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COMPARATIVE EFFECTS OF VARIOUS SEROTONIN RELEASING AGENTS IN MICE*

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Abstract—Five serotonin (5-HT) releasers, *p*-chlorophenylethylamine (*p*-Cl-PEA), *p*-methoxyphenylethylamine (*p*-CH_3O-PEA), 4-methyl-alpha-ethyl-meta-tyramine (H75/12), fenfluramine and *p*-chloroamphetamine (*p*-CA) were compared as to their effects on mouse brain 5-HT metabolism. All 5-HT releasers had similar ED_{50} values (0.34–0.70 μM) for tritiated 5-HT ([^{3}H]5-HT) release from brain synaptosomes. With the exception of H75/12, they had similar potencies for the production of the 'serotonin syndrome,' which probably reflect their *in vivo* 5-HT releasing actions. H75/12 was also the only 5-HT releaser that did not elevate brain tryptophan concentration. *p*-CH_3O-PEA and H75/12 did not have any long-term effects on brain 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), or tryptophan hydroxylase activity, in contrast to the reported neurotoxic effects of *p*-CA and fenfluramine. *p*-Cl-PEA and *p*-CH_3O-PEA were the only 5-HT releasers that increased brain 5-HT and 5-HIAA levels. Despite the structural similarities of these five 5-HT releasers, we have found considerable differences in their actions on brain 5-HT metabolism.

Five substituted phenylethylamines have been found to release serotonin (5-HT) from nerve terminals (Fig. 1). The two 5-HT releasers that have been studied most extensively are *p*-chloroamphetamine (*p*-CA) [1–4] and fenfluramine, the *m*-trifluromethyl-*N*-ethyl derivative of amphetamine [2, 5–7]. Both *p*-CA and fenfluramine have been reported to produce long-term reductions in brain 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels, in brain tryptophan hydroxylase activity, and in the high affinity uptake of 5-HT by cortical slices. These long-term effects of *p*-CA and fenfluramine on 5-HT neurons have been postulated to be due to a cytotoxic action [2–4, 7, 8]. Partial recovery of serotonergic function occurs by 2 months after fenfluramine, in contrast to minimal recovery several months after *p*-CA [1, 6, 8]. Another substituted phenylethylamine, 4-methyl-alpha-ethyl-meta-tyramine (H75/12), also releases 5-HT both *in vitro* [9] and *in vivo* [10] and produces depletion of brain 5-HT and 5-HIAA as well as inhibition of tryptophan hydroxylase [11]. We recently reported that *p*-chlorophenylethylamine (*p*-Cl-PEA) and its analog *p*-methoxyphenylethylamine (*p*-CH_3O-PEA) are potent 5-HT releasing agents with ED_{50} values very similar to those of H75/12, *p*-CA, and fenfluramine [9, 12].

5-HT releasers and 5-HT agonists produce a characteristic set of behavioral changes known as the 'serotonin syndrome' [13, 14]. In this paper we compare the *in vivo* potencies of these 5-HT releasing agents by scoring their behavioral effects. In addition, experiments were carried out to characterize the effects of releasing 5-HT on brain serotonergic metabolism. Because *p*-CA, fenfluramine, and H75/12 inhibit brain tryptophan hydroxylase *in vivo* and lower brain 5-HT and 5-HIAA, it seemed important to determine whether these biochemical changes would also occur with other 5-HT releasers (*p*-Cl-PEA and *p*-CH_3O-PEA).

METHODS

Male mice (25–30 g) of the Swiss Webster strain were used throughout the study. Animals were decapitated, and whole brain 5-HT and 5-HIAA were analyzed by the method of Curzon and Green [15].

Tryptophan hydroxylase activity was assayed in

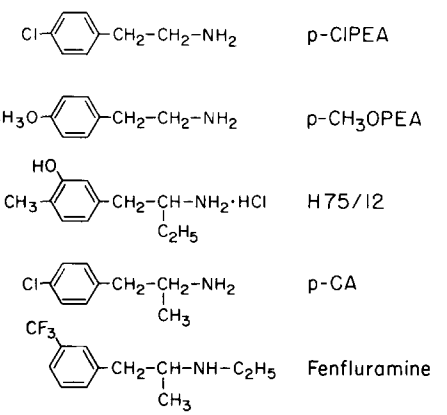

Fig. 1. Structures of 5-HT releasing agents.

