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Structure-activity relations for the inhibition of catecholamine uptake into synaptosomes from noradrenaline and dopaminergic neurones in rat brain homogenates

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Summary

1. The effects of various phenylethylamine analogues on the inhibition of ^{3}H-noradrenaline and ^{3}H-dopamine uptake into homogenates of rat hypothalamus and corpus striatum respectively, were examined.

2. Phenolic hydroxyl groups and α-methylation of the side chain were both found to enhance the affinity for the neuronal uptake sites.

3. Methoxylation, β-hydroxylation and *N*-methylation were all found to reduce the ability of a compound to inhibit catecholamine transport.

4. The noradrenaline and dopamine transport systems responded in a quantitatively different manner to the various phenylethylamine analogues. It was found that, in general, the noradrenaline uptake process was more sensitive to structural changes, both positive and negative, than the dopamine system.

Introduction

In the mammalian central nervous system the putative neurotransmitters, noradrenaline and dopamine, are located in specific neuronal tracts (Hillarp, Fuxe & Dahlstrom, 1966). The principal mechanism for the inactivation of these catecholamines is believed to involve an active transport back into the presynaptic nerve terminal. There is evidence that this occurs in the peripheral sympathetic nervous system and also in the brain (Iversen, 1971).

A detailed structure–activity study of the molecular requirements for the inhibition of noradrenaline uptake has been carried out for catecholamine analogues in the perfused rat heart (Burgen & Iversen, 1965). To date no such systematic study has been performed to determine whether or not these rules apply to noradrenaline uptake in the brain. It is also of interest to see how the transport systems in noradrenergic and dopaminergic neurones differ in this respect, since it is known that these two uptake systems interact differently with the stereoisomers of noradrenaline, amphetamine and tranylcypromine and with various other psychotropic drugs (Snyder & Coyle, 1969 ; Coyle & Snyder, 1969 ; Horn, Coyle & Snyder, 1971 ; Iversen, Jarrott & Simmonds, 1971 ; Horn & Snyder, 1972). Structure–activity relations for the inhibition of catecholamine uptake into homogenates of dopamine and noradrenaline rich areas of rat brain have, therefore, been examined. It has been demonstrated that the uptake of catecholamines by homogenates is predominantly into synaptosomes (Coyle & Snyder, 1969).

Methods

A modification of the method described by Horn & Snyder (1972) was used. Wistar male rats (150–200 g) received intraperitoneal injections of reserpine (2·5 mg/kg) 18 h before being killed by cervical dislocation. Reserpine pretreatment is known to inactivate the vesicular storage mechanism in adrenergic nerves and to deplete the catecholamine stores, thus ensuring that amine accumulation is not affected by exchange with endogenous catecholamines or by intraneuronal binding mechanisms. After the final incubation period the samples were cooled in a bath containing salt-ice-water and a 1 ml portion was removed and filtered under vacuum on a Gooch crucible containing a membrane filter paper (Oxoid, 20 mm, 0·45 μm). Each filter disc was washed with 5 ml of ice-cold 0·9% sodium chloride solution to remove radioactively labelled amine present in the incubation medium adhering to the filter. The filter discs were then placed in a counting vial containing 12 ml of a mixture of 1 volume of Triton X-100 and 2 volumes of 0·6% 2(4-tert-butylphenyl) 5(4-biphenylyl)1-3-4 oxadiazole (Butyl P.B.D.) in toluene and left for at least 30 minutes. Radioactivity in the samples was measured in a Packard 3320 liquid scintillation spectrometer. The small amount of radioactive medium adhering to the filter after washing was determined in each experiment and was subtracted from the tritium counts of each sample. Radioactivity accumulated by tissue samples incubated at 0° C was routinely subtracted as a 'diffusional entry' blank from all experimental samples.

It has been shown previously that, under similar conditions, 85% or more of the synaptosomal content of (±)-^{3}H-noradrenaline or ^{3}H-dopamine was not metabolized in reserpine pretreated preparations (Snyder & Coyle, 1969; Coyle & Snyder, 1969). The uptake of ^{3}H-catecholamines has been shown to be linear for at least 10 min (Coyle & Snyder, 1969). (±)-^{3}H-Noradrenaline was employed for all hypothalamic incubations and ^{3}H-dopamine for all striatal incubations. IC50 values were obtained by incubating a constant concentration of ^{3}H-amine with various concentrations of drug and are expressed as the molar concentration that inhibited 50% of ^{3}H-catecholamine uptake as determined graphically from a logarithmic/probability plot.

