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Pharmacologic Evaluation of the Discriminative Stimulus of Metachlorophenylpiperazine

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BOURSON, A., D. WANNER, R. WYLER, N. PETIT, C. ZWINGELSTEIN, A. RUDLER AND A. J. SLEIGHT. *Pharmacologic evaluation of the discriminative stimulus of metachlorophenylpiperazine.* PHARMACOL BIOCHEM BEHAV **53**(1) 107-114, 1996. – A pharmacologic analysis of the discriminative stimulus of metachlorophenylpiperazine (mCPP) is reported. mCPP and *m*-trifluoromethylphenylpiperazine generalised, whereas 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole, 6-chloro-2-(1-piperazinyl)-pyrazine, and mesulergine partially generalised to the mCPP discriminative cue. However, although mianserin, methiothepin, ritanserin, mesulergine and *N*-(1-methyl-5′-indolyl)-*N*′-(3-pyridyl)urea hydrochloride (SB 200646) all antagonised the effect of 5-hydroxytryptamine (5-HT) on IP_3 formation in the rat choroid plexus, they failed to antagonise the mCPP response in the drug discrimination studies. The 5-HT_3 receptor antagonist MDL 72222 neither generalised nor antagonised the mCPP cue. These data suggest that neither the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_3, 5-HT_5, 5-HT_6, nor 5-HT_7 receptors are involved. The response does appear to be mediated by a postsynaptic 5-HT receptor, however, because fenfluramine generalised to the cue. Haloperidol generalises, and amphetamine partially antagonises the mCPP discriminative cue and low doses of apomorphine partially generalises to the mCPP cue, which suggests that a decrease in dopamine neurotransmission may also be involved.

mCPP   Drug discrimination   PI turnover   5-HT receptors 5-HT_{2C} receptor   Dopamine neurotransmission

METACHLOROPHENYLPIPERAZINE (mCPP) is a metabolite of the antidepressant trazodone and has been claimed to be an agonist at some 5-hydroxytryptamine (5-HT) receptor subtypes (17). It has been used to characterise pharmacologic responses in rats and in numerous human pharmacologic studies investigating the role of 5-HT_{2C} receptors in a number of clinical conditions such as depression and anxiety (16,17). The pharmacologic selectivity of mCPP has been examined using radioligand binding studies and has been shown to have a 10-fold selectivity for the 5-HT_{2C} receptor with respect to other receptors (14). In functional studies, mCPP appears to be an agonist at 5-HT_{2C} receptors (2,8,9,17) and an antagonist at 5-HT_{2A} and 5-HT_3 receptors (8,21).

It has been previously reported that mCPP induces a discriminative stimulus in rats that may be mediated by 5-HT_{2C} receptors (6,24). This classification was based on the ability of *m*-trifluoromethylphenylpiperazine (TFMPP), 5-methoxy-3-(1,2,3,6-tetrahydro-4-pyridinyl)-1H-indole (RU 24969), 6-chloro-2-(1-piperazinyl)-pyrazine (MK-212), 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and *d*-lysergic acid diethylamide (LSD) to substitute for mCPP (11,24) and the ability of the nonselective 5-HT antagonist metergoline to antagonise the discriminative cue (6). The purpose of the present study was to determine whether the mCPP discriminative cue is indeed mediated through a 5-HT_{2C} receptor by attempting to antagonise the discriminative cue with a number of antagonists including the recently characterised 5-HT_{2B}/5-HT_{2C} antagonist *N*-(1-methyl-5′-indolyl)-*N*′-(3-pyridyl)urea hydrochloride (SB 200646) (12,18). The effects of some of these antagonists in the drug discrimination paradigm were compared with their effects on IP_3 production in the choroid plexus, a classical 5-HT_{2C} receptor-mediated response (9). In addition, because mCPP shares behavioural effects with dopamine (DA) receptor agents (1,4,13,19), and as some nonse-

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lective 5-HT antagonists have activity at DA receptors [e.g., mesulergine (7)], we also studied the interaction of mCPP with DA neurotransmission using the amphetamine drug discrimination paradigm.

