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METABOLISM OF TYRAMINE-^{3}H AND OCTOPAMINE-^{3}H BY RAT BRAIN

GEORGE R. BREESE, THOMAS N. CHASE and IRWIN J. KOPIN

Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Md. 20014, U.S.A.

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Abstract—Octopamine-^{3}H or tyramine-^{3}H injected into the cisterna magna of rats is rapidly metabolized and quickly disappears from brain. The disappearance of tyramine-^{3}H is more rapid than octopamine-^{3}H. Monoamine oxidase inhibition diminishes the disappearance of these amines from brain, suggesting that the unaltered amines do not pass freely from brain to blood. The major metabolite formed after intracisternal injection of octopamine-^{3}H is the neutral metabolite, 4-hydroxyphenylglycol, or its conjugate. The principle metabolite formed from tyramine-^{3}H is 4-hydroxyphenylacetic acid. These findings support the contention that β-hydroxylated aldehydes formed by deamination of phenylethanolamines in cerebral tissue are reduced to glycol derivatives, while non-β-hydroxylated intermediates formed by deamination of phenylethylamines are oxidized mainly to acids.

RECENT studies have shown that β-hydroxylated phenylethylamines are deaminated by brain slices to form glycols while phenylethylamines lacking a β-hydroxyl group are deaminated and converted mainly to corresponding acids.[1, 2] These observations indicate that an enzyme for reduction of phenylacetaldehydes is present in cerebral tissue and that the β-hydroxylated derivatives are better substrates for this enzyme than their parent phenylacetaldehydes.

Although tyramine[3] and octopamine[4] have been identified as normal constituents of mammalian tissues, little is known of their disposition and metabolism within the central nervous system. Tyramine has been shown to be converted to octopamine in brain[5, 6] as well as in peripheral sympathetic neurons.[7, 8] However, the fate of octopamine in brain of the living animal has not been studied. The purpose of this investigation was to study the metabolism of these compounds by rat brain *in vivo* after intracisternal injection and to determine whether, as described *in vitro*,[1] tyramine is deaminated and converted mainly to an acid while octopamine is converted primarily to the corresponding glycol.

MATERIALS AND METHODS

Male Sprague–Dawley rats weighing 180–200 g were lightly anesthetized with ether, and 25 μl of Elliott's "B" irrigating solution (Baxter Laboratories) containing either octopamine-2-H^3 (8·3 μc, 15·4 mc/mg) or tyramine-H^3 (8·3 μc, 15·4 mc/mg) was injected into the cisterna magna.[9] Radioactive compounds were purchased from the New England Nuclear Corp. and were checked for purity by thin-layer chromatography (TLC) (butanol:acetic acid:water, 4:1:1, and butanol:isopropyl alcohol:

ammonia, 45:35:20). At various intervals after injection, the animals were killed by decapitation. Their brains were quickly removed, blotted and homogenized in 10 ml of ice-cold 0·4 N perchloric acid. Following centrifugation, a portion of the supernatant was adjusted to pH 5; the amines were adsorbed on to a 20 × 8 mm Dowex-50 (NH_4^+) column and eluted with 6 ml of 3 N ammonium hydroxide. Tyramine-^{3}H and octopamine-^{3}H were assayed as previously described.[7] Recoveries averaged about 90 per cent. After adjustment of an aliquot of the effluent from Dowex-50 columns to pH 7, the neutral metabolites were extracted into ethyl acetate and the tritium in the organic phase was assayed by liquid scintillation spectrometry. The aqueous residue was then brought to pH 1, the acid metabolites were extracted into ethyl acetate and a portion of the ethyl acetate was assayed for tritium. In some experiments, aliquots of the effluent from the Dowex columns and of the ethyl acetate extract were applied to a TLC plate (Silica gel G: Analtech Laboratories) which was developed in butanol: isopropyl alcohol:ammonia (45:35:20). This permitted determination of the partition of metabolites in ethyl acetate at pH 7 and pH 1 and provided an estimate of the percentage of each metabolite in the total radioactivity of the effluent collected from the Dowex-50 column.