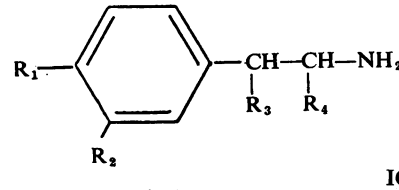
Results

Structural analogues of phenylethylamine having five basic structural variations were tested on homogenates from the hypothalamus and the corpus striatum, areas containing a predominance of noradrenaline and dopamine terminals, respectively (Hillarp *et al.*, 1966).

Effects of phenolic hydroxyl groups (Table 1)

In both brain areas the introduction of phenolic hydroxyl groups enhanced the affinity for the uptake site. The addition of a single hydroxyl group in the *m* or *p* position had a positive effect. The addition of a second hydroxyl group in the vacant *m* position led to a further increase in activity. It was found in both brain areas, however, that the factor for the enhancement was variable, although it was apparent that introduction of these groups generally led to a greater enhancement of affinity in noradrenaline, than in dopamine containing areas of the brain. This is clearly seen by comparing β-hydroxyphenethylamine with noradrenaline. In

TABLE 1. *Effect of the phenolic hydroxyl group on the IC50*



Drug	Substitution	IC50 Hypothalamus	Striatum
β-Phenethylamine*		$5{\cdot}2\times10^{-6}$ M	$1{\cdot}4\times10^{-6}$ M
p-Tyramine	R_1=OH	$1{\cdot}0\times10^{-6}$ M	$5{\cdot}4\times10^{-7}$ M
Dopa mine†	R_1, R_2=OH	Ki $0{\cdot}8\times10^{-7}$ M	Km $3{\cdot}5\times10^{-7}$ M
(±)-β-Hydroxyphenethylamine	R_3=OH	$1{\cdot}3\times10^{-4}$ M	$1{\cdot}1\times10^{-5}$ M
(±)-Octopamine	R_1, R_3=OH	$5{\cdot}0\times10^{-5}$ M	$5{\cdot}3\times10^{-6}$ M
(±)-Noradrenaline†	R_1, R_2, R_3=OH	Km $4{\cdot}1\times10^{-7}$ M	Ki $1{\cdot}8\times10^{-7}$ M
(±)-Amphetamine*	R_4=CH_3	$9{\cdot}2\times10^{-7}$ M	$4{\cdot}0\times10^{-7}$ M
(±)-*p*-Hydroxyamphetamine	R_1=OH, R_4=CH_3	$8{\cdot}4\times10^{-7}$ M	$2{\cdot}8\times10^{-7}$ M
(±)-Norpseudoephedrine	R_3=OH, R_4=CH_3	$6{\cdot}2\times10^{-6}$ M	$3{\cdot}0\times10^{-6}$ M
(−)-Metaraminol	R_2,R_3=OH, R_4=CH_3	$3{\cdot}7\times10^{-7}$ M	$4{\cdot}3\times10^{-7}$ M

* Data from Horn & Snyder, 1972. † Data from Snyder & Coyle, 1969; Coyle & Snyder, 1969. IC50=The concentration of inhibitor required to produce a 50% inhibition of the uptake of ^{3}H-catecholamine. Ki=The concentration of inhibitor required to reduce the apparent affinity of the ^{3}H-catecholamine for the uptake site by 50%. The IC50 is approximately equal to the Ki value since the concentrations of ^{3}H-catecholamine used in both estimations were below that required to saturate the uptake sites. Km=The ^{3}H-catecholamine concentration required to produce a 50% occupation of catecholamine uptake sites. (±)-^{3}H-Noradrenaline and ^{3}H-dopamine were used in the studies on the hypothalamus and striatum, respectively. The data presented in the above table are the mean of 1–3 independent determinations of the IC50 value. Each determination was carried out with 3 concentrations of inhibitor in quadruplicate. In independent determinations, none of the values shown differed by more than 20%.

the hypothalamus the latter was 317 times more potent than the former, compared with a factor of 61 in the striatum. Further examples of this effect are seen in comparisons of octopamine with noradrenaline and β-phenethylamine with dopamine.

Effects of α-methyl groups (Table 2)

There was an increase in affinity for both the hypothalamic and striatal uptake sites upon introduction of an α-methyl group. This effect again appeared to be slightly more pronounced for the hypothalamic homogenates. For example, norpseudoephedrine was 21 times more potent than β-hydroxyphenethylamine in the hypothalamus and only about 4 times more potent in the striatum. Racemic amphetamine was almost 6 times as potent as β-phenethylamine in the hypothalamus and about 4 times as potent in the striatum.

