

Effects of mCPP on the Extracellular Concentrations of Serotonin and Dopamine in Rat Brain

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Intravenous administration of *m*-chloro-phenylpiperazine (mCPP) (0.25 or 2.5 mg/kg) induced a marked and dose-related increase in extracellular concentrations of serotonin in hippocampus (300–1,400% of baseline) as measured using *in vivo* microdialysis in awake male Wistar rats of the spontaneously hypertensive (SH) strain. Indicating that the effect of mCPP was caused by a reversal of the serotonin transporter, it was antagonized by pretreatment with the serotonin re-uptake inhibitor citalopram (10 mg/kg) but was unaffected by local administration of the sodium channel blocker tetrodotoxin (TTX; 1 μ m). mCPP was also shown to induce an increase in extracellular concentrations of dopamine in the nucleus accumbens and the striatum of SH rats and in the nucleus accumbens of rats of the Sprague–Dawley (SD) strain; this effect of mCPP was,

however, much weaker (125–170% of baseline) than the effect on serotonin; moreover, it seems to be TTX-sensitive. In anesthetized SD rats, mCPP induced a moderate reduction of nigral dopamine cell firing rate; supporting the assumption that this effect is secondary to the observed increase in dopamine release, it was blocked by pretreatment either with the dopamine synthesis inhibitor α -methyl-para-tyrosine or with the dopamine D₂ receptor antagonist haloperidol. In conclusion, the results suggest that mCPP induces a marked, TTX-insensitive increase in serotonin release in rat brain, but only a modest and TTX-sensitive increase in the extracellular levels of dopamine.

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m-Chloro-phenylpiperazine (mCPP), the metabolite of the antidepressant drug trazodone, has recently gained marked attention as a putative probe of serotonergic function in clinical psychiatric research. Thus, the prolactin, ACTH, and cortisol responses to mCPP frequently have been used as putative markers of postsyn-

aptic serotonin receptor function in various psychiatric disorders. Moreover, administration of mCPP has been shown to elicit, and/or aggravate, anxiety attacks in patients with panic disorder, obsessions in patients with obsessive compulsive disorder (OCD), elation in alcoholics and cocaine addicts, and positive symptoms in schizophrenic patients (see Murphy et al. 1991; Kahn and Wetzler 1991; Krystal et al. 1993; Buydens-Branchey et al. 1993; Krystal et al. 1994).

mCPP displays high affinity to 5-HT_{2C} receptors and moderate, or low, affinity to 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT₃ receptors; in addition, the compound has some affinity for α -2-adrenoceptors. At the 5-HT_{2C} receptor, mCPP seems to act as a partial agonist with relatively high intrinsic efficacy, whereas, at the other serotonin receptors, the intrinsic efficacy of mCPP is moderate or low (see Murphy et al. 1991; Kahn

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and Wetzler 1991). The behavioral and endocrine effects of mCPP observed in clinical studies are generally attributed to the direct effects of mCPP on various subtypes of serotonin receptors; in contrast, little attention has been paid to the report by Baumann et al. (1993) suggesting that mCPP may also stimulate serotonin release by means of an interaction with the serotonin transporter (see also Baumann et al. 1995).

To further elucidate the possible influence of mCPP on the release of serotonin, we studied the effects of a high dose of mCPP on extracellular, hippocampal concentrations of serotonin in awake, freely moving rats using *in vivo* microdialysis.

Enhanced dopaminergic neurotransmission has been suggested as a mechanism of importance both for the positive symptoms of schizophrenia and for the euphoria induced by such central stimulants as cocaine. The observations that mCPP may aggravate psychotic symptoms in schizophrenic patients and cause elation in cocaine addicts—in conjunction with the data of Benloucif and Galloway (1991) suggesting that local administration of mCPP within the striatum of anesthetized rats leads to a marked increase in dopamine release—prompted us to investigate to what extent extracellular levels of dopamine in awake rats are influenced by systemic administration of mCPP. In addition, the effect of mCPP on the firing rate of dopaminergic neurons was investigated.

We recently found that spontaneously hypertensive (SH) rats are more reactive than other rat strains to stimuli causing anxiety attacks in panic disorder patients (such as sodium lactate and yohimbine) (see Wikander et al. 1995); hence, we also used SH rats for experiments on the mechanism of action of mCPP. Therefore, most of the microdialysis experiments presented in this paper were performed on SH rats; however, for the electrophysiological studies, and for some of the microdialysis experiments, rats of the Sprague–Dawley (SD) strain were used. As stated in the Discussion section, the results obtained in these experiments, and in studies by others, suggest that mCPP exerts qualitatively similar effects on extracellular levels of monoamines in the two strains investigated. The possibility that SH rats and SD rats differ quantitatively with respect to how they respond to mCPP administration cannot be excluded; however, to compare the two strains in this respect was not within the scope of this study.

