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Demonstration and Distribution of Phenylethanolamine in Brain and Other Tissues

(enzyme assay/intraneural synthesis and storage/phenylethylamine/phenylalanine/rat)

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ABSTRACT **A specific and sensitive assay for phenylethanolamine in tissues is described. By this assay, phenylethanolamine was detected in many peripheral tissues and brains of rats. It is unequally distributed in rat brain, with the highest concentration present in hypothalamus and midbrain. Concentrations of brain phenylethanolamine were elevated after administration of phenylethylamine, phenylalanine, *p*-chlorophenylalanine, and monoamine oxidase inhibitors and decreased after administration of dopamine-β-hydroxylase inhibitors. Denervation of sympathetic nerves caused a moderate fall in phenylethanolamine concentrations. These results indicate that phenylethanolamine is synthesized intraneuronally from phenylalanine and phenylethylamine, but that only part of the phenylethanolamine is stored in sympathetic nerves.**

Several biogenic amines occur in mammalian brain and nerves. These include catecholamines: noradrenaline and adrenaline (1, 2), dopamine (3); phenylethylamines: normetanephrine (4), octopamine (5), tyramine and phenylethylamine (6, 7); indoleamines: serotonin (8, 9) and tryptamine (10); and imidazoles: histamine (11). Many of these amines, particularly the catecholamines and indoleamines, have been shown to have important roles in brain function. We describe the occurrence and distribution of another biogenicamine phenylethanolamine in brain and other tissues and a sensitive and specific assay for its measurement in biological material.

MATERIALS AND METHODS

Enzymatic Assay for Phenylethanolamine. The assay depends on the transfer of the [^{3}H]methyl group of [^{3}H]methyl-*S*-adenosylmethionine by phenylethanolamine *N*-methyl-transferase (EC 2.1.1.X) to the amino group of phenylethanolamine. The enzymatically formed [^{3}H]methyl-phenylethanolamine is extracted into heptane containing 5% isoamyl alcohol at pH 10, and the radioactivity is determined. This assay can measure as little as 100 pg of phenylethanolamine in tissues (Fig. 1).

Procedure. Male Sprague–Dawley rats weighing 150–200 g were killed by decapitation. Their organs were rapidly removed, frozen on dry ice, weighed, and homogenized in 5–10 volumes of ice-cold 20 mM Tris·HCl buffer (pH 8.6) containing the monoamine oxidase inhibitor, iproniazid (50 μg/ml). The homogenates were heated at 90° for 3 min, and the proteins were removed by centrifugation at 12,000 rpm in a Sorvall refrigerated centrifuge. A 200-μl aliquot of the supernatant fluid was transferred to a 15-ml glass-stoppered tube and incubated for 20 min at 37° after addition of a mixture containing 10 μl of partially purified phenylethanolamine *N*-methyltransferase, 5 μl (0.54 nmol) of [^{3}H]methyl-*S*-adenosyl-1-methionine (specific activity, 4.54 Ci/mmol) and 35 μl of 20 mM Tris·HCl buffer (pH 8.6). 1 ng of phenylethanolamine was added to another aliquot as an internal standard. The incubation was stopped by addition of 0.5 ml of 0.5 M borate buffer (pH 10) and the radioactive product was extracted with 6 ml of a mixture containing 95% heptane and 5% isoamyl alcohol (v/v), by shaking for 15 sec. After the phases were separated by centrifugation, a 5-ml aliquot of the organic phase was transferred to counting vials and evaporated to dryness under reduced pressure at 40°. 1 ml of ethanol and 10 ml of phosphor containing 40 ml of Liquifluor (New England Nuclear Corp., Boston, Mass.) per liter of toluene were added to the residue, and radioactivity was measured.

Recoveries. There is proportionality between the amount of phenylethanolamine added to the reaction mixture and the amount of *N*-[^{3}H]methylated phenylethanolamine formed. This proportionality was demonstrable when the reaction was performed from an aqueous medium containing different amounts of phenylethanolamine, and also when different amounts of phenylethanolamine were added as internal standards to all the tissues examined (Fig. 2). The recoveries of phenylethanolamine added to tissues ranged from 50–90% when compared to phenylethanolamine added to aqueous medium and carried through the whole procedure. Addition of