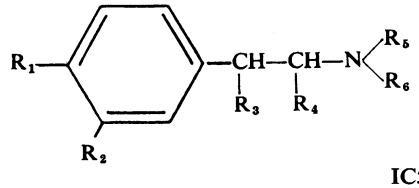
Effects of methoxyl groups (Table 2)

A drastic reduction in affinity was found on methylation of hydroxyl groups. This was again true for both areas of the brain. This is readily seen on comparison of normetanephrine with noradrenaline. In the hypothalamus and striatum the former was 1,800 and 1,300 times, respectively, less potent than the latter. The noradrenaline containing area seemed to be more sensitive to this change.

Effects of N-*methylation* (Table 2)

Although mono or di-*N* methylation decreased the affinity for uptake sites in the two brain areas under study, those sites having a predominance of noradrenaline

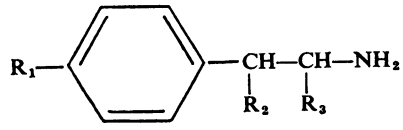
TABLE 2. *Effect of methylation on the IC50*



Drug	Substitution	IC50 Hypothalamus	Striatum
α-Methylation			
β-Phenethylamine*		$5{\cdot}2\times10^{-6}$ M	$1{\cdot}4\times10^{-6}$ M
(±)-Amphetamine*	$R_4=CH_3$	$9{\cdot}2\times10^{-7}$ M	$4{\cdot}0\times10^{-7}$ M
p-Tyramine	$R_1=OH$	$1{\cdot}0\times10^{-6}$ M	$5{\cdot}4\times10^{-7}$ M
(±)-*p*-Hydroxyamphetamine	$R_1=OH$, $R_4=CH_3$	$8{\cdot}4\times10^{-7}$ M	$2{\cdot}8\times10^{-7}$ M
(±)-β-Hydroxyphenethylamine	$R_3=OH$	$1{\cdot}3\times10^{-4}$ M	$1{\cdot}1\times10^{-5}$ M
(±)-Norpseudoephedrine	$R_3=OH$, $R_4=CH_3$	$6{\cdot}2\times10^{-6}$ M	$3{\cdot}0\times10^{-6}$ M
Methoxylation			
p-Tyramine	$R_1=OH$	$1{\cdot}0\times10^{-6}$ M	$5{\cdot}4\times10^{-7}$ M
p-Methoxyphenethylamine	$R_1=OCH_3$	$2{\cdot}4\times10^{-4}$ M	$1{\cdot}5\times10^{-5}$ M
(±)-Noradrenaline†	R_1, R_2, $R_3=OH$	Km $4{\cdot}1\times10^{-7}$ M	Ki $1{\cdot}8\times10^{-7}$ M
(±)-Normetanephrine	R_1, $R_3=OH$, $R_2=OCH_3$	$7{\cdot}4\times10^{-4}$ M	$2{\cdot}4\times10^{-4}$ M
N-*Methylation*			
p-Tyramine	$R_1=OH$	$1{\cdot}0\times10^{-6}$ M	$5{\cdot}4\times10^{-7}$ M
Hordenine	$R_1=OH$, R_5, $R_6=CH_3$	$1{\cdot}6\times10^{-4}$ M	$1{\cdot}3\times10^{-5}$ M
(±)-Octopamine	R_1, $R_3=OH$	$5{\cdot}0\times10^{-5}$ M	$5{\cdot}3\times10^{-6}$ M
(±)-Synephrine	R_1, $R_3=OH$, $R_5=CH_3$	$4{\cdot}0\times10^{-4}$ M	$3{\cdot}0\times10^{-5}$ M

* Data from Horn & Snyder, 1972. † Data from Coyle & Snyder, 1969. IC50=The concentration of inhibitor required to produce a 50% inhibition of the uptake of ^{3}H-catecholamine. Ki=The concentration of inhibitor required to reduce the apparent affinity of the ^{3}H-catecholamine for the uptake site by 50%. The IC50 is approximately equal to the Ki value since the concentrations of ^{3}H-catecholamine used in both estimations were below that required to saturate the uptake sites. Km=The ^{3}H-catecholamine concentration required to produce a 50% occupation of catecholamine uptake sites. (±)-^{3}H-Noradrenaline and ^{3}H-dopamine were used in the studies on the hypothalamus and striatum, respectively. The data presented in the above table are the mean of 1–3 independent determinations of the IC50 value. Each determination was carried out with 3 concentrations of inhibitor in quadruplicate. In independent determinations, none of the values shown differed by more than 20%.

