Pharmacology Biochemistry & Behavior, Vol. 11, pp. 563–566. Printed in the U.S.A.

Properties of Norepinephrine N-Methyltransferase from Pigeon Brain

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Received 23 July 1979

FULLER, R. W., AND S. K. HEMRICK. *Properties of norepinephrine N-methyltransferase from pigeon brain.* PHARMAC. BIOCHEM. BEHAV. 11(5) 563–566, 1979.—Epinephrine concentration in pigeon hypothalamus was higher on an absolute basis and as a percentage of total catecholamine concentration than in other regions of pigeon brain or in rat hypothalamus. Norepinephrine N-methyltransferase (NMT; the epinephrine-forming enzyme) from pigeon brain had Km values for S-adenosyl-L-methionine and L-norepinephrine of 24 ± 1 and 101 ± 4 μM, respectively. The enzyme was inhibited by excess L-norepinephrine and by several arylalkylamines that earlier had been identified as NMT inhibitors with the enzyme from mammalian brain and adrenal medulla. These results indicate that pigeon brain NMT is similar to that in mammalian brain and adrenal glands and can be inhibited by agents previously used to deplete brain epinephrine selectively in rats. The use of pigeons as experimental subjects in studies on the possible role of epinephrine-forming neurons in behavior is suggested.

Norepinephrine N-methyltransferase Epinephrine Pigeon Brain

NOREPINEPHRINE N-methyltransferase (NMT) (EC 2.1.1.28), the last enzyme involved in the biosynthesis of epinephrine, is thought to be present only in epinephrine-forming neurons or adrenomedullary cells. A few years ago Hokfelt *et al.* [7,8] used antibodies to highly purified bovine adrenal NMT to map by immunohistofluorescence techniques neuronal tracts in rat brain that contain NMT and that presumably make and use epinephrine as their neurotransmitter. Although dopamine and norepinephrine neurons in brain have been studied extensively and numerous physiological functions have been elucidated for them, there has been little study of epinephrine-forming neurons in brain.

The ratio of epinephrine to other catecholamines (norepinephrine and dopamine) is somewhat higher in avian brain than in commonly studied mammalian species [5,9]. The possibility that epinephrine plays a more prominent role in brain function of species like chickens and pigeons compared to species like rats, mice and guinea pigs may be considered. Pigeons have been used extensively in operant behavior studies and might be useful subjects for evaluating behavioral consequences of pharmacologic manipulation of epinephrine-forming neurons. NMT inhibitors, for example, should selectively deplete the brain of epinephrine without directly altering dopamine and norepinephrine neurons. We were therefore interested in determining some of the properties of pigeon brain NMT to see if the enzyme was similar to NMT previously studied in rats and some other species. In this paper we describe some of the kinetic characteristics of pigeon brain NMT with respect to its action on substrates and its susceptibility to inhibition.

METHOD

For the preparation of NMT to be used in *in vitro* studies, pigeons of mixed breeds and sex weighing 450–550 g were obtained from the Wilson Small Animal Farm, Vincennes, Indiana. Brains were removed and homogenized in 10 volumes of cold isotonic KCl containing 5 × 10M dithiothreitol. All subsequent operations were done at 4°C. Homogenates were centrifuged at 40,000 × g for 30 min. Ammonium sulfate (199 mg/ml) was dissolved in the supernatant fraction by stirring for 30 min. The precipitated protein was separated by centrifugation at 20,000 × g for 20 min. Additional ammonium sulfate (122 mg/ml) was dissolved in the supernatant fraction by stirring for 30 min. The precipitated proteins were separated by centrifugation at 20,000 × g for 20 min and then dissolved in a small volume of 0.001 M sodium phosphate buffer pH 7.0. This preparation was dialyzed for 24 hours against several changes of 3 liter volumes of the same buffer and was used as the enzyme for NMT assays.

Enzyme activity was measured radiometrically with ^{14}C-S-adenosyl-L-methionine (New England Nuclear) as the methyl-donating substrate and L-norepinephrine bitartrate (Winthrop) as the methyl-accepting substrate, using the methodology of Henry *et al.* [6] in the same way as the enzyme from the brains of rats and other species has been measured previously [2–4]. The concentration of ^{14}C-S-adenosyl-L-methionine was 50 μM, and the concentration of L-norepinephrine was 100 μM, in all experiments except when the concentration of one substrate was varied as described in the Results section.