Some of the behavioural results have been presented in abstract form to the British Pharmacological Society (5).

METHODS

Animals

Male Sprague–Dawley rats (4414 Füllinsdorf, Switzerland), weighing 180–200 g at the beginning of the discrimination training, were used in the drug discrimination experiments. Male Sprague–Dawley rats (180–200 g) were used in the biochemical studies. For behavioural experiments, the animals were caged in groups of four in a room illuminated on a 12 L : 12 D cycle with lights on at 0700 h. The temperature was kept at 20–21°C. Water was available ad lib, but all animals were maintained on a restricted diet of about 10 g of food ("Kliba" standardised pellets, No. 25-343; Kaiseraugst, Switzerland) given after each experimental session.

Drug Discrimination

Training procedure. The behavioural apparatus consisted of standard operant cages housed in sound-attenuating chambers. Each box (30 × 25 × 30 cm) was equipped with a stainless-steel grid floor and two levers, on either side of a food dispenser. First, the rats were trained to press the right- or left-hand lever for food reward (Precision Rodent Food Pellets, 45 mg, Formula A/I; P.J. Noyes Company, Lancaster, NH) according to a fixed ratio (FR1) schedule of reinforcement in which one food pellet was delivered after every lever press. The value of the FR was gradually increased to FR10. Then, the discrimination training started.

Fifteen minutes before daily test sessions lasting 15 min, the rats were administered either the drug (D) or saline (S) according to the following sequences: S, D, D, S, S, D, S, S, D, D. Half of the rats were required to respond on the left lever and half on the right lever for food reward after receiving the drug. In both cases, pressing on the alternative lever was reinforced after receiving saline. Each day, the total number of lever presses made on both levers before receiving the first reward and the total number of responses on each lever during the 15 min training session were recorded. When during at least nine of 10 consecutive sessions the total number of lever presses before receiving the first reward did not exceed 14, the criterion for correct discrimination was reached and the generalisation experiments were initiated.

Testing procedure. For the generalisation experiments, reinforcement was given on both levers (FR10) and the testing session lasted 2 min in the case of mCPP and 5 min in the case of amphetamine. Increasing doses of drugs were administered at 2-h intervals until the drug-appropriate responding was seen in at least 80% of the rats tested or until the total response rate was significantly reduced. The percentage of rats choosing the drug-associated lever (i.e., those pressing no more than nine times on the saline-appropriate lever before completing 10 responses on the drug-appropriate lever) and the response rate (total number of responses on both levers per minute) were recorded.

mCPP drug discrimination. Animals were trained to discriminate mCPP [1 mg/kg, intraperitoneally (IP)] from saline. Generalisation experiments were carried out twice a week with mCPP to ensure stable performance between tests.

For the generalisation tests, all compounds were given 30 min before the 2-min test except mCPP and MK-212 (15 min beforehand).

For the antagonism studies, compounds were administered 30 min before mCPP (1 mg/kg, IP). Then, 15 min later, rats were placed in Skinner boxes for a 2-min testing session. Reinforcement was given on both levers (FR10), and each testing session lasted 2 min.

For all these studies, compounds were administered IP except MDL 72222, yohimbine, 8-OH-DPAT, apomorphine, mesulergine, ritanserin, and haloperidol, which were given subcutaneously (SC).

Amphetamine drug discrimination. Rats were trained to discriminate amphetamine [0.8 mg/kg, IP, (3)] from saline as described before. Generalisation experiments were carried out twice a week with amphetamine to ensure stable performance between tests.

mCPP (0.03–3 mg/kg, IP) was administered 15 min before amphetamine (0.8 mg/kg, IP); then, 15 min later, rats were placed in Skinner boxes for a 5-min testing session. Reinforcement was given on both levers (FR10).

