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SELECTIVE STORAGE OF *p*-HYDROXY-*d*-AMPHETAMINE IN THE DOPAMINERGIC NERVE TERMINALS

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Abstract—Repeated treatments with *p*-hydroxy-*d*-amphetamine (*p*OH*d*A) result in it accumulating on the striatum but not in the brainstem. Agents which destroy dopaminergic nerve terminals (6-hydroxydopamine), or release dopamine (reserpine, tetrabenazine, pimozide) from nerve endings, reduce the accumulation of *p*HO*d*A in the striatum, but do not affect its levels in the brainstem or plasma.

p-Hydroxyamphetamine (*p*-OHA) and *p*-hydroxynorephedrine (*p*-OHNE) are the principal metabolites of amphetamine (A) in hydroxylating animal species [1–3].

p-OHNE has shown to compete with and replace noradrenaline in peripheral and cerebral storage sites [2, 4, 5]. It has thus been tentatively assigned the role of a "false noradrenergic neurotransmitter" suggested as responsible for the development of tolerance to some effects of amphetamine [4, 6].

The role of *p*-OHA in the overall pharmacological effect of A is much less clear although its isomer, α-methyl-*m*-tyramine, has been considered a false dopaminergic transmitter [7, 8]. *p*-Hydroxy-*d*-amphetamine (*p*-OH*d*A) was found specifically localized in dopaminergic brain areas after systemic administration of *d*-amphetamine [9–11] and after administration of *p*-OH*d*A [12–14]. Recently we found that repeated administration of *d*-amphetamine or *p*-OH*d*A results in specific accumulation of *p*-OH*d*A in the striatum [14, 13]. Furthermore, findings after chemical [14] or electrolytic [15] lesion suggest that *p*-OH*d*A is specifically stored in the dopaminergic presynaptic terminals. This suggests that *p*OH*d*A may play some part in the dopaminergic effects of *d*-amphetamine.

To challenge this attractive hypothesis, we investigated whether *p*-OH*d*A concentrated in the striatum was sensitive to pharmacological agents which interact with the dopamine metabolism.

MATERIALS AND METHODS

Female CD-COBS (Charles River, Italy) rats weighing 180 g ± 10 g were used. *p*-Hydroxy-*d*-amphetamine ·HBr (*p*-OH*d*A) (kindly supplied by SKF Laboratories, Philadelphia) was injected i.p. at the daily dose of 40 mg/kg for 4 days.

In one experiment rats were given pargyline (50 mg/kg i.p.) followed 30 min later by 6-hydroxydopamine (6-OHDA) (250 μg/rat i.v.) 6 days before starting the *p*-OH*d*A treatment. In other experiments, reserpine (2.5 mg/kg i.v.) or tetrabenazine (40 mg/kg i.p.) was injected 1 hr after the last *p*-OH*d*A administration in the 4-day *p*-OH*d*A-treated rats.

Finally, in a further experiment pimozide (2 mg/kg i.p.) was injected to rats 30 min before the fourth *p*-OH*d*A injection. Animals were killed by decapitation at various intervals after the last dose of *p*-OH*d*A, as reported in the tables. Blood was collected, brains were removed and brain areas dissected, frozen and stored at −20° until required for determinations.

p-OH*d*A was assayed in plasma, striatum and brainstem (including pons, medulla oblungata, mesencephalon and diencephalon) of each rat. *p*-OH*d*A was measured according to the method of Belvedere *et al.* [16], partially modified as follows: tissue samples were homogenized with acetone–formic acid 1N (85:15). After centrifugation the supernatant phase was washed twice with heptane–chloroform (4:1) and shaken. The aqueous phase was adjusted to pH 10 with NaOH and borate buffer and extracted twice with ethylacetate. The organic extract was again shaken with formic acid and heptane and the acid phase was re-extracted with ethylacetate. The organic extract was evaporated to dryness. *N*-pentafluoropropionyl anhydride was added and the derivatives was left to form for 30 min at 60°. The samples were evaporated to dryness under a nitrogen stream, redissolved in ethylacetate and injected into the gas-chromatographic column. Striatal dopamine (DA) and brainstem noradrenaline (NE) were measured by the method of Laverty and Taylor [17].