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mouse midbrain by the method of Ichiyama *et al.* [16] modified to microscale by Fairman *et al.* [17].

The effects of at least five different concentrations of drug on accumulation and release of tritiated 5-HT ([^{3}H]5-HT), 0.25 μCi, 2.5×10^{-9} M, were studied, using the method of Kuhar *et al.* [18].

In vitro competition of drugs with [^{3}H]5-HT binding to membranes from mice whole brain homogenates was measured by the method of Bennett and Snyder [19].

Stimulation of brain 5-HT receptors is known to produce a characteristic behavioral syndrome consisting of lateral head weaving, Straub tail, hindlimb abduction, tremor, reciprocal forepaw treading, hyperactivity, salivation, and piloerection, which has been called the 'serotonin syndrome' [13, 14]. Behavioral changes were quantified by assigning one point for the presence of each sign. In addition, one point was given for a duration of behavioral changes of more than 15 min and one point for an early onset of less than 5 min after injection, for a total maximum score of 10. Normal behavior was given a zero score. Three or four mice housed in a plastic cage were rated for behavioral changes at one time.

p-Cl-PEA, *p*-CH_3O-PEA, *p*-CA, *p*-chlorophenylalanine, and 5-6 dihydroxytryptamine were purchased from the Sigma Chemical Co. (St. Louis, MO). Other drugs were supplied by the following companies: fluoxetine, Eli Lilly & Co. (Indianapolis, IN); fenfluramine HCl, Robins Research Laboratories (Richmond, VA) and H75/12, Astra (Sodertalje, Sweden).

Table 1. Comparison of 5-HT releasers

	p-Cl-PEA	*p*-CH_3O-PEA	H75/12	*p*-CA	Fenfluramine
		ED_{50}* (μM)			
[^{3}H]5-HT release	0.43	0.70	0.34	0.50	0.35
[^{3}H]5-HT uptake Inhibition	0.51	0.72	0.72	0.75	0.65
[^{3}H]5-HT receptor binding	4.2	62.0	45.0	15.0	16.0
		Behavioral score†			
Serotonin syndrome	9.6 ± 0.3	9.7 ± 0.8	2.0 ± 0.6	8.8 ± 0.4	7.2 ± 0.3
		% of control			
Tryptophan					
1 hr	150 ± 14§	127 ± 8§	106 ± 6	275 ± 27§	152 ± 6§
2 hr	128 ± 12§	122 ± 2§	110 ± 8	369 ± 32§	113 ± 4§
24 hr	104 ± 4	92 ± 4	114 ± 4	128 ± 4§	101 ± 5
Fluoxetine pretreatment					
1 hr	214 ± 14§	154 ± 16§	114 ± 9	249 ± 17§	145 ± 14§
5-HT					
2 hr	128 ± 10§	130 ± 5§	89 ± 6	83 ± 6§	40 ± 3§
24 hr	96 ± 4	112 ± 5	102 ± 4	77 ± 2§	96 ± 3
5-HIAA					
2 hr	139 ± 8§	145 ± 2§	69 ± 2§	70 ± 5§	63 ± 5§
24 hr	79 ± 2§	69 ± 4§	107 ± 2	71 ± 8§	71 ± 6§
Tryptophan hydroxylase					
2 hr	97 ± 16	102 ± 12	122 ± 8	63 ± 6§	73 ± 9
24 hr	94 ± 20	100 ± 4	90 ± 9		64 ± 9§

* These *in vitro* studies were carried out as described in Methods. The concentration producing a 50 per cent effect (ED_{50}) was obtained from a log-dose vs percentage of effect curve constructed by the method of least squares, using the mean of triplicate determinations for each point, with five points for each curve.

† For behavioral studies 0.32 mmole/kg of each compound was injected i.p. and the serotonin syndrome was scored (total maximum score of 10) over the next 20 min as described in Methods. Each value is the mean ± S.E. of five or six mice.

