3}H-dopamine uptake by isolated striatal cells

The isolated striatal cells were preincubated in Krebs' solution at 37°C which contained 10^{-3} M 2,4-dinitrophenol (DNP). After 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 µM or 10 µM) were added. Incubation was terminated after 5 min. Incubation in the presence of DNP caused a significant inhibition of ^{3}H-dopamine uptake at both concentrations;

Fig 38.

Inhibition of the accumulation of ^{3}H-dopamine in isolated striatal cells. Each point is the mean and standard error of at least four determinations.

I. Accumulation at 10 μM dopamine

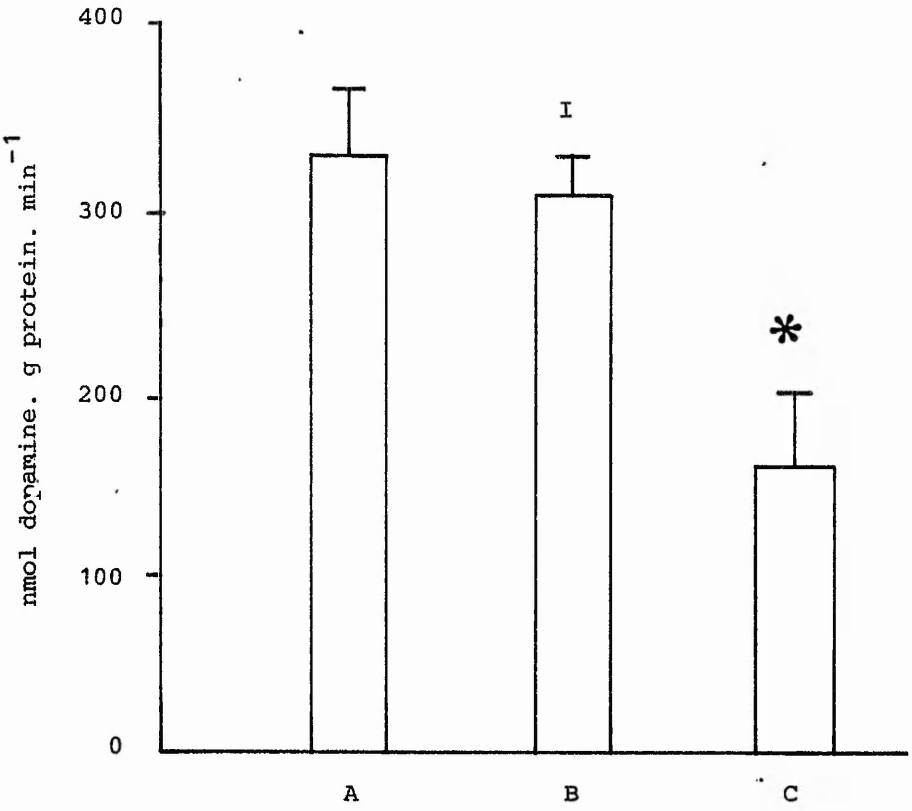
II. Accumulation at 0.1μM dopamine

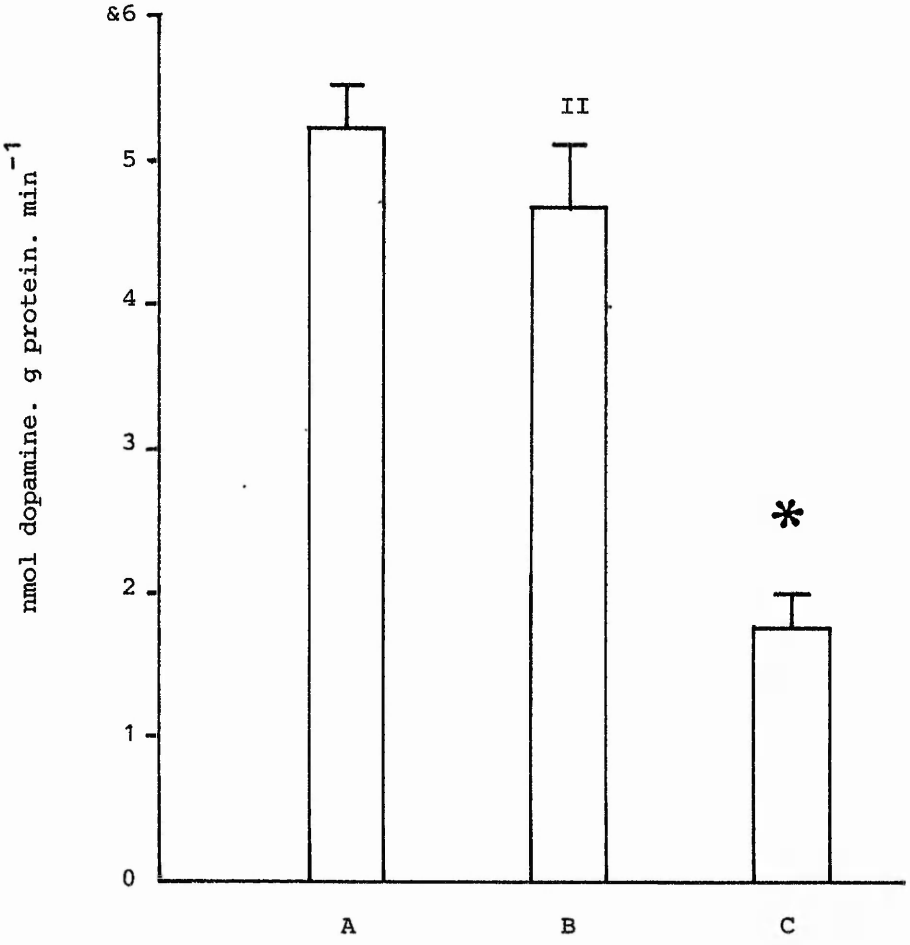
(A) control

(B) low sodium (25mM) medium containing sucrose (220mM)

(C) low sodium (25mM) medium containing choline chloride (118mM)

Points marked * are significantly different from the control.


400
300
200
100
0
nmol dopamine. g protein. min^{-1}
I
*
A
B
C


&6
5
4
3
2
1
0
nmol dopamine. g protein. min^{-1}
II
*
A
B
C

at 0.1 μM 68 ± 4% inhibition and at 10 μM 77 ± 3% inhibition. The results are shown graphically in Fig. 39.

Effect of temperature on ^{3}H-dopamine uptake by isolated striatal cells

Following preincubation in ice-cold Krebs' solution for 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi,ml^{-1}); 0.1, 1.0, 10.0, 100 and 500 μM) were added. Incubation was continued for a further 5 min. The accumulation of ^{3}H-dopamine was significantly reduced at all concentrations studied when the cells were incubated in ice-cold medium. The results are given in Table 18. Incubation in ice-cold medium resulted in signficant inhibition of dopamine uptake at all of the concentrations studied and ranged from 84% at 500 μM to 96% at 0.1 μM dopamine.

Effect of 6-hydroxydopamine pretreatment on ^{3}H-dopamine uptake by isolated striatal cells

Isolated striatal cells were prepared from rat brains treated 5 days previously with 6-hydroxydopamine (6-OHDA). Following 5 min preincubation in Krebs' solution at 37°C ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 μM) were added. Incubation was continued for 5 min. Control experiments using cells from untreated rats were carried out simultaneously. The results for the control animals and the 6-OHDA pretreated animals are presented graphically in Fig.40. Pretreatment with 6-OHDA caused a shift in the position of the curve due to an increase in the S/v values as a result of a reduction in

Fig 39.

Inhibition of the accumulation of ^{3}H-dopamine in isolated striatal cells. Each point is the mean and standard error of at least four values.

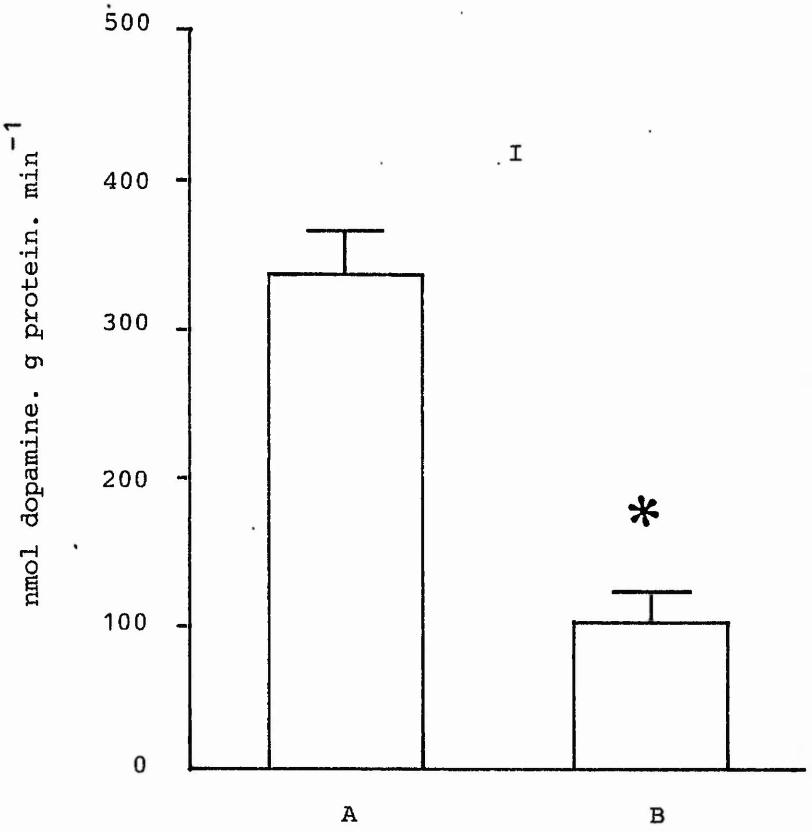
I. Accumulation at 10 μM dopamine

II. Accumulation at 0.1 μM dopamine

(A) control

(B) 2,4-dinitrophenol (10^{-3} M)

Points marked * are significantly different from the control.


500
400
300
200
100
0
nmol dopamine. g protein. min^{-1}
I
*
A
B

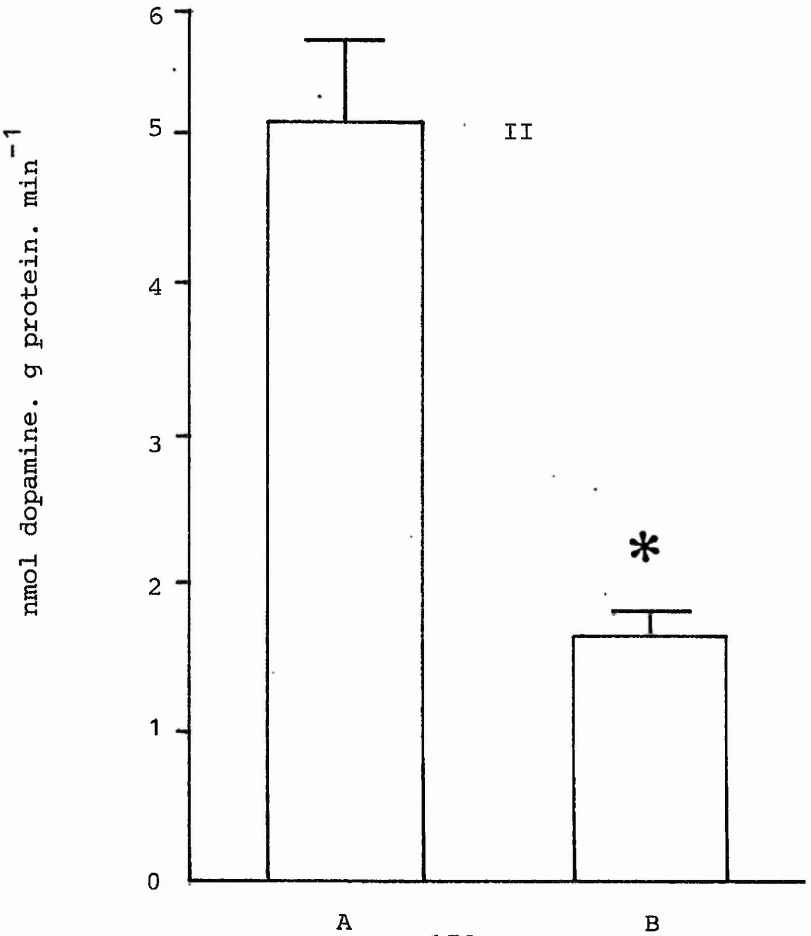

6
5
4
3
2
1
0
nmol dopamine. g protein. min^{-1}
II
*
A
B

Table 18. Values for the accumulation of tritium, from ^{3}H-dopamine, in isolated striatal cells incubated at 0°C and 37°C. Experimental data are mean ± standard error. Percentage inhibition as compared to uptake at 37°C is also presented.

Dopamine conc. (μM)	Uptake at 37°C (nmol.g wet wt^{-1}.min^{-1})	Uptake at 0°C (nmol.g wet wt^{-1}.min^{-1})	Inhibition %
0.1	18.76 ± 0.93	0.75 ± 0.04	96
1.0	30.74 ± 2.41	1.23 ± 0.11	96
10.0	173.96 ± 10.02	8.70 ± 0.90	95
100.0	239.18 ± 18.88	26.27 ± 1.68	89
500.0	503.27 ± 25.13	80.52 ± 7.64	84

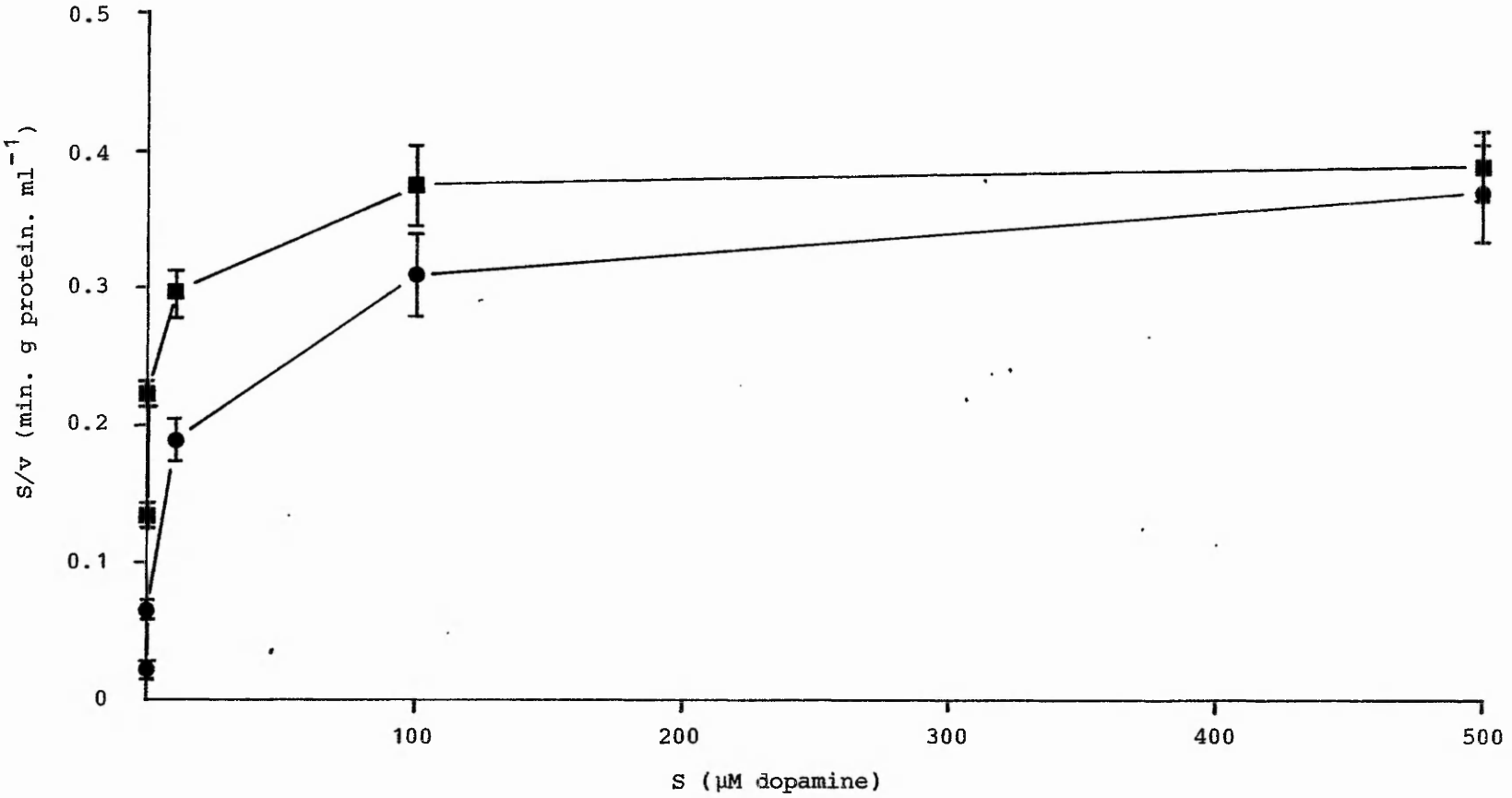
Fig 40.

S/v against S graph of the accumulation of tritium (^{3}H-dopamine) by isolated striatal cells, from:

●——● control

■——■ 6-hydroxydopamine pre-treated

groups of animals. Each point is the mean and standard error of at least six determinations.


0.5
0.4
0.3
0.2
0.1
0
S/v (min. g protein. ml^{-1})
100
200
300
400
500
S (µM dopamine)

uptake. All the values of S/v are significantly different from their respective control values with the exception of the value for incubation in 500 μM dopamine. Both sets of results were analysed kinetically, see Tables 19 and 20. The data was not considered a "good fit" with the three parameter model, but was a "good fit" with the five parameter model; less than 0.2 standard errors for both control and 6-OHDA treated groups. The Vmax value for the high affinity system is significantly reduced for the 6-OHDA treated animals compared to the controls; 0.62 nmol.g protein^{-1}.min^{-1} and 4.86 nmol.g protein^{-1}. min^{-1} respectively. The Km values for the same uptake system are similar; 0.038 μM (control) and 0.077 μM (6-OHDA). The Km and Vmax values for the low affinity system are similar for both the controls and 6-OHDA pretreated animals, as are the values for D.

Time course for the uptake of ^{3}H-dopamine by striatal homogenates

Striatal tissue from a freshly sacrificed rat was homogenised in ice-cold sucrose solution (0.32M). An aliquot of the homogenate was preincubated in Krebs' solution at 37°C for 5 min. At the end of this time ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 μM or 500 μM) was added, and the incubation continued for periods in the range 0.5 - 5.0 min. The time course is presented graphically in Fig. 41. The time course was shown to be linear for at least 5 min at both concentrations studied.

