Pharmacology Biochemistry & Behavior, Vol. 13, pp. 283–286. Printed in the U.S.A.

Para-Halogenated Phenethylamines: Similar Serotonergic Effects in Rats by Different Mechanisms[1]

ROBERT S. SLOVITER, JOHN D. CONNOR, BRUCE P. DAMIANO AND EUGENE G. DRUST

Department of Pharmacology, The Milton S. Hershey Medical Center
The Pennsylvania State University, Hershey, PA 17033

Received 21 March 1980

SLOVITER, R. S., J. D. CONNOR, B. P. DAMIANO AND E. G. DRUST. *Para-halogenated phenethylamines: Similar serotonergic effects in rats by different mechanisms.* PHARMAC. BIOCHEM. BEHAV. 13(2) 283–286, 1980.—The serotonin (5-HT) behavioral syndrome in rats served as a model to test for possible acute serotonergic effects of para-halogenated phenethylamines. *p*-Chloro-, *p*-chloro-β-methyl-, *p*-fluoro-, *p*-bromo-, and *p*-iodophenethylamine produced the same 5-HT behavioral syndrome as did *p*-chloroamphetamine, but unlike the latter did not deplete brain 5-HT 3 days after injection. Pretreatment of rats with the 5-HT depletor *p*-chlorophenylalanine (*p*CPA) prevented the serotonergic effects of both chloro-derivatives, and partially prevented the effects of bromo- and iodophenethylamine. 5-Hydroxytryptophan restored the behavioral responses to these compounds in *p*CPA-pretreated rats. *p*CPA treatment did not prevent the behavioral effects of *p*-fluorophenethylamine. Similarly, zimelidine, a 5-HT uptake inhibitor, prevented the serotonergic behavioral effects of all compounds tested except *p*-fluorophenethylamine. Taken as a group, para-halogenated phenethylamines are short-acting serotonergic compounds which, unlike *p*-chloroamphetamine, do not produce long-lasting depletion of brain 5-HT. *p*-Chlorophenethylamine and its β-methyl analog apparently activate central 5-HT receptors indirectly, i.e., by 5-HT release; *p*-fluorophenethylamine is a direct 5-HT agonist. The *p*-bromo- and *p*-iodo-derivatives apparently possess both properties.

Serotonin behavioral syndrome Halogenated phenethylamines *p*-Chloroamphetamine Zimelidine

p-CHLOROPHENYLALANINE (*p*CPA) and *p*-chloroamphetamine (*p*CA) are widely used experimentally because they decrease brain serotonin (5-HT) concentrations after intraperitoneal injection [3, 9, 11]. However, these closely related compounds also elicit serotonergic behavioral effects acutely in experimental animals. *p*CA produces the serotonin behavioral syndrome (simultaneous forepaw padding, splayed hindlimbs and side-to-side headweaving or head tremor) [6, 12, 18] by releasing endogenous 5-HT [6,18]. Similarly, *p*CPA, in combination with a monoamine oxidase inhibitor, produces the same 5-HT behavioral syndrome as *p*CA [7,14] and by the same mechanism [14]. *p*-Chlorophenethylamine (*p*CPEA), the decarboxylated metabolite of *p*CPA [1] which differs from *p*CA only in that it lacks an α-methyl group, produces the 5-HT behavioral syndrome acutely [7,14] by releasing 5-HT [14], but does not share the 5-HT depleting effects of *p*CPA or *p*CA [3,9]. Other para-halogenated phenethylamine analogs have apparently not been studied for possible serotonergic effects. Therefore, it seemed worthwhile to determine if these compounds represent a class of serotonin agonists which could be used to alter central 5-HT function acutely without producing long-term depletion of brain 5-HT.

