Stereochemical Aspects of Phenylethanolamine Analogues as Substrates of Phenylethanolamine *N*-Methyltransferase

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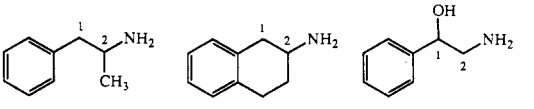
Phenylethylamines and phenylethanolamines represent two major classes of ligands for the epinephrine synthesizing enzyme, phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). Phenylethylamines are usually competitive inhibitors and the isomers with the relative configuration as in (2*S*)-amphetamine (**1**) and (2*S*)-2-aminotetralin (**3**) are better inhibitors than their enantiomers. Phenylethanolamines are usually substrates of PNMT and the enzyme prefers the 1*R* isomers, such as (1*R*)-phenylethanolamine (**5**), in this class. Optically active norephedrines (**7** and **8**), norpseudoephedrines (**9** and **10**), and 2-amino-1-tetralols (**13–16**) were used to study the stereochemical requirements of phenylethanolamines for PNMT active site binding. Although the norephedrines (**7** and **8**) and the norpseudoephedrines (**9** and **10**) were poorer ligands for PNMT than were the 2-amino-1-tetralols (**13–16**), (1*R*,2*S*)-(−)-norephedrine (**7**) showed some activity as a PNMT substrate (K_m = 1310 μM, V_{max} = 0.22, 100 × V_{max}/K_m = 0.017). In the 2-amino-1-tetralols (**13–16**), the isomers with the 2*S* configuration (**13** and **15**) showed higher affinity to PNMT (**13**, K_m = 4.5 μM; **15**, K_i = 4.6 μM) and those with the 1*R* configuration (**13** and **16**) were substrates for the PNMT-catalyzed methyl transfer (**13**, K_m = 4.5 μM, V_{max} = 0.16, 100 × V_{max}/K_m = 3.6; **16**, K_m = 195 μM, V_{max} = 0.12, 100 × V_{max}/K_m = 0.062); the combination of 1*R* and 2*S* configurations, such as in (1*R*,2*S*)-2-amino-1-tetralol (**13**), was required for a good substrate. These stereochemical requirements derived from the norephedrines (**7** and **8**), the norpseudoephedrines (**9** and **10**), and the 2-amino-1-tetralols (**13–16**) complement those for phenylethylamines (**1–4**) and for phenylethanolamines (**5** and **6**) and strongly suggest that phenylethylamine inhibitors bind to PNMT in the same orientation as do phenylethanolamine substrates.

The last step in the biosynthesis of epinephrine is catalyzed by phenylethanolamine *N*-methyltransferase (PNMT; EC 2.1.1.28). The establishment of epinephrine neurons[1] in the central nervous system (CNS) has attracted intensive studies on the functions of epinephrine in the CNS. The inhibition[2] of PNMT could regulate epinephrine levels in the CNS without affecting those of other biogenic amines (i.e., dopamine and norepinephrine). However, the currently available PNMT inhibitors interact with other biomolecules (notably, the α_2-adrenoreceptor) at the concentration required for inhibiting PNMT.[3] We have been using analogues of PNMT inhibitors and substrates to study the binding requirements at the active site of the enzyme in order to design a more selective inhibitor.

Amphetamines (**1** and **2**) and 2-aminotetralins (**3** and **4**) represent one major class of competitive PNMT inhibitors containing a phenylethylamine moiety. The substitution at the amino bearing carbon (C-2) with an alkyl group in phenylethylamines introduces a chiral center and offers an opportunity to study the stereochemical requirements of these inhibitors for PNMT active site binding. Studies[4–6] with optically active amphetamines (**1** and **2**), 2-aminotetralins (**3** and **4**), and other phenylethylamines showed that the isomers with the relative configuration as in (2*S*)-amphetamine (**1**) and (2*S*)-2-aminotetralin (**3**) are consistently better inhibitors for PNMT than their enantiomers.