MATERIALS AND METHODS

In Vivo Microdialysis/High-Performance Liquid Chromatography

Male SH rats (250–300 g) were purchased from Møllegaard, Denmark; male SD rats (250–300 g) were purchased from ALAB (Sollentuna, Sweden). Before the ex-

periments, the rats were kept under controlled conditions (temperature 21–22°C, humidity 55–65%, light on from 5 a.m. to 7 p.m.) with five animals in each cage.

The *in vivo* microdialysis was performed essentially as described by Waters et al. (1993) (dopamine) and Tao and Hjorth (1992) (serotonin). For the analysis of dopamine release in the nucleus accumbens and the striatum, an I-shaped probe was used (Santiago and Westerink, 1990), whereas, for the investigation of serotonin release in hippocampus, the probe used was U-shaped (Tao and Hjorth, 1992). The length of the exposed tip of the dialysis membrane was 3 mm (I-type) and 6 mm (U-type), respectively. The probes were implanted during anesthesia with the rats mounted into a stereotaxic instrument using the following coordinates, relative to bregma, according to Paxinos and Watson (1986): A/P +1.0, L/M –2.6, and V/D –6.2 (striatum), A/P +1.85, L/M –1.6, and V/D –7.8 (nucleus accumbens), and A/P –4.6, L/M +4.6, V/D –8.6 (hippocampus). During anesthesia, immediately before the implantation of the dialysis probe, a cannula was implanted into the vena jugularis. The rats were allowed to recover individually in spherical cages for 24–48 h before the experiment.

On the day of experiment, the rat was placed in an open cage, allowing it to move about freely. The sealed inlet and outlet of the probe were cut open with a scalpel, and the inlet cannula was connected to a perfusion pump. In the striatum and accumbens dialysis experiments, the probe was perfused with a Ringer solution, containing NaCl 140 mm, CaCl₂ 1.2 mm, KCl 3.0 mm, and MgCl₂ 1.0 mm (pH 5.6) (Moghaddam and Bunney 1989), at a rate of 2 µl/min. In the hippocampal dialysis experiments, the probe was perfused with artificial CSF (pH 7.4) (for composition, see Tao and Hjorth, 1992) at a rate of 1.3 µl/min. Samples of dialysate were collected for high-performance liquid chromatography (HPLC) analysis at 20 min intervals.

The dialysate was split and analyzed using two different chromatographic systems; a cation-exchange system for dopamine and serotonin and a reverse-phase system for the metabolites (DOPAC and 5-HIAA) according to a method developed by Lagerkvist (1991). On a 10-port injector with two sample loops, the main part of the sample was introduced to the cation-exchange system (26 µl), and a smaller fraction (11 µl) was introduced to the reverse-phase system. Following the chromatography, the detections were made by two separate electrochemical detectors. The cation-exchange system consisted of an HPLC-pump (LKB 2150, Pharmacia LKB Biotechnology Sverige AB, Sollentuna, Sweden), a pulse damper (Tillquist Analys AB, Kista, Sweden), a stainless steel column (0.21 × 15 cm) packed with Nucleosil, SA, 5 µm (Macherey-Nagel, Düren, FRG), and an amperometric detector (Waters 460, Millipore Waters, Milford, MA, USA) operated at 0.55 V versus Ag/AgCl.

The resulting current was monitored by an integrator (Hewlett-Packard integrator 3395A, USA). The mobile phase consisted of citric acid 0.049 M, NaOH 0.114 M, methanol 20%, and Na₂-EDTA 0.012 M. The flow rate was 0.25 ml/min. The reverse-phase system consisted of an HPLC pump (Milton Roy miniPump VS, Riviera Beach, FL, USA), a stainless steel column (0.21 × 5 cm) packed with Nucleosil, RP-18, 3 μm (Macherey-Nagel, Düren, FRG), and an amperometric detector (Toli Elektronik, Göteborg, Sweden) operated at 0.80 V versus Ag/AgCl. The detections were carried out by a thin layer cell (TL-3 BAS, West Lafayette, IN, USA), a dual glassy carbon working electrode (TL-5A, BAS), and an Ag/AgCl reference electrode. The resulting current was monitored by an integrator (Spectra-Physics Sp 4270, San Jose, CA, USA). The mobile phase consisted of 0.010 M K₂HPO₄, 0.040 M citric acid, 0.012 M Na₂-EDTA and 5% methanol. The flow rate was 0.20 ml/min.

After serotonin and dopamine levels in the dialysate had been stable for at least 1 hour, mCPP (2.5 mg/kg) (Research Biochemicals International, Natick, MA, USA; dissolved in saline) or NaCl was administered intravenously in a volume of 0.5 ml. In one experiment, citalopram (10 mg/kg; H. Lundbeck A/S, Copenhagen; dissolved in saline) was administered subcutaneously 100 min before mCPP. In some experiments, the sodium channel blocker tetrodotoxin (TTX) was added to the perfusion fluid (1 μM).

