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RELEASE OF OCTOPAMINE AND α-METHYLOCTOPAMINE BY L-DOPA*

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Abstract—The effect of L-dopa on the distribution of ^{3}H-octopamine and ^{3}H-α-methyloctopamine in rat was examined. ^{3}H-tyramine or ^{3}H-α-methyltyramine was administered to normal rats and the labeled octopamine recovered by column chromatography. L-Dopa released greater than 95 per cent of the octopamine from both the synaptosomes and the supernatant and greater than 80 per cent of the α-methyloctopamine from the same fractions. Other experiments using inhibitors of dopa-decarboxylase as well as inhibitors of β-hydroxylation suggest that the active agent in releasing these β-hydroxylated phenylethanolamines was dopamine.

The administration of L-dopa has been shown to affect the metabolism of different amines in several ways. L-Dopa both prevents the synthesis of serotonin and brings about its release [1, 2]. L-Dopa also increases the turnover of cardiac norepinephrine [3, 4] and recent evidence suggests that this is accomplished by the release of endogenous norepinephrine from sympathetic nerve endings [5]. These effects of L-dopa are blocked by dopa-decarboxylase inhibition, and dopamine is presumed to be the active agent [6].

Recent work has suggested that the accumulation of some phenylethylamines, notably octopamine, may be involved in the pathogenesis of the symptomatology of hepatic coma [7–9]. L-Dopa has been shown to be efficacious in the treatment of hepatic coma in the presence of chronic liver disease and protein overload of gastrointestinal bleeding [7–14]. It was originally suggested that one mode of action of L-dopa may be by replenishment of brain norepinephrine, which is decreased in acute experimental hepatic coma [15, 16]. In analyzing the serial urine collections of patients in hepatic coma treated with L-dopa, it was noted that after the initial doses of L-dopa there was a great increase in the excretion of phenylethylamines, suggesting that L-dopa may also act by releasing phenylethylamine false transmitters. In the following experiments, L-dopa release of exogenous labeled phenylethylamines is demonstrated.

METHODS

Female rats, 160–200 g, from Charles River Laboratories were used in these experiments. ^{3}H-alpha-methyltyramine (3·2 mCi/μmole) and ^{3}H-tyramine (7·5 mCi/μmole) were purchased from New England Nuclear Corp., purified by paper chromatography and injected without carrier. Dopamine HCl was purchased from Mann Research Laboratory; solutions of dopamine were made in 0·9% NaCl and were calculated to contain the stated amount of the free amine. L-Dopa (kindly provided by Dr. Clifford Joseph of Hoffman-LaRoche) was suspended in 1% carboxymethylcellulose in 0·9% NaCl and injected intraperitoneally in a dose of 100 mg/kg in a volume of 1·0 ml. Disulfiram (kindly provided by Ayerst Pharmaceuticals) was suspended in the same diluent as that used for L-dopa and injected intraperitoneally in a dose of 400 mg/kg in a volume of 1·0 ml; controls received diluent alone. Ro4-4602/1 (kindly provided by Hoffman-LaRoche) was dissolved in 0·9% NaCl in a dose of 100 mg/kg and injected into a tail vein simultaneously with ^{3}H-alpha-methyltyramine in a total volume of 1·0 ml. ^{3}H-alpha-methyltyramine and ^{3}H-tyramine were administered to animals without anesthesia by tail vein after dilution in 0·9% NaCl in a dose of 25–100 μCi/kg. Animals were killed by guillotine and the hearts rapidly removed and homogenized in ice-cold 0·4 M $HClO_4$.

After centrifugation in the cold at 12,000 *g* for 10 min, the supernatant was adjusted to pH 6·1 with KOH and applied to a 3 × 0·7 cm column of Amberlite CG-50 ion-exchange resin (H^+ form, 100–200 mesh) similar to the technique of Fischer *et al.* [17]. The column was washed with 10 ml of 0·05 M sodium acetate buffer, pH 6·1, and amines were eluted with 5 ml of 3 N NH_4OH. Beta-hydroxylated tritiated amines were determined by reacting an aliquot of the ammonia eluate with sodium periodate to oxidize the beta-hydroxylated amines. The resultant ^{3}H-*p*-hydroxybenzaldehyde was extracted into toluene after acidification of the reaction mixture [17]. Radioactivity was counted in a Packard Tri-Carb liquid scintillation spectrometer and corrections were made for quenching.