Phenylethanolamines comprise another major class of PNMT ligands and they are usually substrates. The difference between the phenylethanolamine substrates and the phenylethylamine inhibitors is the presence of hydroxyl functionality at the benzylic position (C-1). (−)-Norepinephrine, the natural substrate of PNMT, has the 1*R* configuration. Stereochemical studies[5] with phenylethanolamines (**5** and **6**) and other phenylethanolamine substrates revealed that PNMT interacts better with the 1*R* isomer, such as (1*R*)-phenylethanolamine (**5**), for the methyl transfer.



1 2*S* **2** 2*R* **3** 2*S* **4** 2*R* **5** 1*R* **6** 2*S*

We recently reported[7] that racemic *cis*-2-amino-1-tetralol (**11**) was a PNMT substrate while racemic *trans*-2-amino-1-tetralol (**12**) was a competitive inhibitor. Optical resolution of these amines (**11** and **12**) should uncover not only the binding affinity for the individual isomers (**13–16**) at the active site of PNMT but also the stereochemical requirements of phenylethanolamine substrates. One unique feature in 2-amino-1-tetralols (**13–16**) is that one (C-2) of the two chiral centers corresponds to the chiral center in phenylethylamine inhibitors (e.g., amphetamine and 2-aminotetralin) and the other (C-1) corresponds to the chiral center in phenylethanolamine substrates. The exploration of the stereochemical requirements could give direct evidence for the binding mode of phenylethanolamine substrates and phenylethylamine inhibitors. Norephedrines (**7** and **8**) and norpseudoephedrines (**9** and **10**) have the same stereochemical feature and were also included in this study.

Chemistry. Both *cis*- and *trans*-2-amino-1-tetralol (**11** and **12**) were synthesized in this laboratory according to the method of Thrift.[8] The resolution of *cis*-2-amino-1-

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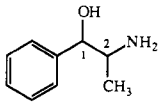
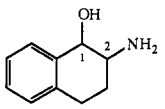


7 (−) erythro; 1*R*,2*S*
8 (+) erythro; 1*S*,2*R*
9 (+) threo; 1*S*,2*S*
10 (−) threo; 1*R*,2*R*



11 (±) cis
12 (±) trans
13 (−) cis; 1*R*,2*S*
14 (+) cis; 1*S*,2*R*
15 (−) trans; 1*S*,2*S*
16 (+) trans; 1*R*,2*R*

tetralol (**11**) followed the procedure of Meyer et al.[9] by recrystallization of the tartarate salts from ethanol/water. The resolution of *trans*-2-amino-1-tetralol (**12**) through camphorsulfonates as reported by Meyer et al.[9] was not successful in our hands. The two enantiomers (**15** and **16**) of *trans*-2-amino-1-tetralol (**12**) were resolved by repeated recrystallization of their dibenzoyltartarates from 50% ethanol. The absolute configurations for **13**–**16** were established by Zymalkowski and Dornhege.[10]

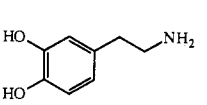
(1*R*,2*S*)-(−)-Norephedrine (**7**), (1*S*,2*R*)-(+)-norephedrine hydrochloride (**8**·HCl), and (1*R*,2*R*)-(−)-norpseudoephedrine hydrochloride (**10**·HCl) were obtained from a commercial source. Fodor et al.[11] reported a procedure for epimerizing the benzylic hydroxyl group in racemic norephedrine to obtain racemic norpseudoephedrine. By applying the same principle, (1*S*,2*S*)-(+)-norpseudoephedrine hydrochloride (**9**·HCl) was obtained from (1*R*,2*S*)-(−)-norephedrine (**7**). The absolute configurations for **7**–**10** were established by Leithe.[12]

Biochemistry. The optically active norephedrines (**7** and **8**), norpseudoephedrines (**9** and **10**), and 2-amino-1-tetralols (**13**–**16**) were evaluated for activity as both substrates for PNMT and inhibitors of PNMT-catalyzed methylation. Bovine adrenal PNMT[13] was used, which had been purified according to the method of Connett and Kirshner through the isoelectric precipitation step.[14] In vitro activity was assessed by use of a standard radiochemical assay that has been previously described for both substrates[15] and inhibitors.[16] For the determination of kinetic constants for substrates, at least five concentrations of the variable substrate were employed in the assay. Inhibition constants were determined by using at least three different concentrations of the inhibitor with phenylethanolamine as the variable substrate.