Table 19. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine in an isolated cell preparation of striatal tissue from control rats on the 6-OHDA study. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models, are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (nmol.g protein.min^{-1})	Calc S/v No. of parameters	
		3	5
0.1	0.021 ± 0.002	0.021	0.021
1.0	0.065 ± 0.006	0.058	0.066
10.0	0.188 ± 0.017	0.210	0.188
100.0	0.310 ± 0.032	0.321	0.330
500.0	0.371 ± 0.032	0.337	0.359
(n = 6)			
Vmax (nmol.g protein^{-1}.min^{-1})		19.072	26.337
Km (μM)		0.331	2.34
Vmax' (nmol.g protein^{-1}.min^{-1})			4.858
Km' ((μM)			0.038
D (ml.g protein^{-1}.min^{-1})		2.953	2.747
Fit (s.e.)		>1	<0.2

Table 20. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in an isolated cell preparation of striatal tissue from 6-OHDA pre-treated rats. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models, are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

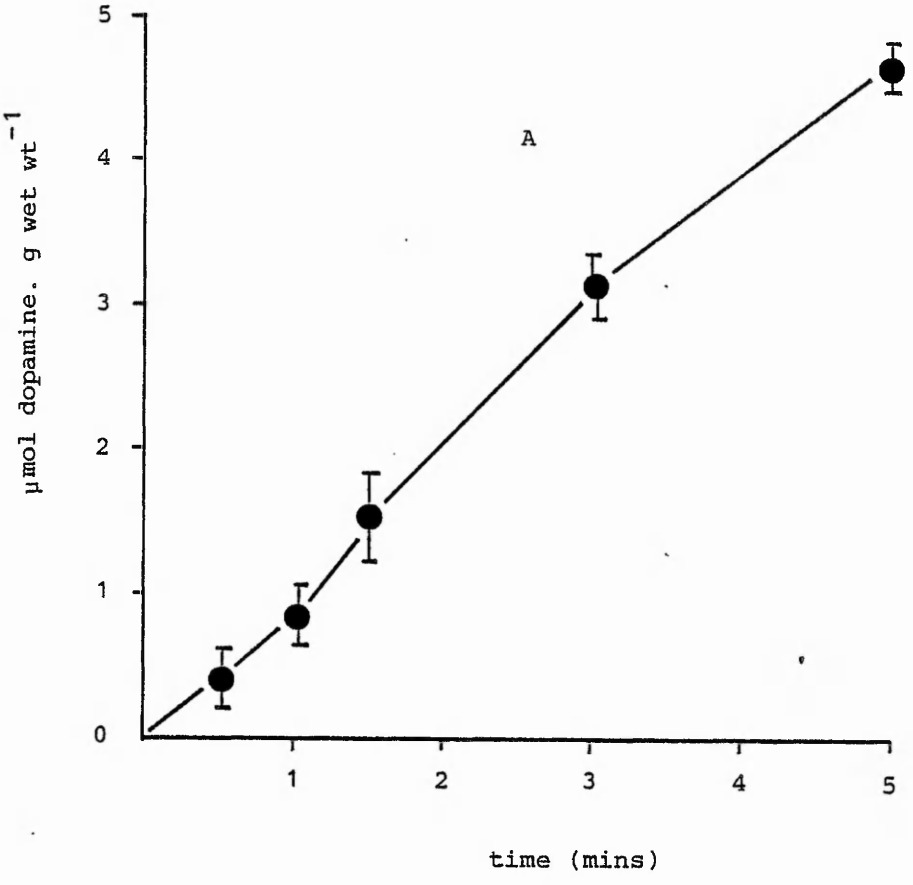
Conc. (μM)	Exp. S/v (min.g protein.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.132 ± 0.014	0.137	0.133
1.0	0.223 ± 0.012	0.217	0.224
10.0	0.299 ± 0.017	0.329	0.301
100.0	0.376 ± 0.029	0.357	0.378
500.0	0.391 ± 0.026	0.360	0.394
(n = 6)			
Vmax (nmol.g protein^{-1}.min^{-1})		2.804	15.177
Km (μM)		0.515	9.906
Vmax' (nmol.g protein^{-1}.min^{-1})			0.624
Km' (μM)			0.077
D (ml.g protein^{-1}.min^{-1})		2.790	2.523
Fit (s.e.)		>1	<0.2

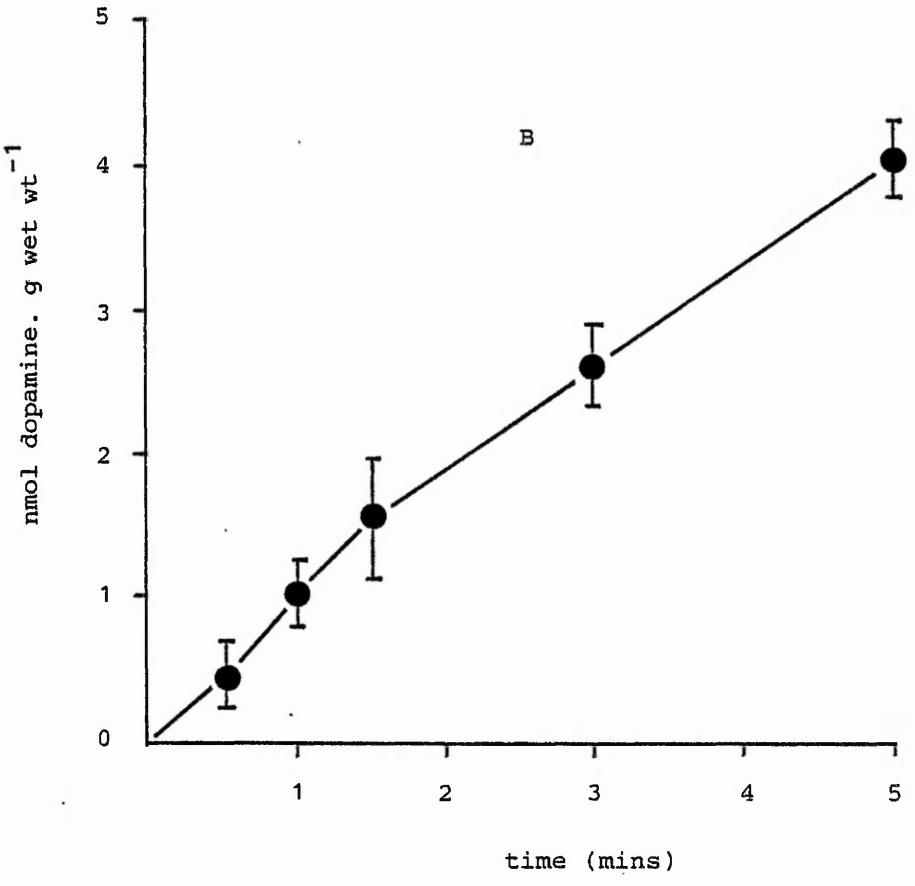
Fig 41.

Time course for the accumulation of ^{3}H-dopamine by striatal homogenates. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 μM

B. Accumulation at 0.1 μM


A
µmol dopamine. g wet wt^{-1}
0
1
2
3
4
5
time (mins)


B
nmol dopamine. g wet wt^{-1}
0
1
2
3
4
5
time (mins)

Kinetics of the uptake of ^{3}H-dopamine by striatal homogenates

The striatal homogenates were prepared as in the time course experiments. After 5 min preincubation in Krebs' solution at 37°C, ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM) were added. Uptake was measured at all concentrations using the homogenate from a single animal. The incubation period was 5 min, as determined by the time course experiments. Values of S/v were calculated from the rate of uptake (nmol.g wet wt^{-1}) for all dopamine concentrations investigated. Plotting S/v against S produced the graph given in Fig.42. The graph shows the characteristic curved shape seen in the previous kinetic experiments. The results were analysed and the S/v value for each value of S investigated are presented in Table 21, together with their relevant kinetic parameters. Neither of the models used to analyse the kinetic parameters were considered to be a "good fit" to the experimental data.

Time course for the uptake of ^{3}H-dopamine by striatal synaptosomes

Synaptosomes were prepared from rat striatal tissue and pre-incubated in Krebs' solution at 37°C. After 5 minutes ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}; 0.1 µM or 500 µM) were added. The incubation was continued for times in the range 0.5 to 5.0 min. At the end of this period an aliquot of the synaptosomal suspension was taken and the synaptosomes separated from the medium by the use of a nitro-cellulose filter. The tritium content of the synaptosomes was measured and the radioactivity present in the medium also assessed. Tissue

Fig 42.

S/v against S graph for the accumulation of tritium (^{3}H-dopamine) by striatal homogenates. Each point is the mean and standard error of at least nine determinations.

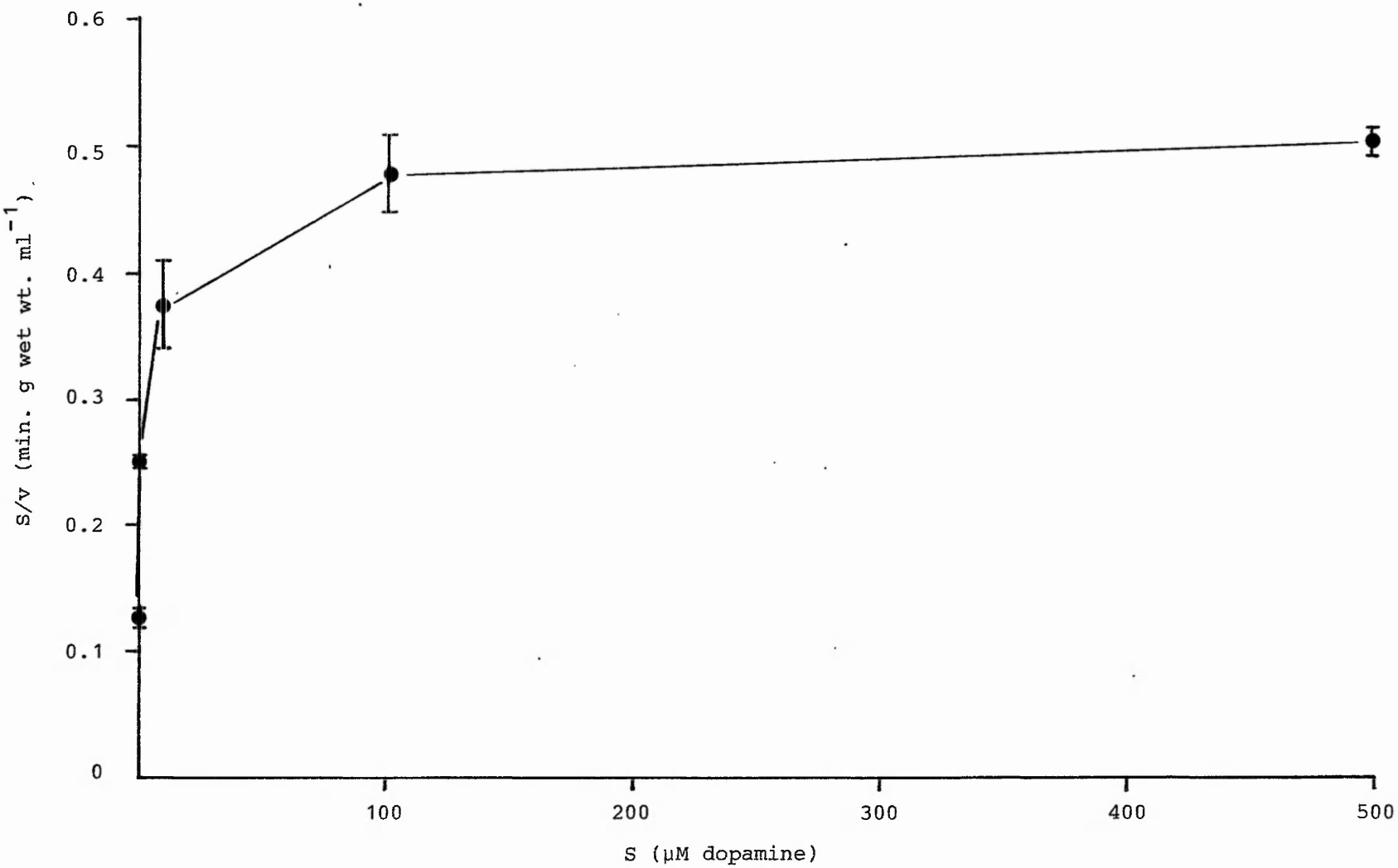

0.6
0.5
0.4
0.3
0.2
0.1
0
S/v (min. g wet wt. ml^{-1})
100
200
300
400
500
S (μM dopamine)

Table 21. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine in a striatal homogenate. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g protein.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.129 ± 0.005	0.130	0.130
1.0	0.252 ± 0.004	0.253	0.254
10.0	0.376 ± 0.036	0.443	0.445
100.0	0.472 ± 0.033	0.494	0.494
500.0	0.502 ± 0.011	0.499	0.500
(n = 9)			
Vmax (nmol.g wet wt^{-1}.min^{-1})		2.688	9.576
Km (μM)		0.371	5.616
Vmax' (nmol.g wet wt^{-1}.min^{-1})			0.553
Km' (μM)			0.035
D (ml.g wet wt^{-1}.min^{-1})		2.014	1.983
Fit (s.e.)		>1	>1

pooled from groups of 4 rats was investigated for all the dopamine concentrations studied.

The time course is presented graphically in Fig. 43 and was shown to be linear for at least 5 min.

Kinetics of the uptake of ^{3}H-dopamine by striatal synaptosomes

Striatal synaptosomes were prepared as in the time course experiments. After 5 min preincubation ^{14}C-sorbitol (final concentration 100 nCi.nl^{-1}) and ^{3}H-dopamine (final concentration 100 nCI,ml^{-1}; 0.1, 1.0, 10.0, 100 and 500 µM) were added. Uptake was measured at all concentrations in synaptosomes produced from pooling the striatal tissue of groups of 4 rats. The incubation period was 5 min as determined by the time course experiments. The value of S/v was calculated (min.g protein^{-1}.ml^{-1}) for all dopamine concentrations studied. The results are presented graphically in Fig. 44. The data was analysed kinetically and found to be a "good fit" to the three parameter model; less than 1 standard error different from the experimental data. The five parameter model produced a value for the fit in excess of one standard error. The Vmax value was found to be 8.65 nmol.g protein^{-1}.min^{-1} and the Km value 0.083 µM. See Table 22.

Effect of temperature on the extracellular space

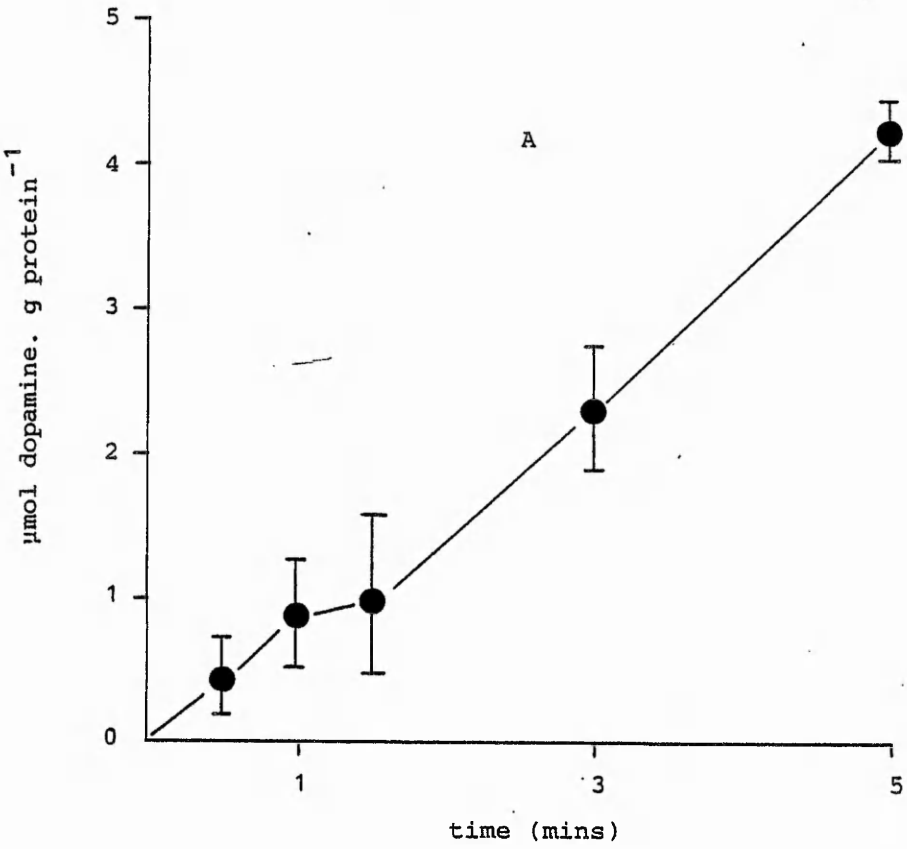
The extracellular space as defined by sorbitol was measured at 37°c and 0°C. Striatal slices and isolated cells were preincubated

Fig 43.

Time course for the accumulation of ^{3}H-dopamine by striatal synaptosomes. Each point is the mean and standard error of at least six determinations.

