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Inhibition of PNMT activity in the adrenal glands and brain stem of rats*

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Phenylethanolamine *N*-methyltransferase (PNMT) catalyzes the formation of epinephrine from norepinephrine and *S*-adenosylmethionine (SAM) serves as a methyl donor [1]. The enzyme is localized primarily in the adrenal medulla and small amounts of a PNMT enzyme have been detected in the CNS [2, 3]. Using immunofluorescence techniques, we have mapped out the PNMT neuronal system in the CNS of rats [4, 5]. Subsequently, these results were confirmed by measuring biochemically, PNMT activity in specific regions of the CNS [6, 7]. Several inhibitors of adrenal PNMT have been described [8, 9], but their inhibitory potency on the PNMT activity in the brain were not elucidated. This report describes the effects of several PNMT inhibitors on the *in vivo* and *in vitro* activity of PNMT in the brain.

[^{3}H]Methyl-SAM (sp. act. 12.3 Ci/m-mole) was purchased from New England Nuclear. 3-methyl-1-2,3,4-tetrahydro(1)benzothieno(3,2-c) pyridine hydrochloride (SK&F 7698) was obtained from Smith Kline & French Laboratories, 2-amino-5-6,7-trifluoro-methyl benzimidazole and 1-methyl-4 or 5-(4-trifluoromethylphenyl) imidazole from Merck Sharp & Dohme Research Laboratories and 2,3-dichloro-α-methyl benzylamine (DCMB) from Eli Lilly & Co.

The rats were killed by decapitation and the tissues were immediately frozen. The tissues were homogenized with 10 vols of isotonic KCl solution containing 5×10^{-4} M dithiothreitol, and the homogenate was centrifuged at 40,000 *g* for 30 min. The supernatant solution was used for the enzymatic assay.

PNMT was assayed by a previously described procedure using phenylethanolamine as substrate [10]. Each incubation mixture consisted of 50 μl of the supernatant solution and 50 μl of a solution containing 50 nmoles of phenylethanolamine, 0.15 nmoles of [^{3}H]methyl-SAM (1 μCi; sp. act. 12.3 Ci/m-mole), 4 μmoles of Tris–HCl, pH 8.0 buffer. The reaction mixture was incubated for 60 min at 37° and terminated by addition of 0.4 ml of 0.32 M borate, pH 10 buffer. The radioactive product was extracted into 3 ml of 3% isoamyl alcohol intoluene and re-extracted into 1 ml of 0.1 N HCl. The aqueous extract was taken to dryness in a vacuum oven at 70° under slightly reduced pressure. The dried residue was dissolved in Bray's counting solution and assayed for radioactivity. The re-extraction procedure was necessary to remove the non-specific radioactive products.

The kinetic studies were carried out under conditions in which the reaction was found to be linearly dependent on time (for 60 min) and substrate concentrations (for phenylethanolamine, 4×10^{-5} M–4×10^{-4} M and for SAM, 10^{-7} M–3×10^{-5} M). The K_m values were determined by the method of Lineweaver and Burke [11] and the K_i values by the method of Dixon [12]. Kinetic data were computed by fitting data to linear functions by the method of least square analysis. Statistics were calculated by paired Student's *t*-tests.

The effects of several PNMT inhibitors on the activity of the enzyme isolated from rat adrenals and the brain stem are summarized in Table 1. Among the tested compounds SK&F 7698 was found to be the most potent inhibitor of PNMT activity in the adrenals and in the brain stem. At various concentrations SK&F 7698 was equipotent in inhibiting the enzyme activity obtained from both tissues. DCMB was a less potent inhibitor of PNMT activity than SK&F 7698. The per cent of inhibition by DCMB and the other tested compounds is the same for the enzyme isolated from the adrenals as from the brain stem. The kinetic studies reveal that both SK&F 7698 and DCMB alter the appaaent K_m for the substrate, phenylethanolamine, (K_m for phenylethanolamine was found to be 4×10^{-4} M in absence of the inhibitor and 1×10^{-3} M when the concentration of the inhibitor was 5×10^{-7} M) without affecting the V_{max} values. This kinetic pattern with the enzyme isolated from the brain stem indicates a competitive type inhibition with respect to phenylethanolamine and is consistent with the results obtained with the enzyme obtained from the adrenals [8, 9]. The K_i values for both inhibitors (SK&F 7698 and DCMB) are summarized in Table 2. It is evident that approximately the same K_i values were obtained for both inhibitors when measured with the enzyme isolated from the adrenal glands of rats or from brain stems of various species.