To determine subcellular distribution of labeled amines, differential centrifugation was performed as described by Iverson *et al.* [18]. Finely minced hearts were homogenized in ice-cold 0·25 M sucrose in a loose-fitting, all-glass homogenizer and centrifuged at 12,000 *g* for 10 min to remove debris. The supernatant was decanted and spun at 100,000 *g* in a Beckman model L ultracentrifuge for 1 hr to separate the particulate fraction, containing nerve ending particles, from the supernatant. The high speed pellet was resuspended in 0·4 N $HClO_4$, homogenized and centrifuged. The high speed supernatant was mixed with

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1/10 vol. of 4·0 N $HClO_4$ and centrifuged. The perchloric acid extracts of both the pellet and supernatant were then applied to ion-exchange columns as described above.

RESULTS

Table 1 shows the distribution of ^{3}H-octopamine and ^{3}H-alpha-methyloctopamine between the synaptosome-containing fraction and supernatant, and the effect of L-dopa on this distribution. Considerably less ^{3}H-alpha-methyloctopamine than ^{3}H-octopamine is released after one dose of L-dopa. This amount of release, in turn, is considerably greater than the release of ^{3}H-norepinephrine reported by Landsberg and Bruno [6] from rat heart under the same conditions, and presumably reflects less tenacious binding of non-catecholamines as compared to norepinephrine.

To determine whether dopa-decarboxylase inhibition prevents the release of ^{3}H-alpha-methyloctopamine by L-dopa, rats were treated with the inhibitor, Ro4-4602/1. Rats received either ^{3}H-alpha-methyltyramine (50 μCi/kg) or the combined solution of ^{3}H-alpha-methyltyramine and Ro4-4602/1 as described above. One hr later, animals received L-dopa and 1 hr later were killed. Whereas 87 per cent of the ^{3}H-alpha-methyloctopamine was released from hearts of control rats, only 33 per cent was released from the experimental rats (Fig. 1). This result suggests that an amine product of L-dopa rather than dopa itself is the releasing agent.

To test whether synthesis of norepinephrine from dopamine was required for release of ^{3}H-alpha-methyloctopamine, rats receiving ^{3}H-alpha-methyltyramine were treated with 400 mg/kg of disulfiram, an inhibitor of dopamine beta-hydroxylase, and were given L-dopa 1 hr later (100 mg/kg). Inhibition of beta-hydroxylation had no effect on the release of amines by L-dopa, suggesting that dopamine was the responsible agent (Table 2).

That dopamine itself in the presence of a beta-hydroxylase inhibitor was capable of releasing ^{3}H-alpha-methyloctopamine was shown in another experiment. One hr after injection of ^{3}H-alpha-methyltyramine, rats received either disulfiram or diluent as above. One hr later, half of each pretreatment group received dopamine intraperitoneally (5 mg/kg); controls received 0·9% NaCl. Beta-hydroxylase inhibition did not affect the ability of dopamine to release ^{3}H-alpha-methyloctopamine (Table 3).

Table 1. Effect of L-dopa on the distribution of ^{3}H-octopamine and ^{3}H-α-methyloctopamine in the heart*

Treatment	Synaptosome-containing fraction	Supernatant
	^{3}H-octopamine (nCi/g in heart)	
Saline	11·9 ± 1·9	15·4 ± 2·5
L-Dopa	0·018 ± 0·004†	0·032 ± 0·004†
	^{3}H-α-methyloctopamine (nCi/g)	
Saline	117·7 ± 12·2	245·6 ± 19·3
L-Dopa	21·3 ± 3·6†	61·9 ± 7·2†

* Rats received either ^{3}H-tyramine (100 μCi/kg) or ^{3}H-α-methyltyramine (100 μCi/kg) i.v. One hr later, half the rats received L-dopa (100 mg/kg) suspended in saline i.p., and the controls received diluent. One hr later, all were killed. Results are given as mean ± S.E.M. for groups of six animals (Student's *t*-test, Yates correction).

† $P < 0·001$.