Tissue Preparation and Incubation for Measurement of IP_3 Production

5-HT_{2C}-mediated stimulation of IP_3 production was measured in the choroid plexus of the rat. The choroid plexus was removed, placed in 200 μl of oxygenated Krebs solution, and incubated with 0.35 nmol myoinositol and 0.35 nmol [^3H]myoinositol for 60 min at 37°C. During this incubation, the tubes were gassed with 95% oxygen/5% CO_2 every 20 min. A mixture of LiCl and pargyline was then added (final concentration: LiCl = 10 mM, pargyline = 10 mM), and 10 min later, the test compounds (final incubation vol. 250 ml). Dose-response curves were constructed from data obtained from three separate measures per data point. The mixture was incubated for a further 30 min at 37°C. The assay was stopped by the addition of 25 ml of a stopping solution ($HClO_4$ 2.64 N + EDTA 40 mM). All assays were performed in the presence of 2% DMSO. Assay tubes were frozen on dry ice for 15 min, thawed, and then kept on ice for 60 min. The tubes were then centrifuged for 20 min at 24,000 × g. Then, 250 μl of the supernatant was removed and placed in Eppendorf tubes precoated with maleic acid together with 25 μl 4 M KOH. The samples were mixed well and kept on ice for 15 min. These samples were then recentrifuged for 15 min at 14,000 rpm (Eppendorf centrifuge 5415C). We removed 230 μl of supernatant and added 30 μl phytic acid.

Isolation of IP_3

We used disposable polystyrene columns containing 1 ml of BioRad (BioRad Laboratories AG, Switzerland) AG1-X8 resin formate form (100–200 mesh) equilibrated and stored in 1 M formic acid. On the day of use, the resin was washed with 4 × 10 ml of distilled water. Then, 4 ml of water was added to the 260 μl obtained from the assay mixture, mixed, and applied to the column without disturbing the resin bed. The eluent was discarded. The columns were then washed with 8 × 5 ml of distilled water followed by 8 ml of a solution consisting of 5 mM tetraborate and 60 mM sodium acetate, and then with 3 × 5 ml of a solution consisting of 0.1 M formic acid and 0.4 M ammonium formate. The resin was then washed with 2 × 5 ml of a solution consisting of 0.1 M formic acid and 0.8 M ammonium formate, and the eluent was collected in 20-ml counter vials. Next, 10 ml of Ultima-Flo AF (Canberra Packard, Netherlands) was added to each vial, which was then sealed and analysed using scintillation spectro-

photometry. The columns were washed in 8 ml 2 M ammonium formate followed by 4 × 10 ml distilled water, and finally, with 2 × 10 ml 1 M formic acid.

Initially, a concentration-response curve to 5-HT was constructed; then, the ability of increasing concentrations of the antagonists was used to reverse the IP_3 stimulation caused by 100 nM 5-HT, a concentration producing a large but submaximal stimulation. The antagonists that were tested were mianserin, ritanserin, methiothepin, mesulergine, and SB 200646. The ability of 10 μM concentrations of these antagonists were also tested to determine whether the compounds themselves would stimulate IP_3 formation and had any intrinsic activity.

Drugs

The following drugs were used: amphetamine, apomorphine, mCPP, MK-212, fenfluramine·HCl, 8-hydroxy-2-(di-*n*-propylamino)tetralin·HBr (8-OH-DPAT), MDL 72222, methi-

