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Phenylethanolamine *N*-Methyltransferase Kinetics: Bovine Versus Recombinant Human Enzyme

Gary L. Grunewald,[a,*] Michael J. McLeish[b] and Kevin R. Criscione[a]

[a]*Department of Medicinal Chemistry, University of Kansas, 4060 Malott Hall, 1251 Wescoe Hall Drive, Lawrence, KS 66045-7582, USA*

[b]*College of Pharmacy, University of Michigan, Ann Arbor, MI 48109, USA*

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Abstract—Epinephrine (Epi) acts as a neurotransmitter in the brain, but its function therein is not well understood. Phenylethanolamine *N*-methyltransferase (PNMT) catalyzes the final step in the biosynthesis of Epi and is thus a pharmacological target to investigate the function of Epi in the central nervous system. The kinetic differences between bovine adrenal PNMT and human brain PNMT for a number of substrates and inhibitors are examined and the results reported.

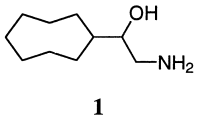
Phenylethanolamine *N*-methyltransferase (PNMT) (EC 2.1.1.28) is the enzyme that catalyzes the final step in the biosynthesis of epinephrine (Epi). Epi comprises 5–10% of the total catecholamine content of the brain, but its function therein is not well understood.[1]

This laboratory has published a number of studies on substrates and inhibitors of PNMT obtained from bovine adrenal glands as part of our research to define the structure–activity relationships required for the design of a potent and selective inhibitor of Epi biosynthesis in the central nervous system (CNS). While earlier studies have shown similarity between PNMT isolated from different animal sources, these results were based on a limited number of closely related substrates or inhibitors.[2,3] What is now required is a comparison of the kinetics of a number of structurally diverse substrates and inhibitors using the bovine adrenal enzyme versus human brain PNMT (hPNMT), which we have prepared through overexpression in *Escherichia coli*.[4] These results will be used to extend conclusions made using prior data obtained on bovine adrenal PNMT to hPNMT.

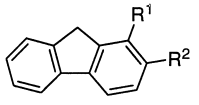
In vitro PNMT activity of the compounds in this study[5] was assessed by use of a standard radiochemical assay[6] that has been described previously for both substrates[7] and inhibitors.[8] Bovine adrenal PNMT was purified according to the procedure of Connett and Kirshner[9] through the isoelectric precipitation step. Recombinant human PNMT was overexpressed in *E. coli* and purified according to the procedure of Caine et al.[4] For internal consistency, the bovine PNMT values reported in Tables 1–5 were determined in assays run concurrently with the hPNMT assays,[10] rather than those reported previously.[7,8,11–24] The chiral compounds in Tables 1, 3, and 5 were tested as their racemates.

The compounds in Table 1 were chosen to examine the kinetics of structurally diverse and sterically bulky substrates. A non-ethanolamine substrate (**5**) has been

Table 1. Substrates: probes for bulk tolerance

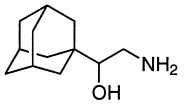


1



2: $R^1 = CH(OH)CH_2NH_2$, $R^2 = H$

3: $R^1 = H$, $R^2 = CH(OH)CH_2NH_2$



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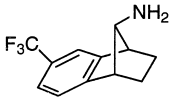

5

Compd	Human PNMT (H) K_m (±SEM) μM	Bovine PNMT (B) K_m (±SEM) μM	Ratio (H/B)	Ref
1	39 (±7)	13 (±2)	3.0	7
2	420 (±100)	270 (±40)	1.6	11
3	110 (±10)	26 (±3)	4.2	11
4	720 (±100)	280 (±30)	2.6	12
5	47 (±5)	27 (±4)	1.7	13

*Corresponding author. Tel.: +1-785-864-4497; fax: +1-785-864-5326; e-mail: ggrunewald@ukans.edu

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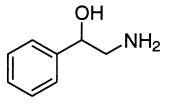
included, which does not possess the β-hydroxyl group once thought to be necessary for substrate activity.[25]

All of the K_m ratios (human/bovine) are greater than 1, which is consistent with hPNMT having less activity with these substrates than the bovine enzyme. This may be due, in part, to hPNMT having less steric bulk tolerance than the bovine enzyme. Such an area of steric bulk tolerance could also have directionality, as indicated by the ratio of the K_m values of **2** and **3**, both between enzymes, and with the same enzyme.