TABLE 3. *Effect of the β-hydroxyl group on the IC50*



Drug	Substitution	IC50 Hypothalamus	Striatum
β-Phenethylamine*		$5{\cdot}2\times10^{-6}$ M	$1{\cdot}4\times10^{-6}$ M
(±)-β-Hydroxyphenethylamine	$R_2=OH$	$1{\cdot}3\times10^{-4}$ M	$1{\cdot}1\times10^{-5}$ M
p-Tyramine	$R_1=OH$	$1{\cdot}0\times10^{-6}$ M	$5{\cdot}4\times10^{-7}$ M
(±)-Octopamine	R_1, $R_2=OH$	$5{\cdot}0\times10^{-5}$ M	$5{\cdot}3\times10^{-6}$ M
(±)-Amphetamine*	$R_3=CH_3$	$9{\cdot}2\times10^{-7}$ M	$4{\cdot}0\times10^{-7}$ M
(±)-Norpseudoephedrine	$R_2=OH$, $R_3=CH_3$	$6{\cdot}2\times10^{-6}$ M	$3{\cdot}0\times10^{-6}$ M

* Data from Horn & Snyder, 1972. IC50=The concentration of inhibitor required to produce a 50% inhibition of the uptake of ^{3}H-catecholamine. (±)-^{3}H-Noradrenaline and ^{3}H-dopamine were used in the studies on the hypothalamus and striatum, respectively. The data presented in the above table are the mean of 1–3 independent determinations of the IC50 value. Each determination was carried out with 3 concentrations of inhibitor in quadruplicate. In independent determinations, none of the values shown differed by more than 20%.

seemed more sensitive. Hordenine was 160 times less potent than *p*-tyramine in the hypothalamus but only 24 times less potent in the striatum. A less marked, but similar effect, was seen for synephrine and octopamine.

Effects of the β-hydroxyl group (Table 3)

Introduction of a β-hydroxyl group reduced the affinity for the uptake site but not as dramatically as the introduction of the methoxyl group. Once again this was true of both brain areas and the hypothalamic homogenates, generally, appeared to be influenced more strongly. Octopamine was 50 times less potent than *p*-tyramine in the hypothalamus but there was only a factor of 10 for this comparison in the striatum.

Discussion

In the perfused rat heart Burgen & Iversen (1965) have shown that the following changes in the basic β-phenethylamine structure lead to an increase in affinity of the molecule for uptake sites at the external neuronal membrane:

1. Phenolic hydroxyl groups in the *meta*- and *para*-positions of the benzene ring.
2. α-Methylation of the side chain.

The following changes lead to a decrease in affinity

3. Methylation of the phenolic hydroxyl groups.
4. β-Hydroxylation of the side chain.
5. Mono or di-*N*-methylation of the primary amino group.

Bearing in mind the fact that only a limited number of compounds have been studied, it is, nevertheless, clear from the results that the structural rules obtained in the perfused rat heart study (Burgen & Iversen, 1965) for the inhibition of the uptake of noradrenaline by structural analogues, appear to be true for catecholamines uptake in both the rat hypothalamus and corpus striatum. It was found, as in the rat heart, that the introduction of phenolic hydroxyl and/or α-methyl groups lead to an increase in affinity for both the noradrenaline and dopamine uptake sites in brain. Methylation of the hydroxyl groups, β-hydroxylation or *N*-methylation lead to a decrease in affinity for these uptake sites.

It was found, however, that nerve terminals in the homogenates from the two brain areas responded in a quantitatively different manner to the various structural changes. In general, homogenates of hypothalamic tissue containing predominantly noradrenaline uptake sites were more sensitive to the various structural changes than homogenates from the corpus striatum, which contain a predominance of dopaminergic nerve terminals. Thus, with a few exceptions structural changes leading to an increase or a decrease in affinity affected noradrenaline uptake sites to a greater extent than the corresponding dopamine sites. It is perhaps possible to rationalize this on the grounds that noradrenaline has an asymmetric centre whilst dopamine does not. Thus, the steric and stereochemical requirements of the noradrenergic uptake sites are, *a priori*, likely to be more stringent, not only because of the requirement for an extra binding site for the additional hydroxyl group, but also because this group has produced an asymmetric centre at the β carbon atom, so that uptake site may be expected to interact optimally with the naturally occurring (−) isomer. This is supported by the fact that the noradrenergic nerve terminals exhibit stereochemical selectivity for the transport of (+) and (−) noradrenaline, whilst dopaminergic nerve terminals do not (Snyder & Coyle, 1968; Coyle & Snyder, 1969; Iversen *et al.*, 1972). This is also true for inhibitors of uptake such as (+) and (−) amphetamine (Coyle & Snyder, 1969).