Electrophysiology

Male Sprague–Dawley rats (250–300 g) were purchased from ALAB (Sollentuna, Sweden) and kept under the same conditions as described above. At the day of the experiment, the rats were anesthetized (chloral hydrate, 400 mg/kg, IP) and mounted in a conventional stereotaxic frame. mCPP and additional anesthetic were given through a lateral tail vein. Throughout the experiments, the body temperature of the animals was maintained at 37°C by means of a heating pad. The skull surface was exposed, and a 3-mm burr hole was drilled with its center located approximately 2 mm anterior to lambda and 2 mm lateral to the midline. The dura was carefully removed and a micropipette (tip diameter approximately 1–2 μm) filled with 2 M NaCl saturated with pontamine sky blue was lowered by means of a hydraulic microdrive (David Kopf Instr., Tujunga, CA, USA) into the region of substantia nigra, zona compacta. The in vitro impedance of the electrodes were 3 to 8 MΩ, measured in saline at 135 Hz. Single unit potentials were passed through a high input impedance amplifier and filters. The impulses were discriminated from background noise and fed into a digital counter, which was reset every 10 s, and finally displayed on a storage oscilloscope (TRIO, Tokyo, Japan), a computer (Macintosh II), an audiomonitor (Grass Instr., Quincy,

MA, USA), and a strip chart recorder (Gould Instr., Cleveland, Ohio, USA). The neurophysiological characteristics of the cells were identical to those previously described for dopaminergic neurons of substantia nigra, zona compacta (Grace and Bunney, 1983). The position of the electrode was marked at the end of each experiment by iontophoretic ejection of pontamine sky blue. The rats were then perfused with 10% buffered formalin solution and the brains were subjected to conventional histological procedures. Only results derived from rats with a correctly positioned recording electrode were included in the statistical analysis. Some rats were pretreated with *α*-methyl-para-tyrosine (*α*-MT, dissolved in saline, 250 mg/kg, SC, 40–80 min) or haloperidol (dissolved in a few drops of glacial acetic acid and diluted with 5% glucose solution, 0.1 mg/kg, IV, 5 min) before the administration of mCPP.

Presentation of Data/Statistics

The difference between the mean concentration of the two samples obtained after the intravenous injection (NaCl/mCPP/citalopram) and the mean concentration of the two samples obtained immediately before the injection was calculated and used as a measure of drug-induced change in extracellular transmitter concentrations (= "delta values"); in addition, when possible, the mean concentration of the two samples obtained after drug administration was expressed as a percentage of the mean of the two baseline values (= "% of baseline"). Different treatment groups were compared with respect to delta values using nonparametric statistics (>2 groups: Kruskal–Wallis test followed by Mann–Whitney U-test; 2 groups: Mann–Whitney U-test); repeated measures within a group were compared using Wilcoxon signed rank test. For clarity, in the figures group data are presented as means with standard errors.

RESULTS

Microdialysis/Hippocampus (Serotonin)

Baseline levels of serotonin in dialysate samples obtained from the hippocampus varied between 0.2 and 1 fmol/μL. Whereas intravenous administration of 0.9% NaCl (0.5 ml) did not influence the extracellular concentrations of serotonin (percentage of baseline: 120 ± 18), infusion of mCPP (2.5 mg/kg; IV; 0.5 ml) elicited a marked increase in the dialysate serotonin concentrations (percentage of baseline: 996 ± 302) (Figure 1A). Administration of citalopram (10 mg/kg; SC) also caused a significant increase in the dialysate concentrations of serotonin (percentage of baseline: 413 ± 44); when mCPP was administered (2.5 mg/kg; IV, 0.5 ml) 100 min after the injection of citalopram, no further increase in dialysate serotonin levels was observed (per-

centage of baseline: 107 ± 7) (Figure 1B). In rats given the sodium channel blocker TTX in the dialysis perfusion fluid, baseline dialysate serotonin concentration was below the detection limit; the mCPP (2.5 mg/kg IV; 0.5 ml)-induced increase in dialysate serotonin concentrations was, however, comparable to that observed in rats not given TTX (Figure 1C). For statistical differences between groups, see legend to Figure 1.

In a separate experiment, examining two different doses of mCPP (0.5 and 2.5 mg/kg; 0.5 ml), the effect of mCPP on dialysate serotonin concentrations was found

to be dose-related (Figure 2) (percentage of baseline: $315 \pm 44\%$ and $1420 \pm 447\%$, respectively) (for statistics, see legend to Figure 2). No consistent effects of mCPP on the dialysate concentrations of the serotonin metabolite 5-HIAA were observed (data not shown).