TABLE 1

GENERALISATION STUDIES WITH THE mCPP DISCRIMINATIVE STIMULUS

Drug	Dose (mg/kg)	Route of Administration	% of Rats Responding	% of Rats Choosing Drug Lever	Response Rate (% of Control)
mCPP	0.25	IP	100	40	81.33 ± 12.70
	0.50	IP	100	80	99.83 ± 10.14
	1.00	IP	100	100	105.14 ± 9.47
MK-212	0.25	IP	100	75	84.11 ± 12.19
	0.50	IP	100	75	68.10 ± 13.14
	1.00	IP	12.5	100	7.70 ± 6.34
TFMPP	0.10	IP	100	33.33	85.54 ± 7.47
	0.30	IP	83.33	20	90.17 ± 22.26
	1.00	IP	100	100	89.89 ± 15.67
RU 24969	0.10	IP	100	20	100.41 ± 14.75
	0.30	IP	100	60	118.98 ± 16.75
	1.00	IP	100	80	76.12 ± 17.20
	3.00	IP	0	–	1.43 ± 0.51
8-OH-DPAT	0.01	SC	100	11.11	106.93 ± 15.46
	0.03	SC	100	22.22	81.59 ± 14.69
	0.10	SC	22.22	0	18.76 ± 12.72
MDL 72222	0.3	SC	100	0	80.88 ± 22.35
	1.0	SC	100	0	77.03 ± 20.00
	3.0	SC	100	0	71.89 ± 26.56
	10.0	SC	20	0	5.28 ± 4.00
Fenfluramine	0.3	IP	87.50	14.29	76.67 ± 19.27
	1.0	IP	100	37.50	75.24 ± 27.65
	3.0	IP	37.50	100	13.11 ± 14.28
Methiothepin	0.1	IP	100	0	56.21 ± 15.94
	0.3	IP	85.71	16.67	59.12 ± 14.51
	1.0	IP	28.57	0	10.58 ± 4.21
Mesulergine	5	IP	88.89	0	67 ± 15.67
	10	IP	77.78	42.86	51.27 ± 15.80
	20	IP	77.78	66.67	28.68 ± 5.81
	30	IP	44.44	50	20.98 ± 8.70
Yohimbine	1	SC	100	0	86.04 ± 17.94
	3	SC	100	0	34.41 ± 13.12
Mianserin	1	IP	100	20	101.69 ± 8.02
	3	IP	100	20	125.76 ± 10.34
	10	IP	80	0	59.49 ± 15.25
Amphetamine	0.1	IP	100	20	94.31 ± 12.42
	0.3	IP	100	20	86.62 ± 9.71
	1	IP	80	0	62.71 ± 18.79
	2	IP	60	0	30.94 ± 12.52
Apomorphine	0.1	SC	100	40	36.59 ± 7.42
	0.3	SC	80	50	25.58 ± 6.30
	1.0	SC	0	–	0.18 ± 0.18
Haloperidol	0.01	SC	100	25	80.9 ± 20.03
	0.03	SC	100	25	72.61 ± 17.88
	0.1	SC	62.50	80	26.93 ± 11.68

Groups of five to eight animals were used for each dose. The response rate was given as the percent of the control group (vehicle-treated animals).

othepin, RU 24969, mianserin·HCl, pindolol, SB 200646·HCl, and TFMPP. When available, the drugs were purchased from either Sigma or Research Biochemicals, Inc., and when this was not possible they were synthesized at Roche. All drugs were dissolved in sterile saline solution. Mesulergine and yohimbine were dissolved in water. Haloperidol was diluted from 5 mg/ml Haldol® (Janssen Pharmaceutica, Switzerland) ampoules to the required doses using sterile saline solution. If the drug used was in the form of the salt, then the dose refers to the weight of the salt; however, if the drug was only available in the base form, the dose refers to the weight of the base.

Statistical Analysis

The response rate for each antagonism study was subjected to analysis of variance (ANOVA) with repeated measures followed, when appropriate, by Dunnett's *t*-test.

RESULTS

Generalisation Studies on the mCPP Discriminative Stimulus

Treatment of rats with mCPP during training sessions caused a decrease in the number of responses compared with saline. Approximately 50 sessions were required for the animals to learn the mCPP discriminative cue. Mean response rates were 73.8 ± 7.3/min and 64.58 ± 5.42/min for saline and mCPP treatments, respectively.

