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STRUCTURE-ACTIVITY RELATIONS FOR THE INHIBITION OF 5-HYDROXYTRYPTAMINE UPTAKE BY TRICYCLIC ANTIDEPRESSANTS INTO SYNAPTOSOMES FROM SEROTONINERGIC NEURONES IN RAT BRAIN HOMOGENATES

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1 The inhibitory effects of various analogues of imipramine on [^{3}H]-5-hydroxytryptamine (5-HT) uptake into homogenates of rat hypothalamus were examined.

2 For structures with a three carbon side chain the tertiary amine derivative was more potent than the compound with a secondary amine function.

3 Potency was reduced by increasing or decreasing the length of the three carbon side chain by one carbon atom.

4 Substitution of a methyl group in the α or β position in the side chain reduced potency.

5 Replacement of the dimethylene bridge in imipramine by a sulphur atom or substitution of a C=C double bond for the exocyclic N–C bond of imipramine both led to a fall in potency.

6 3-Chlorimipramine was the most potent inhibitor of [^{3}H]-5-hydroxytryptamine uptake of the compounds tested.

Introduction

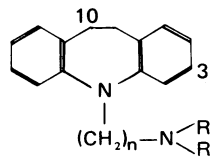
There is considerable evidence that a functional abnormality of one or more of the biogenic amine systems in the brain is involved in the aetiology of the affective disorders. The amines that have received the most attention in this respect are noradrenaline (Schildkraut & Kety, 1967) and 5-hydroxytryptamine (5-HT) (Coppen, 1972). The rat brain has separate neuronal uptake systems for noradrenaline (Dengler, Michaelson, Spiegel & Titus, 1962; Snyder & Coyle, 1969; Horn, 1973a) and 5-HT (Blackburn, French & Merrills, 1967; Shaskan & Snyder, 1970) and the clinically efficacious tricyclic antidepressants are known to inhibit both these transport processes (Iversen, 1971). It has been suggested that this effect may be responsible for their ameliorative action (Schildkraut & Kety, 1967), however, this theory is complicated by the time interval required to bring about a reversal in mood (Oswald, Brezinova & Dunleavy, 1972). The effect of the molecular structure of the tricyclic antidepressants on the noradrenaline uptake process has received more detailed attention (Callingham, 1966; Horn, Coyle & Snyder, 1971) than the corresponding 5-HT neuronal uptake system. The structure-activity relations of inhibitors of 5-HT uptake into blood platelets, however, has received considerable attention (Todrick & Tait, 1969; Born, Juengjaroen & Michal, 1972). In the present study we have examined the effects on 5-HT uptake into synaptosomes from rat hypothalamic homogenates of several molecular modifications of the imipramine molecule. The hypothalamus of the rat brain is known to have a high concentration of 5-HT (Erspamer, 1966).

Methods

The method employed has been described previously in detail (Horn, 1973b), and is based on the incubation of a crude synaptosome fraction from rat brain with radioactive 5-HT. IC_{50} values are expressed as the molar concentration of drug that inhibited 50% of [^{3}H]-5-HT uptake as determined graphically from a logarithmic/probability plot.

[^{3}H]-5-HT (G-[^{3}H]) was obtained from the Radiochemical Centre, Amersham and had a specific activity of 500 mCi/mmol. Imipramine

Table 1 Inhibition of 5-hydroxytryptamine uptake by compounds possessing the imipramine nucleus