Microdialysis/Nucleus Accumbens (Dopamine and Serotonin)

Baseline levels of dopamine in dialysate samples obtained from the nucleus accumbens of SH rats varied between 0.6 and 4 fmol/ μ l. Whereas intravenous administration of 0.9% NaCl (0.5 ml) did not markedly influence the extracellular concentrations of dopamine (percentage of baseline: 107 ± 4), infusion of mCPP (2.5 mg/kg; IV; 0.5 ml) induced a moderate but significant increase in the dialysate dopamine concentration (percentage of baseline: $171 \pm 28\%$); this effect was completely counteracted when TTX was added to the dialysis perfusion fluid (percentage of baseline: 107 ± 11) (Figure 3A). For statistical significances between groups, see legend to Figure 3.

After administration of mCPP, concentrations of the dopamine metabolite DOPAC in dialysate obtained from the nucleus accumbens were moderately, but significantly, increased (mean \pm SEM of two samples obtained before and after mCPP, respectively: 339 ± 20 and 378 ± 24 ; $n = 12$; $z = 2.7$; $p < .01$; Wilcoxon signed rank test).

A moderate, but significant, increase in extracellular dopamine levels following administration of mCPP was also observed in rats of the SD strain (percentage of baseline: mCPP: 138 ± 8 , NaCl: 105 ± 3) (Figure 3B). Baseline levels of dopamine in dialysate samples ob-

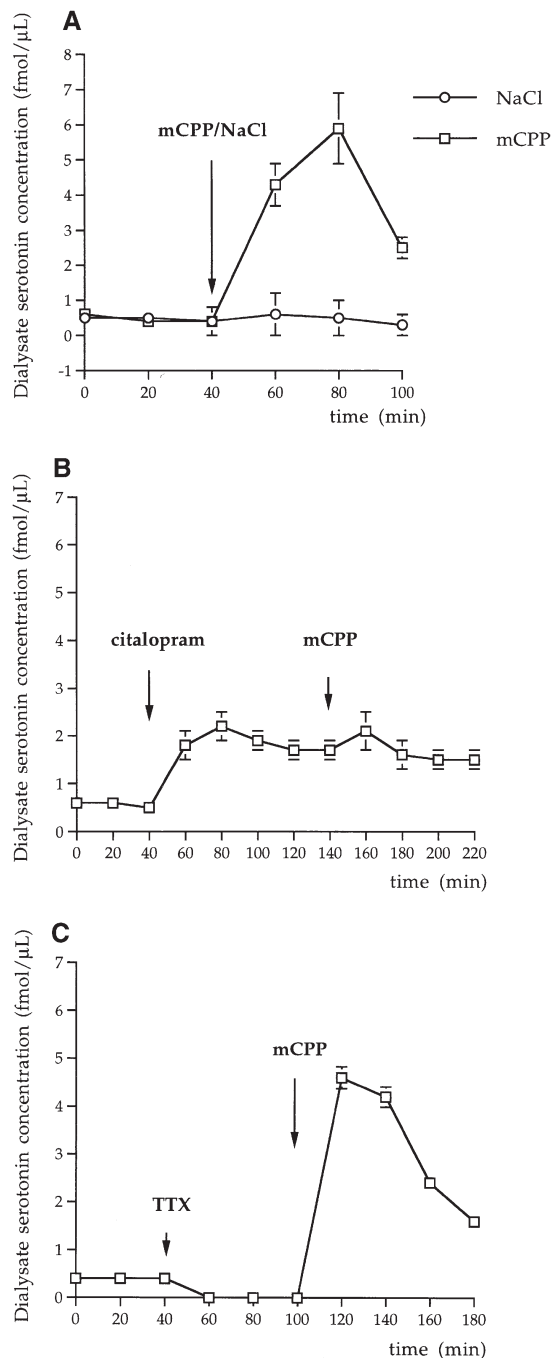


Figure 1. mCPP induces a citalopram-sensitive but TTX-insensitive increase in extracellular concentrations of serotonin in rat hippocampus. Shown is the concentration of serotonin in dialysate samples obtained every 20 min from the hippocampus of awake SH rats administered (A) NaCl ($n = 6$) or mCPP (2.5 mg/kg, IV) ($n = 8$), (B) citalopram (10 mg/kg SC) + mCPP (2.5 mg/kg, IV) ($n = 6$), or (C) TTX (in the dialysate probe) + mCPP (2.5 mg/kg, IV) ($n = 4$). Values represent means \pm SEM. The mCPP- (or NaCl)-induced changes in dialysate serotonin concentrations in the different groups were expressed as the difference between the mean serotonin concentration of the two samples obtained after the administration of mCPP (or NaCl) and the mean serotonin concentration of the two samples obtained immediately before the administration of mCPP (or NaCl), and compared by means of Kruskal-Wallis test followed by Mann-Whitney U-test. H-value: 17.4 ($p = .0006$). NaCl vs. mCPP: U-value 0.0 ($p = .002$). NaCl vs. citalopram + mCPP: U-value 1.5 (ns). NaCl vs. TTX + mCPP: U-value 0.0 ($p = .01$). mCPP vs. citalopram + mCPP: U-value 0.0 ($p = .002$). mCPP vs. TTX + mCPP: U-value 12.0 (ns).

