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Inhibition *in vitro* of norepinephrine *N*-methyltransferase by 2-aminotetralins, analogs of phenylethylamines with rigid conformation

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Norepinephrine *N*-methyltransferase (EC 2.1.1.28) is the terminal enzyme in epinephrine biosynthesis. This enzyme, which was referred to as phenylethanolamine *N*-methyltransferase (PNMT) prior to the 1972 recommendations of the International Union of Biochemistry [1], catalyzes the transfer of the methyl group from *S*-adenosylmethionine to norepinephrine. Although a variety of other amines [2–4] are methylated by the enzyme *in vitro*, conversion of norepinephrine to epinephrine may be its only physiological function. For several years inhibitors of norepinephrine *N*-methyltransferase have been sought as pharmacologic tools for interrupting epinephrine biosynthesis without altering the biosynthesis of dopamine and norepinephrine. Known inhibitors of the enzyme include various phenylethylamines and amphetamines [5–7], which are not substrates because they lack the requisite β-hydroxyl group (or similar functional group such as the β-amino); although these amines cannot accept a methyl group they nonetheless combine with and, therefore, inhibit the enzyme. We earlier studied the effect of ring substitution on norepinephrine *N*-methyltransferase inhibition by amphetamines (α-methyl-phenylethylamines) and found that 3, 4-dichloroamphetamine was the most active inhibitor among 33 derivatives that were compared [7]. This paper deals with studies *in vitro* on some phenylethylamine amphetamine analogs having rigid conformation which provides a greater degree of norepinephrine *N*-methyltransferase inhibition.

Norepinephrine *N*-methyltransferase from rabbit adrenal glands was prepared by ammonium sulfate fractionation of the supernatant fluid obtained by high speed centrifugation of tissue homogenates as described previously [8]. Enzyme activity was assayed with 40 μM l-norepinephrine bitartrate (Winthrop) as substrate in the assay method we previously devised using reineckate to precipitate unreacted *S*-adenosylmethionine-[methyl-^{14}C] (New England Nuclear) after incubation [8]. Inhibitors were tested at four to six concentrations, and pI_{50} values (negative logarithm of the molar concentration required for 50 per cent inhibition) were determined by interpolation between points on both sides of 50 per cent inhibition. All of the inhibitors were synthesized in the Lilly Research Laboratories, and their identity and purity were verified by appropriate physicochemical methods.

We started by studying several rigid conformational derivatives of phenylethylamine that had the amino-bearing carbon of the side chain connected to the ortho position of the ring (Table 1). The compound (II) having the carbons directly connected was slightly more active as an inhibitor than was phenylethylamine (I). The derivative (III) with an added methylene unit connecting the carbons had markedly increased inhibitory activity, and a second methylene unit increased the activity still more (IV). Further expansion of the ring then sharply reduced inhibitor activity (V). Thus, the optimum size for the second ring structure was six carbons, inhibitor activity decreasing mark-

Table 1. Inhibition of norepinephrine *N*-methyltransferase by phenylethylamine and related bicyclic compounds

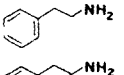
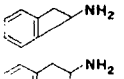
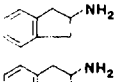
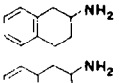
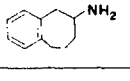
	Structure	Per cent inhibition, Micromolar concentration 3	10	32	100	317	1000	3170	pI_{50}
I						22	40	69	2.83
II				0	5	35	74	95	3.31
III		9	33	66	87	95			4.74
IV		18	47	76	91	96			4.95
V						5	31	70	2.76

Table 2. Norepinephrine *N*-methyltransferase inhibition influence by chlorine substituents on the aromatic ring of 2-aminotetralin

Ring substituent	pI_{50}	Difference in pI_{50} from corresponding amphetamine*
None	4.95	+ 1.69
5-Chloro	5.28	+ 1.05
6-Chloro	5.06	+ 1.46
7-Chloro	5.27	+ 1.04
8-Chloro	4.76	+ 1.52
5,6-Dichloro	6.03	+ 0.93
6,7-Dichloro	5.60	+ 0.50

* Comparison to data in Ref. 7.

edly when this ring size was larger and decreasing less abruptly when the ring size was smaller. The bicyclic compound (IV) containing two six-membered rings, 2-aminotetralin (1,2,3,4-tetrahydronaphthalene-2-amine), was chosen for further study as an inhibitor. This compound was at least 100-fold more active than phenylethylamine.

Table 2 shows the effect of chlorine substitution on the inhibitory activity of 2-aminotetralin. The addition of a single chlorine atom to any position of the aromatic ring increased inhibitor activity, and the addition of a second chlorine substituent further increased inhibitor activity. The most active compound, 5,6-dichloro-2-aminotetralin, inhibited norepinephrine *N*-methyltransferase by 50 per cent at a concentration of 9.3×10^{-7} M. The less potent inhibitors among the 2-aminotetralins were 30–50 times more active than the corresponding amphetamines, whereas the most potent inhibitors among the 2-aminotetralins were 3–10 times more active than the corresponding amphetamines. This finding suggests that the conformation of these phenylethylamine derivatives as fixed in the tetralin ring is more suitable for combination with norepinephrine *N*-methyltransferase than is the conformation usually assumed by amphetamines and phenylethylamines, an observation that may be useful in designing other inhibitors of this enzyme.

The inhibition of norepinephrine *N*-methyltransferase by 5,6-dichloro-2-aminotetralin, the most active inhibitor among those listed in Table 2, showed competitive kinetics with l-norepinephrine as the variable substrate (Fig. 1). The apparent K_m value for the control reaction calculated according to the method of Wilkinson [9] was 14.4 ± 2.7 μM. This figure was increased to 65.0 ± 6.1 by the presence of 1 μM inhibitor. From these apparent K_m values in the presence and absence of inhibitor, the K_i for 5,6-dichloro-2-aminotetralin was estimated [10] to be 2.9×10^{-7} M. Thus, by restricting the conformation we have been able to obtain an inhibitor with about 10 times

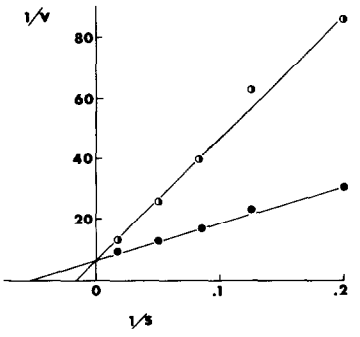


Fig. 1. Competitive kinetics in the inhibition of norepinephrine *N*-methyltransferase by 5,6-dichloro-2-aminotetralin: Lineweaver–Burk plot with l-norepinephrine as the variable substrate. l-Norepinephrine concentrations (*S*) were 5, 8, 12, 20 and 60 μM. Velocity (*V*) is expressed in units of nmoles product formed/30 min of incubation. Inhibitor concentration was zero (●) or 1 μM (◑).

the affinity for norepinephrine *N*-methyltransferase as the most active inhibitor among the amphetamine series, 3,4-dichloroamphetamine [7].

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