Results and Discussion

The observed in vitro activities as either substrates or inhibitors of PNMT for the optically active norephedrines (**7** and **8**), norpseudoephedrines (**9** and **10**), and 2-amino-1-tetralols (**13**–**16**) are summarized in Table I. For reference, the data for the optically active amphetamines (**1** and **2**), 2-aminotetralins (**3** and **4**), and phenylethanolamines (**5** and **6**) are included.

The norephedrines (**7** and **8**) and the norpseudoephedrines (**9** and **10**) were poor ligands for PNMT as indicated by their K_m or K_i values. Although the benzylic hydroxyl group did not improve the binding affinity, the (1*R*,2*S*)-norephedrine (**7**) did show some activity as a PNMT substrate (K_m = 1310 μM, V_{max} = 0.22, 100 × V_{max}/K_m = 0.017) while (2*S*)-amphetamine (**3**) did not. It has been noted[17] that dopamine (**17**) is not a PNMT substrate. The benzylic hydroxyl group is necessary, although not sufficient, for the PNMT-catalyzed methyl transfer reaction, especially for molecules with a flexible side chain.[18] These results for **7**–**10** are in agreement with the previous finding that α-methylnorepinephrine (**18**) was only a weak PNMT substrate.[19] The presence of the α-methyl group could alter the distribution among possible conformations of the aminoethyl side chain[20] and make the binding to PNMT unfavorable.



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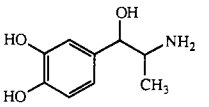

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Restriction of the side-chain conformation of the norephedrines (**7** and **8**) and the norpseudoephedrines (**9** and **10**) to the 2-amino-1-tetralols (**13**–**16**) resulted in a much higher affinity for PNMT. The 1*R*,2*S* isomer (**13**) and the 1*R*,2*R* isomer (**16**) were substrates of PNMT (**13**, K_m = 4.5 μM, V_{max} = 0.16, 100 × V_{max}/K_m = 3.6; **16**, K_m = 195 μM, V_{max} = 0.12, 100 × V_{max}/K_m = 0.062) while the other two isomers (**14** and **15**) were PNMT inhibitors (**14**, K_i = 293 μM; **15**, K_i = 4.6 μM). Close examination of the data revealed that substrates **13** and **16** have the 1*R* configuration while the two isomers with the 2*S* configuration (**13** and **15**) were better ligands than their 2*R* counterparts (**14** and **16**). These two observations suggest that in the binding and catalysis steps of the methyl transfer process, the 2*S* configuration is more important in binding while the 1*R* configuration is required for PNMT catalysis. The combination of the 1*R* and 2*S* configurations such as in **13** is required for a good PNMT substrate.

We previously reported[7] that racemic *cis*-2-amino-1-tetralol (**11**) was a PNMT substrate (K_m = 22 μM, V_{max} = 0.15, 100 × V_{max}/K_m = 0.68) and racemic *trans*-2-amino-1-tetralol (**12**) was a PNMT inhibitor (K_i = 9.4 μM). In fact, only the cis isomer with the 1*R*,2*S* configurations (**13**) was a substrate; its enantiomer **14** (the 1*S*,2*R* configurations) was an inhibitor. On the basis of the earlier results,[7] it was unexpected that the trans 1*R*,2*R* isomer **16** would show activity as a PNMT substrate. However, it is understandable because the *trans*-(1*S*,2*S*)-2-amino-1-tetralol (**15**) was a potent inhibitor (K_i = 4.6 μM) and could inhibit the activity as a substrate from *trans*-(1*R*,2*R*)-2-amino-1-tetralol (**16**, K_m = 195 μM, V_{max} = 0.12, 100 × V_{max}/K_m = 0.062) in the racemic mixture.

