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SEROTONIN-RELEASING EFFECTS OF SUBSTITUTED PIPERAZINES *IN VITRO*

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Abstract—The effects of various piperazine-containing compounds on the release of endogenous serotonin (5-HT) from rat hypothalamic slices were evaluated. Incubation of hypothalamic slices with *m*-chlorophenylpiperazine (mCPP) or *m*-trifluoromethylphenylpiperazine (mTFMPP) evoked a potent, dose-dependent release of endogeous 5-HT that was similar in magnitude to that seen with tryptamine, *p*-chloroamphetamine, or fenfluramine. In the presence of the 5-HT uptake blockers fluoxetine or chlorimipramine, this release was reduced dramatically. Furthermore, removal of calcium from the incubation medium had little effect on the drug-induced release, suggesting that the release mechanism involved displacement of 5-HT stores and not depolarization-induced exocytosis. Trazodone, MK-212, and quipazine had only small effects on release. These studies show that several piperazine-containing compounds can evoke a potent release of endogenous stores of hypothalamic 5-HT *in vitro*, actions which should be considered together with their direct agonist activity when interpreting the CNS effects *in vivo*.

Several substituted piperazines have potent serotonin-mimetic effects in the central nervous system. For example, quipazine [2-(1-piperazinyl) quinoline] and MK-212 [2-(1-piperazinyl)-6-chloropyrazine] exert behavioral and biochemical changes in brain associated with enhanced serotonergic activity. Both compounds induce head twitches in mice [1, 2], reduce food intake [3, 4], and decrease serotonin turnover [5–10].

The addition of a halogenated phenyl ring to the piperazine nucleus also results in compounds with strong serotonin-mimetic activity (Fig. 1). Thus, *m*-trifluoromethylphenylpiperazine (mTFMPP; [11]) and *m*-chlorophenylpiperazine (mCPP; [3, 12–14]) are serotonin-mimetics thought to act primarily by direct stimulation of serotonin receptors in the CNS. mCPP is also of interest because it is thought to be an active metabolite of the atypical antidepressant trazodone [15, 16], the formation of which from trazodone *in vivo* may impart the serotonin-agonist activity observed with higher doses of the antidepressant [14–18]. The recent finding that mCPP is also formed in man after a single oral dose of trazodone [19] lends support to the speculation that this metabolite may be involved in the clinical effects of trazodone.

To further examine the pharmacological characteristics of trazodone and mCPP as well as other piperazine-containing compounds, the effects of several substituted piperazines on 5-HT release from rat hypothalamic slices were investigated.

MATERIALS AND METHODS

Animals. Adult, male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), having free access to standard rat chow and tap water, were used in all experiments.

Release of endogenous 5-HT. Hypothalami were dissected on an ice-chilled glass plate and then minced by a hand-held razor blade to homogeneity. The slices were suspended in ice-cold Ca^{2+}-free Krebs–Ringer bicarbonate buffer (basic composition: 118 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 10 mM glucose, 1 μM clorgyline, 100 μM ascorbic acid, oxygenated to pH 7.4 with 95/5 O_2/CO_2) in which $MgSO_4$ was raised to 2.4 mM to substitute for the removal of calcium. The slices were transferred in

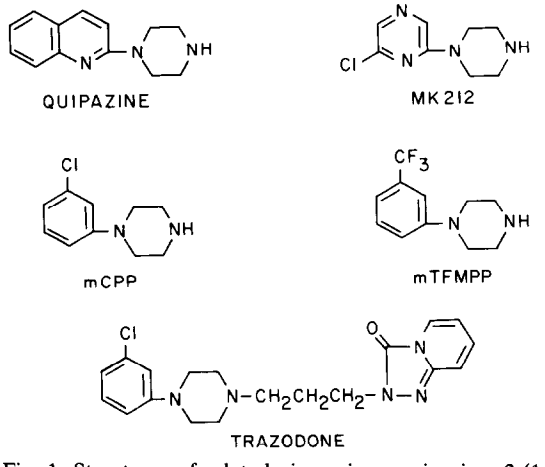

Fig. 1. Structures of related piperazines: quipazine, 2-(1-piperazinyl) quinoline; MK-212, 2-(1-piperazinyl)-6-chloropyrazine; mCPP, *m*-chlorophenylpiperazine; mTFMPP, *m*-trifluoromethylphenylpiperazine; and trazodone, 2-(3-[4-(*m*-chlorophenyl)-1-piperazinyl]propyl)-5-triazolo(4,3-*a*)pyridin-3-(2*H*)-one.