RESULTS

6-OHDA. After repeated daily treatments, *p*-OH*d*A accumulates in the striatum. The levels were 2.59 μg/g (see Table 1) in comparison with 1.86 ± 0.11 μg/g in the acutely treated rats. In brainstem the levels attained after a single or repeated treatments were comparable to those in plasma. When rats were pretreated 10 days before with pargyline + 6-OHDA the catecholminergic nerve terminals were lesioned, as shown by a decrease in catecholamine concentrations, to 78.3 per cent for NE in the brainstem and 76.1 per cent for DA in the striatum. In this experimental condition *p*-OH*d*A in the striatum was markedly reduced, while the level in brainstem and in plasma were unaffected (Table 1).

Reserpine and tetrabenazine. Reserpine (2.5 mg/kg i.v.) or tetrabenazine (40 mg/kg i.p.) were given to rats 1 hr after the fourth daily dose of *p*-OH*d*A. These agents, which release catecholamines, also markedly

Table 1. Effect of 6-OHDA on *p*-OH*d*A levels in rat striatum, brainstem and plasma

Time after last treatment with *p*-OH*d*A (hr)	Striatum μg/g ± S. E.		Brainstem μg/g ± S. E.		Plasma μg/ml ± S. E.	
	Control	6-OHDA	Control	6-OHDA	Control	6-OHDA
1	2.59 + 0.17	1.01 ± 0.17*	0.486 ± 0.09	0.453 ± 0.10	0.517 ± 0.009	0.509 ± 0.025
5	1.12 ± 0.11	0.28 ± 0.12*	0.030 ± 0.006	0.036 ± 0.004	0.015 ± 0.001	0.020 ± 0.001

6-OHDA (250 μg/rat intraventricularly) was injected 30 min after pargyline (50 mg/kg i.p.) and 6 days later rats received 4 daily administrations of *p*-OH*d*A (40 mg/kg/i.p.). Each figure is the average of 4 determinations.
* $P < 0.01$ compared to the corresponding control group (Student's *t* test).

Table 2. Effect of tetrabenazine and reserpine on concentrations of noradrenaline, dopamine and *p*-OH*d*A

Levels (μg/g or ml ± S. E.)	Striatum	Brainstem	Plasma	Striatum	Brainstem	Plasma
		Saline			Tetrabenazine (40 mg/kg i.p.)	
p-OH*d*A	1.07 ± 0.1	0.032 ± 0.006	0.014 ± 0.001	0.37 ± 0.03*	0.020 ± 0.004	0.013 ± 0.001
DA	6.88 ± 0.2	—	—	0.59 ± 0.12*	—	—
NE	—	0.40 ± 0.05	—	—	0.16 ± 0.04*	—
		Saline			Reserpine (2.5 mg/kg i.v.)	
p-OH*d*A	0.88 ± 0.09	0.038 ± 0.003	—	0.32 ± 0.04*	0.040 ± 0.008	—
DA	6.88 ± 0.2	—	—	0.34 ± 0.05*	—	—
NE	—	0.40 ± 0.051	—	—	0.04 ± 0.003*	—

Determinations were made 4 hr after reserpine or tetrabenazine, which were given 1 hr after the fourth dose of *p*-OH*d*A (40 mg/kg i.p.).
Each figure is the average of at least 4 determinations.
* $P < 0.01$ compared to the corresponding saline group (Student's *t* test).

reduced the concentration of *p*-OH*d*A in the striatum, but did not modify levels in the brainstem and plasma (Table 2).