‡ For *in vivo* biochemical studies, the following doses were used: *p*-Cl-PEA (0.32 mmole/kg), *p*-CH_3O-PEA (0.32 mmole/kg), H75/12 (0.12 mmole/kg), *p*-CA (0.12 mmole/kg), and fenfluramine (0.11 mmole/kg). Absolute values of controls are: tryptophan 4.02 ± 0.25 μg/g; 5-HT 0.60 ± 0.04 μg/g; 5-HIAA, 0.40 ± 0.02 μg/g; and tryptophan hydroxylase activity, 0.165 ± 0.016 nmole $^{14}CO_2 \cdot g \cdot hr^{-1}$. Each value is the mean ± S.E. of five or six mice.

§ $P < 0.05$, compared to control.

RESULTS AND DISCUSSION

As can be seen in Table 1, the *in vitro* potencies of all five compounds (*p*-Cl-PEA, *p*-CH_3O-PEA, H75/12, *p*-CA, and fenfluramine) in releasing previously accumulated [^{3}H]5-HT from mouse whole brain synaptosomes were remarkably similar to each other. The ED_{50} for inhibition of [^{3}H]5-HT uptake and the ED_{50} for release of [^{3}H]5-HT were also very similar for each drug. This suggests that the predominant action of each of these five compounds is release of [^{3}H]5-HT from mouse brain synaptosomes rather than inhibition of uptake. All releasing agents necessarily cause an apparent inhibition of accumulation of [^{3}H]amine during the uptake incubation periods, but it has been demonstrated that, to be an effective uptake inhibitor, the ED_{50} for uptake inhibition must be lower than that for release [20, 21].

p-Cl-PEA, *p*-CH_3O-PEA, *p*-CA, and fenfluramine, injected in equimolar doses (0.32 mmole/kg i.p.), each produced, between 5 and 20 min after injection, the 'serotonin syndrome' (Table 1) [13, 14]. This syndrome is due to increased activation of central 5-HT receptors and can be used as a behavioral assay of serotonergic stimulation in the brain. Since all of the 5-HT releasers examined in this paper had relatively weak affinity for 5-HT receptors (Table 1), it can be assumed that the 'serotonin syndrome' was produced by these compounds via 5-HT release from pre-synaptic nerve terminals and not by direct stimulation of post-synaptic serotonergic receptors. This assumption is supported by the observation that the behavioral changes produced by *p*-CA and fenfluramine were attenuated or prevented by depleting brain 5-HT with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine, or by pretreatment with specific 5-HT uptake inhibitors [22, 23]. We previously reported that pretreatment of mice with 5-HT uptake inhibitors only partially inhibited *p*-Cl-PEA- and *p*-CH_3O-PEA-induced serotonin syndrome [9, 12]. Furthermore, pretreatment with the tryptophan hydroxylase inhibitor *p*-chlorophenylalanine did not markedly reduce the behavioral effect of *p*-Cl-PEA and *p*-CH_3O-PEA [9, 12]. We therefore concluded that these two phenylethylamine derivatives induce the serotonin syndrome by a combination of 5-HT release and direct stimulation of 5-HT receptors. Since *p*-chlorophenylalanine only partially depletes endogenous brain 5-HT, we examined the behavioral effects of *p*-Cl-PEA and *p*-CH_3O-PEA after combined treatment with 5,6-dihydroxytryptamine, which produces degeneration of 5-HT neurons, and *p*-chlorophenylalanine. In mice injected intracisternally with 5,6-dihydroxytryptamine (50 μg, free base) during the first post-natal day and administered *p*-chlorophenylalanine (300 mg/kg, i.p., daily for 3 days) at 5 weeks of age, *p*-Cl-PEA and *p*-CH_3O-PEA produced very much lower serotonin syndrome scores compared with their controls (Table 2). Fluoxetine (50 mg/kg), at a dose five times greater than we previously used [9, 12], also greatly reduced the behavioral effects of *p*-Cl-PEA and *p*-CH_3O-PEA (Table 2). These data demonstrate that the behavioral effects of *p*-Cl-PEA and *p*-CH_3O-PEA, like those of *p*-CA and fenfluramine, are due to the 5-HT releasing properties of these drugs. Thus, it appears that the endogenous content of brain serotonin may have to be depleted to a greater degree to attenuate the serotonin syndrome produced by *p*-Cl-PEA and *p*-CH_3O-PEA compared with the syndrome produced by *p*-CA and fenfluramine.