A. Accumulation at 500 μM

B. Accumulation at 0.1 μM


A
µmol dopamine. g protein^{-1}
0
1
2
3
4
5
1
3
5
time (mins)

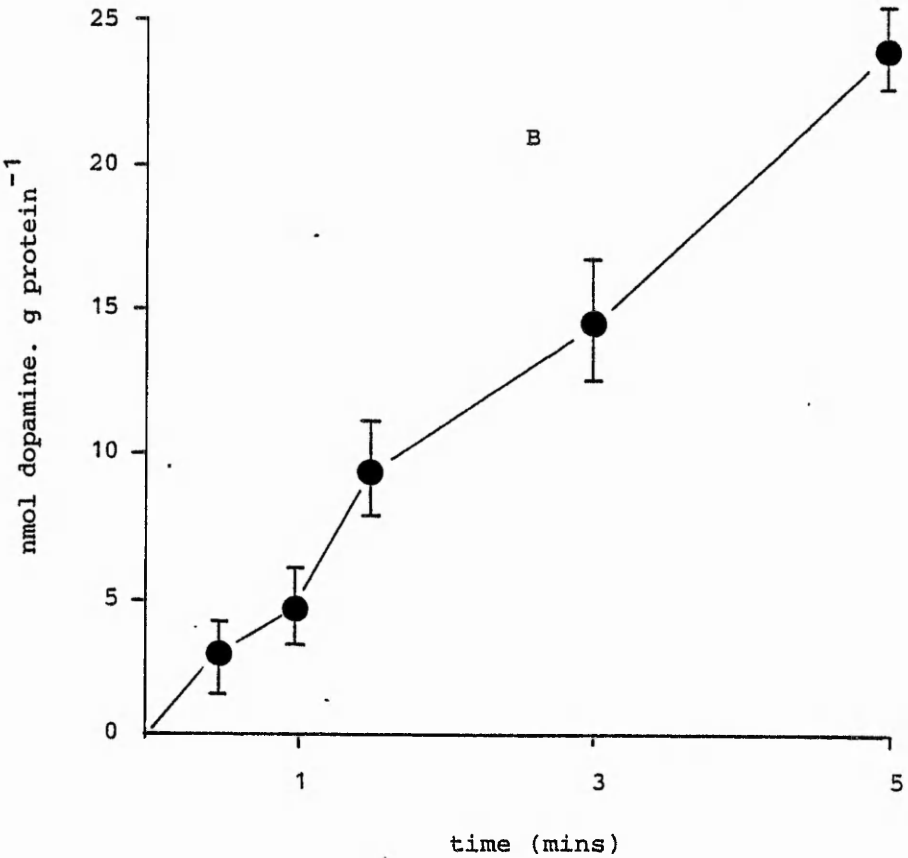

B
nmol dopamine. g protein^{-1}
0
5
10
15
20
25
1
3
5
time (mins)

Fig 44.

S/v against S graph for the accumulation of tritium (^{3}H-dopamine) by striatal synaptosomes. Each point is the mean and standard error of at least six determinations.

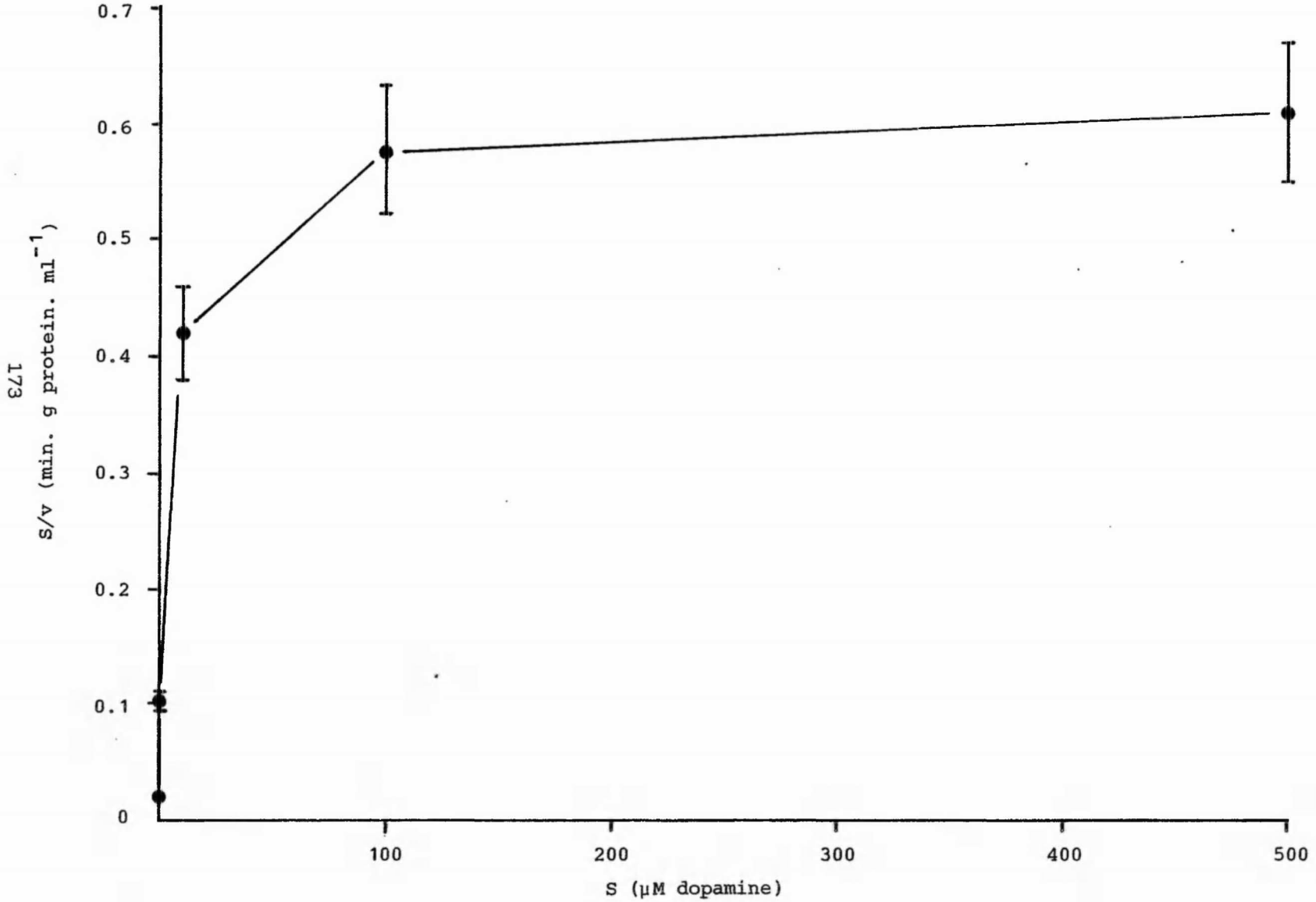

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
S/v (min. g protein. ml^{-1})
100
200
300
400
500
S (μM dopamine)

Table 22. Values of S/v for the accumulation of tritium, from ^{3}H-dopamine, in striatal synaptosomes. Experimental data are mean ± standard error. Values of S/v, at the different concentrations, calculated using the kinetic parameters from the two curve fitting models are given. The largest difference between experimental and calculated values is given as the fit in terms of standard error.

Conc. (μM)	Exp. S/v (min.g protein.ml^{-1})	Cal S/v No. of parameters	
		3	5
0.1	0.020 ± 0.001	0.021	0.021
1.0	0.103 ± 0.009	0.104	0.104
10.0	0.423 ± 0.048	0.403	0.406
100.0	0.577 ± 0.055	0.588	0.591
500.0	0.610 ± 0.059	0.613	0.616
(n = 6)			
Vmax (nmol.g protein^{-1}.min^{-1})		8.653	7.475
Km (μM)		0.083	0.908
Vmax' (nmol.g protein^{-1}.min^{-1})			1.263
Km' (μM)			0.054
D (ml.g protein^{-1}.min^{-1})		1.631	1.619
Fit (s.e.)		<1	>1

in Krebs' solution at either 37°C or 0°C. After 5 min ^{14}C-sorbitol (final concentration 100 nCi.ml^{-1}) and ^{3}H-dopamine (final concentration 100 nCi.ml^{-1}; 0.1 µM or 100 µM) were added. The slices were incubated for 2 min, the isolated cells for 5 min. The incubations were terminated as previously described for the two preparations and the radioactivity measured. The accumulation of ^{14}C-sorbitol, in terms of the tissue: medium ratio (T/M), by the two preparations are given in Table 23.

Lowering the temperature of incubation from 37°C to 0°C was shown to reduce the extracellular space in both slices and isolated cells of the striatum. This occurred at both concentrations of dopamine studied.

H.P.L.C. ANALYSIS OF CATECHOLAMINES AND THEIR METABOLITES

Metabolism of dopamine accumulated by striatal slices

Following a 5 min preincubation in Krebs' solution at 37°C the striatal slices were incubated in medium containing 100 µM dopamine. The tissue was removed after 2, 10 or 30 min periods of incubation and homogenised in $HClO_4$. After centrifugation, samples of the supernatant were analysed by HPLC. Samples of the incubation medium (at the end of the incubation period) were also analysed by this method. Slices of striatal tissue which had not been incubated in dopamine containing media were also assayed in order to act as controls.

Dopamine accumulation appears to be approaching a maximum by 10 minutes, 271.7 ± 43.9 nmol.g wet wt^{-1}. The increase in tissue

Table 23. The accumulation of ^{14}C-sorbitol (T/M) by striatal tissue preparations: Effect of temperature. Each value is the mean and standard error; the group size is given in parenthesis.

Striatal slices

Dopamine conc. (μM)	T/M ratio 37°C	0°C
0.1	0.317 ± 0.024 (36)	0.164 ± 0.016 (6)
100.0	0.301 ± 0.022 (36)	0.140 ± 0.015 (6)

Striatal cells

Dopamine conc. (μM)	T/M ratio 37°C	0°C
0.1	26.48 ± 1.36 (12)	16.47 ± 1.76 (4)
100.0	22.00 ± 1.59 (12)	13.41 ± 1.95 (4)

content of DOPAC also appears to have reached a maximum by this time 26.7 ± 5.7 nmol.g wet wt^{-1}, however the production of HVA is linear over the time period studied. The level of HVA rising from 1.9 ± 0.05 nmol.g wet wt^{-1} to 4.5 ± 0.5 nmol.g wet wt^{-1} between 0 and 30 minutes. The levels of 3-MT present in the tissue are too small to measure until the 30 minute sample when 0.7 ± 0.2 nmol.g wet wt^{-1} were detectable. See Fig. 45.

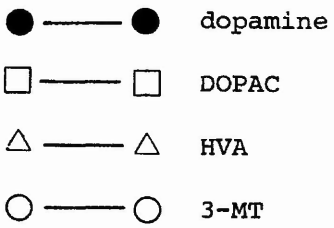
Due to the presence of dopamine in the incubation media it was not possible to estimate the amount of dopamine released from the tissue. DOPAC was detected after 10 min 1.2 ± 0.1 $nmol.ml^{-1}$ and 30 min 2.6 ± 0.2 $nmol.ml^{-1}$. No signal corresponding to either HVA or 3-MT was detected.

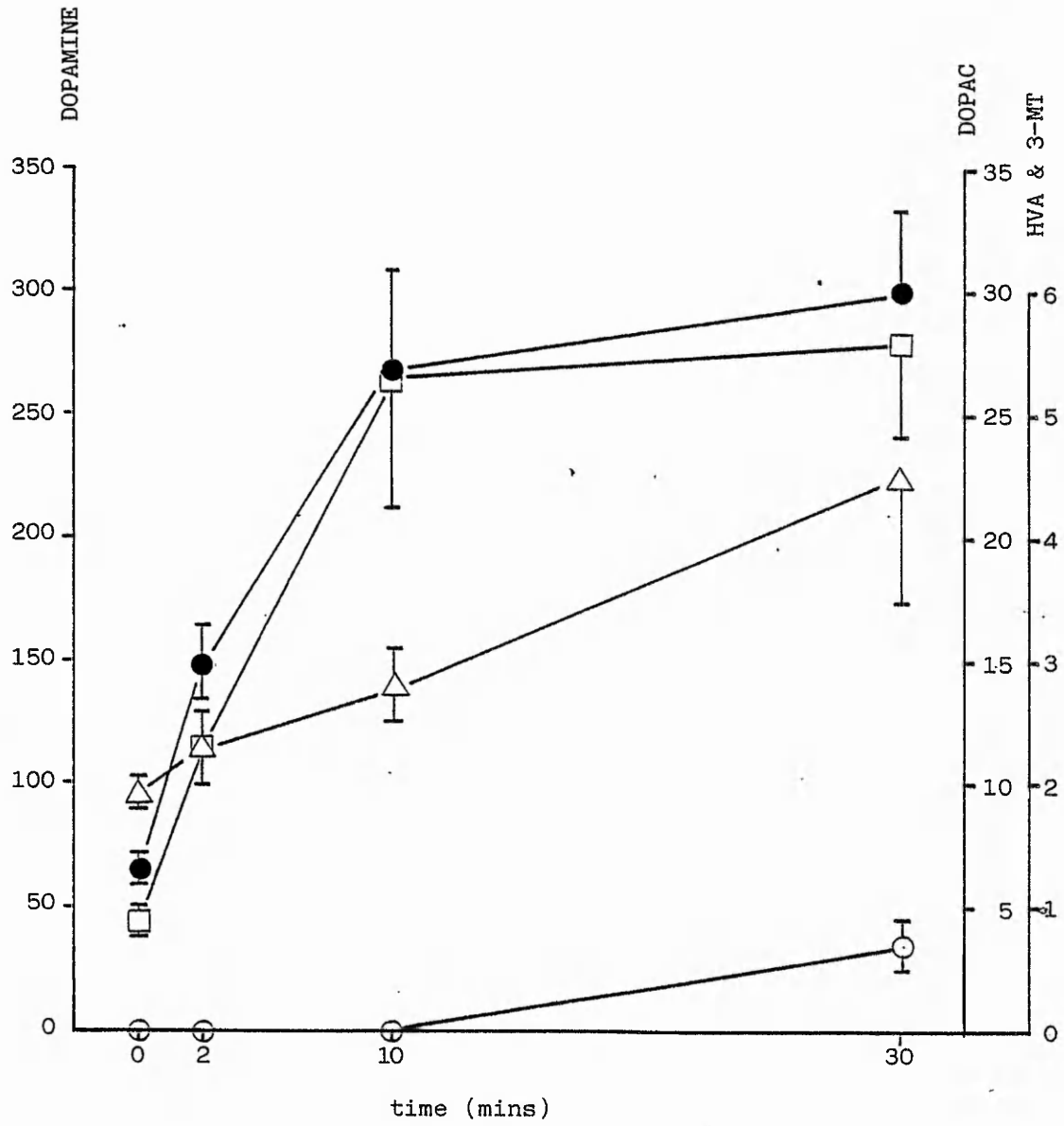
Effect of 6-hydroxydopamine pretreatment on the metabolism of dopamine accumulated by striatal slices

Striatal slices were prepared from rats that had been treated 5 days previously with i.c.v. administered 6-OHDA (250 ng). The slices were preincubated for 5 min in Krebs' solution at 37°C. A further 10 minute incubation was then carried out in the presence of 100 µm dopamine. Following homogenisation in $HClO_4$ and centrifugation samples were analysed by HPLC. Slices of striatum prepared from untreated animals were also incubated in Krebs' solution in the presence of dopamine (100 µM). Slices from both 6-OHDA treated and untreated rats were also taken for HPLC analysis. These slices were not incubated.

Fig 45.

Time course for the accumulation of dopamine and its metabolites by striatal slices, following incubation in a medium containing 100 µM dopamine. Each point is the mean and standard error of at least six determinations.





All values are in terms of nmol.g wet wt^{-1}

Pretreatment with 6-OHDA resulted in a significant reduction of the dopamine content of the striatum in tissue which did not undergo incubation in dopamine. See Table 24. DOPAC levels were reduced by 42%, a significant reduction, but HVA levels were apparently unaffected by the treatment. 3-methoxytyramine could not be detected.

The tissue from animals pretreated with 6-OHDA and incubated for 10 min in the presence of 100 μM dopamine accumulated 206.0 ± 35.7 nmol.g wet wt^{-1} of the catecholamine. A level not significantly different from that found in untreated tissue which had been similarly incubated. Levels of DOPAC, 29.9 ± 6.0 nmol.g wet wt^{-1}, were not significantly different from those of untreated tissue that had been incubated in 100 μM dopamine; however HVA, 4.8 ± 0.9 nmol.g wet wt^{-1}, was significantly increased. 3-MT was not detected. See Table 24.

Metabolism of noradrenaline accumulated by striatal slices

Slices of striatal tissue were preincubated for 5 min in Krebs' solution at 37°C. Noradrenaline (100 μM) was added and the incubation continued for a further 2, 10 or 30min. After incubation the tissue was homogenised in $HClO_4$ and, following centrifugation, samples of supernatant were analysed by HPLC. Samples of the medium (at the end of the incubation) were also analysed by this method. Striatal tissue which had not been incubated in the presence of noradrenaline was also assayed for catecholamine content.

The levels of noradrenaline and its metabolites were too low to be detectable in the non-incubated tissue. The accumulation of

Table 24. Concentration of dopamine and its metabolites present in striatal tissue and incubation medium following incubation in 100 µM dopamine. Values for control (untreated) and 6-OHDA pretreated animals are given. Each value represents the mean and standard error of at least 6 results.

Treatment	Incubation time (min)	Concentration in striatum (nmol.g wet wt^{-1})			
		DA	DOPAC	HVA	3-MT
Control	0	66.1 ± 4.0	4.6 ± 0.4	1.9 ± 0.05	≠
	2	147.5 ± 16.0	11.6 ± 0.4	2.3 ± 0.3	≠
	10	271.7 ± 43.9	26.7 ± 5.7	2.8 ± 0.3	≠
	30	303.4 ± 32.4	28.1 ± 4.2	4.5 ± 0.5	0.7 ± 0.2
6-OHDA	0	27.9 ± 3.6	2.4 ± 0.4	1.8 ± 0.1	≠
	10	206.0 ± 35.7	29.9 ± 6.0	4.8 ± 0.9	≠
		Concentration in medium (nmol.ml^{-1})			
Control	2	≠≠	≠	≠	≠
	10	≠≠	1.2 ± 0.1	≠	≠
	30	≠≠	2.6 ± 0.2	≠	≠
6-OHDA	10	≠≠	2.2 ± 0.1	≠	≠

≠ below detectable level

≠≠ medium contained excess dopamine therefore released dopamine from slices could not be measured

the compounds following incubation in 100 μM noradrenaline is presented graphically in Fig. 46. An unidentifiable peak was to be seen present in the chromatograms having a retention time of about 6.3 mins. The accumulation of noradrenaline appears to be approaching a maximum by 30 min, 216.6 ± 25.9 nmol. g wet wt^{-1}. The level of DHMA/DOPEG accumulation parallels noradrenaline accumulation, reaching a level of 34.6 ± 1.8 nmol. g wet wt^{-1} by 30 min. VMA levels were 3.7 ± 0.8 nmol. g wet wt^{-1} by this time and normetanephrine levels 0.8 ± 0.1 nmol. g wet wt^{-1}. Only DOPEG/DHMA was detectable in the incubation media at levels of 0.8 ± 0.05 nmol. ml^{-1} by 30 min.