METHOD

Animal Treatment

Male Sprague-Dawley descendant rats (Charles River Laboratories, Inc.; 200–400 g) were used in all experiments. Rats were maintained on a 12 hr light/dark cycle and allowed free access to food and water. On the day of the experiments, rats were removed from the animal quarters, weighed in the laboratory and placed in individual metal cages (30×30×13.5 cm high and with 1–2 cm of ground corncob bedding). Rats were given 30 min to become acclimated to the cages and were handled only for injection or sacrifice (by decapitation). Brains were removed from the skull and separated into right and left halves. The right half was routinely taken for monoamine assays. Tissues were placed in vials on dry ice within 2 min of decapitation and stored at - 80°C for subsequent analysis.

Compounds

The following compounds were used in these experiments: DL-*p*-chlorophenylalanine methylester HCl (*p*CPA), 5-hydroxy-L-tryptophan (5-HTP), *p*-chlorophenethylamine

[1]Research supported by USPHS Grant DA 02007 from the National Institute on Drug Abuse.

(*p*CPEA), *p*-chloro-β-methylphenethylamine HCl (*p*CMPEA), *p*-chloroamphetamine HCl (*p*CA), *p*-bromophenethylamine (*p*BPEA), *p*-fluorophenethylamine HCl (*p*FPEA) (all from Sigma Chemical Co.); *p*-iodophenethylamine (*p*IPEA) (ICN-K and K Laboratories); and zimelidine HCl (Astra). The compounds were dissolved in 0.9% w/v sodium chloride (saline) with the following exceptions: *p*CPEA and *p*BPEA were dissolved in 1% w/v citric acid in saline; *p*IPEA was suspended in 1% carboxymethylcellulose. Doses of drugs in salt form refer to the weight of the salt. Control rats received the appropriate vehicle(s). All injections were by the intraperitoneal route.

Behavioral Evaluation

The behavioral syndrome in rats caused by 5-HT receptor stimulation was evaluated as described previously [15]. It was considered present, in all-or-none fashion, if rats exhibited simultaneously forepaw padding, splayed hindlimbs and side-to-side headweaving or head tremor at any time during the observation period. The terms "serotonin behavioral syndrome" or "syndrome" refer specifically to these behavioral signs. Pilot experiments provided information on the latency and duration of the syndrome and on other drug effects, e.g., different behaviors, convulsions, death. Rats whose brains were assayed for amine concentrations were sacrificed at times coincident with manifestation of the syndrome or three days after injection. Acute behavioral responses were judged continuously by an observer unaware of the treatment, from one minute after injection until sacrifice.

Monoamine Assay

Frozen brain tissue was homogenized in 0.4 N $HClO_4$, then assayed for norepinephrine (NE), dopamine (DA), and 5-HT by a spectrofluorometric method [13]. When the drugs were tested for interference in this assay, *p*BPEA and *p*FPEA interfered with 5-HT-ninhydrin fluorescence values. Therefore, brain tissues from rats treated with these compounds were assayed for 5-HT by an o-phthalaldehyde-5-HT fluorescence method described previously [17].

The average standard errors within a single assay were 4% for NE and DA, and 6% for 5-HT. Amine recoveries (internal standard/external standard) were in the range of 85%-90% for all amines in the Shellenberger and Gordon method [13]. In the OPT modified assay, the recovery of 5-HT was 60–70%. Values were corrected for recovery.

RESULTS

Effects of Phenethylamines on Behavior and Brain Monoamine Concentrations

All five halogenated phenethylamines (*p*CPEA, *p*CMPEA, *p*FPEA, *p*BPEA, and *p*IPEA) caused the serotonin behavioral syndrome (simultaneous side-to-side headweaving or head tremor, forepaw padding, splayed hindlimbs). The onset of the syndrome was between 3–10 min for all compounds; the durations were 10–30 min depending on the dose. Dose-response relationships and acute effects of the drugs on brain NE, DA and 5-HT concentrations are presented in Table 1.