Table 2. Effect of serotonin depletion and high dose fluoxetine pretreatment on *p*-Cl-PEA- and *p*-CH_3O-PEA-induced serotonin syndrome*

	Behavioral score	
	p-Cl-PEA	*p*-CH_3O-PEA
Control	9.6 ± 0.3	9.7 ± 0.3
5,6-DHT + *p*-CPA	2.6 ± 0.5†	1.1 ± 0.5†
Fluoxetine	2.0 ± 0.4†	1.5 ± 0.3†

* For the serotonin depletion study, newborn mice were pretreated with desmethylimipramine (25 mg/kg, i.p.) and 1 hr later were injected intracisternally with 5,6-dihydroxytryptamine (5,6-DHT) (50 μg, free base). At 5 weeks of age these mice were given *p*-chlorophenylalanine (*p*-CPA) (300 mg/kg, i.p., daily for 3 days). Another group of mice was pretreated with 50 mg/kg fluoxetine, i.p. Twenty-four hours after the last *p*-CPA injection and 1 hr after fluoxetine, either *p*-Cl-PEA (0.32 mmole/kg, i.p.) or *p*-CH_3O-PEA (0.32 mmole/kg, i.p.) was injected and behavioral effects were scored over the next 20 min. Each value is the mean ± S.E. of four to seven mice.

† $P < 0.0001$, compared to control.

H75/12, in an equimolar dose, produced only minimal behavioral changes compared to the other 5-HT releasing agents (Table 1). Even a further increase of H75/12 to 100 mg/kg (0.465 mmole/kg) produced a behavioral score of only 3.3. At 200 mg/kg of H75/12, mice developed a complete serotonin syndrome. This dose, however, was lethal to most of the animals. Since the intensity of the serotonin syndrome is presumably proportional to the *in vivo* release of brain 5-HT, H75/12 may be a less potent *in vivo* 5-HT releaser than the other compounds. There are some reports in the literature that indicate that a higher percentage of injected *p*-CA and fenfluramine get into the brain compared to H75/12 [24–26].

With the exception of H75/12, the 5-HT releasers increased brain tryptophan 1 and 2 hr after injection, as shown in Table 1. By 24 hr brain tryptophan had returned to normal, except after *p*-CA. Tryptophan is the immediate precursor of 5-HT, and agents that increase brain tryptophan levels have been shown to enhance brain 5-HT biosynthesis [27]. Tryptophan-induced enhancement of 5-HT synthesis could explain the increase in brain 5-HT and 5-HIAA observed 2 hr after *p*-Cl-PEA and *p*-CH_3O-PEA administration (Table 1). At 30 min, however, *p*-Cl-PEA (0.32 mmole/kg) decreased brain 5-HT and concurrently increased 5-HIAA, which presumably is due to 5-HT release and correlates with the time of behavioral changes [12]. We have observed that brain tryptophan is significantly increased as early as 15 min after *p*-Cl-PEA injection (unpublished observation). This elevation of brain tryptophan may cause an early enhancement of brain 5-HT turnover, which could explain why the serotonin syndrome produced by *p*-Cl-PEA is more resistant to pretreatment with *p*-chlorophenylalanine and fluoxetine.

Table 3. Effect of multiple injections of p-CH_3O-PEA, H75/12, and fenfluramine on brain serotonin metabolism*

Treatment (mmole/kg)	5-HT	5-HIAA	Tryptophan hydroxylase
		% of control	
p-CH_3O-PEA (0.32)	101 ± 4	91 ± 6	105 ± 4
H75/12 (0.12)	103 ± 3	97 ± 6	108 ± 10
Fenfluramine (0.07)	82 ± 3†	80 ± 6†	96 ± 12

* p-CH_3O-PEA, H75/12, or fenfluramine was injected i.p. twice daily for 5 days and whole brain 5-HT and 5-HIAA and midbrain tryptophan hydroxylase activities were measured 1 week after the last injection. For absolute values of controls see Table 1. Each value is the mean ± S.E. of five or six mice.