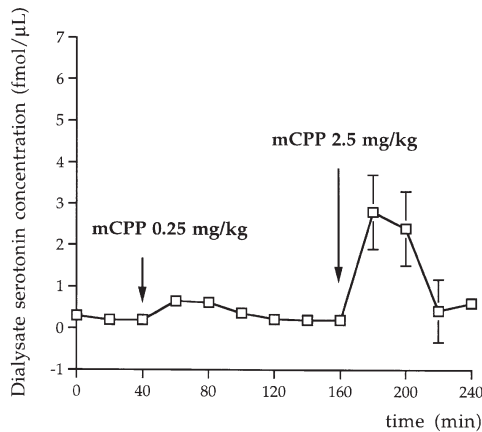


Figure 2. The effect of mCPP on extracellular concentrations of serotonin in hippocampus is dose-related. Shown is the effect of intravenous administration of two different doses of mCPP (0.25 and 2.5 mg/kg, respectively) on the concentration of serotonin in dialysate samples obtained every 20 min from the hippocampus of awake SH rats ($n = 5$). Values are given as means \pm SEM. The difference between the mean of the two samples obtained after the administration of the low and high dose of mCPP, respectively, was statistically evaluated by means of the Wilcoxon Signed Rank Test ($z = 2.0, p = .04$).

tained from the nucleus accumbens of SD rats varied between 0.8 and 4.5 fmol/ μ L. In the same experiment, mCPP was shown to induce a marked increase in serotonin levels in the nucleus accumbens of SD rats (mCPP: baseline levels: 0.13 ± 0.02 fmol/ μ L, post-treatment levels: 0.81 ± 0.1 fmol/ μ L, percentage of baseline: $693 \pm 81, n = 10$; NaCl: baseline levels: 0.16 ± 0.02 fmol/ μ L, post-treatment levels: 0.14 ± 0.02 fmol/ μ L, percentage of baseline: $95 \pm 6, n = 13$; difference between groups with respect to Δ values: $p < .0001$) (rats displaying nondetectable levels of serotonin before drug administration were excluded). The maximal baseline level of serotonin in a dialysate sample obtained from the nucleus accumbens of SD rats was 0.4 fmol/ μ L.

Microdialysis/Striatum (Dopamine)

Baseline levels of dopamine in dialysate samples obtained from the striatum of SH rats varied between 4 and 10 fmol/ μ L. Whereas intravenous administration of 0.9% NaCl (0.5 ml) did not significantly increase the extracellular concentrations of dopamine, administration of mCPP (2.5 mg/kg) moderately but significantly enhanced dialysate dopamine concentrations (mCPP: baseline levels: 4.9 ± 0.7 fmol/ μ L, post-treatment levels: 5.8 ± 0.6 fmol/ μ L, percentage of baseline: $123.8, n = 10$; NaCl: baseline levels: 6.4 ± 1.4 fmol/ μ L, post-treatment levels: 6.0 ± 3.0 fmol/ μ L, percentage of base-