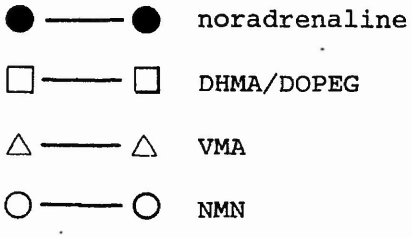
Effect of 6-hydroxydopamine pretreatment on the metabolism of noradrenaline accumulated by striatal slices

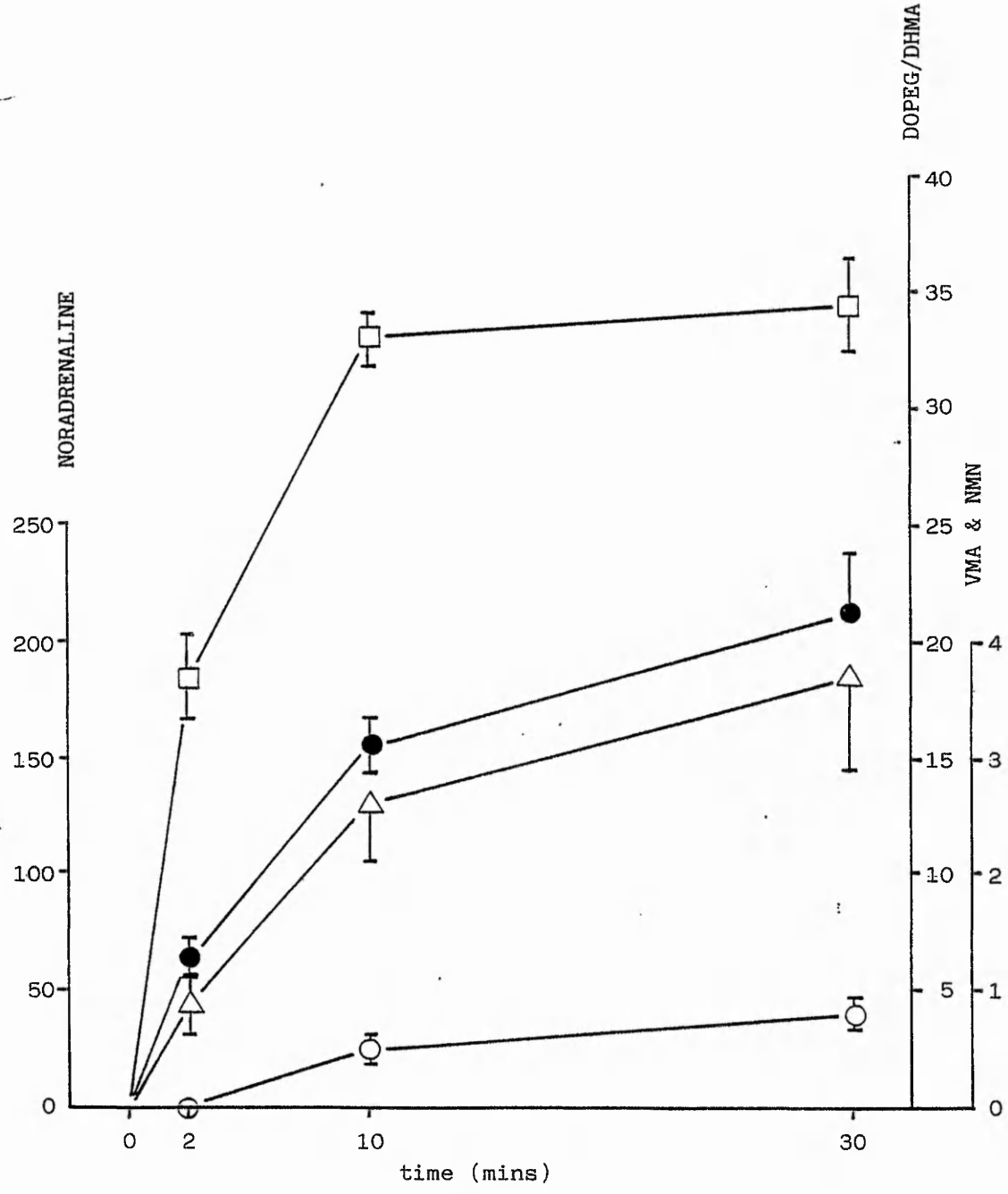
Five days after i.c.v. administration of 6-OHDA, slices of rat striatum were prepared. Following preincubation in Krebs' solution at 37°C for 5 min noradrenaline (100 μM) was added. The catecholamines were extracted by homogenisation in $HClO_4$. After centrifugation samples of supernatant were analysed by HPLC along with samples of the incubation media collected at the end of the incubation. Striatal slices from untreated animals were also incubated in noradrenaline (100 μM). Striatal slices from 6-OHDA treated and untreated animals were also analysed by HPLC without being incubated in noradrenaline beforehand.

Pretreatment with 6-OHDA produced no significant differences in the amount of noradrenaline taken up in 10 minutes, when compared to the levels accumulated in this time by untreated striatal tissue

Fig 46.

Time course for the accumulation of noradrenaline and its metabolites by striatal slices, following incubation in a medium containing 100 µM noradrenaline. Each point is the mean and standard error of at least six determinations.





All values are in terms of nmol.g wet wt^{-1}

154.0 ± 13.4 nmol.g wet wt^{-1} (control) and 190.2 ± 17.9 nmol. g wet wt^{-1} (6-OHDA). The level of DOPEG/DHMA found after incubation in noradrenaline was reduced, 33.2 ± 1.2 nmol.g wet wt^{-1} in untreated tissues compared to 29.0 ± 1 6 nmol. g wet wt^{-1} in 6-OHDA treated striata. The production of VMA was slightly increased from 2.6 ± 0.5 nmol. g wet wt^{-1} in control tissue to 3.1 ± 0.5 nmol.g wet wt^{-1} in 6-OHDA treated tissue. See Table 25.

The unidentified peak (retention time 6.3 min) found to be present in the chromatograms from untreated rats was evident in the chromatograms from 6-OHDA pretreated animals following incubation in the presence of 100 µM noradrenaline. It was not seen to be significantly different in area.

Table 25. Concentrations of noradrenaline and metabolites present in striatal tissue and incubation medium following incubation in 100 μM noradrenaline. Values for control (untreated) and 6-OHDA pretreated animals are given. Each value represents the mean and standard error of at least 6 results

Treatment	Incubation time (mins)	Concentration in striatum (nmol.g wet wt^{-1})			
		NA	DOPEG/DHMA	VMA	NMN
Control	0	≠	≠	≠	≠
	2	61.3 ± 7.0	18.6 ± 1.8	0.9 ± 0.3	≠
	10	154.0 ± 13.4	33.2 ± 1.2	2.6 ± 0.5	0.4 ± 0.1
	30	216.6 ± 25.9	34.6 ± 1.8	3.7 ± 0.8	0.8 ± 0.1
6-OHDA	0	≠	≠	≠	≠
	10	190.2 ± 17.9	29.0 ± 1.6	3.1 ± 0.5	
		Concentration in medium (nmol.ml^{-1})			
Control	2	≠≠	≠	≠	≠
	10	≠≠	0.6 ± 0.05	≠	≠
	30	≠≠	0.8 ± 0.05	≠	≠
6-OHDA	10	≠≠	0.9 ± 0.1	≠	≠

≠ below detectable level

≠≠ medium contained excess noradrenaline, therefore noradrenaline released from slices could not be measured

DISCUSSION

The extracellular space

In studying uptake using tissue slices in vitro, the observed uptake of substrate into the slice is a combination of the accumulation into the extracellular space and the intracellular compartment. Therefore when estimating the intracellular concentration a knowledge of the size of the extracellular space is essential. Whilst studying the uptake of tritiated catecholamines the extracellular space for each slice was measured simultaneously by including carbon-14 labelled sorbitol in the incubation medium as an extracellular marker. This is important as earlier studies using brain slices showed that the tissue enlarges during incubation (Stern, Eggleston, Hems and Krebs, 1949). This was considered to be caused by fluid adhering to the tissue and the expansion of the extracellular compartment. The size of this compartment and the swelling of the tissue are not constant, but depend on the incubation medium, experimental conditions and method of tissue preparation (Bachelard, Campbell and McIlwain, 1962).

In experiments using slices the tissue was rinsed in ice-cold, amine free solution and blotted dry immediately after incubation. This removed as much labelled medium adhering to the external surface of the slice as possible. The process was rapid in order to minimise the loss of amine which had been taken up but not firmly bound. The technique was designed to remove the minimum amount of radioactive substance present in the extracellular space following incubation.

The amount of tritiated catecholamine accumulated by the cellular compartment of the tissue was estimated from the total tissue content after applying a correction factor representing the amount of tritiated catecholamine in the extracellular space. This factor was estimated using a substance which dissolves and diffuses freely in the incubation medium and hence the extracellular space, but is unable to cross the cellular membrane. Neither should the substance bind to the tissue or a false result would be obtained. Several compounds have been described as being suitable for measuring extracellular space including, inulin, sucrose, D-mannitol, D-sorbitol and sulphate (Cohen et al, 1970a). Sorbitol was selected as the marker for these experiments as it has a similar molecular weight to that of the catecholamines.

The time course for the uptake of ^{14}C-sorbitol in the striatum is shown in Fig. 5. The curve shows a rapid initial accumulation of the substance by the tissue. The rate of accumulation appears to be declining by the end of the study i.e. after 6 minutes. It has been shown that there is a further slow increase in sorbitol accumulation (Cohen et al, 1968). It was concluded that this may be due to sorbitol entering a cellular compartment by diffusion (Cohen et al, 1968) or into an extracellular compartment which has a barrier to free diffusion (Cohen et al, 1970a).

Investigating inulin accumulation by mouse cerebrum slices Cohen et al (1968), described three compartments: the inulin space, permeable to inulin at 37°C and 0°C, a second inulin space permeable only at 37°C and a compartment impermeable to inulin, the non-inulin space.

A similar second marker space has been found for sucrose, mannitol and sorbitol (Cohen et al, 1970b). This space was independent of the tonicity of the medium but the total marker space increased if the medium were hypertonic due to a shift in fluid from the cells to the extracellular space. The total marker space was inversely proportional to the size of the marker molecule but the size of the second marker space was only partially dependent on this factor. It was concluded that this second marker space was due to microtubules running through the cell bodies and connected to the extracellular space via micropores. The size of these microtubules was considered to be temperature dependent.

Mireylees (1975), described three wash out phases for sorbitol following its accumulation in rat brain slices. He concluded that of these the 'fast phase' was due to the rinsing of sorbitol from the slice surface the 'medium phase' was caused by the wash out of the marker from the extracellular space and the 'slow phase' to sorbitol accumulated in the microtubules. He also concluded that sorbitol was an acceptable extracellular space marker for incubation of a limited duration.

The size of the extracellular space was shown to be reduced in both striatal slices and isolated cell preparations when the incubation temperature was reduced from 37°C to 0°C. This result would be consistent with the theory of microtubules the size of which is temperature dependent. The results also suggest that these microtubules represent approximately 50% of the extracellular space

available to ^{14}C-sorbitol.

Kinetics of the uptake of tritiated catecholamines in slices from the rat striatum and cerebellum

It has previously been demonstrated that the mechanism of uptake varies between the different regions of the rat brain (Shaskan and Snyder, 1970; Snyder and Coyle, 1969; Snyder et al, 1968). In this study uptake was investigated in slices of the striatum. The technique used to measure uptake involves the estimation of the initial rate of accumulation of substrate, measured in terms of the radiolabel, by the tissue. Accumulation is taken as an accurate representation of uptake, although they cannot strictly be defined as identical. The substrates, in this case the catecholamines noradrenaline and dopamine, were labelled with a radioactive isotope. This should not affect their function as a substrate for the uptake system but enables the compound to be detected and quantified (Ross and Renyi, 1964; Snyder et al, 1968). Catecholamines have been shown to be metabolised by enzymes located intracellularly (Lightman and Iversen, 1969; Sharman, 1973) and hence the measurement of total radioactivity in the tissue includes all the catecholamine accumulated. It does not discriminate between catecholamine which had been taken up and subsequently metabolised and that taken up but not metabolised. A measure of the initial rate of accumulation is necessary as an increase in the intracellular content of catecholamine leads to the passage of the label from the cell until a state of equilibrium is reached between substrate entering and leaving the cell. Radiolabel that

escapes the cells may be present as part of a catecholamine molecule or part of a catecholamine metabolite. As no radiolabel is present initially in the cells the initial rate of accumulation equals the rate of uptake.

Time courses were constructed to estimate the initial rate of accumulation. The cellular accumulation of radiolabel in slices of striatum was shown to be linear for at least two minutes for concentrations of 500 µM, for both dopamine and noradrenaline. At concentrations of 0.1µM the time course was linear for at least 6 minutes for both catecholamines. As the time courses pass through the origin this suggests that the correction for extracellular space is valid. The rate of uptake of tritiated catecholamine in striatal slices was obtained by estimating the intracellular content of tritium following a 2 minute incubation.

The kinetics of the rate of uptake of the catecholamines in striatal slices were investigated by incubating at 5 concentrations, 0.1, 1.0, 10, 100 and 500 µM. This range of concentrations was selected as it included that range of concentrations necessary for an uptake system which demonstrates Michaelis-Menten kinetics and subsequent saturation at higher concentration (Snyder et al, 1968a,b; Snyder and Coyle, 1969; Salama et al, 1971; Mireylees, 1975) plus diffusion which occurs at all concentrations. A system possessing kinetic parameters similar to those of the low affinity system described by Mireylees (1975) would also be demonstrable at this range of concentrations.

The S/v against S graphs for the uptake of both catecholamines in the range 0.1 to 500 μM were similar. Plotting the values of S/v (min.g wet wt.ml^{-1}) against S (μM) produced a curve. The line approaching a parallel to the abscissa at higher concentrations (100 to 500 μM). The shape of the graph indicates that the uptake of catecholamines involves more than simple diffusion. A saturable system produces a line with a positive slope, diffusion a horizontal line. The slope of the graph therefore suggests that at least one saturable system for uptake is present.

To further analyse the kinetic data the experimental results were subjected to a curve fitting technique. The data when analysed by the three parameter model produced kinetic parameters which were considered to be a fit that was 'not good'. Analysis by the five parameter model produced a 'good fit' for the kinetic parameters calculated for both catecholamines. The values of Km for the high affinity mechanism in the striatum are very different for the two catecholamines. The Km value for dopamine was 0.081 μM and for noradrenaline 0.604 μM. The Vmax values obtained using striatal slices were 0.088 nmol.g wet wt^{-1}.min^{-1} and 0.086 nmol.g wet wt^{-1}.min^{-1}, for dopamine and noradrenaline respectively.

The Km for dopamine uptake is lower than those previously reported for the striatum, e.g. 0.4 μM (Coyle and Snyder, 1969; Snyder and Coyle, 1969; Shaskan and Snyder, 1970) or 0.1 to 0.2 μM (Harris and Baldessarini, 1973; Tuomisto et al, 1974; Mireylees, 1975). Similarly, the Km value for the high affinity uptake of noradrenaline in the

striatum is lower than that previously reported; 4.1 μM (Mireylees, 1975).

The Km values for the low affinity uptake system in the striatum were 40 μM and 59 μM for dopamine and noradrenaline respectively. These values are of the same order of magnitude as those reported by Mireylees (1975): dopamine, 24 μM and noradrenaline, 80 μM, for the low affinity uptake of catecholamines in the striatum. Shaskan and Snyder (1970), using striatal slices, reported a low affinity mechanism for the uptake of dopamine with a Km value in the range 1.0 to 1.3 μM. The Vmax values for the low affinity uptake of the two catecholamines, in the striatum, were, 22 nmol.g wet $wt^{-1}.min^{-1}$ for dopamine and 21 nmol.g wet $wt^{-1}.min^{-1}$ for noradrenaline.

The diffusion constant (D) for the uptake of the two catecholamines in the striatum are very similar: 0.144 ml.g wet $wt^{-1}.min^{-1}$ and 0.148 ml.g wet $wt^{-1}.min^{-1}$ for dopamine and noradrenaline respectively. These are in good agreement with those obtained by Mireylees (1975) in similar experiments: 0.138 ml.g wet $wt^{-1}.min^{-1}$ for dopamine and 0.137 ml.g wet $wt^{-1}.min^{-1}$ for noradrenaline.

The uptake of dopamine and noradrenaline was also investigated in slices of rat cerebellum. Accumulation of both catecholamines were shown to be linear for at least two minutes. Kinetic studies of dopamine uptake in this region of the brain showed that at least one saturable uptake system was present as well as diffusion. Analysis of the experimental uptake data using the three parameter model suggests that the model and data were not compatible. Using the five parameter

model a 'good fit' of experimental data and model was obtained. Kinetic studies of noradrenaline uptake in the cerebellum resulted in similar findings, with regard to the number of uptake systems shown to be present.

A Km value of 0.16 μM was obtained for the high affinity uptake of dopamine, and a Km of 0.46 μM for the high affinity uptake of noradrenaline, in slices of rat cerebellum. These values compare with Km values of 0.08 μM for dopamine, and 0.4 μM for noradrenaline obtained by Snyder and Coyle (1969), using non-striatal homogenates. Mireylees (1975), using slices of cerebellum, obtained values for the Km of the high affinity systems of 0.071 μM for dopamine and 0.57 μM for noradrenaline.

The Vmax values for the high affinity systems in the cerebellum were shown to be 0.008 nmol.g wet $wt^{-1}.min^{-1}$ for dopamine and 0.127 nmol.g wet $wt^{-1}.min^{-1}$ for noradrenaline. The corresponding values obtained by Mireylees (1975) were 0.028 nmol.g wet $wt^{-1}.min^{-1}$ and 0.17 nmol. g wet $wt^{-1}.min^{-1}$ for dopamine and noradrenaline respectively.

Analysis of the data produced a Km value of 18 μM for the low affinity uptake of dopamine in the cerebellum; the Km value for the low affinity uptake of noradrenaline in this brain region was 49 μM. The corresponding values for Vmax were 2.6 nmol.g wet $wt^{-1}.min^{-1}$ for dopamine and 3.5 nmol.g wet $wt^{-1}.min^{-1}$ for noradrenaline. The Km and Vmax values obtained by Mireylees (1975) for the low affinity

systems were 31 μM and 4 nmol.g wet $wt^{-1}.min^{-1}$ for dopamine and 453 μM and 11 nmol.g wet $wt^{-1}.min^{-1}$ for noradrenaline.

In the cerebellum the values for the diffusion constants were 0.194 ml.g wet $wt^{-1}.min^{-1}$ for dopamine and 0.088 ml.g wet $wt^{-1}.min^{-1}$ for noradrenaline. These results are similar to those of Mireylees (1975), 0.117 ml.g wet $wt^{-1}.min^{-1}$ for dopamine and 0.08 ml.g wet $wt^{-1}.min^{-1}$ for noradrenaline. The reason for the difference between the diffusion constants for dopamine and noradrenaline are not obvious. A possible explanation is that the greater the difference in the kinetic parameters for the two uptake systems and diffusion the more reliable a fit can be made.