	Terminal amino group	*Side chain length*	*IC_{50} (M)*	*Potency relative to imipramine (=100)*
Imipramine	NMe_2	C_3	2.85×10^{-7}	100
Desmethylimipramine	NHMe	C_3	1.37×10^{-6}	21
Desdimethylimipramine	NH_2	C_3	2.65×10^{-7}	105
N-methylimipramine	N^+Me_3	C_3	1.40×10^{-7}	205
N-(dimethylaminobutyl)-iminodibenzene	NMe_2	C_4	1.98×10^{-6}	14
N-(dimethylaminoethyl)-iminodibenzene	NMe_2	C_2	2.45×10^{-6}	12
N-(methylaminoethyl)-iminodibenzene	NHMe	C_2	2.35×10^{-6}	12
α-Methyl imipramine	NMe_2	C_3	4.80×10^{-6}	6.0
β-Methyl imipramine	NMe_2	C_3	9.00×10^{-6}	3.2
3-Chloroimipramine	NMe_2	C_3	5.60×10^{-8}	510
3-Dimethylamino imipramine	NMe_2	C_3	2.70×10^{-7}	105
10-Methyl imipramine	NMe_2	C_3	3.75×10^{-7}	76

IC_{50} = The concentration of inhibitor required to produce a 50% inhibition of the uptake of [^{3}H]-5-HT. The potency is the direct ratio of the IC_{50} of the inhibitors where imipramine is given the value of 100. Each drug was tested at three concentrations and a mean value with standard error was obtained for four determinations at each concentration. Values for s.e. mean were less than ±10%. The IC_{50} values were then obtained by a graphical method (Horn, 1973b).

Table 2 Inhibition of 5-hydroxytryptamine uptake by various tricyclic compounds

	IC_{50} (M)	*Potency relative to imipramine (=100)*
Imipramine	2.85×10^{-7}	100
Amitriptyline	4.90×10^{-7}	58
Nortriptyline	1.60×10^{-6}	18
Promazine	8.30×10^{-6}	3.4
Chlorpromazine	2.85×10^{-6}	10
Iprindole	1.00×10^{-5}	2.9

See footnote to Table 1.

analogues were obtained from Geigy. Other drugs and their sources were: iprindole and promazine (John Wyeth & Brother), chlorpromazine (May & Baker), amitryptyline (Merck, Sharpe & Dohme) and nortriptyline (Eli Lilly).

Results

Analogues of imipramine having the following five basic structural changes were examined.

Effects of N-*methylation of the terminal amino group (Tables 1 and 2)*

Potency was maximal for the quaternary salt of imipramine ($IC_{50} = 1.40 \times 10^{-7}$ M) and decreased by approximately 50% on removal of one of the three methyl groups. The monomethyl compound desipramine was the least potent compound of the four in this group, it being approximately five times less potent than imipramine. The primary amine, however, was more potent than the monomethyl compound, desipramine, by a factor of about 5. The same pattern of activity, i.e. the tertiary amino compound being more potent than the drug with a secondary function, also held in compounds with an unsaturated side chain such as amitriptyline and nortriptyline. In the compounds with a two carbon side chain there was no difference, however, in the potency of *N*-(dimethylaminoethyl)-iminodibenzene and *N*-(monomethylaminoethyl)-iminodibenzene.

Effects of changes in the length of the side chain (Table 1)

The two and four carbon side chain compounds

were 8 and 7 times, respectively, less potent than the parent compound imipramine.

Effects of α- and β-methylation of the side chain (Table 1)

Imipramine was about 17 times as potent as the α-substituted compound and 33 times more potent than the β-substituted drug.

Effects of substitution on the nucleus (Table 1)

Substitution of a chlorine atom at the 3-position increased potency more than five-fold, whereas substitution of a dimethylamino group hardly affected the potency.

Effects of changes within the nucleus (Tables 1 and 2)

Substitution of a methyl group at the 10-position of the 10, 11 dimethylene bridge reduced activity by about 25%. Replacement of this two carbon bridge by a sulphur atom as in promazine produced a fall in potency to 3% of the activity of imipramine. Substitution of a chlorine atom in the 3-position of promazine to give chlorpromazine increased the potency three-fold but in spite of this chlorpromazine had only about 1/50 of the potency of its structural analogue chlorimipramine. When the central ring of imipramine was replaced by a pyrrole ring and one of the benzene rings by an eight membered alicyclic ring to give iprindole, potency was reduced 33-fold. Replacement of the N–C bond in the imipramine side chain by a C=C double bond, as in amitriptyline and nortriptyline, led to a large fall in activity.