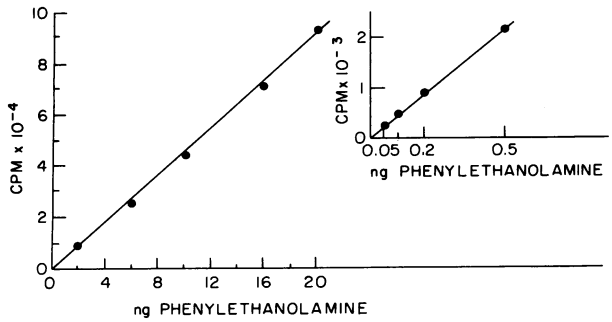

FIG. 1. Sensitivity of the phenylethanolamine assay. Authentic phenylethanolamine was incubated in 20 mM Tris·HCl buffer (pH 8.6), together with [^{3}H]methyl-*S*-adenosylmethionine and phenylethanolamine *N*-methyltransferase as described in text. Results are expressed in cpm after the blank is subtracted (110 cpm).

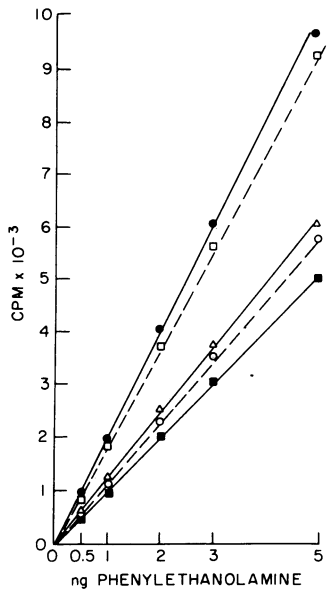

FIG. 2. Recoveries of phenylethanolamine added to rat tissues. Phenylethanolamine was added to rat tissue supernatant fractions and carried through the entire procedure as described in *text*. ●, Buffer; □, vas deferens; △, heart; ○, brain; ■, salivary glands.

an internal standard of phenylethanolamine served to correct for the differences in recoveries amongst various tissues.

Specificity. Normally occurring β-hydroxylated compounds such as metanephrine, octopamine, catecholamines, and other phenylethylamines (dopamine, phenylethylamine, and tryamine), present at 100-times the concentration of phenylethanolamine, gave negligible interference (less than 3%).

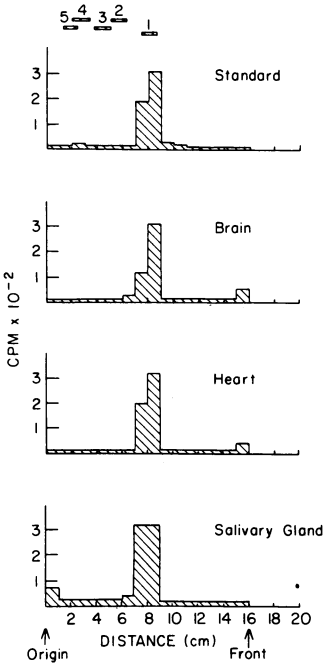

FIG. 3. Specificity of the phenylethanolamine assay. Rat tissues were assayed for phenylethanolamine as described in *Methods*. The solvent system used was *n*-butanol saturated with 1 N HCl. The R_Fs of *N*-methylphenylethanolamine (*1*), synephrine (*2*), metanephrine (*3*), *N*-methylsynephrine (*4*), and *N*-methylmetanephrine (*5*) are also shown.

Thin-Layer Chromatography. Identification of *N*-[^{3}H]-methylated phenylethanolamine in tissues was made by means of thin-layer chromatography on precoated Eastman chromagram sheets. [^{3}H]Methyl-phenylethanolamine was prepared from endogenous phenylethanolamine in tissues as described above. After extraction, the organic solvent was dried under reduced pressure, the contents were redissolved with 50 μl of ethanol, containing authentic *N*-methyl-phenylethanolamine (2 μg) as a carrier, and applied to chromatography sheets. Radioactive standards were prepared with 10 ng of authentic phenylethanolamine with phenylethanolamine *N*-methyltransferase. The solvents used were (*a*) acetone–ammonium hydroxide (99:1); (*b*) *n*-butanol–acetic acid–water (12:3:5); (*c*) tertiary amyl alcohol–methylamine–water (80:10:10); (*d*) isopropanol–10% ammonium hydroxide–water (200:10:20); (*e*) *n*-butanol saturated with 1 N HCl; and (*f*) toluene–acetic acid–ethyl acetate–water (80:40:20:5). After developing, the sheets were cut into 1-cm sections and transferred to vials containing 1 ml of ethanol. 10 ml of toluene phosphor were added to each vial, and radioactivity was counted. Fig. 3 shows a typical chromatography of *N*-[^{3}H]methyl phenylethanolamine isolated from various tissues.