Comparing the parameters obtained using striatal tissue with those using cerebellar tissue it can be seen that the Km for the high affinity uptake system is lower for dopamine in the striatum than in the cerebellum: 0.081 μM (striatum), 0.16 μM (cerebellum). The reverse is true for the Km values for the high affinity uptake of noradrenaline 0.60 μM (striatum) and 0.46 μM (cerebellum). The Km values for dopamine are lower than those for noradrenaline in both brain regions, a pattern also noted by Snyder and Coyle (1969) and Mireylees (1975).

The Vmax value for the high affinity mechanism for the uptake of dopamine in the striatum is a factor of ten higher than its counterpart in the cerebellum, 0.085 nmol.g wet $wt^{-1}.min^{-1}$ compared to 0.008 nmol.g wet $wt^{-1}.min^{-1}$. In the case of noradrenaline the Vmax for

the high affinity uptake mechanisms is slightly larger in the cerebellum 0.127 nmol.g wet $wt^{-1}.min^{-1}$ compared to the striatum 0.086 nmol.g wet $wt^{-1}.min^{-1}$.

The Km values for the low affinity systems for the uptake of dopamine in the two brain regions were of the same order of magnitude 40 µM (striatum) and 18 µM (cerebellum). The values for the corresponding systems for noradrenaline uptake were also similar, both to each other and to those for dopamine, 59 µM (striatum) and 49 µM (cerebellum). The Vmax values for the low affinity system for the uptake of dopamine were 22 nmol.g wet $wt^{-1}.min^{-1}$ (striatum) and 2.6 nmol.g wet $wt^{-1}.min^{-1}$ (cerebellum). The corresponding values for the Vmax for the low affinity mechanism for noradrenaline uptake were 21 nmol.g wet $wt^{-1}.min^{-1}$ (striatum) and 3.5 nmol.g wet $wt^{-1}.min^{-1}$ (cerebellum). The similarity of the kinetic parameters for the low affinity uptake of the two catecholamines in the striatum suggests that a single system may operate for both dopamine and noradrenaline at high concentrations. This system shows no obvious preference for either of the two molecules. A similar picture seems probable for the events occurring in the cerebellum. However, the differences in the kinetic parameters for low affinity uptake between the striatum and cerebellum make it appear unlikely that an identical system is operating in both regions.

Inhibition of the uptake of dopamine in slices of striatum

The uptake of catecholamines into brain tissue can be selectively inhibited by a wide range of compounds (Glowinski and Axelrod, 1964; Haggendal and Hamberger, 1967; Horn, 1973; Raiteri et al, 1977;

Waldmeier, 1982). In order to further investigate the low affinity uptake system previously shown to be present in the striatum, inhibition studies were carried out. The initial experiments involved the screening of a number of compounds related to phenylethylamine. Several known inhibitors of peripheral $Uptake_1$ and $Uptake_2$ were also screened for activity.

The effect of these compounds on the uptake of ^{3}H-dopamine at high concentration, by slices of striatum was investigated. The compounds screened were present at a concentration of 10^{-3}M Table 7.

The compounds catechol and methylamine showed no significant inhibitory effect on the uptake of ^{3}H-dopamine (100 µM). This result suggests that the low affinity uptake system involves more than the simple binding of the aromatic nucleus or amine portion of the molecule to the site of uptake.

17-β-oestradiol, a potent inhibitor of peripheral $Uptake_2$ (Salt, 1972), had no significant effect on the uptake of ^{3}H-dopamine (100 µM). This suggests that the low affinity uptake system in the central nervous system differs from that found in the peripheral nervous system, i.e. it is not the same as $Uptake_2$.

Methoxylated phenylethylamines, for example 3-methoxyisoprenaline, have been shown to inhibit peripheral $Uptake_2$(Mireylees and Foster, 1973). However in this study none of the compounds containing a methoxy group produced a significant effect on uptake. The compounds studied

were methoxamine, metanephrine, normetanephrine, dimethoxyphenylethylamine and 3-methoxyisoprenaline.

Other compounds which demonstrated no significant effect on the uptake of ^{3}H-dopamine (100 μM) were ephedrine, norephedrine, oxedrine and β-phenylethylamine. Zarrindast (1981) has shown ephedrine to inhibit the uptake of ^{3}H-dopamine in striatal synaptosomes; at a dopamine concentration of 0.1 μM the IC_{50} value of ephedrine was 120×10^{-9}M. Equally, β-phenylethylamine has also been shown to inhibit ^{3}H-dopamine (0.1 μM) uptake in striatal synaptosomes (Raiteri et al, 1977), with an IC_{50} value of 45×10^{-7}M. In comparison to ephedrine and norephedrine, pseudo(ψ)ephedrine and nor-pseudo(ψ)ephedrine do significantly effect the uptake of ^{3}H-dopamine (100 μM), therefore, suggesting a possible stereoselective mechanism for the low affinity uptake system.

The remaining compounds which significantly inhibited ^{3}H-dopamine (100 μM) uptake in striatal slices were: adrenaline, atropine, benztropine, desipramine, dopamine, isoprenaline, hordenine, 6-hydroxydopamine, metaraminol, noradrenaline, octopamine and tyramine. These compounds were further investigated.

The log concentration inhibition curves for the inhibition of the uptake of ^{3}H-dopamine (0.1 μM or 100 μM) in striatal slices were investigated for the various compounds tested above. At 100 μM dopamine concentration the maximum inhibition obtained was approximately 60% of the total uptake - this value was obtained with desipramine and benztropine (Figs. 15 and 16). These figures compare favourably with

those derived from the kinetic experiments. The Km, Vmax and diffusion values predict that at a dopamine concentration of 100 μM, 53% of the accumulated dopamine is taken up by the uptake systems. The slight difference observed could be due to a variety of reasons. The theoretical maximum value for percentage inhibition is calculated from the theoretical uptake value derived from the kinetic parameters produced by the curve fitting analysis. These are expressed as a percentage of the theoretical uptake. If the curve fitting analysis is not a particularly good fit with the experimental data at this concentration of substrate under investigation then the theoretical percentage inhibition cannot be regarded as accurate. In this study however the experimental S/v value of 3.281 (min.g wet $wt^{-1}.ml^{-1}$) compared well with calculated S/v (for the five parameter model) value of 3.301 (min.g wet $wt^{-1}.ml^{-1}$).

Even so, the true uptake system may be more complicated than the one presented by the model used to describe it; the portion of the total uptake ascribed to diffusion in the five parameter model masking one or more other saturable uptake systems. Therefore more carriers may be capable of being inhibited and the apparent theoretical maximum inhibition may be lower than that observed in the experiments.

Another explanation for the discrepancy between observed and calculated results is that the compounds known to act as inhibitors of uptake can also act to release catecholamines from the tissue (de Belleroche and Bradford, 1976; Baumann and Maitre, 1976). Thus these compounds by releasing catecholamines from the tissue together with

inhibiting their uptake from the incubation medium appear to possess a greater potency for the inhibition of uptake than is in fact the case.

The rank order of decreasing potency of the test drugs to inhibit the uptake of 100 μM ^{3}H-dopamine in slices of rat striatum is desipramine > benztropine = tyramine > adrenaline = metaraminol > noradrenaline = 6-hydroxydopamine > isoprenaline > octopamine = hordenine. See Table 8 for values of $E_{30}IC_{50}$. Isoprenaline, octopamine and hordenine produced only slight inhibition even at high concentrations and as such the $E_{30}IC_{50}$ value calculated by the GLIM programme possessed a high standard error. Therefore the rank order of potency of these weak compounds is difficult to assess accurately.

If the concentration of dopamine used for incubation is reduced the rank order of decreasing potency would be expected to remain the same if only a single system was in operation, the value for the IC_{50} would however also be reduced. However, if the rank order changes this gives an indication that a different uptake system predominates at the new incubation concentration. The second concentration selected for the study of dopamine uptake was 0.1 μM. Previous workers have assumed the uptake at this concentration is predominantly neuronal (Ross, Renyi and Brunfelter, 1968). However assuming that the kinetic parameters from previous experiments in this study are correct then at this concentration the two uptake systems contribute 41% (high affinity) and 47% (low affinity) of the total uptake, i.e. approximately

88% of the total uptake can be inhibited (Table 3). The maximum inhibition obtained experimentally was approximately 90% - using desipramine at a concentration of 10^{-2}M. The value is therefore in close agreement with the value for the percentage of total uptake which can be inhibited as derived from the kinetic analysis experiments. The rank order of decreasing potency of the test drugs to inhibit the uptake of 0.1 μM ^{3}H-dopamine in slices of rat striatum was benztropine > noradrenaline = desipramine = metaraminol = tyramine > hordenine > adrenaline = octopamine > isoprenaline > 6-hydroxydopamine.

The main differences between the rank order of potency of the test drugs for the inhibition of uptake of ^{3}H-dopamine at 100 μM compared with 0.1 μM are that desipramine is more potent than benztropine at inhibiting the uptake of 100 μM but the reverse is true at 0.1 μM. The potency of desipramine as an inhibitor of dopamine uptake (0.1 μM) compares well with the values found by other workers (Ross and Renyi, 1967).

Desipramine is regarded as an inhibitor of uptake into noradrenergic neurones, and the striatum is considered to be a dopaminergically innervated region (Glowinski and Iversen, 1966). A specific uptake mechanism for dopamine has been demonstrated in this brain area (Coyle and Snyder, 1969; Snyder and Coyle, 1969). The neuronal dopamine uptake mechanism has different inhibition properties from that of the noradrenergic neurones (Horn, 1973). Values of the IC_{50} for inhibiton of dopamine uptake in striatal synaptosomes (0.1 μM dopamine) were 5.9 x 10^{-6}M for desipramine and 4.2 x 10^{-7}M for

benztropine (Friedman et al, 1977). These compare with values of 3.7×10^{-6}M for desipramine and 7.4×10^{-7}M for benztropine obtained in this study using slices, suggesting that these are values for neuronal uptake.

The IC_{50} values for the drugs used to inhibit the accumulation of dopamine at the two concentrations studied (0.1 and 100 µM) are presented in alphabetical order in Table 8.

It is known that various compounds among those investigated are themselves accumulated by brain tissue, these include, tyramine (Ross and Renyi, 1966a); metaraminol (Ross and Renyi, 1966b) and octopamine (Ross et al, 1968). Some of these other compounds have also been shown to inhibit dopamine uptake in rat striatal synaptosomes: tyramine ($IC_{50} = 2.5 \times 10^{-6}$M), noradrenaline ($1.9 \times 10^{-6}$M) and octopamine ($1.6 \times 10^{-5}$M) as reported by Raiteri et al (1977), using dopamine at a concentration of 0.1 µM. These values are of the same order of magnitude but smaller than the corresponding results obtained in this study.

Isoprenaline it should be noted is not taken up by neuronal tissue in the periphery but only in to the extraneuronal structures (Callingham and Burgen, 1966) and was not observed to be actively accumulated by cortical slices (Ross and Renyi, 1966a). Tritiated isoprenaline was shown to be taken up by striatal slices (Mireylees, 1975), but not it was concluded into the dopaminergic neurones, but by binding to a non-specific binding site. Isoprenaline was also shown to be

a poor inhibitor of dopamine uptake at both 0.1 μM or 100 μM by Mireylees (1975).

The differences in the rank orders of decreasing potency of the ten compounds at the two dopamine concentrations studied suggest that two different uptake mechanisms predominate at the dopamine concentrations investigated.

It should be stated that the position of 6-hydroxydopamine in the rank order of potency may be anomalous. This substance was unique in that it was the only compound studied in which the IC_{50} value at the lower dopamine concentration was greater than that at the higher dopamine concentration. This would seem to suggest that 6-hydroxydopamine may not be taken up by the neurones at all. However, evidence opposes this idea, 6-hydroxydopamine is known to produce selective and permanent degeneration in catecholaminergic neurones (Breese and Traylor, 1970; Mendez and Finn, 1975) when injected into the brain. Desipramine (Breese and Traylor, 1971) and nomifensine (Samanin et al, 1975) have been shown to antagonise this effect. This antagonism has been attributed to the blockade of 6-hydroxydopamine uptake into the neurones.

A possible explanation of events in this study is that the five minute 'contact time' for 6-hydroxydopamine with the tissue was not sufficient for the drug to exert a maximum 'blocking effect' on the dopaminergic system. It should also be remembered that 6-hydroxydopamine is rapidly oxidised; in the incubation medium oxygen is readily

available and it is probable that the compound is converted to its oxidation product well before the incubation period is over. Hence the IC_{50} value given in the tables is likely to be a product of the values for 6-hydroxydopamine, its leucochrome and any intermediate forms.

If a great difference in the susceptibility to inhibition by the test drugs exists between the low and high affinity mechanism for the uptake of dopamine in slices of striatum, it would be expected that the graphs of log concentration against percentage inhibition for the uptake at 0.1 μM dopamine would show a plateau somewhere along the curve. This would be followed by a further rise in the curves when the mechanism which is less susceptible to the test drug starts to be affected. There is an indication that a plateau may exist in only one graph, that describing the pattern of inhibition by 6-hydroxydopamine for the uptake of dopamine at a concentration of 100 μM.

The rank order of decreasing potency, for inhibiting dopamine uptake at 0.1 μM, for these compounds compares well with the information already known with regard to inhibition of neuronal uptake i.e. (a) phenolic hydroxyl groups in the para and/or meta positions enhance uptake site affinity; (b) α-methylation increases affinity; (c) β-hydroxylation decreases affinity; (d) mono- or di-N-methylation decreases affinity; and (e) O-methylation of phenolic hydroxyls produces a marked decrease in affinity (Horn, 1978).

The screening experiments carried out at 100 µM provide ample evidence that points (a) and (e) are also true for the lower affinity uptake site. The IC_{50} values and the rank order of potency suggests that no particular substituents of the alkyl side chain are important in the binding of the compound to the uptake site, i.e. points (b), (c) and (d) are not valid for uptake by the low affinity uptake site, more emphasis being placed on the substituents of the ring structure. For the structures of the compounds used in the study see Appendix.

Incubation of the striatal slices in a modified Krebs' solution where sodium ions were replaced with choline ions significantly inhibited dopamine uptake at both 100 µM and 0.1 µM dopamine concentrations, Fig. 25. In experiments where sodium ions were replaced by sucrose significant inhibition of uptake was only achieved in slices incubated in 0.1 µM dopamine. Incubation of striatal slices at 0°C or in the presence of 2,4-dinitrophenol (10^{-3}M) produced significant inhibition of uptake at both dopamine concentrations investigated. Incubation at 0°C produced an inhibition of dopamine uptake of 95% and 88% for 0.1 µM and 100 µM dopamine respectively. Both of these values are in excess of the theoretical value for maximum inhibition derived from the kinetic date. However it should be noted that this reduction in the incubation temperature also causes a reduction in the size of the extracellular space, as measured with ^{14}C-sorbitol. The size of the space is reduced by approximately 50% (see Table 23). The apparently excessive inhibition of uptake may be brought about by a number of different factors including effects upon diffusion; the reduction of extracellular space causing a reduction of the

surface area of the cell membrane available for passive diffusion of dopamine into the cell. Mireylees (1975) also presented evidence that the size of the extracellular space is significantly reduced in the presence of 2,4-dinitrophenol (10^{-3}M). This would therefore contribute to the overall inhibition of dopamine uptake caused by this compound. However it is impossible to calculate the percentage inhibition produced by this effect.

This series of experiments would normally distinguish whether or not the uptake systems were energy or sodium dependent. However, the sorbitol space results indicate a reduction in the extracellular space, and thus a reduction in the availability of uptake sites. This makes some of the results obtained ambivalent. The conclusion that can be drawn is that at the lower dopamine concentration the uptake system appears to be both energy and sodium dependent, while at a high concentration no clear answer is obtained but the system may well be energy and sodium dependent.

Effect of 6-hydroxydopamine pre-treatment on the uptake of tritiated catecholamines by striatal slices

Pretreatment of the rats with the neurotoxin 6-hydroxydopamine injected into the lateral ventricles was shown to significantly reduce the uptake of dopamine in striatal slices at dopamine concentrations of 0.1, 1.0 and 10μM. Sham-operated animals injected with vehicle (0.9% saline plus 1 mg/ml ascorbic acid) showed no significant differences in the amount of uptake when compared to the untreated

control group of animals (Fig. 27).

After analysing the results using the curve fitting programme the results from all three treatments i.e. control, sham operated and 6-hydroxydopamine treated animals were shown to be a 'good fit' to the five parameter model. The Km values for the control and sham operated rats were similar for both the high and low affinity systems; 0.19 μM (control), 0.10 μM (sham) for the high affinity systems; 39.4 μM (control), 42.6 μM (sham) for the low affinity system. The values for the 6-hydroxydopamine treated animals were 0.97 μM for the high affinity system and 134.3 μM for the low affinity system. The Vmax values for the control group were 0.13 nmol.g wet $wt^{-1}.min^{-1}$ and 21.5 nmol.g wet $wt^{-1}.min^{-1}$ for the high and low affinity systems, the corresponding values in the sham operated and 6-hydroxydopamine treated groups were 0.08 nmol.g wet $wt^{-1}.min^{-1}$ and 24.4 nmol.g wet $wt^{-1}.min^{-1}$ for the sham group; 0.43 nmol.g wet $wt^{-1}.min^{-1}$ and 38.2 nmol.g wet $wt^{-1}.min^{-1}$ for the 6-hydroxydopamine treated group.

The main differences between the control and 6-hydroxydopamine treated animals are in the Km and Vmax values of the high affinity system. The destruction of the neurones with a toxic compound such as 6-hydroxydopamine would be expected to produce a fall in the Vmax value, Km remaining constant, due to a reduction in the number of high uptake sites. However as can be seen from the experimental results both the Vmax and Km values are increased for the high affinity mechanism. A possible explanation of these results is that the number of uptake systems involved is in fact greater than the two systems

originally predicted. In this case destruction of the neuronal uptake sites would allow the kinetic analysis using the curve fitting programme, to reveal another of the uptake systems, previously concealed by the low affinity system or diffusion coefficient. This would result in the five parameter model once more fitting the experimental data, as observed, with concomitant changes in either the low affinity parameters and/or the diffusion coefficient.