Discussion

Although only a limited number of compounds have been studied, it is possible, however, to propose some tentative structure-activity generalizations with regard to the ability of tricyclic antidepressant drugs to inhibit 5-HT uptake into synaptosomes from the rat hypothalamus.

(1) For structures with a three carbon side chain the tertiary amino compound was more potent than the corresponding secondary amine.

(2) Potency was reduced by increasing or decreasing the length of the three carbon side chain by one carbon atom.

(3) α- or β-substitution of methyl groups in the three carbon side chain led to a fall in potency.

(4) Replacement of the dimethylene bridge by a sulphur atom decreased the affinity for the uptake site.

(5) Replacement of the N–C bond in the side chain of imipramine by a C=C double bond decreased uptake site affinity.

It is of interest that the results of Todrick & Tait (1969) on the effects of a comparable though more extensive range of analogues on 5-HT uptake into the human blood platelet are very similar. There is now good *in vivo* and *in vitro* evidence that tricyclic antidepressants having a tertiary amine function are more potent inhibitors of 5-HT uptake than those with a secondary amino group (Carlsson, Corrodi, Fuxe & Hökfelt, 1969; Ross & Renyi, 1969; Carlsson, 1970; Shaskan & Snyder, 1970: Lidbrink, Jonsson & Fuxe, 1971; Kannengiesser, Hunt & Raynaud, 1973). The fact that the quaternary methyl amino compound of imipramine was twice as potent as the parent drug indicates that the active form of the terminal amino group at the uptake site is probably the positively charged species.

The effect of chain length on potency possibly indicates that the binding site for terminal amino groups lies at a critical distance from the tricyclic nucleus. The substitution of methyl groups in the α and β positions of the three carbon side chain will affect both the conformation of the side chain and the nature of the interaction at the uptake site. These structural changes could lead to preferred conformations not having an optimal fit for the uptake site or steric factors could inhibit effective binding. The replacement of the dimethylene bridge, as in imipramine and chlorimipramine, by a sulphur atom as in promazine and chlorpromazine, led to large falls in activity. If it is assumed that the two types of drug have a similar distribution in the CNS then it is possible that the dopamine receptor blocking activity of the phenothiazines such as chlorpromazine (Van Rossum, 1967) and the very much weaker effects of the tricyclic antidepressants in this respect may be explicable in terms of the sulphur atom which may act either to affect the conformation of the ring system and hence the side chain or have some direct receptor blocking activity. In the imipramine series, only two compounds with a substituent in the 3-position were examined and one, chlorimipramine, was found to be five times more potent than imipramine whilst the other, 3-dimethylamino imipramine, had a similar potency to imipramine. This indicates that the nature of the 3-substituent is important. Replacement of the N–C single bond in the imipramine side chain by a C=C double bond will lead to a restriction on the number of possible conformations the side chain can adopt, therefore reducing the possible number of chances for a favourable interaction with the uptake site. Iprindole, which is in clinical use as an

antidepressant, (El Deiry, Forrest & Littman, 1967), in confirmation of earlier work (Ross, Renyi& Ogren, 1971), was found to be a very weak inhibitor of 5-HT uptake. It is also known to be a weak inhibitor of noradrenaline uptake in the rat heart and brain (Gluckman & Baum, 1969; Lemberger, Sernatinger & Kuntzman, 1970; Lahti & Maickel, 1971; Ross, Renyi & Ogren, 1971). Iprindole, however, is structurally an atypical 'tricyclic' antidepressant in that one of the rings is not aromatic but consists of a six membered carbon chain fused to an indole system and secondly there are no bridging atoms in the top portion of the molecule between the two outer rings. Although the three ring system of iprindole is not flat, which is apparently a requirement for activity (Maxwell, Keenan, Chaplin, Roth & Eckhardt, 1969; Horn *et al.*, 1971), the above two molecular changes may account for its lack of activity as an inhibitor of biogenic amine uptake.

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