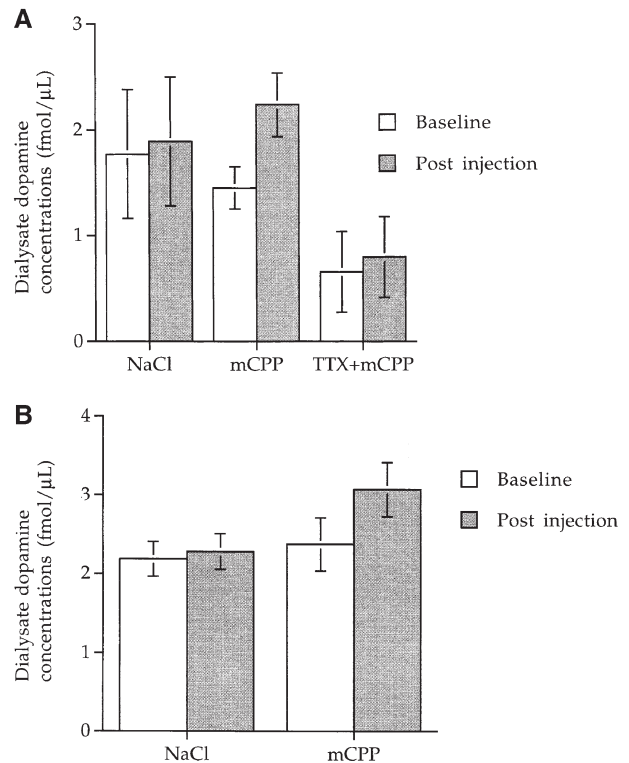


Figure 3. mCPP induces a weak increase in extracellular concentrations of dopamine in rat nucleus accumbens and striatum. Shown is the effect of administration of mCPP (2.5 mg/kg, IV, 0.5 ml) or NaCl (0.5 ml) on the concentration of dopamine in dialysate samples obtained every 20 min from the nucleus accumbens of SH (A) or SD (B) rats. Bars represent the mean dopamine concentration of the two samples obtained before the administration of mCPP (or NaCl) and the mean dopamine concentration of the two samples obtained after the injection (group means \pm SEM). In the experiment shown in graph A, a third group was given TTX-containing dialysate solution + mCPP. In SH rats (graph A), the delta value (= the difference between the two samples obtained after and before injection, respectively) was significantly larger in rats receiving mCPP ($n = 15$) than in those treated with NaCl ($n = 5$) ($p = .008$) or with TTX + mCPP ($n = 4$) ($p = .03$); the groups given NaCl and TTX + mCPP, respectively, did not differ significantly. In SD rats (graph B), the delta value was significantly larger in rats receiving mCPP ($n = 12$) than in those treated with NaCl ($n = 21$) ($p = .0006$). Statistics: Kruskal–Wallis test followed by the Mann–Whitney U-test (A), or Mann–Whitney U-test only (B).

line: $95 \pm 3, n = 13$; difference between groups with respect to Δ values: $p = .03$).

After administration of mCPP, concentrations of the dopamine metabolite DOPAC in dialysate obtained from the striatum were moderately but significantly increased (mean \pm SEM of two samples obtained before and after mCPP, respectively: 567 ± 69 and $633 \pm 66; n = 9; z = 2.5; p < .05$; Wilcoxon signed rank test).

Electrophysiology

Intravenous administration of mCPP was associated with a moderate, but dose-dependent, inhibition of all nigral dopaminergic neurons recorded from (Figure 4). Maximal effect (30% inhibition) was obtained following a dose of 0.5 mg/kg. Intravenous administration of mCPP in doses higher than 0.5 mg/kg was associated with a high degree of mortality, probably caused by an interaction between the drug and chloral hydrate. The mCPP-induced inhibition of dopamine cell firing rate was effectively antagonized by pretreatment either with the dopamine synthesis inhibitor α -methyl-para-tyrosine (α -MT, 250 mg/kg, 40–80 min) or with the dopamine D₂ receptor antagonist haloperidol (0.1 mg/kg, IV, 5 min) (Figure 4).

DISCUSSION

The present study shows that systemic administration of mCPP effectively increases extracellular concentrations of serotonin in the hippocampus of SH rats. As expected (Sabol et al. 1992b; Rutter and Auerbach, 1993), a high dose of the serotonin reuptake inhibitor (SRI) citalopram also caused increased extracellular concentrations of serotonin in the hippocampus; however, this effect was less pronounced than that of mCPP.

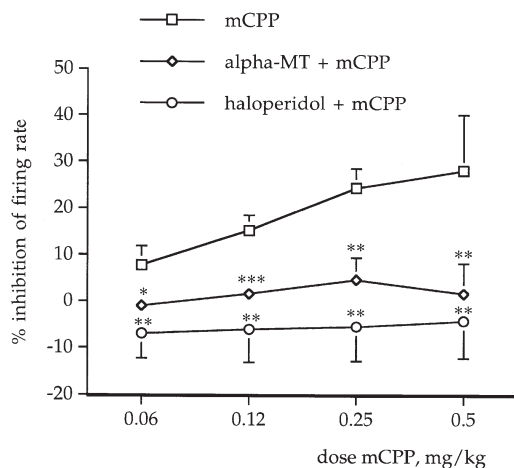


Figure 4. mCPP-induced reduction in the firing rate of nigral dopamine neurons is antagonized by dopamine synthesis inhibition. Cumulative dose response curve showing the inhibitory action of intravenously administered mCPP on the firing rate of nigral dopamine neurons in control rats and in rats pretreated with α -methyl-p-tyrosine (α -MT) (250 mg/kg, IP, 40–80 min) or haloperidol (0.1 mg/kg, IV, 5 min). Each point represents the mean \pm SEM; $n = 4$ –10 in each group. The three groups were statistically compared using the Kruskal–Wallis test followed by the Mann–Whitney U-test (* $p < .05$; ** $p < .001$; *** $p < .001$).

