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THE ACTION OF FENFLURAMINE AND *p*-CHLORAMPHETAMINE ON SEROTONERGIC MECHANISMS: A COMPARATIVE STUDY IN RAT BRAIN NUCLEI

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A single injection of fenfluramine or *p*-chloroamphetamine (PCA) (100 μmol/kg intraperitoneally) decreases the serotonin (5HT) content and the tryptophan hydroxylase activity in various areas of the rat brain. Other reports have shown that a single injection of fenfluramine or PCA causes cytopathological changes in a serotonergic midbrain nucleus which was termed B9 by Dahlstrom and Fuxe (1). Despite this cytopathological change, fenfluramine fails to reduce the tryptophan hydroxylase activity in B9. In hippocampus the decrease of tryptophan hydroxylase elicited by fenfluramine persists for less than 21 days; in contrast PCA reduces the tryptophan hydroxylase activity in hippocampus, striatum, septal nuclei and B9 for longer than 21 days. Probably the decrease of tryptophan hydroxylase elicited by PCA in B9 is due to retrograde degeneration; the intensity and duration of the biochemical lesion elicited by fenfluramine and PCA in serotonergic terminals are a factor in determining the extent of the biochemical lesion in serotonergic cell bodies.

INTRODUCTION

Fenfluramine (F) and *p*-chloroamphetamine (PCA) deplete the 5-hydroxytryptamine (5HT) content (2–4) and decrease the tryptophan hydroxylase activity (TPH) in rat brain (4). The neurochemical changes

elicited by PCA last longer than those caused by F (4). Both compounds induce cytopathological changes in a midbrain region which includes a group of serotonergic cell bodies which was termed B9 by Dahlstrom and Fuxe (1); similar changes were not observed in the dorsal (B7) and median (B8) raphe nuclei (5, 6). Harvey and associates suggested that this selective degeneration of B9 neurons could be useful to study the axonal projections of these serotonergic cell bodies.

We have recently shown that a single injection of PCA preferentially reduces the 5HT content and the TPH activity in the B9 area (7, 8). However, the 5HT depletion elicited by PCA in brain areas containing 5HT nerve terminals was more rapid and pronounced than that of brain areas containing cell bodies. From these results it was inferred that PCA acted primarily on the 5HT nerve terminals; this lesion by an as yet unknown mechanism triggers a secondary retrograde degeneration in the cell bodies.

The present report describes the effect of a single injection of F (100 μmol/kg intraperitoneally) on the TPH activity and 5HT content of several brain nuclei, containing either 5HT terminals or cell bodies.

EXPERIMENTAL PROCEDURE

Sprague Dawley male rats (Zivic Miller, Allison Park, Pennsylvania; 150–200 g) were injected intraperitoneally with 100 μmol/kg of *dl*-F or *dl*-PCA hydrochlorides in a volume of 5 ml saline/kg. Rats used as controls received an equal volume of saline. All the animals were housed in standard laboratory conditions (5–7 animals per cage; environmental temperature 21–24°C; 10 hr dark and 14 hr light per day; free access to Purina rat chow and water).

TABLE I

CONCENTRATION OF 5HT AND ACTIVITY OF TRYPTOPHAN HYDROXYLASE IN BRAIN NUCLEI OF UNTREATED RATS

	5HT (pmol/mg protein)[a]	Tryptophan hydroxylase (nmol 5HTP/mg prot./hr)[a]
N. raphe dorsalis (B7)	173 ± 3 (35)	105 ± 6 (17)
N. raphe medialis (B8)	208 ± 10 (34)	41 ± 4 (17)
B9	105 ± 4 (35)	13 ± 1 (17)
N. septum med. and lat.	61 ± 4 (12)	1.2 ± 0.1 (18)
N. caudatus	44 ± 3 (12)	1.7 ± 0.1 (18)
Hippocampus	34 ± 1 (4)	1.7 ± 0.1 (5)

[a] Number of determinations in parenthesis. Values are the mean ± SEM.

TABLE II

TRYPTOPHAN HYDROXYLASE ACTIVITY AND 5HT CONCENTRATION IN DIFFERENT NUCLEI AT VARIOUS TIMES FOLLOWING ADMINISTRATION OF FENFLURAMINE[a]

	Tryptophan hydroxylase			5HT	
	1 day	7 days	21 days	21 days	60 days
B7	98 ± 6 (5)	109 ± 13 (5)	91 ± 10 (8)	93 ± 8 (5)	99 ± 10 (5)
B8	90 ± 12 (5)	86 ± 19 (5)	76 ± 8 (10)	59 ± 4 (5)[b]	91 ± 7 (4)
B9	94 ± 8 (5)	90 ± 10 (5)	74 ± 10 (6)	83 ± 5 (4)	89 ± 8 (5)
N. septum med. & lat.	ND	ND	100 ± 5 (13)	ND	95 ± 9 (4)
N. caudatus	ND	ND	91 ± 9 (13)	ND	82 ± 2 (4)
Hippocampus	8 ± 4 (5)[b]	57 ± 13 (5)[c]	91 ± 8 (12)	ND	67 ± 2 (4)[d]

[a] Values are expressed as percent of saline treated controls (mean ± SEM). Significance of the difference was computed by the two-tailed t test using the original nonnormalized values. Number of determinations in parenthesis.
[b] $P < 0.01$.
[c] $P < 0.05$.
[d] $P < 0.001$.

At various times after injection, the rats were decapitated and the brains were rapidly removed and frozen in dry ice. The frozen brains were serially sectioned (400 μm) from the caudal end in a cryostat (−8°C). Various brain nuclei were punched out with a hollow steel tube (0.8–1.2 mm ID) using coordinates and reference points derived from the atlas of Konig and Klippel (9) and from Dahlstrom and Fuxe (1). A cut was made through the optic chiasm perpendicular to the longitudinal axis of the brain, and the tissue anterior to the cut was designated "whole forebrain."