Purification of Phenylethanolamine N-Methyltransferase. Phenylethanolamine *N*-methyltransferase was purified as described (12), including the ammonium sulfate and acid precipitation, and dialysis. The enzyme preparation had a protein concentration of 10–20 mg/ml and about 500–600 units/ml with phenylethanolamine as a substrate. One unit of enzymatic activity is defined as the amount of enzyme that forms 1 nmol of product per mg of protein per hr.

Drugs. [^{3}H]Methyl-*S*-adenosylmethionine (4.54 Ci/mmol) was purchased from New England Nuclear Corp. Other chemicals were obtained from commercial sources.

RESULTS

Distribution of phenylethanolamine in tissues

Phenylethanolamine was present in brain and other tissues of rats. The highest concentration was found in the pineal gland, and the lowest in the brain (Table 1). The distribution

TABLE 1. *Endogenous phenylethanolamine concentration in rat tissues*

Tissue	Phenylethanolamine concentration (ng/g)
Brain	7 ± 1
Vas deferens	51 ± 8
Spleen	49 ± 5
Heart	45 ± 2
Submaxillary gland	47 ± 10
Lung	87 ± 7
Pineal gland	480 ± 217

Groups of 10 adult Sprague-Dawley rats were used. Each determination was made in duplicate samples from individual tissues. Tissues were assayed as described in *Methods*. Results are presented as means ±SEM.

of phenylethanolamine was also examined in several regions of rat brain. Highest concentrations of the amine were present in the hypothalamus and midbrain, and lowest in cerebellum and cerebral cortex (Table 2).

Effect of precursors and drugs on phenylethanolamine concentrations in brain

Administration of a monoamine oxidase inhibitor resulted in an eightfold rise in phenylethanolamine concentrations in brain (Fig. 4). These results indicate that monoamine oxidase is a major enzyme in the metabolism of this amine. Phenylethylamine also increases brain phenylethanolamine (Fig. 4). In a previous study, formation of phenylethanolamine was also demonstrated in several tissues after administration of phenylethylamine together with a monoamine oxidase inhibitor (14). The most likely enzyme to catalyze the formation of phenylethanolamine from phenylethylamine is dopamine-β-hydroxylase. To examine this possibility, rats were pretreated with a dopamine-β-hydroxylase inhibitor, sodium diethyldithiocarbamate, (15) together with phenylethylamine and iproniazid, a mono amine oxidase inhibitor. In the absence of dopamine-β-hydroxylase inhibition there was a 15-fold increase in brain phenylethanolamine, but there was a marked reduction in the formation of this amine when the animals were pretreated with the dopamine-β-hydroxylase inhibitor (Fig. 4).

Dopamine-β-hydroxylase is present mainly in sympathetic nerve vesicles (16, 17). To examine whether phenylethanolamine, which is presumably made in sympathetic nerves, is stored there, the sympathetic nerves in rat salivary glands were destroyed by removal of the superior cervical ganglion. Unilateral denervation of the sympathetic nerves of the salivary gland caused a variable fall in the endogenous phenylethanolamine concentrations on the denervated side (Table 3). Administration of exogenous phenylethanolamine together with iproniazid resulted in a 5-fold greater uptake of the amine in the innervated side of the salivary gland than in the denervated side (Table 3). These observations indicate that phenylethanolamine synthesized in the sympathetic nerve is only partially stored there and that circulating phenylethanolamine is selectively taken up by sympathetic nerves.

TABLE 2. *Regional distribution of phenylethanolamine in rat brain*

Region	Phenylethanolamine content (ng/g)
Whole brain	6.2 ± 0.2
Hypothalamus	25 ± 2.2
Midbrain	22 ± 1.5
Cervical spinal cord	9 ± 0.8
Hippocampus	7.7 ± 0.3
Brainstem	6.7 ± 1.0
Striatum	6.6 ± 0.4
Cerebral cortex	2.7 ± 0.1
Cerebellum	2.6 ± 0.1

Rat brains were dissected according to the method of Glowinski and Iversen (13) and assayed as described in text. Each determination was made in duplicate samples from individual brain regions. Results are expressed as means ±SEM for groups of 10 rats.