The substrates in Table 2 were chosen to compare the enantioselectivity of the two enzymes. On the basis of structure–activity studies, we hypothesized that the amine of benzylamine inhibitors can be involved in a hydrogen bond interaction in the same region of space as the β-hydroxyl group of phenylethanolamine substrates (Fig. 1).[26] Using this model, we predicted that **7** would be a better substrate than **6**,[26] and this was found to be the case for both enzymes.

Table 2. Substrates: enantioselectivity



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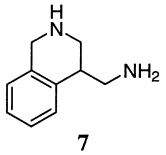


7

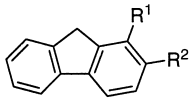
Compd	Human PNMT (H) K_m (±SEM) μM	Bovine PNMT (B) K_m (±SEM) μM	Ratio (H/B)	Ref
6				
±	120 (±10)	66 (±7)	1.8	7
S-(+)	57 (±5)	34 (±4)	1.7	14
R-(−)	170 (±20)	110 (±10)	1.6	14
±	73 (±1)	26 (±3)	2.8	15
7				
R-(+)	32 (±4)	14 (±2)	2.3	16
S-(−)	150 (±10)	92 (±6)	1.6	16

As in Table 1, the ratio of K_m values shows that hPNMT has less activity with these substrates than the bovine enzyme. However, both compounds show similar enantioselectivity at each enzyme, with a (+)/(−) ratio of ca. 3 for **6** at both enzymes, and ca. 5 for **7** at both enzymes.

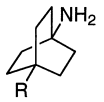
The compounds in Table 3 were chosen to examine the kinetics of structurally diverse and sterically bulky competitive inhibitors.

The inhibitors in Table 3 show lower activity at hPNMT than at the bovine enzyme. The similar ratios shown by **8** and **3** lend support to previous conclusions that (a) the nitrogen of benzylamine-type inhibitors binds near the β-hydroxyl of substrates, and (b) the directionality of steric bulk tolerance of benzylamine-type inhibitors and ethanolamine-type substrates is

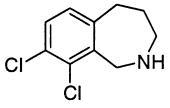
Table 3. Inhibitors: probes for bulk tolerance



8: $R^1 = CH_2NH_2$, $R^2 = H$
9: $R^1 = H$, $R^2 = CH_2NH_2$



10: R = Cyclohexyl
11: R = Phenyl



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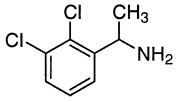


13

14

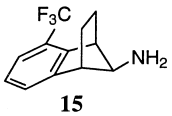
Compd	Human PNMT (H) K_i (±SEM) μM	Bovine PNMT (B) K_i (±SEM) μM	Ratio (H/B)	Ref
8	10 (±1)	2.3 (±0.2)	4.3	11
9	530 (±30)	120 (±10)	4.4	11
10	180 (±10)	51 (±3)	3.5	
11	51 (±2)	27 (±2)	1.9	
12	0.28 (±0.03)	0.22 (±0.03)	1.3	17
13	0.81 (±0.09)	0.56 (±0.08)	1.4	18
14	730 (±70)	470 (±40)	1.6	8

similar. The ratio of 1.6 exhibited by **2**, which is different from the ca. 4.3 shown by **3**, **8**, and **9**, indicates that **2** binds in a different manner at the two enzymes than the other fluorenes.

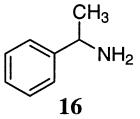
The compounds in Table 4 were chosen to explore the enantioselectivity of competitive inhibitors at the two enzymes.