Catecholamine concentrations in brain regions were de-

TABLE 1

CATECHOLAMINE CONCENTRATION IN PIGEON BRAIN REGIONS AND IN RAT HYPOTHALAMUS DETERMINED BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION

Brain region	Catecholamine concentration, μg		
	Epinephrine	Norepinephrine	Dopamine
Pigeon hypothalamus	0.185 ± 0.021	2.69 ± 0.16	0.50 ± 0.02
Pigeon brain stem	0.020 ± 0.002	0.56 ± 0.02	0.09 ± 0.004
Pigeon midbrain	0.027 ± 0.006	0.79 ± 0.10	0.18 ± 0.02
Rat hypothalamus	0.025 ± 0.002	1.25 ± 0.06	0.18 ± 0.01

All values shown are mean ± standard errors for 5 animals.

termined by high performance liquid chromatography with electrochemical detection [3]. In this experiment White Carneaux pigeons weighing 550–650 g obtained from the Palmetto Pigeon Plant, Sumter, SC or male Wistar rats weighing approximately 50 g obtained from Harlan Industries, Cumberland, Indiana were used.

RESULTS

Table 1 shows the concentration of catecholamines in pigeon brain as measured by high performance liquid chromatography with electrochemical detection. As with rats [10], the highest concentration of epinephrine was found in hypothalamus. There the concentration of epinephrine was about one-fifteenth that of norepinephrine and one-third that of dopamine. In contrast, epinephrine concentration in rat hypothalamus is typically found to be about one-fiftieth or less the concentration of norepinephrine; some representative data are included in Table 1 for comparison. The absolute concentration of epinephrine in pigeon hypothalamus was also several-fold higher than that in rat hypothalamus.

Figures 1 and 2 show Lineweaver-Burk plots for pigeon brain NMT with L-norepinephrine and S-adenosyl-L-methionine, respectively, as the variable substrates. From these data, apparent Km values were calculated according to the statistical method of Wilkinson [11] to be 101 ± 4 μM for L-norepinephrine and 24 ± 1 μM for S-adenosyl-L-methionine. Excess concentrations of L-norepinephrine were inhibitory to substrate activity, and the constant (Km') associated with the inhibition by excess L-norepinephrine was determined graphically to be 1450 μm (Fig. 3).

Table 2 shows IC_{50} concentrations for six inhibitors of NMT previously shown to inhibit the enzyme from rat, chicken and cat brain (2; R. W. Fuller and S. K. Hemrick, unpublished data). All of these compounds were potent inhibitors of pigeon brain NMT. The (+) stereoisomer of 2,3-dichloro-α-methylbenzylamine was approximately 10 times more potent than the (−) stereoisomer as had been found with brain NMT from other species [2].

DISCUSSION

Our data on epinephrine concentration in pigeon brain as determined by high performance liquid chromatography with electrochemical detection agree reasonably well with data previously obtained by others using fluorometric or bioassay techniques [9]. Epinephrine concentration is higher in pigeon

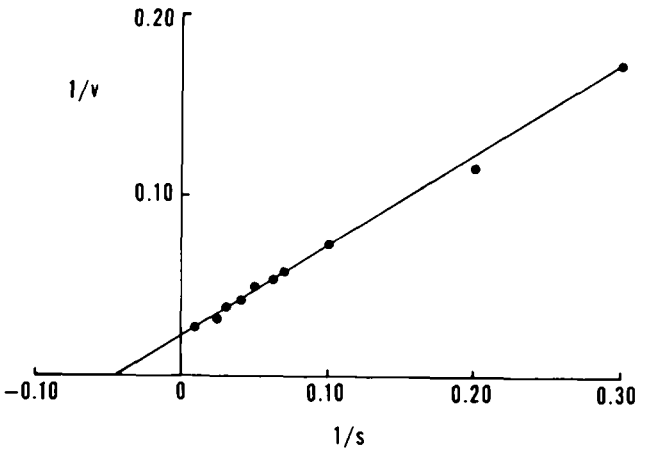


FIG. 1. Lineweaver-Burk plot with ^{14}C-S-adenosyl-L-methionine concentrations (s) varied over a 3.3–100 μM range and L-norepinephrine concentration fixed at 100 μM. Units of velocity (v) were nanomoles product formed per hour.