TPH activity was assayed by high-pressure liquid chromatography as previously described (10) 5HT in brain nuclei was determined by mass fragmentography as previously reported (7, 11, 12). Protein was measured by the method of Lowry et al. (13).

RESULTS

TPH activity and 5HT content of several brain nuclei in rats receiving saline are reported in Table I. These values are used as a reference for the calculation of the changes caused by F at various times after injection (Table II). The TPH activity of hippocampus, a brain structure containing 5HT terminals, is only 8% of control at 24 hr after F. It is still significantly lower than control at 7 days, but at 21 days the TPH activity has returned to control values. Also the TPH activity of the other brain areas containing 5HT terminals was completely recovered 21 days after F injection. In contrast the TPH in brain areas of rats treated with an equimolar amount of PCA is still lower than controls 21 days

TABLE III

TRYPTOPHAN HYDROXYLASE ACTIVITY AND 5HT CONCENTRATION IN DIFFERENT NUCLEI 21 DAYS AFTER EQUIMOLAR AMOUNTS OF FENFLURAMINE OR PCA[a]

	Tryptophan hydroxylase		5HT	
	Fenfluramine	PCA	Fenfluramine	PCA
B7	91 ±10 (8)	96 ± 16 (9)	93 ± 8 (5)	98 ± 5 (5)
B8	76 ± 8 (10)	80 ± 12 (8)	59 ± 4 (5)[c]	79 ± 8 (5)
B9	74 ± 10 (6)	53 ± 4 (9)[b]	83 ± 5 (4)	52 ± 4 (5)[c]
N. septum med. & lat.	100 ± 5 (13)	52 ± 2 (6)[c]	ND	ND
N. caudatus	91 ± 9 (13)	27 ± 3 (6)[c]	ND	ND
Hippocampus	91 ± 8 (12)	9 ± 5 (4)[b]	ND	ND

[a] Values are expressed as percent of saline treated controls (mean ± SEM). Significance of the difference was computed by the two-tailed t test using the original nonnormalized values. Number of determinations in parenthesis.

[b] $P < 0.001$.

[c] $P < 0.01$.

TABLE IV
EFFECT OF EQUIMOLAR AMOUNTS OF FENFLURAMINE OR PCA ON FOREBRAIN TRYPTOPHAN HYDROXYLASE ACTIVITY 1–4 HR AFTER DRUG ADMINISTRATION[a]

	1 hr	4 hr
Fenfluramine	66 ± 8	53 ± 4
PCA	60 ± 10	68 ± 11

[a] Values are expressed as percent of saline treated controls (mean ± SEM). Tryptophan hydroxylase activity in saline-treated controls was 2.7 ± 0.3 nmol 5-hydroxytryptophan/mg protein/hr. There were 5 animals per group, and the enzyme activity in all treatment groups was decreased ($P < 0.05$; two-tailed t test).

after PCA injection (Table III). In rats injected with F the TPH activity of the three brain nuclei containing 5HT cell bodies was never significantly lower than controls (Table II); however, the 5HT content of B8 was significantly lower 21 days after F. In contrast, PCA decreased the TPH activity and 5HT content in area B9 at 21 days postinjection (Table III). At 1 and 4 hr after equimolar amounts (100 μmol/kg) of either F or PCA, the TPH activity of the forebrain was reduced by a comparable extent (Table IV).

DISCUSSION

The decrease in TPH activity and 5HT content elicited by F in nuclei containing serotonergic cell bodies (Tables II and III) is less pronounced than the effect of equimolar amounts of PCA (8). These results are consistent with the report that in the midbrain tegmentum fenfluramine is less neurotoxic than PCA (5, 6). While PCA induced degeneration in approximately 80% of the cell bodies in the B9 region, F had an intense effect only on the posterior 25% of this cell body group (5). Assuming that the cell body degeneration elicited by F and PCA occurs by a retrograde mechanism, we tested whether the neurotoxicity elicited by F is less severe than that elicited by PCA because the impairment of nerve

terminal function is slower after F than after PCA. If the rate of decrease of TPH activity and amine content elicited by F in axon terminals were slower than that elicited by PCA, we reasoned that the cell bodies might have enough time to respond to the cell damage by changing protein synthesis without suppressing the synthesis of TPH. However, it appears that F and PCA reduce forebrain TPH activity by approximately the same degree at 1 and 4 hr after injection (Table IV). The extent of the maximal reduction of TPH activity in the hippocampus is similar in rats receiving F (Table II) and PCA (8).

Although F reduces the enzyme activity and the 5HT content from axon terminals as quickly and to the same degree as PCA, its effect is of shorter duration (Table III). Our data show that the neurotoxic effects of F on 5HT terminals are terminated 21 days after a single injection of the drug; this finding is consistent with other reports (4). Perhaps the time duration of the 5HT depletion can explain why the 5HT cell bodies are for the most part spared from the neurotoxic action of F.

If the neurotoxicity of PCA and F depends only on their primary effect on 5HT terminals, as the data presented here and elsewhere (3, 8) would suggest, then the persistence of the decrease in enzyme activity and amine content in terminals might determine the degree of neurotoxicity in cell bodies. Whereas the signs of F neurotoxicity are minimal at 3 weeks after a single injection and decline in severity from 7 to 21 days, the decrease in TPH activity elicited by PCA persist longer than 60 days in hippocampus and longer than 30 days in the caudate (8). Although these data indicate that the molecular nature of the process involved in the action of F may differ from the process involved in PCA neurotoxicity, the precise cause for this difference is not clarified by the present experiments.

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