Pimozide. Table 3 shows that when pimozide was given to rats 30 min before the fourth *p*-OH*d*A injection, striatal *p*-OH*d*A levels were lower than in untreated rats. The effect of pimozide was specific on the striatum, as plasma and brainstem levels of *p*-OH*d*A during 5 hr of observation, were not modified.

DISCUSSION

Previous data have shown that *p*-OH*d*A is not distributed uniformly in the brain [9, 11, 12] and that a preferential storage site is the striatal dopaminergic area [14]. The present data substantiate these observations, showing that after a few repeated doses, *p*-OH*d*A accumulates in the striatum to a greater extent than in the brainstem. Moreover, disappearance from the striatum is very slow. Thus, 5 hr after the last administration, the striatal concentration is about 50 times the brainstem level.

The fact that destruction of the dopamine nerve terminals obtained by intracerebral 6-OHDA treatment markedly reduces the accumulation of *p*-OH*d*A after repeated treatments, shows that in the striatum *p*-OH*d*A accumulates in dopaminergic structures. 6-OHDA has been used as a tool to study where drugs end up in the catecholaminergic system for several other compounds, for example, reserpine [18–19], and amphetamine [19, 20]. However, while amphetamine was stored in both the noradrenergic and dopaminergic

Table 3. Effect of pimozide on *p*-OH*d*A in rat plasma, striatum and brainstem

Time after last dose of *p*-OH*d*A (hr)	Plasma μg/ml ± S. E.		Striatum μg/g ± S. E.		Brainstem μg/g ± S. E.	
	Saline	Pimozide	Saline	Pimozide	Saline	Pimozide
1	0.46 ± 0.03	0.43 ± 0.03	2.70 ± 0.09	1.86 ± 0.09*	0.47 ± 0.05	0.47 ± 0.08
2	0.13 ± 0.04	0.13 ± 0.02	1.75 ± 0.08	1.18 ± 0.09*	0.15 ± 0.03	0.12 ± 0.03
5	0.02 ± 0.004	0.02 ± 0.08	1.01 ± 0.09	0.58 ± 0.07*	0.04 ± 0.005	0.04 ± 0.01

Pimozide (2 mg/kg i.p.) or saline was given 30 min before the fourth dose of *p*-OH*d*A (40 mg/kg i.p.).
Each figure is the average of at least 4 determinations.
* $P < 0.01$ compared to the corresponding saline group (Student's *t* test).

nerve terminals [19], *p*-OH*d*A seems to be found only in the dopaminergic nerve endings. In the brainstem, where noradrenaline terminals prevail, 6-OHDA treatment fails to reduce the level of *p*-OH*d*A although it causes widespread destruction in the noradrenergic system. Similar conclusions have been drawn by Danielson *et al.* [15], who employed electrolytic lesions of the substantia nigra to reduce striatal accumulation of *p*-OH*d*A in proportion to the degree of neuronal degeneration. In addition we show now that *p*-OH*d*A accumulated in the striatum can be released by pharmacological agents such as tetrabenazine and reserpine which are known as catecholamine depletors. In contrast, these agents are unable to modify the *p*-OH*d*A concentration in other areas such as the brainstem. Furthermore pimozide, a powerful, specific and long-lasting DA receptor blocker [21] which, by feedback response, causes release of DA [22, 23], also facilitates the disappearance of *p*-OH*d*A accumulated in striatal dopaminergic structures without affecting *p*-OH*d*A in the brainstem.

The above manipulations of the dopaminergic system (6-OHDA, tetrabenazine, reserpine and pimozide) are not likely to modify the disposal of *p*-OH*d*A in general because they do not affect plasma levels of the compound.

In conclusion, our findings that *p*-OH*d*A levels can be reduced by agents able to destroy dopamine nerve terminals or to release dopamine from the storage sites, or to increase its turnover, are compatible with the hypothesis that *p*-OH*d*A is stored in the striatal dopaminergic system. Whether *p*-OH*d*A plays a role in tolerance to some dopaminergic effects of amphetamine [13, 24, 25] remains to be established.

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