The effect of 6-hydroxydopamine pre-treatment on the uptake of noradrenaline by striatal slices was slightly different, the only significantly different point is that for 0.1 µM noradrenaline where the uptake of ^{3}H-noradrenaline is significantly decreased compared to the control: 0.037 nmol.g wet $wt^{-1}.min^{-1}$ compared to 0.062 nmol.g wet $wt^{-1}.min^{-1}$. The graph of S/v against S produced by the experimental data for ^{3}H-noradrenaline uptake by striatal slices following 6-OHDA pretreatment has the characteristic shape until this point where the S/v value is greater than that observed for the two points corresponding to 1 µM and 10 µM noradrenaline.

The decrease in noradrenaline uptake at what is thought to be a concentration corresponding to neuronal uptake is expected following treatment with 6-hydroxydopamine. However the S/v against S graph is more consistent with the kinetics which would be observed for an allosteric binding site (Monod, Changeux and Jacob, 1963). This may involve some control of uptake by a feedback system or alternatively cooperativity involving multiple binding sites.

The effect of pargyline on the uptake of ^{3}H-dopamine by striatal slices

Pargyline was shown not to exhibit any effect on the uptake of dopamine at either 0.1 or 100 µM dopamine concentration. This result does not agree with the results of Azzaro and Demarest (1982), who obtained an ID_{50} value of 378 ± 30 µM for the inhibition of ^{3}H-dopamine uptake by synaptosomes of the rat forebrain following incubation in 0.1 µM dopamine for five minutes. However their IC_{50} value obtained for deprenyl (a type B inhibitor) was greatly different from that obtained in a similar study (Lai et al 1980); 23 ± 1.6 µM compared to 530 µM, respectively. Azzaro and Demarest suggested that this discrepancy may be due to the fact that in the earlier study the animals were not treated with nialamide, another MAO inhibitor, or reserpine. These two drugs were introduced in order to eliminate the catabolism and storage components of the accumulation process, thus ensuring that the effects observed were primarily through the interaction of the drug with the high affinity neuronal membrane transport system for dopamine. As the animals in this study were not pretreated in this manner it may be a partial explanation for the observation of a lack of inhibitory effect at the lower concentration of dopamine. It is interesting to note that both type A and type B MAO inhibitors have been shown toinhibit dopamine uptake, but neuronal dopamine is predominantly a substrate for MAO type A (Waldmeier et al, 1976; Demarest et al, 1980; Schoepp and Azzaro, 1981).

Effect of dopamine metabolites on the uptake of ^{3}H-dopamine by striatal slices

Homovanillic acid (HVA) was shown to have no significant effect on dopamine accumulation by striatal slices at either dopamine concentration studied. However, dihydroxyphenylacetic acid (DOPAC) was shown to significantly inhibit ^{3}H-dopamine (100 µM) uptake in this preparation. Assuming that catechol-O-methyl transferase is located extraneuronally (Kaplan et al, 1979) and is responsible for the conversion of DOPAC to HVA, DOPAC being formed intraneuronally then DOPAC must be transferred from one site to the other. It is possible that DOPAC and dopamine are taken up by the same system prior to O-methylation. Therefore, this may be an example of competitive inhibition. Unfortunately, it is impossible to determine whether or not this is the case from the data available from these experiments. However it is more likely that the HVA is produced from dopamine taken up by non-neuronal cells and initially converted to DOPAC by MAO type B (Demarest et al, 1980).

Kinetics of the accumulation of ^{3}H-catecholamines by isolated striatal cells

The technique used for the measurement of the initial rate of uptake of ^{3}H-dopamine by isolated cells of the striatum was basically the same as that used to study uptake in striatal slices. The marker ^{14}C-sorbitol was again used to measure the amount of substrate adhering to the filter, used to separate the cells from the incubation medium, or any other remaining incubation medium. Time courses were constructed

to estimate the initial rate of accumulation. This was shown to be linear for at least five minutes at both 0.1 μM and 500 μM concentrations of dopamine. This period was therefore chosen as the incubation period for the kinetic studies. The same range of concentrations of dopamine was used in these studies as in those previously described i.e. 0.1, 1.0, 10.0, 100 and 500 μM. The sorbitol time course was similar to that obtained in the experiments using slices.

The S/v against S graph was also similar in appearance to those obtained for ^{3}H-catecholamine accumulation in slice preparations. A curve was produced, flattening out toward a line approaching a parallel to the abscissa. This type of curve is characteristic of a system involving more than diffusion. Further analysis of the data using the curve fitting programme produced values that were not a 'good fit' to the three parameter model. The five parameter model was considered a 'good fit' as the values derived from the programme were less than 0.5 standard errors different from the experimental values. This result indicates that at least two saturable uptake systems plus diffusion operate for the uptake of dopamine in the striatum. The result provides confirmation of the models obtained using the slice preparation and suggests that the two uptake systems are present and are not an artefact produced by using the tissue slices.

The uptake of ^{3}H-dopamine by striatal cells was shown to be temperature dependent at the five concentrations studied ranging from 84% inhibition of uptake at 500 μM dopamine to 96% inhibition at 0.1 μM dopamine. These values like those obtained in similar experiments

with slices greatly exceed the percentage inhibition values predicted from the kinetic analysis data. But as described previously the reduction in temperature also reduces the 'extracellular space' and may influence diffusion and thereby produce this exaggerated reduction in accumulation. As the tissue preparation being used in these experiments is one of isolated cells rather than slices the term extracellular space obviously refers to a different compartment, this must be taken as some form of micropores or invaginations of the cellular surface.

The metabolic inhibitor 2,4-dinitrophenol (10^{-3}M) significantly decreased the accumulation of ^{3}H-dopamine by the cells, as did incubation in a low sodium medium, containing choline chloride. Incubation in a low sodium medium, containing sucrose, had no significant effect on dopamine uptake by isolated striatal cells. All the above experiments were performed at dopamine concentrations of 0.1 µM and 10 µM. These incubation concentrations were chosen as at 0.1 µM the high affinity uptake system predominates and at 10 µM the lower affinity uptake system predominates, Table 17. These results suggest that the uptake systems in operation at both dopamine concentrations are energy dependent and probably sodium dependent, the results in low sodium media being ambiguous. Similar results were obtained when striatal slices were incubated in these different media.

Four compounds were investigated in order to study their effect on dopamine uptake by isolated striatal cells at dopamine concentrations of 0.1 µM and 10 µM. All of the four benztropine, desipramine,

noradrenaline and octopamine significantly inhibited dopamine uptake by the cell preparation when incubated in 0.1 μM dopamine. The maximum inhibition for this dopamine concentration obtained experimentally was 92%. At a dopamine concentration of 10μM all the drugs except benztropine produced inhibition of dopamine uptake. The maximum experimental inhibition of uptake was 81%. The values for the theoretical maximum inhibition of uptake derived from the kinetic parameters were 96% for 0.1 μM dopamine and 55% for 10 μM dopamine. The possible reasons for the discrepancy between the theoretical and experimental value for maximum inhibition at 10 μM dopamine have been given previously.

IC_{50} values, fitted by eye, were based on a similar system to that used for the inhibition studies using striatal slices in that the IC_{50} is taken as the value representing 50% of the maximum inhibition of uptake. At a dopamine concentration of 0.1 μM the value for benztropine was less than 10^{-8}M, desipramine approximately 10^{-7}M, noradrenaline and octopamine approximately 10^{-5}M. At 10 μM dopamine, benztropine could not be fitted, desipramine was approximately 10^{-5}M and noradrenaline and octopamine were less than 10^{-8}M.

A rank order of potency could be derived from these results: at 0.1 μM dopamine benztropine > desipramine > noradrenaline = octopamine, but at 10 μM dopamine the rank order of potency would be virtually meaningless. The absence of any inhibitory effect on dopamine uptake (10 μM) by benztropine could indicate that the uptake system investigated in isolated cells at this concentration is not

the same as that studied in the slice preparation using 100 µM dopamine. The rank order of potency at 0.1 µM dopamine is similar to that obtained for the inhibition of dopamine uptake using striatal slices, at 0.1 µM dopamine, except that noradrenaline appears less potent in the study using isolated striatal cells. No obvious explanation for this difference is apparent but it is possible that the uptake system is different from the one investigated in the striatal slice experiments.

Kinetics of the accumulation of ^{3}H-dopamine by striatal homogenates and synaptosomes

The time course experiments using striatal homogenates or synaptosomes resulted in a period of five minutes being selected for the kinetic studies. The time courses were carried out at the same dopamine concentrations used on previous occasions 0.1 and 500 µM. The kinetic studies were carried out by incubating the striatal preparations at five concentrations of dopamine 0.1, 1.0, 10, 100 and 500 µM. Plotting the values of S/v against S for the uptake of ^{3}H-dopamine in striatal homogenates produced the familiar curve shaped graph seen in the previous experiments. However, analysis of the experimental data failed to provide a 'good fit' to either of the normal three or five parameter models. The value for the fit being in excess of one standard error in both cases.

Plotting S/v against S for the uptake of ^{3}H-dopamine in striatal synaptosomes produced a curve of similar shape. Analysis of the data indicates that the experimental results are a 'good fit' to the three parameter model. As expected the values also fit the five parameter

model. If the three parameter model is taken as correct this would suggest that only the high affinity system is present in the synaptosomes, the value for Km being of the same order of magnitude as that found in experiments using isolated striatal cells, 0.083 μM in synaptosomes compared to 0.015 - 0.038 μM in striatal cells. The Km value for the high affinity uptake system in homogenates is also of the same order, 0.035 μM but as the data did not fit the model the validity of the kinetic parameters is suspect. Vmax values are 8.65 nmol.g protein^{-1}.min^{-1} in synaptosomes and 4.75 - 4.86 nmol.g protein^{-1}.min^{-1} in isolated striatal cells. The equivalent Vmax value in striatal homogenates cannot be directly compared as it is in the form of nmol.g wet wt^{-1}.min^{-1}.

The kinetic parameters for the high affinity uptake system in synaptosomes obtained from the five parameter model are also of the same order of magnitude with a Km value of 0.054 μM and Vmax 1.26 nmol.g protein^{-1}.min^{-1}.

The Km value for the high affinity uptake of dopamine in striatal slices was 0.081 - 0.193 μM. The reason for the difference in the magnitude of the Km between a preparation in which the cells are dispersed and the slices which are still intact may well be the accessibility of the uptake sites. Far more uptake sites are immediately available to the dopamine molecules in the isolated cell, homogenate and synaptosomal preparations than in the slice preparation.

The kinetic parameters for the low affinity uptake of dopamine are again similar for the isolated cells and homogenates, Km values being 3.29 μM and 5.62 μM respectively. The value for the Km of low affinity system in synaptosomes, derived from the five parameter model is slightly lower, 0.91 μM. These values are much lower than the Km for low affinity uptake obtained using slices which was 39.4 - 40.3 μM. This may again be as a result of the ease of accessibility of the uptake sites for dopamine or alternatively due to a different low affinity uptake site being investigated.

The Vmax value for the low affinity site in synaptosomes was lower than that in the isolated striatal cells: 7.5 nmol.g protein^{-1}.min^{-1} compared to 26.3 - 36.3 nmol.g protein^{-1}.min^{-1}. Together with the difference in the Km values this suggests that if a low affinity site does exist in the synaptosomes it is different from that obtained in the experiments with isolated cells. The Vmax value for the low affinity site in homogenates was 9.6 nmol.g wet wt^{-1}.min^{-1} obtained in striatal slices.

The diffusion coefficient is much greater in the homogenates than in the slices, 1.98 or 2.01 ml.g wet wt^{-1}.min^{-1} compared to 0.14 ml.g wet wt^{-1}.min^{-1}. This is to be expected as the surface area available for diffusion is much greater in the homogenates. The values for D in the isolated cells and synaptosomes are also different 2.65 - 2.75 ml.g protein^{-1}.min^{-1} compared to 1.62 - 1.63 ml.g protein^{-1}.min^{-1}.

Metabolism of catecholamines accumulated by striatal slices

The accumulation of dopamine and its metabolites by striatal slices were studied over a period of 30 minutes. A concentration of 100 µM dopamine was chosen as at this concentration both the high and low affinity uptake systems are assumed to be in operation. The pattern of metabolism is similar to that described by Rutledge and Jonason (1967) for the metabolism of dopamine in the rabbit brain. The major metabolite produced was DOPAC. The accumulation of this compound more or less paralleled that of dopamine accumulation. DOPAC is produced by the action of MAO type A or B (Hall et al, 1969). MAO type A has been shown to located mainly in the dopaminergic neurons of the striatum (Demarest et al, 1980); type B is thought to be present extraneuronally in the glial cells (Strolin Benedetti and Keane, 1980; Fowler et al, 1980). However, only a small percentage of the accumulated dopamine is converted to DOPAC; 303.4 ± 32.4 nmol dopamine.g^{-1} was present after thirty minutes incubation in 100 µM dopamine compared to 28.1 ± 4.2 nmol DOPAC.g^{-1}. The amount of HVA accumulated in the same period was even smaller 4.5 ± 0.5 nmol.g^{-1} and 3-MT accumulation almost negligible 0.7 ± 0.2 nmol.g^{-1}. These compare with values before incubation of 66.1 ± 4.0 nmol dopamine.g^{-1}, 4.6 ± 0.4 nmol.g^{-1} DOPAC and 1.9 ± 0.05 nmol.g^{-1} HVA, 3-MT levels were below those detectable.

It is probable that under the conditions of the experiment the majority of the dopamine accumulated during the incubation period remains in the tissue without being metabolised. Another explanation

for the accumulation of DOPAC in greater amounts than the other metabolites is that MAO acts on its substrate (dopamine) more rapidly than COMT and as such the deaminated rather than the O-methylated metabolite is more readily produced. This may be due to the amount of enzyme present, or the accessibility of the substrate for the enzyme.

The presence of HVA in the brain (and 3-MT at 30 min) indicates that at least part of the accumulated dopamine is located extra-neuronally prior to metabolism. This assumes that MAO and COMT activity occur in the same cell and that DOPAC formed intraneuronally does not pass to extraneuronal elements for further metabolism. However, this does not necessarily suggest that the extraneuronal elements are the site of an uptake system as the dopamine could have entered these cells by passive diffusion.

It is also possible that metabolites were rapidly released into the medium, attempts were made to measure metabolites in the medium but this proved difficult due to the large concentration of dopamine and dilution of the metabolites in the medium. Small amounts of DOPAC were detected at ten and thirty minutes but neither of the other metabolites could be measured.

Pretreatment of the animals with intracerebroventricularly administered 6-hydroxydopamine led to a reduction in the levels of dopamine found to be present in the striatum. The control samples were shown to contain 271.7 ± 43.9 nmol dopamine.g^{-1} after ten

minutes incubation, whereas the samples from rats pretreated with 6-hydroxydopamine contained 206.0 ± 35.7 nmol dopamine.g^{-1} after a similar period of incubation. These dopamine values are not however significantly different. The amount of dopamine found to be present in striatal samples that had not been incubated in 100 μM dopamine were significantly decreased by pretreatment with 6-hydroxydopamine. The amount of dopamine present being reduced from 66.1 ± 4.0 nmol.g^{-1} in the control samples to 27.9 ± 3.6 nmol.g^{-1} in samples from the pretreated rats.

There was no evidence of an increase in 3-MT accumulation following incubation of the slices from rats pretreated with 6-hydroxydopamine, but the amount of HVA present increased from 2.8 ± 0.3 nmol.g^{-1} to 4.8 ± 0.9 nmol.g^{-1} after ten minutes incubation, control and 6-hydroxydopamine results respectively. Taking into account that 1.9 nmol HVA.g^{-1} was found to be present in the control slice prior to incubation and 1.8 nmol HVA.g^{-1} in the non-incubated pretreated slice, this shows that an increase in HVA production of approximately three-fold has occurred.

The high level of DOPAC, 29.9 ± 6.0 nmol.g^{-1} found in the 6-hydroxydopamine treated tissue following a ten minute incubation seems anomalous as it has been shown that MAO activity is significantly reduced following lesioning of the striatum with 6-hydroxydopamine (Agid et al, 1973). This however will only affect MAO type A. In tissue which has not been incubated in 100 μM dopamine the amount of DOPAC present is significantly lower in the striata from the rats treated with 6-hydroxy-

Following incubation in 100 μM noradrenaline the accumulation of noradrenaline within the slices is closely followed by the accumulation of DHMA/DOPEG, VMA and NMN. The amount of DHMA/DOPEG was far in excess of the other metabolites. Based on the work of Rutledge and Jonason (1967) it can be assumed that the major component of this peak is due to DOPEG, which together with MOPEG (this compound could not be separated from the solvent front using this method) constitute 53% of the total metabolites of noradrenaline. The COMT product NMN was found in very small amounts, 0.8 ± 0.1 nmol.g^{-1} after thirty minutes. This again suggests that the majority of the accumulated noradrenaline is metabolised by MAO.

Tissue from rats pretreated with 6-hydroxydopamine was incubated in the presence of 100 μM noradrenaline and then subjected to analysis by H.P.L.C. These appeared to be little effect on the pattern of metabolism. Normetanephrine was the only exception, as it could not be detected after 6-hydroxydopamine treatment. This may be due to the low levels of the compound found to be present normally. There was no significant difference in the uptake of noradrenaline as compared to controls, 190.2 ± 17.9 nmol.g wet wt^{-1} and 154.0 ± 13.4 nmol.g wet wt^{-1}, respectively. DOPEG/DHMA levels were 29.0 ± 1.6 nmol.g wet wt^{-1} and 33.2 ± 1.2 nmol.g wet wt^{-1} for treated and control respectively and the corresponding VMA values 3.1 ± 0.5 and 2.6 ± 0.5 nmol.g wet wt^{-1}. DOPEG/DHMA was found to be present in the incubation medium in both control and 6-OHDA pretreatment studies.

The amount of noradrenaline accumulated by striatal slices during a two minute incubation in medium containing 100 μM noradrenaline was 61.3 ± 7.0 nmol.g^{-1} this compares with a value of 56 nmol.g^{-1} calculated from the radiolabelled noradrenaline uptake data.