The stereochemical requirements for the norephedrines (**7** and **8**), the norpseudoephedrines (**9** and **10**), and the 2-amino-1-tetralols (**13**–**16**) complement those for phenylethylamine inhibitors and phenylethanolamine substrates. The results from this study are consistent with the conclusion that phenylethylamines bind at the active site of PNMT in the same orientation as do phenylethanolamines.

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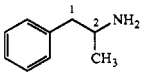
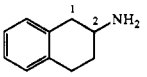
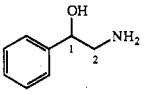
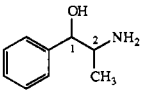
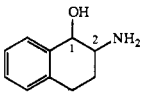
Table I. In Vitro Activities of Phenylethylamine and Phenylethanolamine Analogues as Substrates or Inhibitors of PNMT

	compd	K_i, μM	K_m, μM	V_{max}[a]	100 × V_{max}/K_m
	1 2*S*	422 ± 43[b]			
	2 2*R*	1381 ± 147[b]			
	3 2*S*	4.1 ± 0.1			
	4 2*R*	10.0 ± 0.4			
	5 1*R*		286 ± 130[b]	4.85[b]	1.70[b]
	6 1*S*		96 ± 15[b]	0.92[b]	0.96[b]
	7 1*R*,2*S*		1310 ± 214	0.22 ± 0.02	0.017
	8 1*S*,2*R*	2980 ± 320			
	9 1*S*,2*S*	3700 ± 280			
	10 1*R*,2*R*	2440 ± 140			
	13 1*R*,2*S*		4.5 ± 0.7	0.16 ± 0.02	3.6
	14 1*S*,2*R*	293 ± 12			
	15 1*S*,2*S*	4.6 ± 0.2			
	16 1*R*,2*R*		195 ± 14	0.12 ± 0.01	0.062

[a] Units of V_{max} are nanomoles of product per milligram protein per minute. [b] Taken from ref 5.

Although (−)-norepinephrine is the natural substrate, PNMT accepts many phenylethanolamine analogues without the catechol moiety as substrates.[21] In fact, (−)-norepinephrine was an outlier in a QSAR study of phenylethanolamine substrates.[22] How norepinephrine binds to PNMT with reference to phenylethanolamine substrates and phenylethylamine inhibitors remains to be established. The extension of our work to include (−)-norepinephrine is in progress.

Experimental Section

All compounds made in this laboratory were characterized by spectroscopic methods (IR, MS, ^1H and ^{13}C NMR). Combustion analyses were within 0.4% of the theoretical values.

(1*R*,2*S*)-(−)-2-Amino-1-tetralol Hydrochloride (13·HCl) and (1*S*,2*R*)-(+)-2-Amino-1-tetralol Hydrochloride (14·HCl). Racemic *cis*-2-amino-1-tetralol (11) was resolved to **13** and **14** by following the procedure of Meyer et al.[9] Repeated recrystallization of the (+)-tartrate salt of **11** from ethanol/water gave **13** while repeated recrystallization of the (−)-tartrate salt of **11** from ethanol/water gave **14**. **13**·HCl: mp 223–224 °C (lit.[9] mp 224 °C); $[\alpha]^{23}_D$ −79.3° (*c* 0.3, H_2O) [lit.[9] $[\alpha]^{20}_D$ −79.9° (*c* 3.2, H_2O)]. **14**·HCl: mp 223–224 °C; $[\alpha]^{23}_D$ +81.6° (*c* 0.3, H_2O).