The finding that the serotonin release induced by mCPP was counteracted by pretreatment with citalopram indicates that it is not attributable to an interaction with serotonergic autoreceptors; thus, if the effect of mCPP was attributable to autoreceptor antagonism, it should be potentiated, rather than attenuated, by pretreatment with an SRI. The previous observation that the serotonin release induced by fenfluramine is prevented by pretreatment with reuptake inhibitors (Sabol et al. 1992b) has led to the hypothesis that fenfluramine, in an amphetamine-like manner (Butcher et al. 1988), causes serotonin release by reversing the transport mechanism normally removing the transmitter from the synaptic cleft; the present data indicate that mCPP has a similar mode of action. The concept that mCPP-induced serotonin release is not dependent upon exocytosis lends further support from the finding that it was not modified by co-administration of the sodium channel blocker TTX; similarly, fenfluramine- and amphetamine-induced serotonin release is not affected by TTX (Carboni and Di Chiara 1989; Matos et al. 1990).

The finding that citalopram prevents mCPP-induced serotonin release may offer an explanation to the unexpected finding of Mai and Moryl (1992) that acute pretreatment with either of the two SRIs sertraline or citalopram antagonizes mCPP-induced decrease in locomotion. Of interest in this context is also the observation that the symptom aggravation induced by mCPP in patients with OCD is prevented by subchronic pretreatment with SRIs (Zohar et al. 1988; Hollander et al. 1991); whether also acute pretreatment with an SRI may prevent the clinical effects of mCPP has, to our knowledge, not been investigated.

After we had undertaken the serotonin microdialysis experiments presented in this paper, a fluoxetine-reversible stimulatory effect of mCPP on serotonin release in the diencephalon of SD rats was reported by Bauman and co-workers (1993). The magnitude of the effect of mCPP in that study was comparable to that shown in this report; thus, the effect of mCPP seems to be qualitatively the same in SD rats as in SH rats. The assumption that a marked effect of mCPP on extracellular levels of serotonin may be induced not only in SH rats but also in rats of the SD strain lends further support from the present finding of a marked mCPP-induced increase in extracellular concentration of serotonin in the nucleus accumbens of SD rats.

A serotonin-releasing effect of mCPP, mediated by the serotonin transporter, also gains support from previous *in vitro* studies by Pettibone and Williams (1984); thus, in hypothalamic slices, mCPP, as did fenfluramine, was shown to cause enhanced release of serotonin. Moreover, the mCPP analog 1-(*m*-trifluoromethylphenyl)piperazine (TFMPP) also has been shown to induce serotonin release both *in vitro* (Benloucif and Galloway 1991) and *in vivo* (Auerbach et al. 1991). In

contrast, mCPP does not induce serotonin release from human platelets (Carver et al. 1993).

When interpreting the data obtained in clinical studies using mCPP as a pharmacological tool, the possibility that the effects of the drug, at least partly, are caused by the release of endogenous serotonin should be considered. Thus, whereas previous discussions on the mode of action of mCPP have focused on the serotonin receptor subtypes for which the compound displays affinity, the finding that mCPP is an indirect serotonin agonist implies that *all* serotonin receptors situated in the vicinity of serotonergic nerve terminals, and not concomitantly antagonized by mCPP, may be activated by the drug. That an influence of mCPP on the serotonin transporter may be of importance for the functional effects of the drug was supported by the observation of Baumann and co-workers (1993) that mCPP-induced increase in prolactin release is partially counteracted by acute pretreatment with fluoxetine. Confirming that the effects of mCPP on the serotonin transporter may be of significance not only in rat but also in humans, mCPP was recently reported to display affinity for the human serotonin transporter (Baumann et al. 1995).

Given the similarity in pharmacological profile between mCPP and fenfluramine, it is of interest that fenfluramine, as mCPP, has been reported to induce anxiety attacks in panic disorder patients (Targum and Marshall 1989) and to aggravate psychotic symptomatology (Marshall et al. 1989; Soper et al. 1990). Of interest in this context is also the fact that high doses of serotonin reuptake inhibitors may cause an initial aggravation of anxiety when administered to patients with panic disorder (George et al. 1995; see also Eriksson and Humble 1990). Thus, although long-term facilitation of serotonergic neurotransmission effectively reduces panic attacks and obsessive compulsive symptomatology (see Eriksson and Humble 1990), an abrupt increase in synaptic serotonin concentrations may exert the opposite effects.

Despite its marked serotonin-releasing effect, mCPP, in contrast to fenfluramine (Trulson and Jacobs 1976), does not induce the so-called serotonergic behavioral syndrome in rats (Lucki et al. 1989; Eriksson and Nissbrandt, unpublished observation) unless given at doses tenfold higher than those used in the present study (Freo et al. 1990). Possibly, this failure of mCPP to elicit serotonin-related behavior is attributable to the fact that the drug acts as an antagonist, or a partial agonist, at several postsynaptic receptor subtypes (such as 5-HT_{1A} and 5-HT₂) (Murphy et al. 1991; Kahn and Wetzler 1991), hence protecting these receptors from the increased synaptic concentration of transmitter.