Conjugated metabolites in the effluent solutions from the Dowex-50 columns were hydrolyzed by incubation with 0·05 vol. of "glusulase" (Endo Laboratories, Garden City, N. Y.) at pH 5·5 and 37° for 20 hr. Protein was precipitated with the addition of perchloric acid (60%) to a final concentration of 0·4 N. Hydrolyzed samples as well as unhydrolyzed samples, to which "glusulase" had been added at the same time as perchloric acid, were extracted with 6 vols. of ethyl acetate. Portions of the extracts were chromatographed and assayed for labeled metabolites as described above. In addition, an aliquot of the hydrolyzed and unhydrolyzed effluents were chromatographed directly and the radioactive peaks were determined.

RESULTS

Disappearance of DL-*octopamine-^{3}H from brain.* After intracisternal injection of octopamine-^{3}H, the cerebral content of labeled amine fell rapidly at first and then more slowly (Fig. 1). During the first hr, the half-life was approx. 24 min; between 2 and 6 hr, the half-life was 2·9 hr. Most of the radioactivity found in brain was not octopamine. One hr after the injection, octopamine-^{3}H accounted for only 17 per cent of the total recoverable radioactivity (Table 1).

Pretreatment with pargyline markedly diminished the rate of disappearance of radioactivity from brain. Following treatment with this monoamine oxidase inhibitor, nearly all of the tritium recovered was found to be octopamine-H^3 (Fig. 1).

Metabolites of octopamine-^{3}H. One hr after injection of octopamine-^{3}H, little radioactivity was recovered in the amine fraction (Table 1). Chromatography of the unhydrolyzed effluent from the Dowex-50 columns revealed three distinct peaks of radioactivity (Fig. 2). The peak closest to the origin corresponded to 4-hydroxymandelic acid (4-HMA); the peak nearest the solvent front corresponded to 4-hydroxyphenylglycol (4-HPG). After hydrolysis with "glusulase," however, only two peaks of radioactivity remained (Fig. 2). The radioactivity which was present in the middle peak was now found in the area corresponding to 4-HPG. This indicated that the second peak was the conjugate of 4-HPG, presumably the sulfate conjugate.[10]

The proportions of the three metabolic products of intracisternally injected octopamine-^{3}H varied with time. Six min after injection, nearly all the radioactivity could be accounted for as either octopamine or its unconjugated acidic and neutral metabolites (Table 1). The major deaminated metabolite was a neutral compound, presumably the glycol. One hr after injection, 46 per cent of the radioactivity occurred as

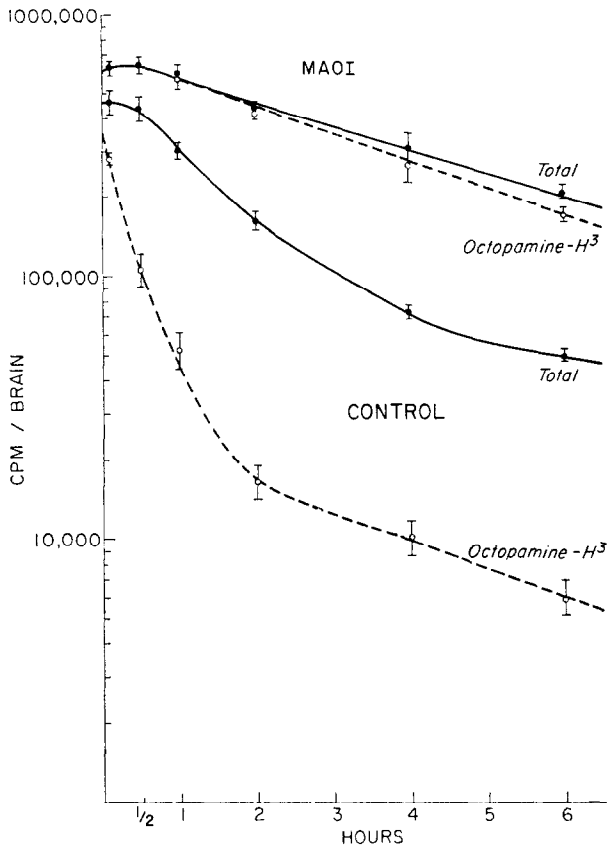

FIG. 1. Total radioactivity and octopamine-^{3}H were assayed at various times after intracisternal injection of octopamine-^{3}H. Pargyline (75 mg/kg, i.p.) or saline (control) was injected 30 min before the injection of labeled octopamine. Each point represents the mean ±S.E.M. of four to six determinations.