(1*S*,2*S*)-(−)-2-Amino-1-tetralol Hydrochloride (15·HCl) and (1*R*,2*R*)-(+)-2-Amino-1-tetralol Hydrochloride (16·HCl). Racemic *trans*-2-amino-1-tetralol (**12**) was resolved to **15** and **16** by repeated recrystallization of the dibenzoyltartrate salts from 50% ethanol. By use of L-(−)-dibenzoyltartaric acid, **15**·HCl was obtained: mp 239–240 °C; $[\alpha]^{23}_D$ −66.6° (*c* 0.4, H_2O) [lit.[9] $[\alpha]^{20}_D$ −65° (*c* 1, H_2O)]. **16**·HCl was obtained by using D-(+)-dibenzoyltartaric acid: mp 243–244 °C; $[\alpha]^{23}_D$ +66.4° (*c* 0.4, H_2O).

(1*R*,2*S*)-(−)-Norephedrine Hydrochloride (7·HCl), (1*S*,2*R*)-(+)-Norephedrine Hydrochloride (8·HCl), and (1*R*,2*R*)-(−)-Norpseudoephedrine Hydrochloride (10·HCl). Compounds **7**, **8**·HCl, and **10**·HCl were purchased from Aldrich Chemical Co., Milwaukee, WI. **8**·HCl: mp 172–174 °C (lit.[23] mp 171–172 °C); $[\alpha]^{23}_D$ +33.9° (*c* 7.3, H_2O) [lit.[23] $[\alpha]^{27}_D$ +33.4° (*c* 6, H_2O)]. **10**·HCl: mp 180–182 °C (lit.[23] mp 180–181 °C); $[\alpha]^{23}_D$ −41.8° (*c* 7.2, H_2O) [lit.[23] $[\alpha]^{20}_D$ −42.7° (*c* 7, H_2O)]. **7**·HCl was prepared in ether with anhydrous HCl and recrystallized from ethanol/ether: mp 172–174 °C (lit.[23] mp 171–172 °C); $[\alpha]^{23}_D$ −33.8° (*c* 3.0, H_2O) [lit.[23] $[\alpha]^{20}_D$ −33.3° (*c* 3, H_2O)].

(1*S*,2*S*)-(+)-Norpseudoephedrine Hydrochloride (9·HCl). Similar to the procedure of Fodor et al.,[11] a mixture of (1*R*,2*S*)-(−)-norephedrine (**7**, 8.0 g) and 14% HCl (200 mL) was heated under reflux for 2 days. After removal of solvent, the residue was recrystallized from ethanol/ether several times, giving **9**·HCl as white crystals (0.85 g): mp 179–181 °C (lit.[23] mp 180–181 °C), $[\alpha]^{20}_D$ +42.7° (*c* 4.9, H_2O) [lit.[23] $[\alpha]^{20}_D$ +42.5° (*c* 7, H_2O)].

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Registry No. **7**, 492-41-1; **8**, 37577-28-9; **9**, 492-39-7; **9**·HCl, 2153-98-2; **10**, 37577-07-4; **11**, 64282-62-8; **12**, 64282-63-9; **13**, 3877-76-7; **13**·HCl, 29365-56-8; **14**, 3809-70-9; **14**·HCl, 29365-58-0; **15**, 21884-39-9; **15**·HCl, 115563-63-8; **16**, 18630-50-7; **16**·HCl, 115563-64-9; PNMT, 9037-68-7.

(21) Because of the high water solubility of [^{3}H]epinephrine, the radiochemical assay[15] with isoamyl alcohol/toluene extraction could not be used for (−)-norepinephrine. A Reineckate salt assay, in which unreacted [^{3}H]-*S*-adenosyl-L-methionine is precipitated and the supernatant is counted, was used to obtain comparison values for (±)-phenylethanolamine and (−)-norepinephrine; for assay procedure, see: Grunewald, G. L.; Pleiss, M. A.; Rafferty, M. F. *Life Sci.* **1982**, *31*, 993. It was found in the Reineckate salt assay that (−)-norepinephrine (K_m = 7.7 ± 3.2 μM, V_{max} = 0.22 ± 0.05, 100 × V_{max}/K_m = 2.9) is a better substrate than (±)-phenylethanolamine (K_m = 239 ± 113 μM, V_{max} = 1.5 ± 0.1, 100 × V_{max}/K_m = 0.63).

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