Another important difference between fenfluramine and mCPP is that the former (Ivernizzi et al. 1991; Sabol et al. 1992a) but not the latter (Fuller et al. 1981; Ulrichsen et al. 1992) drug induces both an acute and a long-

lasting reduction in brain levels of serotonin. The acute reduction of brain serotonin concentration after administration of fenfluramine is probably caused by an increased activation of synthesis-regulating autoreceptors (Gardier et al. 1992); the inherent partial autoreceptor antagonism displayed by mCPP may, thus, explain why this compound does not cause the same effect. The mechanism for the long-lasting neurotoxic effects of fenfluramine is still a matter of controversy. Previous reports have provided indirect support for the assumption that the neurotoxic effects of fenfluramine and related serotonin-releasing compounds are not caused by the serotonin release *per se* (Hekmatpanah et al. 1989; Huang et al. 1992); the finding that mCPP is a forceful serotonin releaser without causing neurotoxic effects lends further support for this concept.

In the present study, using awake and freely moving SH rats, systemically administered mCPP induced a moderate, but significant, increase in the extracellular levels of dopamine in the nucleus accumbens and in the striatum; a similar effect was observed also in rats of the SD strain. Likewise, local perfusion of mCPP has previously been shown to induce an increase in dopamine release in the striatum of anesthetized rats (Benloucif and Galloway 1991). The effect of mCPP on extracellular dopamine levels was modest, as compared to the effect on serotonin; thus, whereas the dialysate serotonin concentrations after mCPP administration (2.5 mg/kg) were always >600% of baseline, dopamine concentrations increased to 120–170% of baseline only. The functional significance of such a weak increase in dopamine levels may be questioned; however, given the putative involvement of dopamine in schizophrenia and reward mechanisms, the possibility that a dopamine-releasing effect of mCPP may be of importance for the behavioral effects observed after administration of the drug to patients with schizophrenia (Iqbal et al. 1991; Kahn et al. 1992; Krystal et al. 1993) and drug addiction (Benkelfat et al. 1991; Buydens-Branchey et al. 1993; Krystal et al. 1994) should not be excluded.

The finding that the effect of mCPP on dopamine release in the nucleus accumbens was abolished by coadministration of TTX suggests that this effect is not caused by a reversal of the dopamine transporter but to a calcium-dependent mechanism. However, the firing rate of dopaminergic neurons in the ventral tegmental area (VTA) (Prisco et al. 1994) and substantia nigra (Kelland et al. 1990; this study) of anesthetized rats is reduced rather than increased after administration of mCPP; thus, if the influence of mCPP on dopamine cell firing is the same in awake rats as in anesthetized animals, the enhanced concentration of extracellular concentrations of dopamine in the nucleus accumbens and the striatum is not attributable to an increase in dopamine cell firing. Rather, it may be suggested that the effect of mCPP on dopamine cell firing is secondary to

the elevated extracellular concentrations of dopamine observed in these experiments and in the report by Benloucif and Galloway (1991). The present observations that the mCPP-induced reduction in nigral dopamine cell firing rate was effectively counteracted by pretreatment either with a dopamine synthesis inhibitor (α -methyl-tyrosine) or with a dopamine D₂ receptor antagonist (haloperidol) lend some support for this assumption.

When comparing the data from the *in vivo* microdialysis experiments with those from the electrophysiological study, the fact that an inhibition of the firing rate of nigral dopamine neurons was obtained with doses of mCPP (0.25–0.5 mg/kg) considerably lower than the dose (2.5 mg/kg) shown to cause an increase in dopamine levels should be taken into consideration. Also, it should be emphasized that the electrophysiology experiments were undertaken in anesthetised animals, whereas, awake rats were used for microdialysis. Indeed, the marked increase in dopamine release observed in a previous study after local administration of mCPP into the striatum of anesthetised rats (Benloucif and Galloway 1991) does suggest that the effect of mCPP on dopamine release may be more pronounced in anesthetised than in awake animals.

The possibility that the effect of mCPP on dopamine concentrations in the terminal region of the dopaminergic nerve cells is attributable to a direct or indirect interaction with serotonergic receptors should not be excluded. Indeed, several previous studies indicate that serotonin may exert a stimulatory influence on dopaminergic synapses in striatum and nucleus accumbens (Benloucif and Galloway 1991; Nissbrandt et al. 1992; Yashimoto and McBride 1992; Parsons and Justice 1993; Yadid et al. 1994; however, see also Ugedo et al. 1989; Dewey et al. 1995).

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