Rats trained to discriminate mCPP from saline showed a dose-dependent selection of the drug lever. TFMPP completely generalised, whereas MK-212 and Ru 24969 partially generalised to the mCPP cue. The 5-HT_{1A} agonist 8-OH-DPAT and the 5-HT_3 antagonist MDL 72222 did not substitute to the mCPP discriminative stimulus up to 0.1 and 10 mg/kg, respectively, doses that produced a strong decrease in the response rate. The 5-HT-releasing compound fenfluramine dose-dependently (0.3–3 mg/kg) generalised to mCPP cue, although this was associated with a decrease in the response rate. Rats administered with methiothepin, yohimbine, mianserin, and amphetamine showed a saline-appropriate lever selection, even at doses that induced a reduction of lever presses by more than 50%. However, both mesulergine, a 5-HT_2 antagonist, and apomorphine, a dopamine receptor agonist, partially generalised to the mCPP cue and caused a decrease in the response rate. The dopamine D_2 antagonist haloperidol generalised to the mCPP discriminative stimulus at low doses (0.01–0.1 mg/kg). A summary of all data from generalisation studies is given in Table 1.

Antagonism Studies on the m-CPP Discriminative Stimulus

Different 5-HT antagonists were tested at appropriate doses. Methiothepin (1 mg/kg, IP) did not antagonise the mCPP cue. Mesulergine (2–10 mg/kg, IP) and MDL 72222 (2 and 3 mg/kg, SC) also did not block mCPP cue, although both compounds reduced the number of animals responding. (±)Pindolol (5 mg/kg, IP), ritanserin (0.5 and 1 mg/kg, SC), SB 200646 (3 mg/kg, SC), mianserin (10 mg/kg, IP), and yohimbine (3 mg/kg, SC) had no effect on the mCPP stimulus. The DA releaser amphetamine (1–3 mg/kg, IP) was the only compound used in the present study that was found partially to antagonise the mCPP discriminative cue; this was associated with a decrease in the number of animals responding. All antagonism data are summarised in Table 2.

Antagonism Studies on the Amphetamine Discriminative Stimulus

A total of 40 training sessions were required for animals to learn the discriminative cue. Mean response rates were 55.09 ± 5.09/min and 62.14 ± 5.92/min for saline and amphetamine treatments, respectively. In rats trained to discriminate amphetamine (0.8 mg/kg) from saline, amphetamine (0.2–0.8

TABLE 2

ANTAGONISM STUDIES WITH THE mCPP CUE

Drug	Dose (mg/kg)	Route of Administration	% of Rats Responding	% of Rats Choosing Drug Lever	Response Rate (% of Control)
Methiothepin	1	IP	100	100	74.63 ± 11.07
Mesulergine	2	IP	66.67	100	73.13 ± 26.48
	5	IP	87.50	83	51.85 ± 9.91*
	10	IP	42.86	100	18.25 ± 5.72*
MDL 72222	2	SC	60	100	32.55 ± 21.64*
	3	SC	20	100	7.47 ± 6.58*
Yohimbine	3	SC	40	100	33.24 ± 24.15
Mianserin	10	IP	40	100	47.91 ± 40.21
Pindolol	5	IP	100	100	50.16 ± 12.34
Amphetamine	1	IP	42.86	66.67	34.38 ± 19.8*
	2	IP	50	50	45.71 ± 20.13*
	3	IP	30	66.67	13.01 ± 8.13*
Apomorphine	0.3	SC	77.78	100	25.37 ± 5.21*
Ritanserin	0.5	SC	87.50	100	92.2 ± 28.77
	1	SC	87.50	85.71	119.15 ± 42.00
SB 200646	3	SC	50	100	84.10 ± 60.02

Groups of five to eight animals were used for each dose. The response rate was given as the percentage of mCPP group.

*$p < 0.05$.