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1-ml aliquots (~2 mg protein) into 1.5-ml Eppendorf tubes, centrifuged briefly (30 sec in Beckman Microfuge at 10,000 rpm), and washed again with the ice-cold Ca^{2+}-free buffer. The supernatant fraction was aspirated and the slices were incubated for 25 min at 37° in 1 ml Krebs buffer (+Ca^{2+}). After brief centrifugation the slices were resuspended in Krebs buffer (500 μl) containing the indicated concentration of drugs and incubated for 10 min (37°) after which the tubes were chilled in ice water for 1 min and centrifuged. The resulting supernatant fraction was assayed directly for serotonin by high performance liquid chromatography (HPLC) with electrochemical detection [20]. Protein was assayed by the method of Lowry *et al.* [21].

Drugs. The following drugs were received as gifts: trazodone HCl (Mead Johnson, Evansville, IN), chlorimipramine HCl (Ciba-Geigy, Summit, NJ), fluoxetine HCl (Eli Lilly, Indianapolis, IN), clorgyline HCl (May & Baker, Dagenham, England), and fenfluramine HCl (A. H. Robins, Richmond, VA); other compounds were obtained commercially: tryptamine HCl (Sigma Chemical Co., St. Louis, MO), *p*-chloroamphetamine HCl (Regis, Morton Grove, IL), and *m*-chlorophenylpiperazine 2HCl (Sigma; Aldrich Chemical Co., Milwaukee, WI). 2-(1-Piperazinyl)-6-chloropyrazine HCl (MK-212), *o*-chlorophenylpiperazine HCl, trifluoromethylphenylpiperazine, and quipazine HCl were synthesized at the Merck Sharp & Dohme Research Laboratories. [^{3}H]Serotonin (5-[1,2-^{3}H](N)]hydroxytryptamine creatinine sulfate, 30.4 Ci/mmole) was obtained from the New England Nuclear Corp. (Boston, MA).

RESULTS

In vitro *release of endogenous 5-HT.* A 10-min incubation of rat hypothalamic slices with any of several known releasers of 5-HT such as tryptamine, *p*-chloroamphetamine (pCA) or fenfluramine elicited a concentration-dependent (0.1 to 10 μM) release of endogenous 5-HT (Tables 1 and 2). mCPP, *o*-chlorophenylpiperazine (oCPP) and mTFMPP exhibited similar activity, evoking 20- to 50-fold increases in release over basal levels at 10 μM (Tables 1 and 2). In comparison, trazodone, quipazine and MK-212 were active but relatively weak, causing only 2- to 4-fold increases in release (Tables 1 and 2).

Preincubation of the slices with the selective 5-HT uptake inhibitors fluoxetine (1 μM) or chlorimipramine (1 μM) elicited slight increases in basal release of

Table 1. Blockade by fluoxetine of stimulated 5-HT release from rat hypothalamic slices

Compound	Concn (μM)	Serotonin released*	
		No fluoxetine (% change)	1 μM Fluoxetine† (% Change)
Tryptamine	0	0.25 ± 0.03	0.56 ± 0.06
	0.1	0.33 ± 0.09 (+32)	0.52 ± 0.03 (−7)
	1	5.46 ± 0.48‡ (+2080)	0.86 ± 0.02 (+54)
	10	17.01 ± 2.30‡ (+6700)	5.98 ± 0.32‡ (+970)
p-Chloroamphetamine	0	0.36 ± 0.11	0.59 ± 0.08
	0.1	0.33 ± 0.04 (−8)	0.62 ± 0.05 (+5)
	1	5.58 ± 0.11‡ (+1450)	0.64 ± 0.08 (+8)
	10	18.20 ± 0.26‡ (+4960)	3.56 ± 0.38‡ (+500)
m-Chlorophenylpiperazine	0	0.22 ± 0.06	0.67 ± 0.03
	0.1	0.33 ± 0.05 (+50)	0.54 ± 0.04 (−19)
	1	4.32 ± 0.78‡ (+1860)	0.60 ± 0.06 (−10)
	10	11.11 ± 0.84‡ (+4950)	1.69 ± 0.26‡ (+151)
m-Trifluoromethylphenylpiperazine	0	0.24 ± 0.05	0.42 ± 0.03
	0.3	0.31 ± 0.07 (+29)	0.47 ± 0.11 (+12)
	1	0.53 ± 0.03 (+121)	0.51 ± 0.04 (+21)
	3	2.13 ± 0.09‡ (+790)	0.66 ± 0.06 (+57)
	10	5.53 ± 0.12‡ (+2200)	0.73 ± 0.10‡ (+74)
Trazodone	0	0.29 ± 0.06	0.33 ± 0.04
	1	0.33 ± 0.05 (+14)	0.37 ± 0.06 (+12)
	10	0.95 ± 0.13‡ (+230)	0.67 ± 0.08 (+103)
Quipazine	0	0.21 ± 0.01	0.27 ± 0.02
	0.1	0.19 ± 0.03 (−10)	0.29 ± 0.05 (+7)
	1	0.26 ± 0.02 (+24)	0.29 ± 0.02 (+7)
	10	0.86 ± 0.09‡ (+310)	0.56 ± 0.05 (+107)
MK-212	0	0.20 ± 0.02	0.71 ± 0.07
	0.1	0.29 ± 0.06 (+45)	0.57 ± 0.06 (−20)
	1	0.42 ± 0.15 (+110)	0.62 ± 0.05 (−13)
	10	0.59 ± 0.16‡ (+195)	0.97 ± 0.11 (+37)