TABLE 1. OCTOPAMINE-^{3}H AND METABOLITES IN RAT BRAIN*

Time (min)	Total radioactivity (cpm/brain)	Per cent total radioactivity			
		Octopamine	Neutral	Acid	Conjugated neutral
6	460,750 ± 22,750	61 ± 3	31 ± 2	5 ± 1	2 ± 2
60	308,710 ± 20,540	17 ± 2	32 ± 2	6 ± 1	46 ± 1

* Octopamine-^{3}H was injected intracisternally; octopamine and metabolites were analyzed in brain 6 or 60 min after injection. Values are the mean ± S.E.M. of four determinations.

the conjugated neutral metabolite; almost 80 per cent of the total radioactivity in brain was either 4-HPG or its conjugate. At most times the conjugate of 4-HPG was found to be the major metabolite (Fig. 3). Thirty min after administration, the conjugate represented approx. 37 per cent of the total radioactivity and increased to 56 per cent at 6 hr.

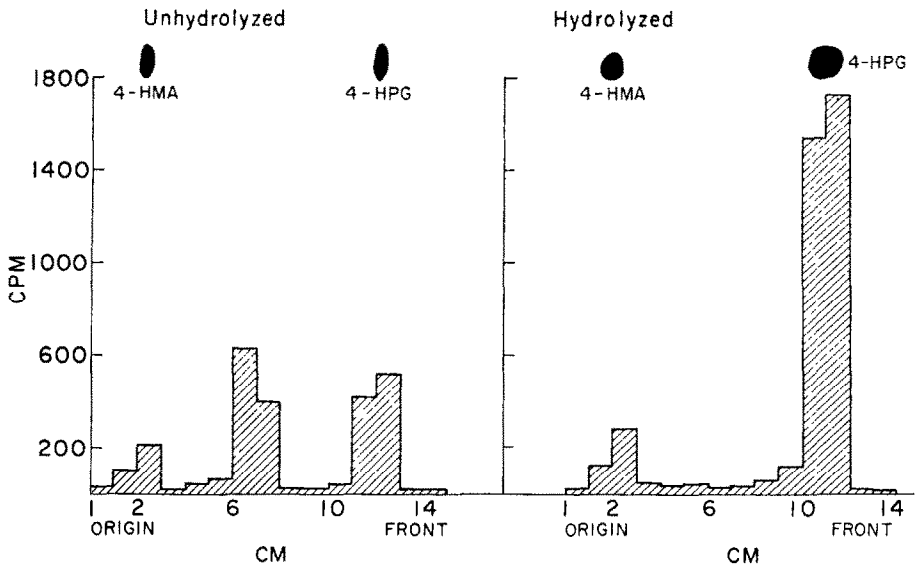

FIG. 2. Octopamine-^{3}H was injected intracisternally and the animals were killed 90 min later. The homogenate was passed through a Dowex-50 column to remove amines and the effluent was treated as described in Methods. Aliquots of both hydrolyzed and unhydrolyzed fractions were chromatographed and the radioactivity was determined. 4-HMA = 4-hydroxymandelic acid; 4-HPG = 4-hydroxyphenylglycol.

Effect of monoamine oxidase inhibitors on the disappearance of octopamine-^{3}H. As noted earlier, pargyline substantially diminished the disappearance of labeled octopamine from brain (Fig. 1). Pheniprazine also had an inhibitory effect but to a significantly lesser degree than pargyline (Table 2).