Compounds (+)-**15**[29] and (+)-**18**, in contrast to the results in Tables 1–3 and those for **16** and **17**, may

Table 4. Inhibitors: enantioselectivity



15



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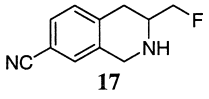
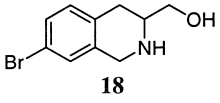


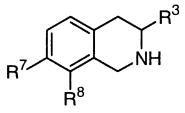
17



18

Compd	Human PNMT (H) K_i (±SEM) μM	Bovine PNMT (B) K_i (±SEM) μM	Ratio (H/B)	Ref
15				
±	13 (±1)	13 (±2)	1.0	13
+	10 (±1)	13 (±1)	0.77	29
−	16 (±2)	14 (±1)	1.1	29
±	830 (±100)	280 (±20)	3.0	14
16				
R-(+)	4800 (±300)	1600 (±100)	3.0	14
S-(−)	430 (±40)	140 (±10)	3.1	14
±	2.6 (±0.2)	1.7 (±0.1)	1.5	19
17				
R-(+)	1.5 (±0.1)	0.97 (±0.08)	1.5	20
S-(−)	4.5 (±0.4)	3.5 (±0.2)	1.3	20
±	Not available	Not available	—	
18				
R-(+)	0.30 (±0.03)	0.38 (±0.05)	0.79	20
S-(−)	1.1 (±0.1)	0.48 (±0.05)	2.3	20

Table 5. Inhibitors: 1,2,3,4-tetrahydroisoquinolines



19–31

Compd	R^3	R^7	R^8	Human PNMT (H) K_i (±SEM) (μM)	Bovine PNMT (B) K_i (±SEM) (μM)	Ratio (H/B)	Ref
19	H	Cl	Cl	0.30 (±0.04)	0.22 (±0.05)	1.4	17
20	H	H	H	15 (±1)	9.7 (±0.4)	1.5	21
21	H	SO_2NH_2	H	0.58 (±0.04)	0.56 (±0.04)	1.0	22
22	H	SO_2NHCH_3	H	20 (±2)	4.6 (±0.3)	4.3	23
23	CH_2F	Br	H	1.2 (±0.1)	0.64 (±0.08)	1.9	20
24	CH_2F	I	H	0.66 (±0.07)	0.37 (±0.07)	1.8	20
25	CH_2F	N_3	H	3.5 (±0.2)	1.7 (±0.1)	2.1	20
26	CH_2F	SO_2NH(*p*-Cl-Ph)	H	0.95 (±0.09)	0.74 (±0.07)	1.3	20
27	CH_2F	SO_2NH(*p*-Br-Ph)	H	1.0 (±0.1)	0.59 (±0.1)	1.7	20
28	CH_2F	SO_2NHCH_2Ph	H	62 (±0.4)	6.5 (±0.2)	9.5	20
29	CH_2Cl	H	H	13 (±1)	7.5 (±0.5)	1.7	20
30	CH_2OH	SO_2CH_3	H	2.0 (±0.1)	0.64 (±0.04)	3.1	24
31	CH_2OH	SO_2NH_2	H	2.1 (±0.1)	0.34 (±0.06)	6.2	24

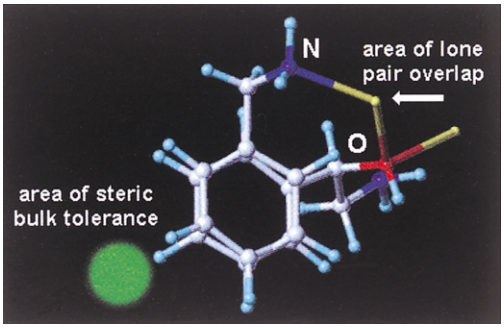


Figure 1. Molecular overlay of benzylamine and phenylethanolamine (**6**), showing how the lone pairs (yellow) on the benzylamine nitrogen (N) and the phenylethanolamine β-hydroxyl (O) can reach the same area of space, albeit from different directions. The global minimum energy conformations (Tripos force field with MOPAC charges in SYBYL[27] were superimposed using both ends of 2 Å long normals through the centroids of the aromatic rings and the ends of the lone pairs (2.4 Å). An area of steric bulk tolerance, determined to be at the *para*-position of phenylethanolamine substrates and the *meta*-position of benzylamine inhibitors,[28] is shown in green.