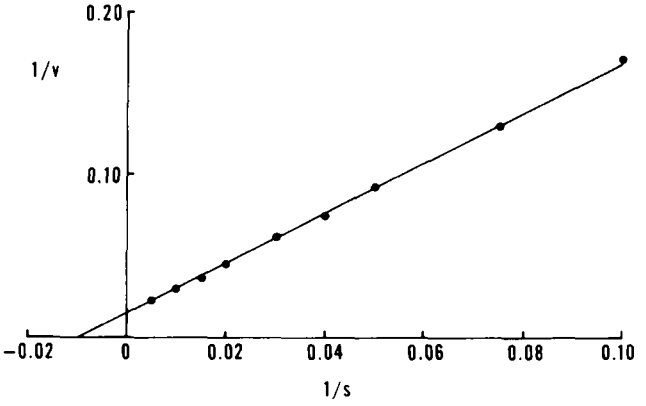
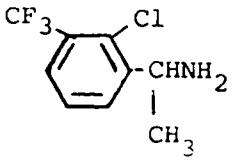
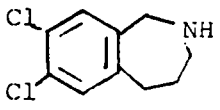
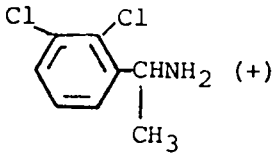
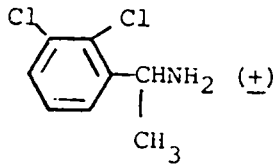
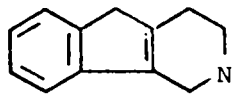
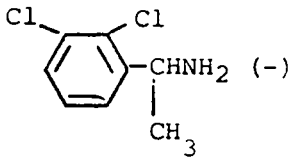


FIG. 2. Lineweaver-Burk plot with L-norepinephrine concentration (s) varied over a 10–200 μM range and ^{14}C-S-adenosyl-L-methionine concentration fixed at 50 μM. Units of velocity (v) were nanomoles product formed per hour.

TABLE 2
INHIBITION OF PIGEON BRAIN NMT

Inhibitor	IC_{50}
	2.8×10^{-7} M
	1.4×10^{-6} M
	3.7×10^{-6} M
	4.7×10^{-6} M
	6.9×10^{-6} M
	4.2×10^{-5} M

Each inhibitor was studied at 7–9 concentrations from which the IC_{50} (molar concentration producing 50% inhibition of enzyme activity) was determined by interpolation on a graph of percent inhibition versus inhibitor concentration.

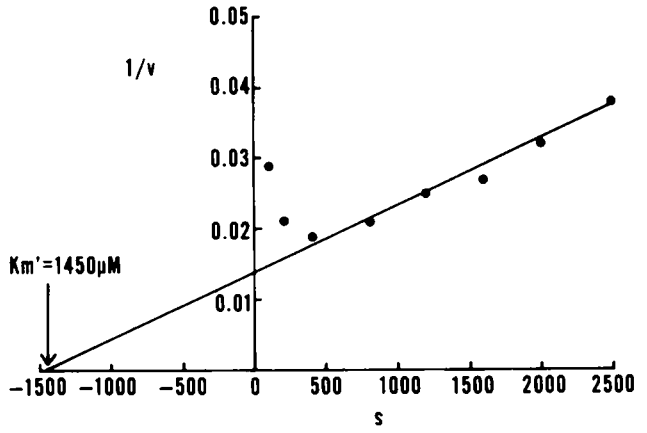


FIG. 3. Dixon plot of inhibition by excess L-norepinephrine. The concentration of L-norepinephrine (s) is shown in micromolar units. The concentration of ^{14}C-S-adenosyl-L-methionine was fixed at 50 μM. Units of velocity (v) were nanomoles product formed per hour. The lower two points (100 and 200 μM concentrations) were below the region of inhibition by excess substrate and were not considered in drawing the line through the remaining data points.

brain both absolutely and relative to the concentration of other catecholamines than in rat brain.

The properties of the epinephrine-forming enzyme, NMT, in pigeon brain are similar at least in some respects to those of brain NMT from other species that have been studied. Apparent Km values for L-norepinephrine and S-adenosyl-L-methionine were in the same concentration range as found earlier for other species [2]. Pigeon brain NMT was inhibited by excess L-norepinephrine as has been found with other species [2]. Several compounds previously shown to inhibit NMT from the brain of rats, cats and chickens [1,2] also inhibited pigeon brain NMT. This finding suggests that epinephrine synthesis in pigeon brain can be selectively inhibited as demonstrated earlier with rat brain [1, 3, 4]. Neither NMT nor epinephrine has been proven to be localized in neurons in pigeon brain, but a reasonable hypothesis would be that some neurons do use epinephrine instead of norepinephrine or dopamine as their neurotransmitter. If so,inhibition of epinephrine synthesis and other means of pharmacologic modification of epinephrine neuron function should be useful in evaluating physiological roles of brain epinephrine neurons in pigeons.

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