Disappearance of tyramine-^{3}H from brain. Tyramine-^{3}H disappears from brain more rapidly than octopamine-^{3}H (Fig. 4). One hr after injection of tyramine-^{3}H, amines accounted for only about 7 per cent of the total radioactivity while amines accounted for 17 per cent of the radioactivity present after the administration of octopamine-H^3 (Table 1). As with octopamine-H^3, pretreatment of the animals with pargyline or pheniprazine markedly diminished the rate of disappearance of radioactivity from brain after labeled tyramine. The effect of pargyline was greater than that of pheniprazine. Most of the radioactivity was found to be labeled amines (Fig. 4). A large portion of the tyramine appeared to have been β-hydroxylated since octopamine represented 58 per cent of the radioactive amines present in brain 1 hr after injection. After treatment with pargyline and 1 hr after administration, octopamine represented 72 per cent of the labeled amines. The major metabolite of tyramine exhibited the same chromatographic characteristics as authentic *p*-hydroxyphenylacetic acid (Table 3).

Although not found 6 min after injection, a neutral conjugated metabolite of tyramine-H^3 appeared later. Since octopamine-^{3}H was identified in brain extracts (Table 2), it is possible that most of the neutral metabolites were derived from this compound.

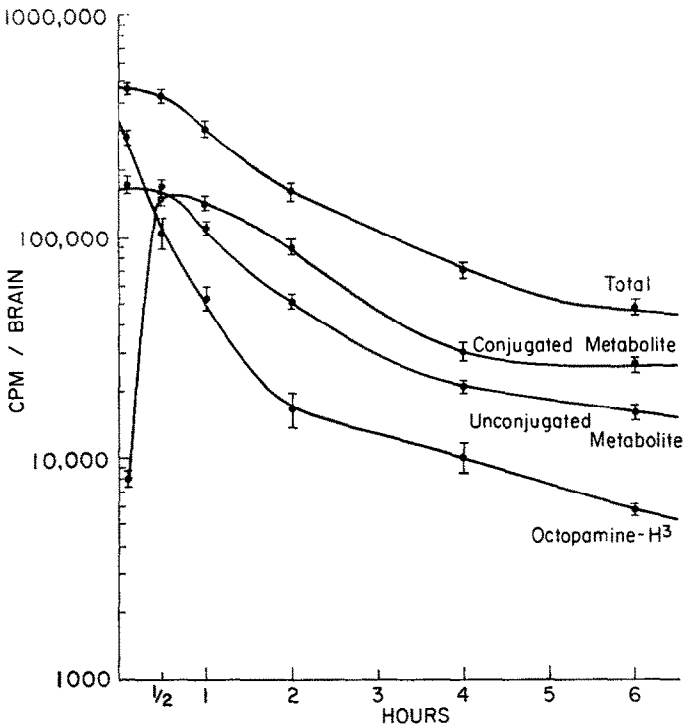

FIG. 3. Octopamine-^{3}H was injected intracisternally. Octopamine-^{3}H and metabolites in brain were analyzed at various times after injection. Each point represents the mean ±S.E.M. of four determinations.

TABLE 2. EFFECTS OF PARGYLINE AND PHENIPRAZINE ON THE DISAPPEARANCE OF OCTOPAMINE-^{3}H IN RAT BRAIN*

Treatment	Octopamine-^{3}H
Control	5700 ± 500
Pargyline	123,200 ± 7200
Pheniprazine	72,900 ± 7400†

* Pargyline (75 mg/kg, i.p.) and pheniprazine (15 mg/kg, i.p.) were injected 30 min prior to the intracisternal injection of octopamine-^{3}H. Animals were killed 4 hr after the administration of the labeled amine. Each value represents the mean cpm ± S.E.M. of five to seven determinations.

† $P < 0{\cdot}01$ when compared to pargyline-treated animals.

