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INHIBITION OF NEURONAL UPTAKE OF ^{3}H-BIOGENIC AMINES INTO RAT CEREBRAL CORTEX BY PARTIALLY AND FULLY SATURATED DERIVATIVES OF IMIPRAMINE AND DESIPRAMINE

THE IMPORTANCE OF THE AROMATIC RING IN ADRENERGIC AMINES—PART 3

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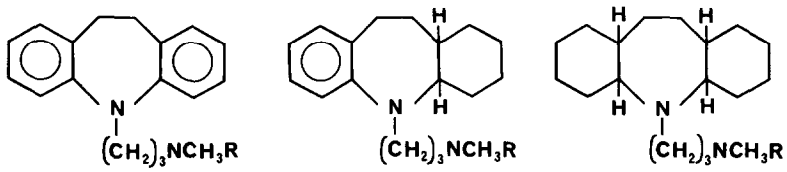
Abstract In order to assess the importance of the aromatic rings in inhibition of neuronal amine uptake produced by tricyclic antidepressant drugs, derivatives of imipramine (IMI) and desipramine (DMI) were prepared in which either one (IMIH, DMIH) or both (IMIH2, DMIH2) of the aromatic rings were fully reduced to cyclohexane rings. The relative abilities of these compounds to inhibit the uptake of *l*-[^{3}H]-norepinephrine (NE), [^{3}H]-dopamine (DA) and [^{3}H]-5-hydroxytryptamine (5-HT) into chopped tissue of rat cerebral cortex were determined. Reduction of one or both aromatic rings did not alter significantly the inhibition of uptake of [^{3}H]-DA or [^{3}H]5-HT produced by either IMI or DMI (IC_{50} values 25–80 μM). However, saturation of one or both rings abolished the selectivity of DMI for inhibition of NE uptake (IC_{50} 0.12 μM), decreasing potency 150-fold (IC_{50} 18.3 μM) and 250-fold (IC_{50} 29.4 μM) respectively. The effect of aromatic ring reduction on the IMI-induced inhibition of NE uptake was much less pronounced. The results suggest that hydrophobic rather than π-electron attractive forces are involved in the interaction of DMI or IMI with DA or 5-HT uptake sites. However, the loss in selectivity for inhibition of NE uptake upon reduction of DMI may reflect loss of π-electron interactions in the binding of DMI to the NE uptake site, or may reflect increased sensitivity to spatial disposition of the hydrophobic binding areas of the drug relative to that found at the DA or 5-HT amine uptake sites.

Although a variety of intermolecular forces (such as hydrophobic, ionic, π-electron overlap and charge transfer) may be major determinants of drug–receptor interaction, direct experimental demonstration of such forces is difficult to achieve. Interest in the role of aromatic rings in drug–receptor interactions is based upon the possibility of charge transfer or π-electron overlap interactions between the drug and receptor.

For instance, it has been assumed that an aromatic ring is essential for substrate binding to phenylethanolamine *N*-methyltransferase (EC 2.1.1) [1, 2]. However, we have demonstrated recently [3] that several non-aromatic amino alcohols are better substrates for methylation than phenylethanolamine itself. This indicates that π-electron overlap interactions are not determinants of enzymatic activity in this case, since these interactions coud not occur in nonaromatic substrates. The data suggest a less specific hydrophobic contribution to the enzyme–substrate complex formation.

Similarly, it has been assumed [4, 5] that an aromatic ring is a determining factor in the binding of sympathomimetic amines to neuronal uptake sites in adrenergic nerve endings. However, examination of a series of partially and fully saturated cyclic ethanolamines for inhibition of neuronal uptake in reserpinized rat vas deferens has shown [6] that affinity for the neuronal uptake site increases with increasing ring saturation.

Tricyclic antidepressants are known to be potent competitive inhibitors of neuronal uptake of norepinephrine in the central nervous system. Maxwell *et al.* [7] examined selected tricyclic antidepressants and their corresponding diphenyl and monophenyl alkylamine analogs in both central and peripheral



IMI: R=CH$_3$ DMI: R=H IMIH: R=CH$_3$ DMIH: R=H IMIH2: R=CH$_3$ DMIH2: R=H

Fig. 1. Imipramine, desipramine and their saturated analogs.

tissue preparations. Their results suggest that, upon binding of the aromatic rings to the receptor, there is a specific alignment between the *N*-methyl group in the drug molecule and an ancillary hydrophobic binding site. With removal of rotational restriction between the two aromatic rings (diphenylalkylamines) or with removal of one ring (monophenylalkylamines), crucial determinants of spatial alignment are lost.