The long term decreases in brain 5-HT concentrations produced by some halogenated α-methylphenethylamines (e.g., *p*-chloroamphetamine) prompted us to investigate whether the compounds we tested also had this property. Rats were injected with the test substance or vehicle 3 days before sacrifice. *p*CA (10 mg/kg) produced the 5-HT behavioral syndrome in 4 out of 4 rats tested. Three days after *p*CA, brain NE and DA concentrations were not significantly different from control; brain 5-HT was reduced to 29% of control (Table 2). In contrast to *p*CA, none of the 5 phenethylamines tested changed brain NE, DA or 5-HT concentrations ($p > 0.05$) 3 days after a single injection of the dose which caused syndromes in 4 out of 4 rats.

TABLE 1

ACUTE EFFECTS OF PARA-HALOGENATED PHENETHYLAMINES ON BEHAVIOR AND BRAIN MONOAMINES

Treatment*	Syndrome response ratios†	Brain monoamine concentrations % of control		
		NE	DA	5-HT
p-Chlorophenethylamine (*p*CPEA)				
(10)	0/4			
(20)	1/4			
(40)	4/4	87‡	100	78‡
p-Chloro-β-methylphenethylamine (*p*CMPEA)				
(20)	0/4			
(30)	1/4			
(40)	4/4	95	97	83§
p-Fluorophenethylamine (*p*FPEA)				
(40)	1/4			
(60)	3/4			
(80)	4/4	71¶	86	106
p-Bromophenethylamine (*p*BPEA)				
(10)	0/4			
(20)	2/4			
(40)	4/4	91	114	101
p-Iodophenethylamine (*p*IPEA)				
(20)	0/4			
(30)	2/4			
(40)	4/4	98	98	98

Four rats in each group; rats sacrificed for assay 15 min after drug or vehicle injection. All drugs given by the intraperitoneal route. Mean control concentrations for 6 separate assays were: NE 368 ± 29, DA 610 ± 15, 5-HT 473 ± 32 (mean ± SEM, ng/g frozen tissue). Each group compared statistically to its respective vehicle control group.

*Doses in parentheses are in mg/kg body weight.

†Number of rats displaying syndrome per number tested.

‡$p < 0.05$, significantly different from vehicle control by Student's *t*-test.

§$p < 0.01$.

¶$p < 0.001$.

Effect of pCPA Pretreatment on the Responses to Phenethylamines

If the phenethylamines produced the 5-HT behavioral syndrome by a mechanism dependent on endogenous 5-HT, e.g., 5-HT release, then depletion of brain 5-HT should prevent this behavioral response. Conversely, if the phenethylamines produced the syndrome by an action independent of endogenous 5-HT, e.g., direct 5-HT receptor ac-

TABLE 2

EFFECT OF PARA-HALOGENATED PHENETHYLAMINES ON BRAIN MONOAMINE CONCENTRATIONS 3 DAYS AFTER INJECTION

Treatment*	Brain monoamine concentrations % of control NE	DA	5-HT
p-Chloroamphetamine (*p*CA) (10)	96	99	29†
p-Chloro-β-methylphenethylamine (*p*CMPEA) (40)	90	99	101
p-Chlorophenethylamine (*p*CPEA) (40)	101	110	100
p-Fluorophenethylamine (*p*FPEA) (80)	99	96	91
p-Bromophenethylamine (*p*BPEA) (40)	105	99	90
p-Iodophenethylamine (*p*IPEA) (40)	89	96	92

Four rats in each group. All drugs given by intraperitoneal route. Mean amine concentrations for the 6 control groups were: NE 350 ± 2, DA 687 ± 15, 5-HT 475 ± 8 (mean ± SEM, ng/g frozen tissue). Each group compared to its respective vehicle control group.

*Doses in parentheses are in mg/kg body weight and represent the doses which cause the 5-HT behavioral syndrome in all 4 rats in each group (Table 1).

†$p < 0.001$; significantly different from vehicle control group by Student's *t*-test.

tivation, then depletion of 5-HT should not prevent the behavioral response. Pretreatment of rats with *p*-chlorophenylalanine (*p*CPA methylester HCl, 400 mg/kg daily for 3 days) reduced brain 5-HT concentrations to 10% of control; NE and DA concentrations were 65 and 78% of control, respectively (Table 3).