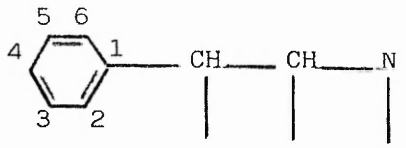
Conclusions

This study has provided evidence that at least two separate uptake systems for dopamine are present in the striatum: one of these systems appears to be neuronal and the other probably extraneuronal in origin. Work performed by other authors suggests that the glial cells are a possible site of monoamine uptake (Henn and Hamberger, 1971 ; Liesi et al, 1981). Glial cells are known to accumulate other putative neurotransmitters for example γ-amino butyric acid (Henn and Hamberger, 1971 ; Schon and Kelly, 1974; Sellstrom and Hamberger, 1975) and glutamate (Currie and Kelly, 1981). Several attempts were made during this study to prepare isolated neurones and isolated glial cells by the methods of Norton and Poduslo (1970) and also by the method of Chao and Rumsby (1977). However, neither method resulted in the isolation of sufficient viable cells for experimental purposes. Successful isolation of these cells and kinetic analysis of their uptake of monoamines may help to resolve the problem of the site of the second uptake system. Another possibility is to study catecholamine uptake in cultured cells.

The fate of the catecholamines following uptake was also investigated. This revealed that COMT, considered to be an enzyme found predominantly, if not solely, in the glial cells, was responsible

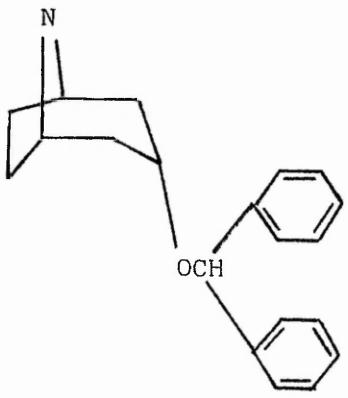
for little of the direct metabolism of the catecholamines. The majority of the compounds are metabolised by MAO of either type A or possibly type B. Further studies using specific inhibitors of these iso-enzymes, such as deprenyl a type B inhibitor or clorgyline a type A inhibitor may reveal more about the metabolism of accumulated catecholamines. More extensive studies of the metabolic pathways following chemical lesioning of the striatum with compounds such as 6-hydroxydopamine or 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) a neurotoxin selective for dopaminergic neurones may help to unravel the complex problems of the sites for uptake and the fate of the compounds accumulated by these systems.

APPENDIX

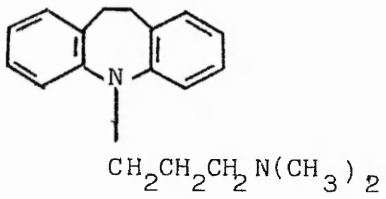


ADRENALINE	3-OH 4-OH	OH	H	H, CH_3
DOPAMINE	3-OH 4-OH	H	H	H_2
HORDENINE	3-OH	H	H	$(CH_3)_2$
6-HYDROXYDOPAMINE	3-OH 4-OH 6-OH	H	H	H_2
ISOPRENALINE	3-OH 4-OH	OH	H	$CH(CH_3)_2$, H
METARAMINOL	3-OH	OH	CH_3	H_2
NORADRENALINE	3-OH 4-OH	OH	H	H_2
OCTOPAMINE	4-OH	OH	H	H_2
TYRAMINE	4-OH	H	H	H_2

BENZTROPINE



DESIPRAMINE



REFERENCES

Agid. Y., Javoy, F. and Youdim, M.B.H. (1973) Monoamine oxidase and aldehyde dehydrogenase activity in the striatum of rats after 6-hydroxydopamine lesion of the nigrostriatal pathway.
Br. J. Pharmac. 48 : 175 - 178.

Avakian, O.V. and Gillespie, J.S. (1968) Uptake of noradrenaline by adrenergic nerves, smooth muscle, and connective tissue in isolated perfused arteries and its correlation with the vasoconstrictor response.
Br. J. Pharmac. 32 : 168 - 184.

Azzaro, A.J. and Demarest, K.T. (1980) Inhibitory effects of type-B monoamine oxidase inhibitors on synaptosomal accumulation of (^{3}H)-dopamine: a reflection of antidepressant potency.
Biochem. Pharmac. 31 : 2195 - 2197.

Bachelard, H.S., Campbell, W.J. and McIlwain, H. (1962) The sodium and other ions of mammalian cerebral tissues, maintained and electrically stimulated in vitro.
Biochem. J. 84 : 225 - 232.

Bauman, P.A. and Maitre. L. (1976) Is drug inhibition of dopamine uptake a misinterpretation of in vitro experiments ?
Nature, 264 : 789 - 790.

Belleau, B. and Burba, J. (1963) Occupancy of adrenergic receptors and inhibition of catechol O-methyl transferase by tropolones.
J. Med. Chem. 6 : 755 - 759.

Borchardt, R.T. (1973) Catechol O-methyltransferase. 1. Kinetics of tropolone inhibition.
J. Med. Chem. 16 : 377 - 381.

Braestrup, C., Andersen, H. and Randrup, A. (1975) The monoamine oxidase B inhibitor deprenyl potentiates phenylethylamine behaviour in rats without inhibition of catecholamine metabolite formation.
Eur. J. Pharmac. 34 : 181 - 185.

Breese, G.R. and Traylor, T.D. (1970) Effect of 6-hydroxydopamine on brain norepinephrine and dopamine: Evidence for selective degeneration of catecholamine neurons.
J. Pharmac. Exp. Ther. 174 : 413 - 420.

Breese, G.R. and Traylor, T.D. (1971) Depletion of brain noradrenaline and dopamine by 6-hydroxydopamine.
Br. J. Pharmac. 42 : 88 - 99.

Burgen, A.S.V. and Iversen, L.L. (1965) The inhibition of noradrenaline uptake by sympathomimetic amines in the rat isolated heart.
Br. J. Pharmac. Chem. 25 : 34 - 49.

Burrows, G.H. , Myers, M.M., Whittemore, S.R. and Hendley, E.D. (1981) Calcium-sensitive accumulation of norepinephrine in rat cerebral cortex.
Eur. J. Pharmac. 69: 301 - 302.

Buu, N.T., Duhaime, J., Savard, C., Truong, L. and Kuchel, O. (1981) Presence of conjugated catecholamines in rat brain: A new method of analysis of catecholamine sulfates.
J. Neurochem. 36 : 769 - 772.

Callingham, B.A. and Burgen, A.S.V. (1966) The uptake of isoprenaline and noradrenaline by the perfused rat heart.
Mol. Pharmac. 2 : 34 - 42.

Carlsson, A., Fuxe, K., Hamberger, B. and Lindqvist, M. (1966) Biochemical and histochemical studies on the effects of imipramine-like drugs and (+)-amphetamine on central and peripheral catecholamine neurones.
Acta. Physiol. Scand. 67 : 481 - 497.

Carlsson, A. and Hillarp, N.A. (1962) Formation of phenolic acids in brain after administration of 3,4-dihydroxyphenyl alanine.
Acta. Physiol. Scand. 55 : 95 - 100.

Chao, S.W. and Rumsby, M.G. (1977) Preparation of astrocytes, neurones and oligodendrocytes from the same rat brain.
Brain. Res. 124 : 347 - 351.

Clarke, D.E. and Jones, C.J. (1969) Are adrenergic nerves required for $Uptake_2$ in the isolated perfused rat heart ?
Br. J. Pharmac. 7 : 121 - 124.

Clarke, D.E., Jones, C.J. and Linley, P.A. (1969) Histochemical fluorescence studies on noradrenaline accumulation by $Uptake_2$ in the isolated rat heart.
Br. J. Pharmac. 15 : 707 - 720.

Cohen, S.R., Blasberg, R., Levi, G. and Lajtha, A. (1968) Compartmentation of the inulin space in mouse brain slices.
J. Neurochem. 15 : 707 - 720.

Cohen, S.R., Stampleman, P.F. and Lajtha, A. (1970a) The temperature dependent compartmentation of the extracellular space in mouse brain slices as revealed by the markers: Inulin, sucrose, D-mannitol, D-sorbitol and sulfate.
Brain Res. 21 : 419 - 434.

Cohen, S.R. and Lajtha, A. (1970b) The effect of tonicity, D-mannitol, D-sorbitol and choline chloride on the water content of mouse brain slices and on the compartmentation of the space indicated by 'extracellular space markers'.
Brain Res. 23 : 77 - 93.

Colbourn, R.W. Goodwin, F.K., Murphy, D.L., Bunney, W.E. and Davis, J.M. (1968) Quantitative studies of norepinephrine uptake by synaptosomes.
Biochem. Pharmac. 17 : 957 - 964.

Cotman, C.W. and Matthews, D.A. (1971) Synaptic plasma membranes from rat synaptosomes: Isolation and partial characterization.
Biochim. Biophys. Acta. 249 : 380 - 394.

Coyle, J.T. and Snyder, S.H. (1969) Catecholamine uptake by synaptosomes in homogenates of rat brain: Stereospecificity in different areas.
J. Pharmac. Exp. Ther. 170 : 221 - 231.

Crews, F.T. and Smith, C.B. (1981) The effects of antidepressants on the retention and metabolism of (^{3}H)-norepinephrine in rat brain.
Neuropharmac. 20: 363 - 369.

Currie, D.N. and Kelly, J.S. (1981) Glial versus neuronal uptake of glutamate.
J. Exp. Biol. 95 : 181 - 193.

De Belleroche, J.S. and Bradford, H.F. (1976) Transport of amino acids and catecholamines in relation to metabolism and transmission. Transport phenomena in the nervous system. Physiological and pathological aspects.
Adv. Exp. Med. Biol. 69 : 395 - 404.

De Jong, A.P., Fesik, S.W. and Makriyannis, A. (1982) Conformational requirements for norepinephrine uptake. Inhibition by phenylethylamines in brain synaptosomes. Effects of α-alkyl substitution.
J. Med. Chem. 25 : 1438 - 1441.

De Paulis, T., Kelder, D., Ross, S.B. and Stjernstrom, N.E. (1978) On the topology of the norepinephrine transport carrier in the rat hypothalamus. The site of action of tricyclic uptake inhibitors.
Mol. Pharmac. 14 : 596 - 606.

Dedek, L., Baumes, R., Tien-Dic, N., Gomeni, R. and Korf, J. (1979) Turnover of free and conjugated 'sulphonyloxy' DOPAC and HVA in rat striatum.
J. Neurochem. 33 : 687 - 695.

Demarest, K.T., Smith, D.J. and Azzaro, A.J. (1980) The presence of the type A form of monoamine oxidase within the nigrostriatal dopamine-containing neurones.
J. Pharmac. Exp. Ther. 215 : 461 - 468

Dengler, H.J., Spiegel, H.E. and Titus, E.O. (1961) Effects of drugs on uptake of isotopic norepinephrine by cat tissues.
Nature 191 : 816 - 817

Dixon, M. and Webb, E.C. (1964) Enzymes 2nd edition, pp. 118 - 128.
Longmans, London.

Duncan, R.J.S. and Sourkes, T.L. (1974) Some enzymatic aspects of the production of oxidised or reduced metabolites of catecholamines and 5-hydroxytryptamine by brain tissues.
J. Neurochem. 22 : 663 - 669.

Elchisak, M.A., Maas, J. and Roth, R.H. (1977) DOPAC conjugate: Natural occurrence and demonstration of probenecid-induced accumulation in rat striatum, olfactory tubercles and frontal cortex.
Eur. J. Pharmac. 41 : 369 - 378.

Farnebo, L-O. and Malmfors, T. (1969) Histochemical studies on the uptake of noradrenaline and α-methyl noradrenaline in the perfused rat heart.
Eur. J. Pharmac. 5 : 313 - 320.

Foldes, A. and Meek, J.L. (1974) Occurrence and localisation of brain phenolsulphotransferase.
J. Neurochem. 23 : 303 - 307.

Fowler, C.J., Wiberg, A., Oreland, L., Marcusson, J. and Winblad, B. (1980) The effect of age on the activity and molecular properties of human brain monoamine oxidase.
J. Neural Transm. 49 : 1 - 20.

Freeman, J.J. and Sulser, F. (1972) Iprindole-amphetamine interactions in the rat: The role of aromatic hydroxylation of amphetamine in its mode of action.
J. Pharm. Exp. Ther. 183 : 307 - 315.

Friedman, E., Fung, F. and Gershon, G. (1977) Antidepressant drugs and dopamine uptake in different brain regions.
Eur. J. Pharmac. 42 : 47 - 51.

George, S.R. and van Loon, G.R. (1982) β-endorphin alters dopamine uptake by dopamine neurons of the hypothalamus and striatum.
Brain Res. 248 : 293 - 303.

Gillespie, J.S. and Hamilton, D.N.H. (1967) A possible active transport system of noradrenaline into arterial smooth muscle cells.
J. Physiol. (Lond.) 192 : 30P.

Gillespie, J.S. Hamilton, D.N.H. and Hosie, R.J.A. (1970) The extra-neuronal uptake and localisation of noradrenaline in the cat spleen and the effect on this of some drugs, of cold and of denervation.
J. Physiol. (Lond.) 206 : 563 - 590.

Gillespie, J.S. and Towart, R. (1973) Uptake kinetics and ion requirements for extraneuronal uptake of noradrenaline by arterial smooth muscle and collagen.
Br. J. Pharmac. 47 : 556 - 567.

Glowinski, J. and Iversen, L.L. (1966) Regional studies of catecholamines in the rat brain. 1. The disposition of (^{3}H)-norepinephrine, (^{3}H)-dopamine and (^{3}H)-DOPA in various regions of the brain.
J. Neurochem. 13 : 655 - 699.

Glowinski, J. and Axelrod, J. (1964) Inhibition of uptake of (^{3}H)-noradrenaline in the intact rat brain by imipramine and structurally related compounds.
Nature 204 : 1318 - 1319.

Goodale, D.B. and Moore, K.E. (1975) Benztropine-induced release of dopamine from brain in vivo.
Neuropharmac. 14 : 585 - 589.

Gordon, E.K., Markey, S.P., Sharman, R.L. and Kopin, I.J. (1976) Conjugated 3,4-dihydroxyphenylacetic acid (DOPAC) in human and monkey cerebrospinal fluid and rat brain and the effects of probenecid treatment.
Life Sci. 18 : 1285 - 1292.

Green, A.D. III and Miller, J.W. (1966) Evidence for the active transport of epinephrine and norepinephrine by the uterus of the rat.
J. Pharmac. Exp. Ther. 152 : 42 - 50.

Gropetti, A., Algeri, S., Cattabeni, F., Di Giulio, A.M., Galli, C.L., Ponzio, F. and Spano, P.F. (1977) Changes in specific activity of dopamine metabolites as evidence of a multiple compartmentation of dopamine in striatal neurones.
J. Neurochem. 28 : 193 - 197.

Häggendal, J. and Hamberger, B. (1967) Quantitative in vitro studies on noradrenaline uptake and its inhibition by amphetamine, desipramine and chlorpromazine.
Acta. Physiol. Scand. 70 277 - 280.

Hall, D.W.R., Logan, B.W. and Parsons, G.H. (1969) Further studies on the inhibition of monoamine oxidase by M&B 9302. 1. Substrate specificity in various mammalian species.
Biochem. Pharmac. 18 : 1447 - 1454.

Hamberger, B. (1967) Reserpine-resistant uptake of catecholamines in isolated tissues of the rat.
Acta. Physiol. Scand. Suppl. 295 : 1 - 56.

Hamberger, B. and Masuoka, D. (1965) Localisation of catecholamine uptake in rat brain slices.
Acta. Pharmac. Tox. 22 : 363 - 368.

Hardebo, J.E. , Emson, P.C., Falk, B., Owman, C. and Rosengren, E. (1980a) Enzymes related to monoamine transmitter metabolism in brain microvessels.
J. Neurochem. 35 : 1388 - 1393.

Hardebo, J.E. and Owman, C. (1980b), Characterisation of the in vitro uptake of monoamines into brain microvessels.
Acta Physiol. Scand. 108 : 223 - 229.

Harris, J.E. and Baldessarini, R.J. (1973) The uptake of (^{3}H)-dopamine by homogenates of rat corpus striatum : effects of cations.
Life Sc. 13 : 303 - 312.

Heikkila, R.E. Orlansky, H. and Cohen, G. (1975) Studies on the distinction between uptake inhibition and release of (^{3}H)-dopamine in rat brain slices.
Biochem. Pharmac. 24 : 847 - 852.

Hendley, E.D., Taylor, K.M. and Snyder, S.H. (1970) (^{3}H)-normetanephrine uptake in rat brain slices. Relationship to extraneuronal accumulation of norepinephrine.
Eur. J. Pharmac. 12 : 167 - 179.

Henn, F.A. and Hamberger, A. (1971) Glial cell function: Uptake of transmitter substances.
Proc. Nat. Acad. Sci. USA. 68 : 2686 - 2690.

Holz, K.W. and Coyle, J.T. (1974) The effects of various salts, temperature and the alkaloids veratridine and bachrotoxin on the uptake of (^{3}H)-dopamine into synaptosomes from rat striatum.
Mol. Pharmac. 10 : 746 - 758.

Horn. A.S: (1973) Structure-activity relations for the inhibition of catecholamine uptake into synaptosomes from noradrenaline and dopamine neurones in rat brain homogenates.
Br. J. Pharmac. 47 : 332 - 338.

Horn, A.S. (1974) The conformation of dopamine at its uptake site; further studies with rigid analogues.
J. Pharm. Pharmac. 26 : 735 - 737.

Horn, A.S. (1978) Characteristics of neuronal dopamine uptake.
Adv. Biochem. Psychopharmac. 19 : 25 - 34.

Horn, A.S., Coyle, J.T. and Snyder, S.H. (1971) Catecholamine uptake by synaptosomes from rat brain. Structure-activity relationships of drugs with differential effects on dopamine and norepinephrine neurones.
Mol. Pharmac. 7 ; 66 - 80.

Horn, A.S. and Snyder, S.H. (1972) Steric requirements for catecholamine uptake by rat brain synaptosomes: Studies with rigid analogues of amphetamine.
J. Pharmac. Exp. Ther. 180 : 523 - 530.

Hunt, P., Kannengeiser, M-H. and Raynaud, J-P. (1974) Nomifensine a new potent inhibitor of dopamine uptake into synaptosomes from rat brain corpus striatum.
J. Pharm. Pharmac. 26 : 370 - 371.

Iversen, L.L. (1963) The uptake of noradrenaline by the isolated perfused rat heart.
Br. J. Pharmac. Chem. 21 : 523 - 537.

Iversen, L.L. (1965a) The uptake of catecholamines at high perfusion concentrations in the rat isolated heart. A novel uptake process.
Br. J. Pharmac. 25 : 18 - 33.

Iversen, L.L. (1965b) The inhibition of noradrenaline uptake by drugs.
Adv. Drug Res. 2 : 1 - 46.

Iversen, L.L., Jarrot, B. and Simmonds, M.A. (1971) Differences in the uptake, storage and metabolism of (+) and (-) noradrenaline.
Br. J. Pharmac. 43 : 845 - 855.