In an attempt to elaborate further the structure–activity relationships for tricyclic antidepressants and to assess the possibility that the orientational aromatic binding sites may be hydrophobic in nature, we have investigated, as competitive inhibitors of neuronal amine uptake, a series of imipramine (IMI) and desipramine (DMI) derivatives (Fig. 1) in which the 2-carbon bridge restricting aromatic ring rotation was maintained, but in which one (IMIH, DMIH) or both (IMIH2, DMIH2) of the aromatic rings would be saturated, thereby preventing any type of π-electron binding interaction of the drug with the neuronal uptake site.

METHODS

Inhibition of uptake of ^{3}H-amines into chopped cortical tissue from rats was measured by the method of Ziance and Rutledge [8]. Briefly, male Sprague–Dawley rats (200–250 g) were killed by decapitation, the brains were quickly removed, and the cerebral cortex (white matter removed) was chopped at 0.3-mm intervals with a mechanical tissue chopper. The tissue samples were preincubated in physiological buffer at 37° with various concentrations of the test drugs.

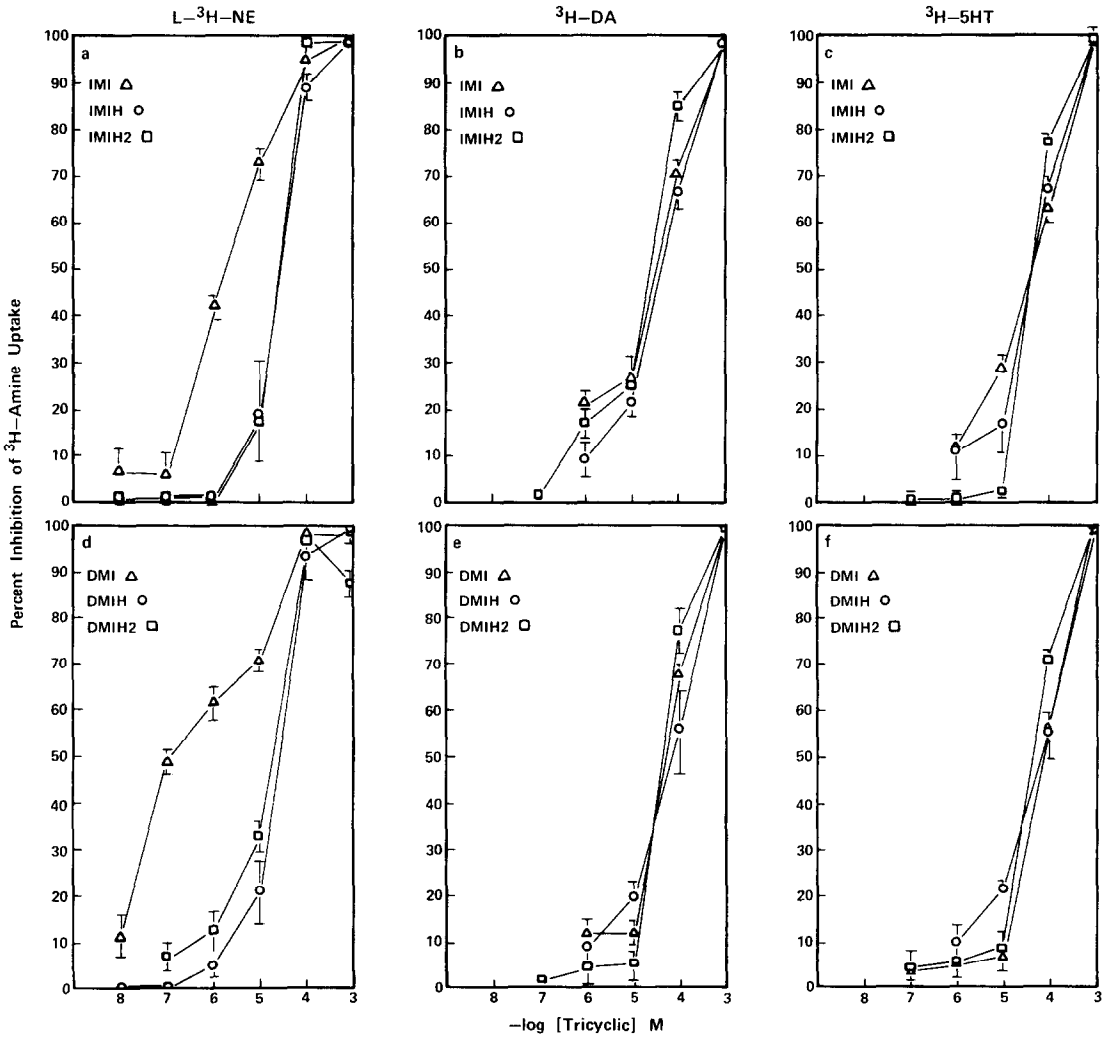


Fig. 2. Inhibition of uptake of ^{3}H-amines into chopped cerebral cortex of rat by tricyclic drugs. Chopped cerebral cortex was incubated for 10 min with various concentrations of the test drugs (10^{-8}–10^{-3} M). [^{3}H]-norepinephrine (10^{-7} M) was then added and the incubation was continued for an additional 20 min. Tissue and medium were separated by centrifugation, and tritium was measured in the tissue and medium. Inhibition of neuronal uptake is expressed as a percentage of control values (mean ± S.E.M.) for three to four experiments, Panel a: inhibition of [^{3}H]norepinephrine uptake by imipramine derivatives. Panel b: inhibition of [^{3}H]dopamine uptake by imipramine derivatives. Panel c: inhibition of [^{3}H]5-hydroxytryptamine uptake by imipramine derivatives. Panel d: inhibition of [^{3}H]norepinephrine uptake by desipramine derivatives. Panel e: inhibition of [^{3}H]dopamine uptake by desipramine derivatives. Panel f: inhibition of [^{3}H]5-hydroxytryptamine uptake by desipramine derivatives.

After 10 min the ^{3}H-amine was added to achieve a final concentration of 10^{-7} M and to give a total incubation volume of 2 ml. After an additional incubation of 20 min, the uptake was terminated by centrifugation (10,000 *g* for 5 min, 4°). The supernatant fluid was removed, and the tissue was washed with 1 ml of physiological buffer. After recentrifugation, the wash was combined with the medium. The tissue was homogenized in ethanol and centrifuged at 10,000 *g* for 10 min. The radioactivity in the media and ethanol extracts was determined by liquid scintillation spectrometry. The protein content of the tissue homogenates was determined spectrophotometrically, using the biuret reagent. The per cent inhibition of uptake was calculated according to the following formula:

$$\text{Per cent inhibition of uptake} = \frac{R_c - R_t}{R_c - R_o} \times 100$$

where R_c is the tissue:medium ratio for control tissue expressed as (dis./min/mg of protein)/(dis./min/ml of media), R_t is the tissue:medium ratio for tissue incubated with the test compound, and R_o is the tissue:medium ratio obtained for control tissue at 0°.

Imipramine HCl was a gift of Geigy Pharmaceuticals, Division of Ciba–Geigy Corp., Summit, NJ, and desipramine HCl was provided by U. S. V. Corp., Tuckahoe, NY. The partially reduced derivatives (DMIH and IMIH) were prepared in our laboratory by catalytic reduction of DMI or IMI using rhodium on alumina as the catalyst. The fully reduced derivatives (DMIH2 and IMIH2) were prepared from DMI or IMI by an initial Birch reduction followed by catalytic hydrogenation with platinum oxide. Satisfactory microcombustion analyses were obtained on all new derivatives. Spectral data (n.m.r., i.r. and mass spectrum) were consistent with the assigned structures.* *l*[7-^{3}H]norephinephrine HCl, [G-^{3}H]dopamine HCl and [G-^{3}H]5-hydroxytryptamine creatinine sulfate (all 5–10 Ci/m-mole) were obtained from Amersham Searle Corp., Arlington Heights, IL.

RESULTS

The concentration–effect curves for inhibition of uptake of [^{3}H]NE, [^{3}H]DA and [^{3}H]5-HT into the chopped cerebral cortex of the rat by various tricyclic compounds are shown in Fig. 2, panels a–f. The IC_{50} values are summarized in Table 1. Partial and complete saturation of the aromatic rings in IMI and DMI had very little effect on the inhibition of uptake of [^{3}H]DA into rat cortex (Fig. 2, panels b and e). Similar data were obtained for inhibition of [^{3}H]5-HT uptake (Fig. 2, panels c and f). The six compounds studied possessed similar potencies in inhibiting [^{3}H]5-HT uptake.

In contrast to the data obtained for [^{3}H]DA and [^{3}H]5-HT uptake, ring saturation of DMI resulted in a marked decrease in potency for inhibition of uptake of [^{3}H]NE. Partial reduction of DMI (Fig. 2d) (DMIH, IC_{50} 18.3 μM) resulted in a 150-fold decrease in potency when compared with DMI (IC_{50} 0.12 μM) for inhibition of [^{3}H]NE uptake in this system, thus abolishing the specificity of the tricyclic compound for the neuronal uptake system.