* Values represent pmoles released · (mg protein)$^{-1}$ · (10 min)$^{-1}$ and are group means ± S.E.M. (N = 3 points/group).
† Fluoxetine was included where indicated during the 25-min preincubation period and the 10-min release period.
‡ $P < 0.05$ vs respective untreated controls (ANOVA, Dunnett's *t*-test).

Table 2. Comparison of the effects of some phenylpiperazines and fenfluramine on the release of 5-HT from hypothalamic slices

Compound	Concn (μM)	Serotonin released* (% change)
Control	0	0.32 ± 0.03
Fenfluramine	0.3	0.68 ± 0.02 (+113)
	1	2.92 ± 0.23† (+810)
	3	6.45 ± 0.19† (+1920)
o-Chlorophenylpiperazine	1	0.93 ± 0.08 (+190)
	3	4.59 ± 0.09† (+1330)
	10	10.68 ± 0.48† (+3240)
m-Chlorophenylpiperazine	1	4.31 ± 0.33† (+1250)
Trazodone	3	0.84 ± 0.01 (+160)
	10	1.51 ± 0.08† (+370)

* Values represent pmoles released · (mg protein)$^{-1}$ · (10 min)$^{-1}$ and are group means ± S.E.M. (N = 3 points/group).
† $P < 0.05$ vs control (ANOVA, Dunnett's *t*-test).

5-HT (Tables 1 and 3) and largely inhibited the enhanced release of 5-HT by tryptamine, pCA or the substituted piperazines (Tables 1 and 3). Removal of calcium from the incubation medium, however, had no significant effect on the magnitude of release (Table 4).

DISCUSSION

The present examination of several piperazine-containing compounds *in vitro* indicates that some halogenated phenylpiperazines (i.e. mCPP, oCPP and mTFMPP) are potent releasers of endogenous stores of hypothalamic 5-HT. The 5HT-releasing activity of these compounds approached that of known releasers of 5-HT as indicated in the potency order: tryptamine = pCA ≥ fenfluramine, mCPP, oCPP ≥ mTFMPP ≫ trazodone, quipazine, MK-212. The large disparity in the releasing activities of mCPP and trazodone *in vitro* is interesting in view of the finding that mCPP is formed from trazodone *in vivo* [15, 16], thereby suggesting that the antidepressant is enzymatically transformed, in part, into a releasing agent after its peripheral administration. The reason for the large difference in releasing activities between these two compounds is unknown, but it may be related to the restricted entry of trazodone into the nerve terminal. Like tryptamine, pCA and fenfluramine [22], mCPP, oCPP and mTFMPP appear to release 5-HT by a non-exocytotic mechanism (see Ref. 23) involving the displacement of neuronal 5-HT; elimination of Ca^{2+} from the incubation system had only minimal effects on the 5-HT release by these compounds, while inclusion of the 5-HT-uptake blockers fluoxetine or chlorimipramine drastically reduced the drug-induced release. It has been suggested recently that 5-HT uptake blockers inhibit displacement-type release (i.e. by pCA) by preventing carrier-mediated efflux of 5-HT from the nerve terminal [22] which contrasts with the more commonly held belief that these compounds act by preventing drug entry into the neuron [24]. From the present results, however, we are unable to determine by which of these mechanisms fluoxetine and chlorimipramine are acting to inhibit the drug-induced release of 5-HT.