Saline- or *p*CPA-treated rats received the dose of halogenated phenethylamine (shown in Table 1) that caused the syndrome in 4 out of 4 rats. *p*CPA pretreatment did not prevent the syndrome caused by *p*FPEA, but did prevent the syndromes caused by *p*CPEA, *p*CMPEA and *p*IPEA. The *p*BPEA syndrome was partially prevented (4 out of 6 rats displaying the syndrome in the *p*CPA group vs 6 out of 6 in the saline group). A separate group of *p*CPA-treated rats was used to determine if these rats were incapable of displaying the syndrome after *p*CPEA, *p*CMPEA or *p*IPEA, or if higher doses of these drugs could overcome *p*CPA inhibition of the syndrome. Even ultimately lethal doses of *p*CPEA or *p*CMPEA (3 times higher than the dose necessary to produce the syndrome in 4 out of 4 normal rats) did not evoke the syndrome in *p*CPA-treated rats. However, all rats in each group of 4 rats that received *p*CPA for 3 days, then *p*IPEA (120 mg/kg) or *p*BPEA (80 mg/kg) did display the 5-HT behavioral syndrome.

Rats received 5-hydroxytryptophan (5-HTP) to determine if acute, partial restoration of brain 5-HT concentrations could reinstate the behavioral effects of the 4 drugs which were prevented by *p*CPA pretreatment. 5-HTP (30 mg/kg, 30 min before the phenethylamine) increased 5-HT concentrations from 10% to 58% of control (Table 3) and restored the behavioral effects of *p*CPEA, *p*CMPEA, *p*BPEA, and *p*IPEA in *p*CPA-pretreated rats. This dose of 5-HTP had no significant effect on brain catecholamine concentrations (Table 3) in saline- or *p*CPA-pretreated rats.

Effect of Zimelidine on the Behavioral Effects of Phenethylamines

Is access to 5-HT neuronal presynaptic terminals by the halogenated phenethylamines necessary for these compounds to elicit the 5-HT behavioral syndrome? To test this possibility, the 5-HT neuronal uptake inhibitor zimelidine was administered 10 min before each test compound. An assumption in this experimental design is that if the phenethylamines enter the presynaptic 5-HT terminal, they do so by way of the 5-HT uptake mechanism. Zimelidine (20 mg/kg) did not prevent the syndrome caused by *p*FPEA (80 mg/kg). However, the same dose of zimelidine did prevent the syndromes evoked by *p*CPEA (40 mg/kg), *p*CMPEA (40 mg/kg), *p*CA (10 mg/kg), *p*BPEA (40 mg/kg) or *p*IPEA (40 mg/kg).

DISCUSSION

The goal in these experiments was to examine the behavioral, as well as the short- and long-term neurochemical effects of para-halogenated phenethylamines. All five haloge-

TABLE 3

EFFECT OF *p*CPA AND 5-HTP ON BRAIN MONOAMINE CONCENTRATIONS

Treatment	NE Conc.	%	DA Conc.	%	5-HT Conc.	%
Saline + saline	365 ± 7	100	713 ± 15	100	450 ± 16	100
Saline + 5-HTP	340 ± 19	93	723 ± 25	101	758 ± 39*	168
*p*CPA + saline	239 ± 20*	65	557 ± 29*	78	47 ± 3*	10
*p*CPA + 5-HTP	221 ± 13*	61	507 ± 18*	71	262 ± 42†‡	58

Six rats in each group. Rats received saline or *p*CPA methylester HCl (400 mg/kg, IP) 72, 48 and 24 hr before injection of saline or 5-HTP (30 mg/kg, IP). Rats were sacrificed 30 min after injection of 5-HTP or saline. Values given are mean concentrations (ng/g of frozen tissue) ± SEM or percentage of control.