*Details of the chemistry will be reported elsewhere.

Table 1. The IC_{50} values (μM) for 50 per cent inhibition of uptake of ^{3}H-amines into chopped cerebral cortex by various cortex by various tricyclic derivatives*

Inhibitor	Norepinephrine	Dopamine	5-Hydroxytryptamine
IMI	1.8 ± 0.2	39.4 ± 5.2	51.9 ± 7.0
IMIH	28.8 ± 0.0	26.8 ± 1.4	41.8 ± 1.4
IMIH2	22.9 ± 4.0	39.5 ± 5.3	45.1 ± 7.6
DMI	0.12 ± 0.03	63.8 ± 5.3	72.4 ± 6.7
DMIH	18.3 ± 1.6	43.7 ± 4.1	47.9 ± 1.3
DMIH2	29.4 ± 4.3	49.1 ± 2.2	82.2 ± 18.6

*Values are the mean ± S.E.M. for three to four experiments.

Reduction of both rings (DMIH2) resulted in an overall 250-fold decrease in activity (IC_{50} 29.4 μM). The effect of saturation on the inhibition of uptake of [^{3}H]NE produced by IMI (Fig. 2a) was less pronounced when compared with DMI, but again, the selectivity for inhibition of NE uptake (IC_{50} 1.8 μM) when compared with DA or 5-HT was lost, and the activity was decreased 12-fold (IMIH IC_{50} 28.8 μM) with saturation of one ring. Saturation of the second ring did not lead to an additional decrease in potency.

DISCUSSION

The specificity of accumulation of the ^{3}H-amines in cortical tissue is a function of the degree of innervation, the occurrence of a high affinity uptake system specific for each neurotransmitter, and the concentration of the ^{3}H-amine employed. In this study the conditions were chosen to maximize the selective accumulation of the ^{3}H-amines into nerve endings containing the corresponding neurotransmitter. It is known that the cerebral cortex receives a diffuse but prominent innervation from noradrenergic, dopaminergic and serotonergic neurons [9–15]. Although there are both high and low affinity uptake systems for the three biogenic amines [16, 17], if the concentration of ^{3}H-amine is less than the K_m for the high affinity uptake system, each ^{3}H-amine appears to be accumulated preferentially into nerve endings containing the corresponding neurotransmitter [18–20]. The uptake systems of the three types of neurons are also differentially inhibited by the tricyclic antidepressants, with nerve endings which accumulate NE being most sensitive [21–26]. Carlsson *et al* [21] first demonstrated that the effect of DMI on the uptake of NE into nerve endings of the hypothalamus is much more pronounced than its effect on the uptake of DA into nerve endings of the corpus striatum. These results have since been replicated in similar studies by other investigators [23, 24]. The results of the present study suggest that the selective effect of tricyclic antidepressants on NE nerve endings also occurs in the cerebral cortex. This is consistent with other studies in which the effect of antidepressants on the uptake of [^{3}H]NE and [^{3}H]DA into cerebral cortex has been measured either directly or indirectly [21, 22, 26]. The saturated and unsaturated tricyclic antidepressants have effects on the uptake of

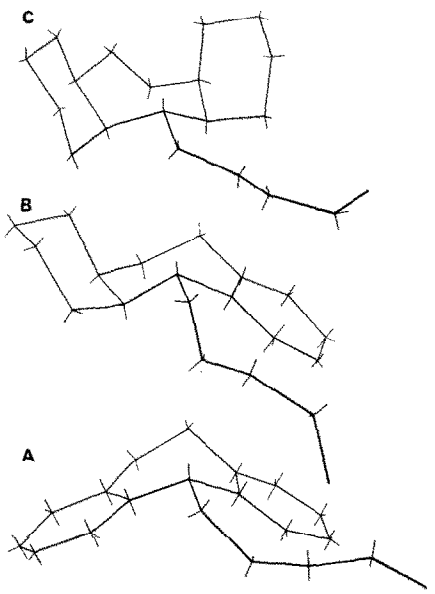


Fig. 3. Change in molecular geometry upon saturation of the aromatic rings in imipramine and desipramine. Key: (a) the fully aromatic nucleus; (b) reduction of one aromatic ring to a cyclohexane ring, with a *cis*-ring junction; and (c) the fully saturated nucleus. These drawings were prepared from photographs of Framework Molecular Models of the ring systems. The carbon atoms of the aromatic rings show additional lines orthogonal to the plane of the ring, which represents the disposition of the p-orbitals which make up the π-electron system of the aromatic ring. The bonds between atoms are reasonably accurate approximations of the interatomic distances found in these structures.