Iversen, L.L. and Kravitz, E.A. (1966) Sodium dependence of transmitter uptake at adrenergic nerve terminals.
Mol. Pharmac. 2 : 360 - 362.

Iversen, L.L. and Salt, P.J. (1970) Inhibition of catecholamine Uptake$_2$ by steroids in the isolated rat heart.
Br. J. Pharmac. 40 : 528 - 530.

Johnston, P. (1968) Some observations upon a new inhibitor of monoamine oxidase in brain tissue.
Biochem. Pharmac. 17 : 1285 - 1297.

Kaplan, G., Hartman, B.K. and Creveling, C.R. (1979) Immunohistochemical demonstration of catechol O-methyl transferase in mammalian brain.
Brain Res. 167 : 241 - 250.

Keen, P. and White, T.D. (1969) The role of ions in the transport of noradrenaline into synaptosomes.
J. Physiol. (Lond.). 203 : 54P - 55P.

Kehr, W. (1976) 3-methoxytyramine as an indicator of impulse-induced dopamine release in rat brain in vivo.
Naunyn-Schmied. Arch. Pharmac. 293 : 209 - 215.

Klein, P.D. and Eisler, W.J. (1966) An improved description of separation and performance capabilities of liquid scintillation counters used in dual isotope studies.
Anal. Chem. 38 : 1453 - 1457.

Komiskey, H.L., Hsu, F.L., Bossart, F.J., Fowbie, J.W., Miller, D.D. and Patil, P.N.(1978) Inhibition of synaptosomal uptake of norepinephrine and dopamine by conformationally restricted sympathomimetic amines.
Eur. J. Pharmac. 52 : 37 - 45

Kopin, I.J. and Gordon, E.K. (1963) Metabolism of administered and drug-released norepinephrine-7-^{3}H in the rat.
J. Pharmac. Exp. Ther. 140 : 206 - 216.

Lai, J.C.K., Leung, T.K.C., Guest, J.F., Lim, L. and Davison, A.N. (1980) The MAO inhibitors clorgyline and l-deprenyl also effect uptake of dopamine, noradrenaline and serotonin by rat brain synaptosomal preparations.
Biochem. Pharmac. 29 : 2763 - 2767.

Law, S-J., Morgan, J.M., Masten, L.W., Borne, R.F., Arana, G.W., Jula, N.S. and Baldessarini, R.J. (1982) Rigid analogues of dopamine: Synthesis and interaction of 6-exo- and 6-endo-(3',4'-dihydroxyphenol)-2-azabicyclo (2.2.2.) octane with dopamine uptake sites and receptors.
J. Med. Chem. 25 : 213 - 216.

Liesi, P., Paetau, A., Rechardt, L. and Dahl, D. (1981) Glial uptake of monoamines in primary cultures of rat median Raphe nucleus and cerebellum.
Histochem. 73 : 239 - 250.

Lightman, S.L. and Iversen, L.L. (1969) The role of Uptake 2 in the extraneuronal metabolism of catecholamine in the isolated rat heart.
Br. J. Pharmac. 37 : 638 - 649.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) Protein measurement with the Folin phenol reagent.
J. Biol. Chem. 193 : 265 - 275.

Mahler, H.R. and Cordes, E.H. (1966) Biological chemistry. Harper International Edition, (Harper and Row), New York.

Marsden, C.A., Broch, O.J. and Guldberg, H.C. (1972) Effect of nigral and raphe lesions on catechol O-methyl transferase and monoamine oxidase activities in the rat striatum. Eur. J. Pharmac. 19 : 35 - 42.

Mendez, J.S. and Finn, B.W. (1975) Use of 6-hydroxydopamine to create lesions in catecholamine neurons in rats. J. Neurosurg. 42 : 166 - 173.

Mireylees, S.E. (1975) Ph.D. thesis. University of Manchester.

Mireylees, S.E. and Foster, R.W. (1973) 3-methoxyisoprenaline: A potent selective Uptake$_2$ inhibitor. J. Pharm. Pharmac. 25 : 833 - 835.

Monod, J., Changeux, J-P and Jacob, F. (1963) Allosteric proteins and cellular control systems. J. Mol. Biol. 6 : 306 - 329.

Norton, W.T. and Poduslo, S.E. (1970) Neuronal soma and whole neuroglia of rat brain: A new isolation technique. Science 167 : 1144 - 1146.

Raiteri, M., Cerrito, F., Cervoni, A.M., Del Carmine, R., Ribera, M.T. and Levi, G. (1978) Studies on dopamine uptake and release in synaptosomes. Adv. Biochem. Psychopharmac. 19 : 35 - 56.

Raiteri, M., Del Carmine, R., Bertollini, A. and Levi, G. (1977) Effect of sympathomimetic amines on the synaptosomal transport of noradrenaline, dopamine and 5-hydroxytryptamine. Eur. J. Pharmac. 41 : 133 - 143.

Rock, G.D., Tong, J.H. and D'Iorio, A.D. (1970) A comparison of brain and liver catechol O-methyl transferase.
Can. J. Biochem. 48 : 1326 - 1331.

Roffler-Tarlov, S., Sharman, D.F. and Tegerdine, P. (1971) Dihydroxyphenylacetic acid and 4-hydroxy, 3-methoxyphenyl acetic acid in mouse striatum: a reflection of intra- and extra-neuronal metabolism of dopamine ?
Br. J. Pharmac. 42 : 343 -351.

Ross, S.B. and Renyi, A.L. (1964) Blocking action of sympathomimetic amines on the uptake of tritiated noradrenaline by mouse cerebral cortex tissues in vitro.
Acta. Pharmac. Tox. 21 : 226 - 239.

Ross, S.B. and Renyi, A.L. (1966a) Uptake of some tritiated sympathomimetic amines by mouse brain cortex slices in vitro.
Acta Pharmac. Tox. 24 : 297 - 309.

Ross, S.B. and Renyi, A.L. (1966b) Active uptake of tritiated metaraminol by mouse brain slices in vitro.
Life Sci. 5 : 639 - 647.

Ross, S.B. and Renyi, A.L. (1967) Inhibition of the uptake of tritiated catecholamines by antidepressants and related agents.
Eur. J. Pharmac. 2 : 181 - 186.

Ross, S.B., Renyi, A.L. and Brunfelter, B. (1968) Cocaine-sensitive uptake of sympathomimetic amines in nerve tissue.
J. Pharm. Pharmac. 20 : 283 - 288.

Rutledge, C.O. and Jonason, J. (1967) Metabolic pathways of dopamine and norepinephrine in rabbit brain in vitro.
J. Pharmac.Exp. Ther. 157 : 493 - 502.

Salama, A.I., Insalaco, J.R. and Maxwell, R.A. (1971) Concerning the molecular requirements for the inhibition of uptake of racemic (^{3}H)-norepinephrine into rat cerebral cortex slices by tricyclic antidepressants and related compounds.
J. Pharmac. Exp. Ther. 178 : 474 - 481.

Salt, P.J. (1972) Inhibition of noradrenaline Uptake$_2$ in the isolated rat heart by steroids, clonidine and methoxylated phenylethylamines.
Eur. J. Pharmac. 20 : 329 - 340.

Salt, P.J. and Iversen, L.L. (1972) Inhibition of the extraneuronal uptake of catecholamine in the isolated rat heart by cholesterol.
Nature New Biol. 238 : 91 - 92.

Samanin, R., Bernasconi, S. and Garattini, S. (1975) The effects of nomifensine on the depletion of brain serotonin and catecholamines induced respectively by fenfluramine and 6-hydroxydopamine in rats.
Eur. J. Pharmac. 34 : 377 - 380.

Schon, F. and Kelly, J.S. (1974) The characterisation of (^{3}H)-GABA uptake into the satellite glial cells of rat sensory ganglia.
Brain Res. 66 : 289 - 300.

Schoepp, D.D. and Azzaro, A.J. (1981) Alteration of dopamine synthesis in rat striatum subsequent to selective type A monoamine oxidase inhibition.
J. Neurochem. 37 : 527 - 530.

Sellstrom, A. and Hamberger, A. (1975) Neuronal and glial systems for γ-aminobutyric acid transport.
J. Neurochem. 24 : 847 - 852.

Sharman, D.F. (1973) The catabolism of catecholamines. Recent studies.
Br. Med. Bull. 29 : 110 - 115.

Shaskan, E.G. and Snyder, S.H. (1970) Kinetics of serotonin accumulation into slices from rat brain: Relationship to catecholamine uptake.
J. Pharmac. Exp. Ther. 175 : 404 - 418.

Snyder, S.H. and Coyle, J.T. (1969) Regional differences in (^{3}H)-norepinephrine and (^{3}H)-dopamine uptake into rat brain homogenates.
J. Pharmac. Exp. Ther. 165 : 78 - 86.

Snyder, S.H., Green, A.I. and Hendley, E.D. (1968a) Kinetics of (^{3}H)-norepinephrine accumulation into slices from different regions of the rat brain.
J. Pharmac. Exp. Ther. 164 : 90 - 102.

Snyder, S.H. Green, A.I., Hendley, E.D. and Gfeller, E. (1968b) Noradrenaline: Kinetics of accumulation into slices from different regions of rat brain.
Nature 218 : 174 - 176.

Stern, J.R., Eggleston, L.V., Hems, R. and Krebs, R.A. (1949) Accumulation of glutamic acid in isolated brain tissue.
Biochem. J. 44 : 410 - 412.

Strolin Benedetti, M. and Keane, P.E. (1980) Differential changes in monoamine oxidase A and B activity in the ageing rat.
J. Neurochem. 35 : 1026 - 1032.

Tissari, A.H. and Bogdanski, D.P. (1971) Biogenic amine transport. VI. Comparison of effects of ouabain and K^+ deficiency on the transport of 5-hydroxytryptamine and norepinephrine by synaptosomes.
Pharmacology 5 : 225 - 234.

Tuomisto, J. (1977) Nomifensine and its derivatives as possible tools for studying amine uptake.
Eur. J. Pharmac. 42 : 101 - 106.

Tuomisto, J. and Voutilainen, R. (1982) Conformation on amine uptake site: Results with Ro-8-4650.
Br. J. Pharmac. 77: 364P

Tuomisto, L. Tuomisto, J. and Smissman, E. (1974) Dopamine uptake in striatal and hypothalamic synaptosomes conformational selectivity of the inhibition.
Eur. J. Pharmac. 25 : 351 - 361.

Tyce, G.M. and Rorie, D.K. (1982) Conjugated dopamine in superfusates of slices of rat striatum.
J. Neurochem. 39 : 1333 - 1339.

Uretsky, N.J. and Iversen, L.L. (1970) Effects of 6-hydroxydopamine on catecholamine containing neurones in the rat brain.
J. Neurochem. 17 : 268 - 278.

Wagner, J. Vitali, P., Palfreyman, M.G., Zraika, M. and Huot, S. (1982) Simultaneous determination of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, dopamine, 4-hydroxy-3-methoxyphenylalanine, norepinephrine, 3,4-dihydroxyphenyl acetic acid, serotonin, and 5-hydroxyindoleacetic acid in rat cerebrospinal fluid and brain by high performance liquid chromatography with electrochemical detection.
J. Neurochem. 38 : 1241 - 1254.

Wakade, A.R. and Furchgott, R.F. (1968) Metabolic requirements for the uptake and storage of norephinephrine by the isolated left atrium of the guinea-pig.
J. Pharmac. Exp. Ther. 163 : 123 - 125.

Waldmeier, P.C. (1982) Effects of antidepressant drugs on dopamine uptake and metabolism.
J. Pharm. Pharmac. 34 : 391 - 394.

Waldmeier, P.C., Delini-Stula, A. amd Maitre, L. (1976) Preferential deamination of dopamine by an A type monoamine oxidase in rat brain. Naunyn-Schmied. Arch. Pharmac. 292 : 9 - 14.

Wang, Y-J., Gurd, J.W. and Mahler, H.R. (1975) Topography of synaptosomal high affinity uptake systems.
Life Sci. 17 : 725 - 734.

Westerink, B.H.C. and Korf, J. (1976) Turnover of acid dopamine metabolites in striatal and mesolimbic tissue of the rat brain.
Eur. J. Pharmac. 37 249 - 255.

Westerink, B.H.C. and Spaan, S.J. (1982) Estimation of the turnover of 3-methoxytyramine in the rat striatum by high performance liquid chromatography with electrochemical detection: Implications for the sequence in the cerebral metabolism of dopamine.
J. Neurochem. 38 : 342 - 347.

White, T.D. and Paton, D.N. (1972) Effects of external Na^+ and K^+ on the initial rates of noradrenaline uptake by synaptosomes prepared from rat brain.
Biochim. Biophys. Acta. 226 : 116 - 127.

Yang, H.Y. and Neff, N.H. (1974) The monoamine oxidases of brain: Selective inhibition with drugs and the consequences for the metabolism of the biogenic amines.
J. Pharmac. Exp. Ther. 189 : 733 - 740.

Zarrindast, M.R. (1981) Dopamine-like properties of ephedrine in rat brain.
Br. J. Pharmac. 74 : 119 - 122.

ASCORBIC ACID AND THE UPTAKE OF CATECHOLAMINES IN RAT BRAIN SLICES

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The reducing agent ascorbic acid is routinely added to the incubation media in pharmacological experiments as catecholamines are susceptible to oxidation (Iversen, 1963). It has recently been shown that ascorbic acid will potently inhibit the binding of dopamine to dopamine receptors in the striatum (Heikkila et al. 1981). It is thus essential to establish whether ascorbic acid has any effect on other processes involving catecholamines, for example uptake. We have therefore investigated the interaction of ascorbic acid with the high affinity uptake systems for catecholamines.

Uptake of [^{3}H]- dopamine ([^{3}H]-DA) and [^{3}H]- noradrenaline ([^{3}H]-NA) were studied using slices (0.5 mm thick) of rat striatum and cerebellum respectively. The tissue was preincubated at 37°C in Krebs solution (pH 7.4) containing different concentrations of ascorbic acid. After 10 min [^{3}H]-DA or [^{3}H]-NA was added to give a final catecholamine concentration of 0.1µM. Incubation was for 2 min during which time uptake was linear. Slices were removed by filtration, washed with 2ml of ice cold Krebs solution, blotted, weighed, digested with Soluene - 350 and the radioactivity measured by liquid scintillation.

Table 1 Catecholamine uptake at different ascorbic acid concentration

Ascorbic acid M	Uptake n mol/g wet wt/min DA	NA
0	0.186 ± 0.019 (5)	0.031 ± 0.004 (6)
10^{-6}	0.197 ± 0.010 (6)	0.028 ± 0.001 (6)
10^{-4}	0.203 ± 0.017 (6)	0.026 ± 0.002 (6)
10^{-3}	0.236 ± 0.024 (6)	0.030 ± 0.003 (6) *
10^{-2}	0.226 ± 0.016 (6)	0.013 ± 0.001 (6)

Values are mean ± s.e.mean (n)
* $p<0.05$

There is no significant difference between the uptake of DA in different concentrations of ascorbic acid. Only NA uptake in an ascorbic acid concentration of 10^{-2}M was significantly different from the others. The results suggesting inhibition.

Ascorbic acid is widely used at concentrations of 20mg/l, (approx 10^{-4}M) (Iversen 1963) to 200mg/l, (approx. 10^{-3}M) (Snyder and Coyle, 1969). It is therefore possible to conclude that the concentrations of ascorbic acid normally employed in studies of DA, and of NA uptake are unlikely to have any inhibitory effect, but care should be exercised in the use of concentrations in excess of 10^{-3}M.

Heikkila, R.E. et al (1981) Res.Commun.Chem.Pathol.Pharmacol. 34, 409-421

Iversen, L.L. (1963) Br.J.Pharmac.Chemother. 21, 523-537

Snyder, S.H. & Coyle, J.T. (1969) J. Pharmacol.Exp.Ther. 165, 78-86

INHIBITION OF DOPAMINE UPTAKE IN RAT STRIATAL SLICES BY PHENYLETHYLAMINE DERIVATIVES

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The presence of at least two saturable systems for the uptake of dopamine in striatal tissue has been demonstrated (Mireylees, 1975). The evidence suggests a high affinity mechanism Km 1.19μM, Vmax 0.16 nmol/g wet wt/min and a low affinity mechanism Km 24.4 μM, Vmax 19.9 nmol/g wet wt/min. The high affinity uptake system has characteristics similar to those of peripheral neuronal uptake (Mireylees, 1975, Horn, 1973). However little is know of the characteristics of the low affinity uptake system. In order to study this system the effect on uptake of a variety of phenylethylamine based compounds was investigated. The accumulation of dopamine in striatal slices was also studied in the presence of benztropine and desipramine.

Uptake of (3H)-dopamine was studied using slices (0.5 mm thick) of striatum from the brain of male Sprague-Dawley rats (175-250g). The slices were preincubated for 5 min at 37 ^{o}C in Krebs' solution containing the compound under investigation. Drug concentrations in the range 10^{-3}M - 10^{-8}M were used. An aliquot of the incubation solution containing (3H)-DA was added (final concentrations 100 nCi/ml). At a total dopamine concentration of 100 μM the low affinity uptake system is the major system of the total uptake mechanism whereas at a dopamine concentration of 0.1 μM the high affinity system predominates. Incubation was for two min. Slices were removed by filtration, washed with 2ml of ice cold solution, blotted, weighed, digested with Soluene-350 and the radioactivity measured by liquid scintillation.

The results indicate that complete inhibition of catecholamine uptake was not possible, the fraction of the total uptake which cannot be inhibited is taken to be due to diffusion or non-specific binding. The IC50 values were determined relative to the maximum inhibitable uptake obtained experimentally; 60% at 100 μM dopamine and 90% at 0.1 μM dopamine. The calculation of IC50 values was performed using the GLIM program on a DEC 20 computer.

At a dopamine concentration of 100 μM the rank order of potency of inhibition these compounds was: desipramine > adrenaline = metaraminol =tyramine = benztropine > noradrenaline =6-hydroxydopamine >isoprenaline =octopamine > hordenine, whereas at a dopamine concentration of 0.1 μM the rank order was: desipramine= benztropine> hordenine =metaraminol= noradrenaline= tyramine> isoprenaline= octopamine >adrenaline> 6-hydroxydopamine.

These results suggest that the low affinity site shows the characteristics of specific binding. The low affinity system exhibits a pattern of inhibition different from that found for the high affinity system.

Mireylees, S.E.(1975) Ph.D Thesis, University of Manchester
Horn, A.S.(1973) Br.J.Pharmac., 47, 332-338