In studies from other laboratories, mCPP has been reported to be inactive [3] or only slightly active [12, 25] in releasing 5-HT *in vitro*. These results therefore differ from the present findings where

Table 3. Inhibition of the drug-induced release of 5-HT from hypothalamic slices by chlorimipramine (CIMI)

Treatment	5-HT released*	
	No CIMI (% change)	1 μM CIMI† (% change)
Control	0.42 ± 0.04	1.31 ± 0.06
p-Chloroamphetamine (1 μM)	7.27 ± 0.36‡ (+1630)	1.38 ± 0.08 (+5)
m-Chlorophenylpiperazine (1 μM)	5.52 ± 0.25‡ (+1310)	1.57 ± 0.13 (+20)
m-Trifluorophenylpiperazine (3 μM)	4.21 ± 0.17‡ (+1000)	1.49 ± 0.11 (+14)

* Values represent pmoles released · (mg protein)$^{-1}$ · (10 min)$^{-1}$ and are group means ± S.E.M (N = 4 points/group).
† CIMI was included where indicated during the 25-min preincubation period and the 10-min release period.
‡ $P < 0.05$ vs respective untreated controls.

Table 4. Calcium dependency of 5-HT release induced by halogenated phenylpiperazines*

Treatment	5-HT released†		
	$+Ca^{2+}$	$-Ca^{2+}$	% Change
Control	0.35 ± 0.04	0.24 ± 0.06	−31
p-Chloroamphetamine (1 μM)	7.07 ± 0.62‡	5.93 ± 0.73‡	−16
m-Chlorophenylpiperazine (1 μM)	5.62 ± 0.24‡	4.73 ± 0.63‡	−16
m-Trifluoromethylphenylpiperazine (3 μM)	4.96 ± 0.30‡	3.62 ± 0.40‡	−27

* Hypothalamic slices were treated as described in Methods except that, in the $-Ca^{2+}$ group, $CaCl_2$ was omitted throughout all washes and incubations, being replaced with Mg_2SO_4.

† Values represent pmoles released · (mg protein)$^{-1}$ · (10 min)$^{-1}$ and are group means ± S.E.M. (N = 3 points/group).

‡ $P < 0.05$ vs respective untreated controls (ANOVA, Dunnett's *t*-test). No significant differences were found between respective groups with or without calcium.

mCPP approached the activity of tryptamine and pCA and was equivalent or slightly higher in activity to fenfluramine. Reasons for this quantitative difference may be related to differences in sample preparation (i.e. whole brain synaptosomes vs hypothalamic slices) or other procedural distinctions (i.e. release of previously taken-up [^{3}H]5-HT or [^{14}C]5-HT vs endogenous 5-HT release). Indeed, we found that, in experiments which were run in parallel under identical conditions, mCPP (1–3 μM) was only about one-half as active in releasing previously taken-up [^{3}H]5-HT (uptake conditions: 0.1 μM [^{3}H]5-HT, 10 min at 37°) when compared to endogenous 5-HT release from hypothalamic slices. When measuring the release of total ^{3}H instead of [^{3}H]5-HT purified by HPLC, mCPP was only one-fourth to one-fifth as active in this respect. This quantitative difference in endogenous 5-HT release vs [^{3}H]5-HT release was observed only with displacement-type releasers such as pCA or mCPP. The magnitude of stimulated release induced by depolarization (i.e. by elevated K^+), however, was the same whether endogenous 5-HT release or release of previously taken-up [^{3}H]5-HT was measured (our unpublished observations).

The extent to which mCPP and other related halogenated phenylpiperazines release 5-HT *in vivo* after their peripheral administration is unknown. Indeed, the available neurochemical evidence suggests that these agents are primarily direct-acting 5-HT agonists in the brain. For instance, acute administration of mCPP or mTFMPP reduces 5-HT turnover in brain without affecting 5-HT concentrations [11, 13, 14]. Furthermore, these compounds inhibit [^{3}H]5-HT binding *in vitro* with K_i values in the 100 nM range [3, 11, 13, 14]. In one study, lesions of the median raphe nucleus which depleted forebrain 5-HT by 75% did not influence the serotonin-dependent anorexia induced by mCPP [3], thus arguing in favor of a mechanism involving direct interaction with 5-HT receptors. This interpretation, however, does not take into account the possible releasing effects of mCPP on the pool of 5-HT not affected by the lesion. In view of the fact that mCPP and mTFMPP do not alter 5-HT metabolism *in vivo* in a way characteristic of 5-HT releasers [11, 13, 14, 26], however, it is unlikely that the potent 5-HT-releasing property of halogenated phenylpiperazines *in vitro* is singularly responsible for their serotonergic effects *in vivo*. The possibility remains, however, that enhanced 5-HT release contributes in part to the overall effect of 5-HT receptor activation by these compounds. In fact, if one assumes single compartmentalization, the concentration of the drugs in the brain after peripheral injection of mCPP (2.5 to 10 mg/kg; [12, 14]) or mTFMPP (15 mg/kg; [11]) easily exceeds that necessary (1–3 μM) for 10-fold increases in release *in vitro*. Direct measurement of 5-HT release *in vivo* after the administration of the halogenated phenylpiperazines will be needed to determine whether, in fact, these compounds are active as 5-HT releasers *in vivo*.