TABLE 3

STUDIES WITH THE AMPHETAMINE CUE

Drug	Dose (mg/kg, IP)	% of Rats Responding	% of Rats Choosing Drug Lever	Response Rate (% of Control)
Amphetamine (generalisation)	0.2	100	50	97.13 ± 16.69
	0.4	100	90	134.87 ± 25.2
	0.8	100	100	149.81 ± 24.66
mCPP (antagonism)	0.03	100	100	88.88 ± 15.42
	0.1	100	71.43	68.00 ± 13.36
	0.3	100	100	69.59 ± 15.93
	1	100	71.43	22.89 ± 5.46
	3	14.28	100	18.49 ± 17.8

Groups of seven animals were used for each dose. The response rate is given as the percentage of amphetamine group.

mg/kg) dose-dependently increased the percentage of animals selecting the amphetamine lever with an enhancement of the response rate (Table 3). Although amphetamine (1–3 mg/kg) showed a partial blockade of the mCPP cue, mCPP (0.03–3 mg/kg) had no effect on the amphetamine discriminative stimulus (Table 3).

Antagonism of 5-HT-Stimulated IP_3 Turnover

5-HT induced a dose-dependent stimulation of inositol phosphate production with an EC_{50} of 33 nM. The nonselective 5-HT antagonists mianserin, ritanserin, methiothepin, and mesulergine and the selective $5\text{-}HT_{2B}/5\text{-}HT_{2C}$ antagonist SB 200646 antagonised the stimulation induced by 100 nM 5-HT. The IC_{50} values for this antagonism are given in Table 4. The dose–response curves of the antagonism of 100 nM 5-HT by each of the antagonists are shown in Fig. 1. None of the compounds tested produced any stimulation of IP_3 turnover in the rat choroid plexus (data not shown). These data indicate that all six of the compounds tested in the present study are silent antagonists at the $5\text{-}HT_{2C}$ receptor in the rat choroid plexus.

DISCUSSION

The major finding of the present study is that although mCPP induces a drug-discriminative stimulus in rats, the response does not appear to be mediated via a $5\text{-}HT_{2C}$ receptor.

TABLE 4

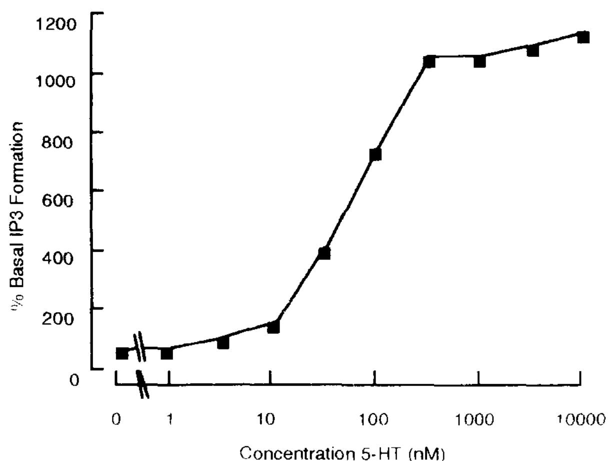
CONCENTRATIONS OF MIANSERIN, RITANSERIN, METHIOTHEPIN, MESULERGINE AND SB 200646 REQUIRED TO REDUCE THE STIMULATION OF IP_3 FORMATION PRODUCED BY 100 nM 5-HT BY 50%

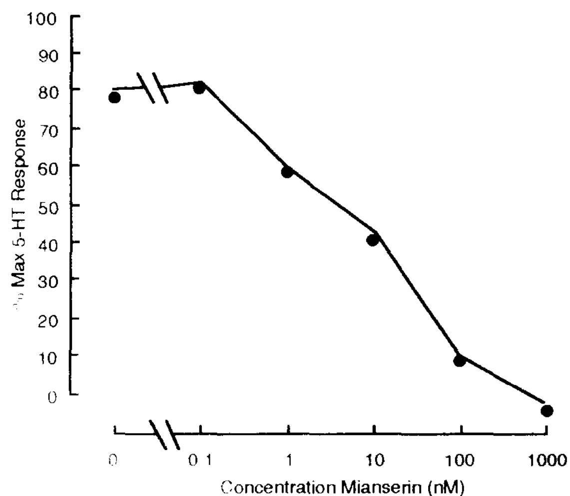
Compound	IC_{50} (nM)
Mianserin	10
Ritanserin	20
Methiothepin	33
Mesulergine	5
SB 200646	210