There are various possible explanations for the enhancement of affinity by phenolic hydroxyl groups and its drastic reduction by methylation of this function. If the aromatic hydroxyl group is taking part in a hydrogen binding interaction with the uptake site it is most likely to be through the hydrogen atom as an acceptor rather than the oxygen atom as a donor. This is supported by the fact that replacement of the hydrogen atom by the methyl group produces a large decrease in affinity. Although the oxygen atom could still act as a hydrogen bond donor this is not so likely, as in the case of an aliphatic alcohol, due to interaction of the lone pairs of electrons on a phenol with the benzene ring. Other factors which must be considered upon the methylation of the aromatic hydroxyl group are that the extra bulk of the methyl group could prevent the correct alignment of the molecule at the uptake site or it could induce an unfavourable conformational change in the site itself on attempting to bind. Methylation will also reduce the polarity of the molecule and make it more lipid soluble, this again might lead to adverse interactions. It would seem reasonable to infer from these results, limited as they are, that it is unlikely that the methoxylated psychotomimetic phenylethylamines and amphetamines, such as mescaline and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (D.O.M.) produce their characteristic effects by inhibition of neuronal catecholamine uptake in the brain.

Turning now to substitutions in the side chain it must be noted that not only will there be different groups interacting with the uptake site but that these substituents will affect the conformer populations of the molecules. The conformers of a molecule are the various shapes it may adopt as a result of changes involving the flexion or torsion, but not the breaking, of ordinary covalent bands. The binding of an alkyl group is usually through hydrophobic and or van der Waals interactions. The group may also induce a favourable change in the binding site leading to greater affinity. A recent study with rigid and semi-rigid analogues of amphetamine (Horn & Snyder, 1972) showed that the most probable conformation of amphetamine at the uptake site of the catecholamine neurones, is with the side chain fully extended and above the plane of the benzene ring, i.e. an *anti* conformation. This has recently been shown to be the preferred conformation in the solid state (Bergin & Carlstrom, 1971) and in solution (Bailey, By, Graham & Verner, 1971; Neville, Deslauriers, Blackburn & Smith, 1971). The α-methyl group may contribute to the predominance of the *anti* conformation.

It is also known from a recent study in which molecular orbital calculations were used (Pullman, Coubeils, Courriere & Gervoiz, 1972) that, in general, the energy barriers separating the various conformations of phenylethylamine analogues is of the order of a few K cal/mole. A difference in free energy as small as 2·7 K cal/mole between two conformations at equilibrium will result in there being only about 1% of the minor component present at room temperature. The solid state conformation of noradrenaline is known to be in an *anti* form (Carlstrom & Bergin, 1967). Molecular orbital calculations on the conformation of noradrenaline from several groups (Pedersen, Hoskins & Cable, 1971; Pullman *et al.*, 1972) have, however, shown that the fully extended *anti* conformation and the folded *gauche* form have very similar stabilities. Thus, the presence of the β-hydroxyl group may contribute to an increase in the proportion of a conformer not having an optional fit for the uptake site, or perhaps the site itself has trouble in 'moulding' the hydroxylated drug to fit its requirements.

At physiological pH the amines tested in this series will exist predominantly in the protonated form. Hence, it is likely that the primary mode of interaction of the amino group with the uptake site is through a cationic–anionic interaction. The introduction of methyl groups will lead to small changes in the basic strength of the amino group. It is probable, however, that the negative effect of this methylation is due to steric hindrance. An effect on conformer populations has also to be considered.

In conclusion, it has been shown that the structure-activity relations determined in the rat heart for the inhibition of noradrenaline uptake apply to both noradrenaline and dopamine uptake in the rat brain. It would appear that, in general, noradrenergic nerve terminals are more sensitive to structural change, both positive and negative, than dopaminergic nerve endings.

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