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REFERENCES

1. J. B. Malick, E. Doreen and A. Barnett, *Pharmac. Biochem. Behav.* **6**, 325 (1977).
2. B. V. Clineschmidt, J. C. McGuffin and A. B. Pflueger, *Eur. J. Pharmac.* **44**, 65 (1977).
3. R. Samanin, T. Mennini, A. Ferraris, C. Bendotti, F. Borsini and S. Garattini, *Naunyn-Schmiedeberg's Archs Pharmac.* **308**, 159 (1979).
4. B. V. Clineschmidt, H. M. Handon, A. B. Pfleuger and J. C. McGuffin, *Psychopharmacology* **55**, 27 (1977).
5. M. Grabowski, L. Antkiewicz and J. Michaluk, *Biochem. Pharmac.* **23**, 3211 (1974).
6. R. W. Fuller, H. D. Snoddy, K. W. Perry, B. W. Roush, B. B. Molloy, F. P. Bymaster and D. T. Wong, *Life Sci.* **18**, 925 (1976).
7. J. H. Jacoby, R. A. Howd, M. S. Levin and R. J. Wurtman, *Neuropharmacology* **15**, 529 (1976).
8. A. R. Green, M. B. H. Youdim and D. G. Grahame-Smith, *Neuropharmacology* **15**, 173 (1976).
9. M. Hamon, S. Bourgoin, A. Enjalbert, J. Bockaert, F. Hery, J. P. Ternaux and J. Glowinski, *Naunyn-Schmiedeberg's Archs Pharmac.* **294**, 99 (1976).
10. B. V. Clineschmidt, *Gen. Pharmac.* **10**, 287 (1979).
11. R. W. Fuller, H. D. Snoddy, N. R. Mason and B. B. Molloy, *Eur. J. Pharmac.* **52**, 11 (1978).
12. R. Samanin, S. Caccia, C. Bendotti, F. Borsini, R. Borroni, R. Invernizzi, R. Pataccini and T. Mennini, *Psychopharmacology* **68**, 99 (1980).

13. R. Invernizzi, S. Cotecchia, A. DeBlasi, T. Mennini, R. Pataccini and R. Samanin, *Neurochem. Int.* **3**, 239 (1981).
14. R. W. Fuller, H. D. Snoddy, N. R. Mason and J. E. Owen, *Neuropharmacology* **20**, 155 (1981).
15. M. Melzacka, J. Boska and J. Maj, *J. Pharm. Pharmac.* **31**, 855 (1979).
16. M. H. Fong, S. Garattini and S. Caccia, *J. Pharm. Pharmac.* **34**, 674 (1982).
17. J. Maj, W. Palider and A. Rawlow, *J. neural Transm.* **44**, 237 (1979).
18. S. Caccia, M. Ballabio, R. Fanelli, G. Guiso and M. G. Zanini, *J. Chromat.* **210**, 311 (1981).
19. S. Caccia, M. H. Fong, M. Garattini and M. G. Zanini, *J. Pharm. Pharmac.* **34**, 605 (1982).
20. J. F. Reinhard, Jr., M. A. Moskowitz, A. F. Sved and J. D. Fernstrom, *Life Sci.* **27**, 905 (1980).
21. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
22. G. Maura, A. Gemignani, P. Versace, M. Martire and M. Raiteri, *Neurochem. Int.* **4**, 219 (1982).
23. M. Raiteri and G. Levi, *Rev. Neurosci.* **3**, 77 (1978).
24. R. W. Fuller, *Neurochem. Res.* **5**, 241 (1980).
25. T. Menini, E. Borroni, R. Samanin and S. Garattini, *Neurochem. Int.* **3**, 289 (1981).
26. E. C. Hwang and M. H. VanWoert, *Biochem. Pharmac.* **29**, 3163 (1980).