DA into the corpus striatum which are quite similar to those of the present study in which cerebral cortex was used.* Thus, it appears that nerve endings in the cerebral cortex are capable of distinguishing among the three biogenic amines and that the tricyclic antidepressants have a selective affinity for NE-containing nerve endings.

The overall effect of successive reduction of the aromatic rings of IMI and DMI upon the structures of the derivatives is shown in Fig. 3. Reduction conditions were chosen to produce a *cis*-ring junction in the partially reduced derivatives (Fig. 3b), and a *cis, syn, cis*-arrangement in the fully saturated derivatives (Fig. 3c). The effect of successive aromatic ring reduction is to increase the angle formed by intersection of the planes of the outer rings, lifting each ring out of the plane which it defined when aromatic.

If the π-electron density of the aromatic rings were a crucial contributing factor in the attractive intermolecular forces resulting in the binding of IMI or DMI to an amine uptake site, one would predict a substantial loss of interaction with the uptake sites upon loss of aromaticity. If hydrophobic interactions were more important for competitive binding, one would expect binding of the derivatives to be decreased only to the extent that the steric fit of the hydrophobic binding areas of the amine uptake site was lost upon ring saturation. In fact, ring saturation would be expected to increase binding to the uptake site were binding governed by hydrophobic interactions alone. The results obtained thus suggest that, in the case of DA and 5-HT uptake in rat cortex, π-electron interactions between the tricyclic amine and the uptake site must not play a significant role in binding, since fully saturated analogs of both IMI and DMI retain their ability to inhibit neuronal uptake in these systems. These results present an interresting contrast to those reported for non-specific binding of tricyclic antidepressants to human serum albumin [27], where binding was found to correlate with charge-transfer properties of the drugs.

A different situation may be evident in the case of interaction with the NE uptake sites, since reduction of one ring of DMI reduced activity of the derivative 150-fold so that the IC_{50} for NE uptake inhibition was in the same range as that for inhibition of DA or 5-HT uptake. There are two possible mechanisms whereby the reduction in activity may have occurred. Optimal interaction with the NE uptake site may require π-electron interactions which would be totally lost upon reduction of DMI to DMIH2. Limited interaction would still be possible with the remaining aromatic ring of DMIH, if the ring were sterically accessible to the binding site. Alternatively, binding to the NE uptake site may require only hydrophobic interactions which would be satisfied with either an aromatic or reduced ring. But unlike the case of DA or 5-HT uptake, the NE uptake site may be considerably more sensitive to spatial disposition of the hydrophobic binding areas of the substrate. The latter situation is quite likely since the relative geometries of the outer rings change considerably upon reduction, as seen in Fig. 3.

Since a racemic mixture was generated in the formation of DMIH, and one enantiomer might feasibly be inactive with respect to inhibition of NE uptake, it is possible that the reduction in activity seen in DMIH might effectively be only 75-fold. However, even in this case the loss of selectivity for inhibition of NE uptake would still be primarily due to loss of aromaticity. This result is especially interesting in light of the structure activity study reported by Maxwell *et al.* [7], in which removal of the 2-carbon bridge constraining the aromatic rings of DMI resulted in a nearly 800-fold reduction in potency for the inhibition of uptake of [^{3}H]NE into cortical synaptosomes. These results and those of the present study clearly suggest that an overall steric fit, secured in part through hydrophobic interactions, is a crucial determinant in the ability of tricyclic compounds to inhibit uptake of [^{3}H]NE. The loss of potency upon removal of the 2-carbon bridge in DMI can be explained by loss of the conformation of the parent antagonist required to interact successfully with the amine uptake sites. This interaction is entropically unfavored in the absence of the restraining ethylene bridge.

Further evidence for the importance of hydrophobic association in the binding of DMI with the NE uptake site is obtained by comparison of our findings with those of Maxwell *et al.* [7]. Whereas partial loss of aromaticity by reduction of one ring in DMI (DMIH) reduces NE uptake inhibition 150-fold, partial loss of aromaticity by complete removal of one aromatic ring, as reported by Maxwell, reduced uptake inhibitory potencies over 2000-fold. The π-

*C. O. Rutledge and S. Vollmer, unpublished observations.

electron interaction with the aromatic rings of DMI may play a role in the high selectivity exhibited by DMI in the inhibition of NE uptake, hence suggesting possible differences in the nature of the NE uptake site as compared with DA or 5-HT uptake sites, where such interactions are not important. However, it is also possible that the loss in selectivity of NE uptake inhibition seen upon ring saturation is a reflection of a high steric specificity on the part of the hydrophobic and/or π-electron binding sites of the NE uptake carrier.

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