(within standard error) show greater activity at hPNMT than at the bovine enzyme. Also, while the enantiomers of **15**[29] possess the same activity at the bovine enzyme, they show definite enantioselectivity at hPNMT. A similar pattern exists for the enantiomers of **18**.

One of the limitations of current PNMT inhibitors as pharmacological tools for the determination of the function of Epi in the CNS is their affinity for other pharmacologically relevant sites, particularly the α_2-adrenoceptor.[1] Some 1,2,3,4-tetrahydroisoquinolines (THIQs) with 3,7-disubstitution have been found to be highly selective inhibitors of bovine adrenal PNMT (vs the α_2-adrenoceptor).[19,20,24] The compounds in Table 5 were chosen to examine the kinetics of some 3- or 7-substituted-, or 3,7-disubstituted-THIQs.

While there are a number of structural differences among the compounds in Table 5 that preclude specific conclusions as to the effects of a single structural change on the binding differences between hPNMT and the bovine enzyme, it is interesting to note that the addition of a 3-hydroxymethyl substituent to **21** enhanced the potency of **31** at bovine PNMT while reducing its activity at hPNMT. This could be the result of a steric interaction at the 3-position of THIQ, but as **20** and **29** show similar ratios (for a 3-H vs a 3-CH_2F moiety), this would seem unlikely. We hypothesized previously that a hydrogen bond interaction was involved in the increased activity of 3-hydroxymethyl-THIQs at the bovine enzyme,[24] and such an interaction would appear to be absent at hPNMT.

In conclusion, although there are some dramatic differences in the activity of substrates and inhibitors at the two enzymes, which is consistent with structural differences in the active site region between hPNMT and the bovine enzyme, the general conclusions of prior studies based on structure–activity relationships with bovine adrenal PNMT are valid for hPNMT as well.

Acknowledgements

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5. The compounds in this study were synthesized in our laboratory with the following exceptions: **10** and **11** (DuPont, Inc., Wilmington, DE, USA), **12** and **13** (Eli Lilly & Co., Indianapolis, IN, USA), **19** and **21** (Smith, Kline & French Laboratories, SmithKline Beecham Corp., Philadelphia, PA, USA), and **20** (Aldrich Chemical Co., Milwaukee, WI, USA).
6. A typical assay mixture consisted of phosphate buffer (pH 8.0, 0.5 M, 50 μL), unlabeled AdoMet (10 mM, 25 μL), [*methyl*-^{3}H]AdoMet, (ca. 3×10^5 dpm, 5 μL), substrate solution (25 μL) [(±)-**6** was used as the variable substrate in inhibitor assays], inhibitor solution (25 μL), enzyme preparation (25 μL), and sufficient water to achieve a final volume of 250 μL. After incubation for 30 min at 37 °C, the mixture was quenched with borate buffer (pH 10.0, 0.5 M, 250 μL) and extracted with toluene/isoamyl alcohol (7:3, 2 mL). A portion of the organic layer (1 mL) was removed, transferred to a scintillation vial and diluted with cocktail (5 mL) for counting. The mode of inhibition was ascertained to be competitive in all cases reported in Tables 3–5 by inspection of the $1/V$ versus $1/S$ plots of the data. All assays were run in duplicate with four substrate concentrations over a 10-fold range or three inhibitor concentrations over a 5-fold range. K_m and K_i values were determined by a hyperbolic fit of the data.
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