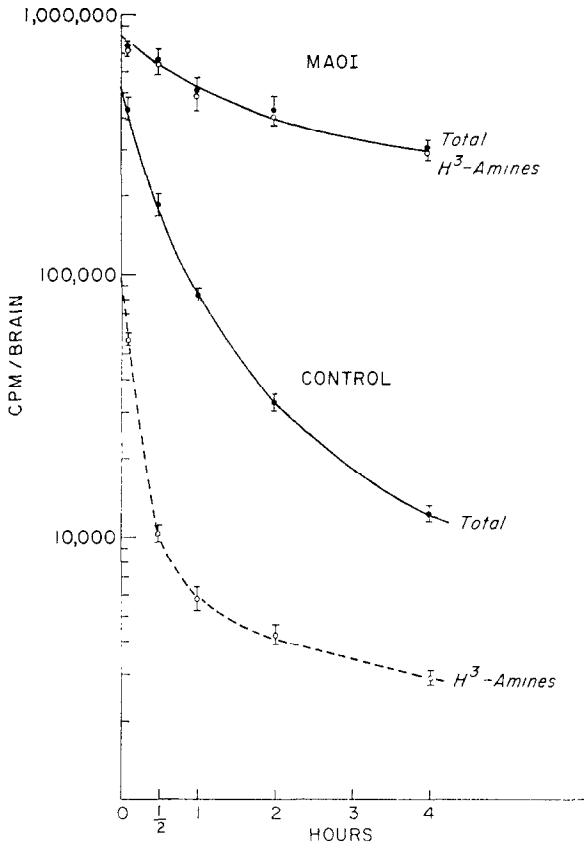

FIG. 4. Tyramine-^{3}H was injected intracisternally. Total tritiated amines and metabolites in brain were analyzed at various times after injection. Each point represents the mean ±S.E.M. of five determinations.

TABLE 3. TYRAMINE-^{3}H AND METABOLITES IN RAT BRAIN*

Time (min)	Total	Tyramine	Octopamine	Metabolites	
				Acid	Neutral
6	428,000 ± 22,300	46,500 ± 1000	10,000 ± 500	352,000 ± 27,000	
30	185,000 ± 14,000	6100 ± 300	4000 ± 250	148,000 ± 2400	6100 ± 370
60	83,000 ± 4000	2500 ± 120	3400 ± 200	52,100 ± 1200	5800 ± 160
120	32,500 ± 2600	1900 ± 290	2200 ± 180	16,600 ± 700	3300 ± 160
240	12,200 ± 300	1400 ± 290	1600 ± 200	1600 ± 50	1050 ± 40

* Tyramine-^{3}H was injected intracisternally. Values are the mean cpm/brain ± S.E.M. of four determinations.

DISCUSSION

Goldstein *et al.*[11] have reported the presence in brain of conjugates of radioactive phenolic alcohols after the systemic administration of Dopa-^{14}C. Intracisternally injected normetanephrine is converted largely to a neutral metabolite, 3-methoxy-4-hydroxyphenylglycol, which is conjugated with sulfate.[10] After injection of octopamine-^{3}H, 4-hydroxyphenylglycol-^{3}H was present in brain mainly as a conjugate. The nature of the conjugate was not conclusively established, but preliminary findings suggest that the conjugate of 4-HPG is also a sulfate.* In contrast, the major metabolite of tyramine was *p*-hydroxyphenylacetic acid. No conjugates of this compound were found. Similar results have been reported when these compounds were incubated with rat brain slices.[1] Although the amount of labeled amine administered in the present experiments is several-fold greater than that normally found in rat brain, the findings are consistent with previous observations *in vitro* and appear to support the contention that after deamination in brain, β-hydroxylated amines are reduced predominantly to glycols while the non-β-hydroxylated aldehyde intermediates are oxidized mainly to acids.

Treatment with pargyline or pheniprazine greatly reduced the disappearance of octopamine-^{3}H and tyramine-^{3}H from brain. Similar results have been reported for normetanephrine.[10] These findings are consistent with the view that amines must be deaminated before leaving brain.[12] However, pheniprazine did not diminish disappearance of octopamine to the same extent as did pargyline. This difference may reflect the higher potency of pargyline to elevate endogenous monoamine levels in rat brain.[13] The possible existence of two distinct monoamine oxidase species in rat brain, each with a different sensitivity to monoamine inhibition,[14] might also explain the disparate results with pargyline and pheniprazine. Recent studies,* however, indicate that pargyline had a similar effect on the disappearance from brain of metaraminol, an amine which is not a substrate for monoamine oxidase. The latter data suggest that pargyline and not pheniprazine may influence amine transport from brain in addition to its effect on monoamine oxidase.

* Unpublished observations.

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