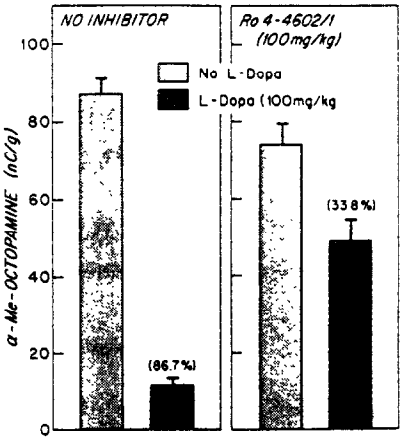

Fig. 1. Rats were treated either with ^{3}H-α-methyltyramine (50 μCi/kg) or with ^{3}H-α-methyltyramine (50 μCi/kg) simultaneously administered with Ro4-4602/1 (100 mg/kg) injected i.v. One hr later, half of each group received L-dopa (100 mg/kg) in saline suspension i.p. One hr later, all were killed. Results are expressed as mean ± S.E.M. for groups of six animals and are significant at $P < 0·01$ by Student's *t*-test.

Table 2. Effect of disulfiram (β-hydroxylase inhibition) on the release of α-methyloctopamine from rat heart by L-dopa*

Treatment		Heart ^{3}H-α-methyloctopamine
Disulfiram	L-dopa	(nCi/g)
–	–	280·3 ± 16·0
–	+	40·0 ± 6·6†
+	–	244·0 ± 19·0
+	+	44·7 ± 3·2†

* Rats received 100 μCi/kg ^{3}H-α-methyltyramine by tail vein; 1 hr later, half of the rats received disulfiram. 400 mg/kg i.p., in 1·0 ml of 1% carboxymethylcellulose in 0·9% NaCl, while the other half received diluent. One hr later, half of each pretreatment group received L-dopa (100 mg/kg i.p.) in saline suspension and half received saline. One hr later, all were killed. Results are given as mean ± S.E.M. for groups of six animals (Student's *t*-test, Yates correction).

† $P < 0·001$.

Table 3. Effect of disulfiram (β-hydroxylase inhibition) on the release of α-methyloctopamine from rat heart by dopamine*

Treatment		Heart ^{3}H-α-methyloctopamine
Disulfiram	Dopamine	(nCi/g)
–	–	40·2 ± 2·6
–	+	3·9 ± 1·1
+	–	35·0 ± 3·0
+	+	5·1 ± 1·0

* Rats received 25 μCi/kg of ^{3}H-α-methyltyramine by tail vein; 1 hr later half of the rats received disulfiram (400 mg/kg i.p.) in 1·0 ml of 1% carboxymethylcellulose in 0·9% NaCl, while the other half received diluent. One hr later, half of each pretreatment group received dopamine (5 mg/kg i.p.) in 1·0 ml of 0·9% NaCl and half received 0·9% NaCl. One hr later, all were killed. Results are given as mean ± S.E.M. for groups of five rats. The difference between the two groups that received dopamine is not significant (Student's *t*-test, Yates correction).

DISCUSSION

L-Dopa is known to provoke the release of brain serotonin in normal animals [1, 2] and in animals with portal flow diversion [19]. With respect to norepinephrine, it has been known that the administration of L-dopa will cause increased turnover of cardiac norepinephrine [3, 4, 6]. Although some have suggested that substrate loading with L-dopa bypasses the rate-limiting step in catecholamine biosynthesis and, by stimulating synthesis, results in increased turnover of norepinephrine, Landsberg and Bruno [6] have recently confirmed that L-dopa releases norepinephrine from sympathetic nerve endings and that, in fact, norepinephrine release is the basis for increased norepinephrine turnover.

Recent work from this laboratory has suggested that the accumulation of phenylethylamines functioning as false neurochemical transmitters may be responsible for some of the symptomatology in hepatic coma [7, 8]. The efficacy of L-dopa in such patients has been ascribed to a variety of mechanisms, including the depletion of potentially toxic levels of *S*-adenosylmethionine [10, 20] and replenishment of norepinephrine and dopamine [7, 8], compounds thought to be reduced in hepatic coma [14–16].

In addition, Brandau and Axelrod [21] have reported the release by L-dopa of heart and brain octopamine. The experiments reported here are confirmatory and suggest that the effect of L-dopa in respect to other beta-hydroxylated phenylethylamines is similar, namely release due to displacement. This effect is blocked by decarboxylase inhibitors, but is unaffected by inhibition of dopamine beta-hydroxylase, suggesting that the active agent is dopamine. Presumably, the mechanism by which L-dopa effects this release is by entering the nerve and undergoing decarboxylation to dopamine which then displaces the accumulated phenylethylamines. The results suggest that the release of accumulated false neurochemical transmitters may be one mechanism of action in the efficacy of L-dopa in hepatic coma.

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