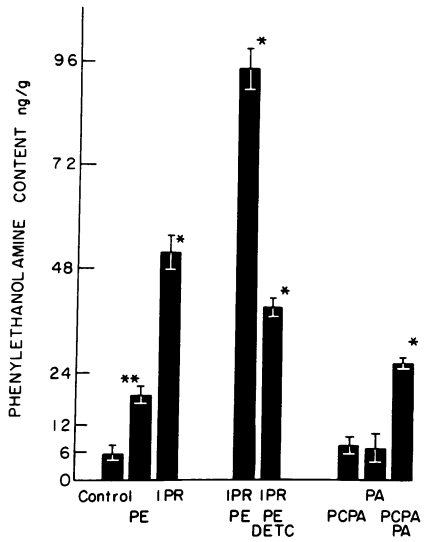

FIG. 4. Effect of enzyme inhibitors and precursors on rat-brain phenylethanolamine. Compounds were dissolved in water and administered intraperitoneally as follows: β-phenylethylamine (*PE*): 50 mg/kg, 90 min; iproniazid (*IPR*) 150 mg/kg, 18 hr; Na diethyldithiocarbamate (*DETC*), 500 mg/kg, 30 min; *p*-chlorophenylalanine methyl ester (*PCPA*), 375 mg/kg, 48 and 24 hr; 1-phenylalanine methyl ester·HCl (*PA*), 800 mg/kg, 90 min, before the rats were killed. * Statistically significant, $P < 0.01$. ** Statistically significant, $P < 0.05$.

The most likely amino-acid precursor for phenylethanolamine is phenylalanine. However, administration of phenylalanine resulted in little change in brain phenylethanolamine.

TABLE 3. *Effects of ganglionectomy on phenylethanolamine content and uptake in the rat submaxillary gland*

Rat no.	Innervated gland	Denervated gland
	No treatment	
1	60	56
2	84	13
3	35	16
4	18	18
5	23	12
6	36	21
Average ±SEM.	42.7 ± 10.2	22.7 ± 6.8
	Phenylethanolamine and iproniazid administered	
7	806	205
8	1352	319
9	1053	262
10	1080	293
11	915	205
12	1197	310
Average ±SEM.	1067 ± 80	265 ± 21

Rats were unilaterally ganglionectomized (right superior cervical ganglia) under ether anesthesia. 1 Week later, one group of rats was injected with iproniazid, 150 mg/kg, 18 hr, followed by phenylethanolamine, 5 mg/kg, 30 min before they were killed. Phenylethanolamine was assayed in the denervated right salivary gland, and in the normal left gland.

When phenylalanine hydroxylase was inhibited by prior treatment with *p*-chlorophenylalanine (18, 19), there was more than a 2-fold increase in brain phenylethanolamine (Fig. 4).

DISCUSSION

The development of a specific and sensitive assay for phenylethanolamine in tissues enabled us to demonstrate and quantitate its distribution in many tissues. Phenylethanolamine was present in all tissues examined, the highest concentration being found in the pineal gland and lowest in the brain. By means of gas-chromatography and mass-spectrometry procedures, phenylethanolamine has been detected in the heart (20) and brain (LeFevre, Wilner, and Costa, unpublished observation). In the brain there is an unequal distribution of phenylethanolamine. Amounts of this amine are about 4 times greater in the hypothalamus and midbrain than in any other brain area. These regions have a high density of noradrenergic terminals and high dopamine-β-hydroxylase activity (21). Thus, it is likely that phenylethanolamine is made in the noradrenergic nerves, since the enzyme dopamine-β-hydroxylase, which synthesizes this amine, is highly localized in these nerves. However, unlike its *p*-hydroxylated congener, octopamine (5), phenylethanolamine is stored in sympathetic nerve terminals only to a limited extent. Though sympathetic nerve terminals apparently do not have a great capacity to store phenylethanolamine, they can selectively take up this amine.

Studies with enzyme inhibitors and precursors suggest that phenylethanolamine is formed as follows:

phenylalanine→phenylethylamine→phenylethanolamine.

The first step is catalyzed presumably by aromatic acid decarboxylase and the second by dopamine-β-hydroxylase. It appears that decarboxylase is a very minor pathway for phenylalanine metabolism, since administration of large amounts of this amino acid results in a negligible elevation of phenylethanolamine. When phenylalanine hydroxylase is inhibited, metabolism of the phenylalanine is diverted to the decarboxylation and β-hydroxylation pathway. In diseases such as phenylketonuria, where there is a deficiency of phenylalanine hydroxylase, an elevated amount of phenylethanolamine would be expected. The very large elevation of phenylethanolamine in brain after monoamine oxidase inhibition suggests that the major route of metabolism of phenylethanolamine is by deamination by monoamine oxidase.

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