† Significantly different from control, $P < 0.02$.

Interestingly, H75/12, which did not produce the serotonin syndrome, also did not change brain tryptophan levels. Perhaps elevation of brain tryptophan by the other releasers may have contributed to their effectiveness in producing the serotonin syndrome. We also examined the effect of fluoxetine (10 mg/kg, i.p.), given 1 hr before the injection of the various 5-HT releasers, on the increase in brain tryptophan levels (Table 1). Fluoxetine, which is known to inhibit the uptake of these drugs and prevent 5-HT release, did not counteract the elevation of brain tryptophan. This finding indicates that the elevation of brain tryptophan is not related to 5-HT release. Brain tryptophan levels of fluoxetine-treated animals were not significantly different from controls (4.29 ± 0.14 vs 4.20 ± 0.02 μg/g).

Aside from the initial 5-HT release and related behavioral changes, these substituted phenylethylamines have delayed effects (2 and 24 hr, Table 1) on 5-HT, 5-HIAA and tryptophan hydroxylase activity. p-CA and fenfluramine inhibited tryptophan hydroxylase activity, resulting in a decrease in brain 5-HT and 5-HIAA. Since p-Cl-PEA and p-CH_3O-PEA do not inhibit tryptophan hydroxylase, they produced an increase in brain 5-HT and 5-HIAA at 2 hr due to the elevated brain tryptophan levels. H75/12 (0.12 mmole/kg) had no effect on either brain tryptophan hydroxylase activity or tryptophan concentration, which may explain why 5-HT levels did not change at 2 or 24 hr. The reason for the H75/12-induced decrease in brain 5-HIAA at 2 hr is not clear, although the possibility that inhibition of monoamine oxidase is causing this effect should be investigated. At a higher dose of H75/12 (0.32 mmole/kg) in rats, Fuller *et al.* [11] observed a decrease in brain 5-HT at 2 and 4 hr and a decrease in tryptophan hydroxylase activity and 5-HIAA at 4 hr after injection. Since these serotonin releasers also alter brain tryptophan concentration and tryptophan hydroxylase activity, it is not possible to determine the effect, if any, of only 5-HT release, alone, on the levels of brain 5-HT and 5-HIAA at 2 hr.

Fenfluramine and p-CA have been reported to produce neurotoxic effects on brain serotonergic neurons [1, 5, 7]. Therefore, we compared the long-term effects of p-CH_3O-PEA and H75/12 with fenfluramine. Table 3 shows that p-CH_3O-PEA (0.32 mmole/kg, i.p.) or H75/12 (0.12 mmole/kg, i.p.) administered twice daily for 5 days had no effects on brain 5-HT, 5-HIAA, and tryptophan hydroxylase activity assayed 1 week after the last injection. The concentrations of brain 5-HT and 5-HIAA however, were reduced 1 week after the last injection of fenfluramine (0.07 mmole/kg, i.p. twice daily for 5 days); tryptophan hydroxylase activity had returned to normal by this time. These findings corroborate the recent paper by Steranaka and Sanders-Bush [24] in which they reported that 2 weeks after a single injection of fenfluramine (80 mg/kg) brain levels of 5-HT and 5-HIAA were significantly reduced, while the activity of tryptophan hydroxylase had returned to control value. During these long-term studies we have observed that each of the ten injections of p-CH_3O-PEA, in the same animal, produced the same intensity of the serotonin syndrome. It is of interest that tolerance develops to serotonin syndrome induction with repeated injections of p-CA [28].

In conclusion, all of the substituted phenylethylamines that we studied had similar ED_{50} values for *in vitro* synaptosomal release of 5-HT, but they had considerably different effects *in vivo*. Of the agents we studied, H75/12 was the least potent in producing the serotonin syndrome and was the only one that did not elevate brain tryptophan levels. Only p-CA and fenfluramine were neurotoxic and inhibited tryptophan hydroxylase. p-Cl-PEA and p-CH_3O-PEA are unique because they increase brain 5-HT and 5-HIAA, which may account for the absence of behavioral tolerance to these compounds.

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