*$p < 0.001$; †$p < 0.01$; significantly different from saline + saline group by Student's *t*-test.

‡$p < 0.001$, significantly different from *p*CPA + saline group.

nated phenethylamines tested caused the same acute serotonergic behavioral effects as *p*CA, but lacked the long-term 5-HT depleting effects of *p*CA. Interestingly, all of the halogenated phenethylamines do not produce the 5-HT behavioral syndrome by the same mechanism. The para-chlorinated compounds (*p*CPEA and *p*CMPEA) apparently release endogenous 5-HT, whereas *p*FPEA seems to be a direct 5-HT agonist. The other two compounds, *p*BPEA and *p*IPEA apparently possess both properties. Evidence in support of these conclusions is as follows: (1) all phenethylamines tested evoked the behavioral syndrome (simultaneous side-to-side headweaving or head tremor, forepaw padding and splayed hindlimbs) shown previously to be due to central 5-HT receptor activation [5, 8, 15]; (2) *p*CPA pretreatment, which depleted brain 5-HT to 10% of control, blocked the syndromes caused by *p*CPEA, *p*CMPEA, *p*BPEA and *p*IPEA, but did not block, the *p*FPEA syndrome. Blockade by *p*CPA could not be overcome by increasing the dose of *p*CPEA or *p*CMPEA. Conversely, higher doses of *p*BPEA or *p*IPEA did overcome the *p*CPA blockade. With these latter compounds, some direct 5-HT agonist activity seems probable, although release of residual 5-HT cannot be ruled out; (3) in *p*CPA treated rats, partial restoration of brain 5-HT concentrations by 5-HTP administration reinstated the behavioral effects of *p*CPEA, *p*CMPEA, *p*BPEA, and *p*IPEA; (4) presynaptic actions for *p*CPEA, *p*CMPEA, *p*BPEA and *p*IPEA are suggested by the observations that zimelidine, an inhibitor of 5-HT neuronal uptake [10], blocked the syndromes caused by these compounds but did not block the *p*FPEA syndrome.

Fuller and colleagues [2,4] compared *p*CA with other para-halogenated amphetamines with respect to long-term effects on brain 5-HT. These workers did not comment on any acute behavioral effects of halogenated amphetamines. They reported that *p*-chloro-, *p*-bromo- and *p*-iodoamphetamine caused long-lasting depletion of 5-HT, but that *p*-fluoroamphetamine did not [2]. Similarly, the present findings show that the fluoro-compound was the only phenethylamine tested which apparently lacks a presynaptic action.

In recent reports we showed that amphetamine [16], β-phenethylamine [13a], and a variety of hallucinogenic phenethylamines [17] all cause the 5-HT behavioral syndrome in rats. These observations, together with the present results, indicate that phenethylamines, as a class of drugs, produce similar serotonergic behavioral effects but do so by different mechanisms, i.e., dependent or independent of endogenous 5-HT. The molecular properties responsible for the different mechanisms of action by which these compounds produce similar behavioral effects remain to be elucidated. To do so may lead to a better understanding of serotonin release processes and receptor mechanisms as well as provide a rationale for the development of drugs which selectively augment or decrease 5-HT release.

ACKNOWLEDGMENTS

We thank Dr. Ulf Henrick Lindberg (Astra Läkemedel AB) for providing zimelidine. The clerical assistance of Diana Barto and Ann McCann is appreciated.