The results in Table 3 show that following a single administration of SK&F 7698 or of DCMB, the PNMT activity in the brain stem is effectively inhibited. SK&F 7698, at a dose of 100 mg/kg (i.p.), and DCMB, at a dose of 45 mg/kg (i.p.), almost completely inhibit PNMT activity in the brain stem. The relative higher potency of DCMB as compared with SK&F to inhibit the brain enzyme *in vivo* might be due to its greater accumulation in the PNMT containing neurons.

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Table 1. The inhibition of PNMT activity obtained from rat adrenal gland and brain stem by various compounds

Inhibitor	Inhibition per cent							
	Adrenal gland				Brain stem			
	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M	10^{-7} M	10^{-6} M	10^{-5} M	10^{-4} M
SK&F 7698	58	75	98	100	61	78	94	100
DCMB	12	31	80	98	30	53	91	100
ATMB	—	14	55	90	—	20	53	92
MTMI	—	9	34	85	—	8	40	85

Abbreviations used: ATMB (2-amino-5-(6)-trifluoro-methyl benzimidazole) MTMI (1-methyl-4 or 5-(4-trifluoro-methyl phenyl) imidazole) [15].

The results are the means of 3 experiments ±S.E.M. (5–10%).

Table 2. The K_i values for SK&F 7698 and for DCMB

Enzyme source	K_i (μM)*	
	SK&F 7698	DCMB
Adrenal glands (rats)	0.315	2.0
Brain stems (rats)	0.314	0.66
Brain stem (monkey)	0.315	0.41
C_1 and C_2 areas (post-mortem human)	0.303	0.425

* Each value is the mean ± S.E.M. (5–12%) generated from intercepts of 5 separate lines. The K_i values were determined at 7 concentrations of phenylethanolamines (ranging from 4×10^{-5} M–4×10^{-4} M), constant SAM concentration (1.4×10^{-6} M) and at 2 concentrations of the respective inhibitors (5×10^{-7} and 1×10^{-6} M).

hibitors of PNMT activity in the adrenal glands have almost the same inhibitory effectiveness in the brain stem. The immunotitration curve of PNMT from rat adrenal glands and from rat brain stem against bovine adrenal PNMT antiserum are similar (Lew and Goldstein, unpublished data), indicating that the enzyme from both tissues have similar antigenic determinants. Unlike the PNMT activity in the adrenal glands, the activity in the brain stem is not altered significantly by hypophysectomy reported (Lew and Goldstein, unpublished data). Thus, although the regulation of PNMT in the adrenal may be different than in the brain stem, the enzymes themselves appear to be similar.

The existence of a PNMT-containing neuronal system which arises from cells in the medulla oblongata (cell groups C_1 and C_2) and projects to the spinal cord, pons, midbrain, hypothalamus and thalamus [4, 5] raises the question on the role of these neurons in the control of various brain functions. It was also shown that small quantities of epinephrine are localized in the PNMT-containing regions of the CNS [13, 14]. The effects of the PNMT inhibitors on epinephrine levels in the specific regions of the CNS are now under investigation and these studies may contribute to the elucidation of the role of epinephrine in the CNS functions.

Table 3. The effect of SK&F 7698 and DCMB administration on PNMT activity obtained from the brain stem of rats

Inhibitor	Inhibition per cent
SK&F 7698 (45 mg/kg; i.p.)	24 ± 5
SK&F 7698 (100 mg/kg; i.p.)	90 ± 8
DCMB (45 mg/kg; i.p.)	94 ± 10

The results are the means ± S.E.M. of 5 experiments. The animals were sacrificed 1 hr after administration of the inhibitor.

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