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LOWERING OF EPINEPHRINE CONCENTRATION IN RAT BRAIN BY 2,3-DICHLORO-α-METHYL-BENZYLAMINE, AN INHIBITOR OF NOREPINEPHRINE N-METHYLTRANSFERASE

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The occurrence of dopamine and norepinephrine in discrete neural tracts in brain has been known for more than a decade, and roles for these catecholamine neurotransmitters in various brain functions have been elucidated. A third catecholamine, epinephrine, is present in brain in much lower concentrations, and recent immunohistochemical fluorescence studies [1,2] have mapped neurons in rat brain containing the epinephrine-synthesizing enzyme, norepinephrine N-methyltransferase (EC 2.1.1.28).

The physiological roles of these epinephrine-containing neurons are unknown, but some possibilities have been suggested based on the anatomic localization of the neuronal projections [2]. Inhibitors of norepinephrine N-methyltransferase should be valuable tools for studying epinephrine neurons, since they would not directly affect norepinephrine or dopamine neurons. In contrast, inhibitors of tyrosine hydroxylase, dopa decarboxylase, or dopamine β-hydroxylase would interrupt the synthesis of norepinephrine and/or dopamine as well as epinephrine. We are reporting here that an inhibitor of norepinephrine N-methyltransferase, (±)-2,3-dichloro-α-methylbenzylamine hydrochloride (DCMB) [3,4], does lower epinephrine content of rat brain without lowering norepinephrine or dopamine content.

For the assay *in vitro* of rat brain norepinephrine N-methyltransferase with L-norepinephrine as the methyl-accepting substrate, the methodology of Henry *et al.* [5] was used. The concentration of L-norepinephrine was 100 μM (K_m was 31 ± 4 μM), and the concentration of S-adenosylmethionine was 50 μM (K_m was 13 ± 2 μM). For the experiment *in vivo*, DCMB was injected i.p. at 25 mg/kg into groups of five male Wistar rats weighing 130-150 g (from Harlan Industries, Cumberland, Ind.). The rats were killed by decapitation and the brains were rapidly excised. A section including the hypothalamus (defined

by the circle of Willis) was punched out with a metal cork borer, 5 mm inside diameter; the tissue sections weighed 60 ± 1 mg (mean of 20 samples ± standard error) and contained hypothalamus, thalamus and subthalamus. The tissue samples were frozen immediately on dry ice and stored frozen overnight prior to analysis. For the quantitative determination of brain catecholamines, a high-pressure liquid chromatographic method using electrochemical detection was developed based on the procedure of Keller et al. [6]. Briefly the procedure involved protein precipitation with perchloric acid and adsorption of the catecholamines on alumina. Routinely the aliquot of alumina eluate applied to the column contained 2-3 picomoles of epinephrine. A Vydac TP cation exchange column (pre-packed, 10 µm particle size, 3.2 mm x 25 cm) completely resolved norepinephrine, epinephrine and dopamine (inset shows typical recorder tracing); the catecholamines were eluted in that order by 0.1 M $NH_4H_2PO_4$ (adjusted to pH 6.5 with 5 N NaOH) at a flow rate of 0.4 ml/min in a total time of 8 min. The pumping system consisted of a high-pressure mini Pump from Laboratory Data Control, stainless steel tubing and a 1000 psi pressure gauge. The electrochemical detector used a carbon paste electrode and was purchased from Bioanalytical Systems. Peak heights compared to internal catecholamine standards included in each experiment were used to calculate catecholamine content.

DCMB inhibited norepinephrine N-methyltransferase from rat brain in vitro by 17, 32, 52 and 64 per cent when added at 0.3, 1, 3 and 10 µM concentrations respectively. This degree of inhibition by DCMB is less than we had reported earlier for the adrenal enzyme [3] but is similar to that observed by Lew et al. [7], who used (±)-phenylethanolamine as substrate for the rat brain enzyme.

Table 1 shows evidence that DCMB inhibited epinephrine synthesis in vivo in rat brain. The concentration of epinephrine was decreased at 2, 4 and 6 hr after drug administration, the maximum decrease being 44 percent from the zero time value at 4 hr. In contrast, neither norepinephrine nor dopamine concentration was significantly influenced. The rate of decline in epinephrine concentration suggests that epinephrine in brain turns over relatively rapidly at a rate comparable to the turnover of dopamine and norepinephrine. In a separate experiment, we have found significant lowering

of brain epinephrine after a 12.5 mg/kg i.p. dose of DCMB but not after a 6.25 mg/kg i.p. dose. Previously we had presented evidence that DCMB was effective in inhibiting epinephrine synthesis in rat adrenal glands [4].

*Table 1. Catecholamine concentration in rat hypothalamus after treatment with DCMB (25 mg/kg, i.p.)**

Hr after injection	Brain catecholamines, pmoles/g		
	Epinephrine	Norepinephrine	Dopamine
0	137 ± 11	7686 ± 450	712 ± 33
2	104 ± 5 (P<.05)	7284 ± 340	915 ± 105
4	77 ± 5 (P<.001)	7544 ± 485	810 ± 98
6	87 ± 5 (P<.005)	7077 ± 521	784 ± 65

*Mean values ± standard errors for five rats per group are shown. Statistical comparisons to the zero time value were made by the Student's *t*-test. Significance of the difference from the zero time group is shown for each value for which P was less than .05.

To our knowledge, these data represent the first published data showing a selective decrease in brain epinephrine concentration after the administration of an inhibitor of norepinephrine N-methyltransferase. Data on epinephrine concentration are crucial to the interpretation of any pharmacologic effects produced by norepinephrine N-methyltransferase inhibitors, since one must be sure that the inhibitor actually accomplishes *in vivo* the purpose for which it was given. For example, we have found that 5,6-dichloro-2-aminotetralin, another inhibitor *in vitro* of norepinephrine N-methyltransferase [8], apparently does not act simply as an inhibitor of this enzyme *in vivo*. This compound inhibited the rat brain enzyme *in vitro* by 27, 35 and 61 per cent at 1, 3 and 10 μM concentrations, respectively, i.e. it was only slightly less potent than DCMB. However, it did not decrease brain epinephrine concentration when injected i.p. at 25 mg/kg (as the maleate salt) into rats. 5,6-Dichloro-2-aminotetralin actually increased epinephrine, norepinephrine and dopamine concentration slightly in brain (perhaps a result of monoamine oxidase inhibition).

With reversible inhibitors, measurement of norepinephrine N-methyltransferase activity in tissue homogenates after drug injection amounts to a bioassay of drug concentration in the homogenate and may not reflect

accurately the inhibition of epinephrine synthesis in vivo. Any pharmacologic effects attributed to norepinephrine N-methyltransferase inhibition in vivo should parallel the duration and dose-dependence of epinephrine lowering.

Currently we are studying in more detail the effects of DCMB and other norepinephrine N-methyltransferase inhibitors to establish their properties as inhibitors of epinephrine synthesis in brain. These inhibitors should be useful to determine the rate of turnover of brain epinephrine as a function of physiologic and pharmacologic variables. The inhibitors--if they are specific--may also represent an excellent means of probing the physiologic functions of epinephrine-containing neurons in brain.

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