REFERENCES

1. Edwards, D. J. and K. Blau. The *in vivo* formation of *p*-chloro-β-phenylethylamine in young rats injected with *p*-chlorophenylalanine. *J. Neurochem.* **19:** 1829–1832, 1972.
2. Fuller, R. W., J. C. Baker, K. W. Perry and B. B. Molloy. Comparison of 4-chloro-, 4-bromo-, and 4-fluoroamphetamine in rats: drug levels in brain and effects on brain serotonin metabolism. *Neuropharmacology* **14:** 739–746, 1975.
3. Fuller, R. W., C. W. Hines and J. Mills. Lowering of brain serotonin level by chloramphetamines. *Biochem. Pharmac.* **14:** 483–488, 1965.
4. Fuller, R. W., H. D. Snoddy, A. M. Snoddy, S. K. Hemrick, D. T. Wong and B. B. Molloy. *p*Iodoamphetamine as a serotonin depletor in rats. *J. Pharmac. exp. Ther.* **212:** 115–119, 1980.
5. Grahame-Smith, D. G. Studies *in vivo* on the relationship between brain tryptophan, brain 5-HT synthesis and hyperactivity in rats treated with a monoamine oxidase inhibitor and L-tryptophan. *J. Neurochem.* **18:** 1053–1066, 1971.
6. Growdon, J. H. Postural changes, tremor, and myoclonus in the rat immediately following injections of *p*-chloroamphetamine. *Neurology* **27:** 1074–1077, 1977.
7. Hwang, E. C. and M. H. Van Woert. Behavioral and biochemical actions of *p*-chlorophenylethylamine (*p*CPEA) in mice. *Life Sci.* **24:** 595–602, 1979.
8. Jacobs, B. L. Minireview: an animal behavior model for studying central serotonergic synapses. *Life Sci.* **19:** 777–786, 1976.
9. Koe, B. K. and A. Weissman. *p*-Chlorophenylalanine, a specific depletor of brain serotonin. *J. Pharmac. exp. Ther.* **154:** 499–516, 1966.
10. Ögren, S-O., S. B. Ross, A-C. Holm and L. Baumann. 5-Hydroxytryptamine and avoidance performance in the rat. Antagonism of the acute effect of *p*-chloroamphetamine by zimelidine, an inhibitor of 5-hydroxytryptamine uptake. *Neurosci. Lett.* **7:** 331–336, 1977.
11. Sanders-Bush, E., J. A. Bushing and F. Sulser. Long-term effects of *p*-chloroamphetamine on tryptophan hydroxylase activity and on the levels of 5-hydroxytryptamine and 5-hydroxyindoleacetic acid in brain. *Eur. J. Pharmac.* **20:** 385–388, 1972.
12. Scheel-Krüger, J. Behavioral and biochemical comparison of amphetamine derivatives, cocaine, benztropine and tricyclic anti-depressant drugs. *Eur. J. Pharmac.* **18:** 63–73, 1972.
13. Shellenberger, M. K. and J. H. Gordon. A rapid simplified procedure for simultaneous assay of norepinephrine, dopamine and 5-hydroxytryptamine from discrete brain areas. *Analyt. Biochem.* **39:** 356–372, 1971.
13a. Sloviter, R. S., J. D. Connor and E. G. Drust. Serotonergic properties of β-phenethylamine in rats. *Neuropharmacology*, in press, 1980.
14. Sloviter, R. S., E. G. Drust and J. D. Connor. Serotonin agonist actions of *p*-chlorophenylalanine. *Neuropharmacology* **17:** 1029–1033, 1978.
15. Sloviter, R. S., E. G. Drust and J. D. Connor. Specificity of a rat behavioral model for serotonin receptor activation. *J. Pharmac. exp. Ther.* **206:** 339–347, 1978.
16. Sloviter, R. S., E. G. Drust and J. D. Connor. Evidence that serotonin mediates some behavioral effects of amphetamine. *J. Pharmac. exp. Ther.* **206:** 348–352, 1978.
17. Sloviter, R. S., E. G. Drust, B. P. Damiano and J. D. Connor. A common mechanism for lysergic acid, indolealkylamine and phenethylamine hallucinogens: serotonergic mediation of behavioral effects in rats. *J. Pharmac. exp. Ther.*, in press, 1980.
18. Trulson, M. E. and B. L. Jacobs. Behavioral evidence for the rapid release of CNS serotonin by PCA and fenfluramine. *Eur. J. Pharmac.* **36:** 149–154, 1976.