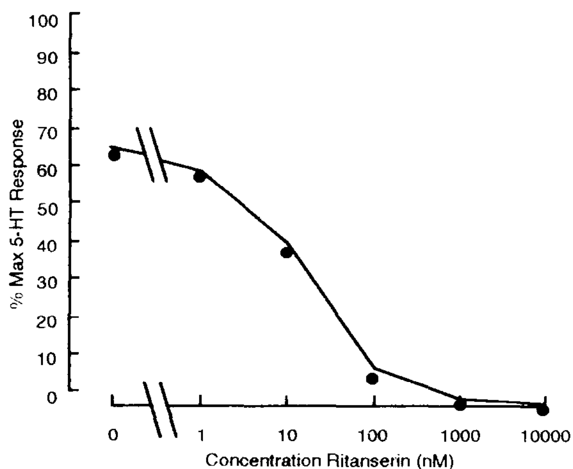
Experiments were performed by incubating rat choroid plexi prelabelled with [^{3}H]myoinositol with 100 nM 5-HT and increasing concentrations of antagonists.

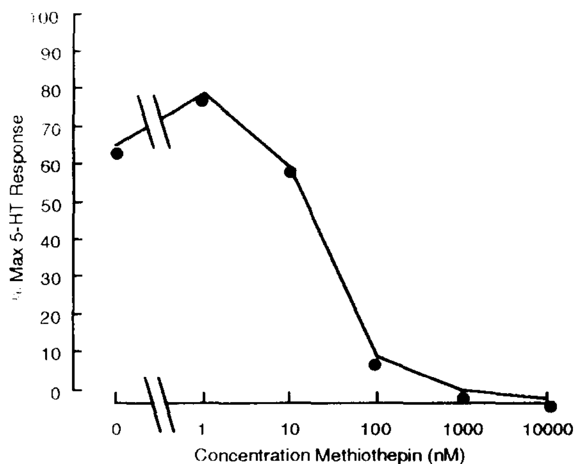
Drug discrimination stimuli are usually mediated by specific receptor populations and specific subtypes of receptors. For example, the drug discrimination stimulus of amphetamine appears to be mediated by DA D_2 receptors in the nucleus accumbens (10,20) and the discriminative stimulus of phencyclidine (PCP) by *N*-methyl-D-aspartic acid (NMDA) receptors (22,23). Clearly, however, this may not always be the case. In agreement with previously published results (6,24), mCPP (1 mg/kg) produced a discriminative cue probably mediated via a postsynaptic 5-HT receptor, because fenfluramine, the 5-HT releaser, generalised to the cue. Generalisation studies also suggest that the response may be mediated by $5\text{-}HT_1$ or $5\text{-}HT_{2C}$ receptors, because TFMPP and RU 24969 generalise to the mCPP cue. Although mCPP and TFMPP have affinity for the $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, and $5\text{-}HT_{2C}$ receptors, RU 24969 is selective for the $5\text{-}HT_{1A}$ and $5\text{-}HT_{1B}$ but not $5\text{-}HT_{2C}$ receptors. This would suggest that the discriminative stimulus is mediated by either $5\text{-}HT_{1A}$ or $5\text{-}HT_{1B}$ receptors. 8-OH-DPAT, however, did not generalise to the mCPP cue, which suggests that the $5\text{-}HT_{1A}$ receptors are not involved in mediating this response, and that it is indeed mediated by $5\text{-}HT_{1B}$ receptors. All of these data are in agreement with previously published data.

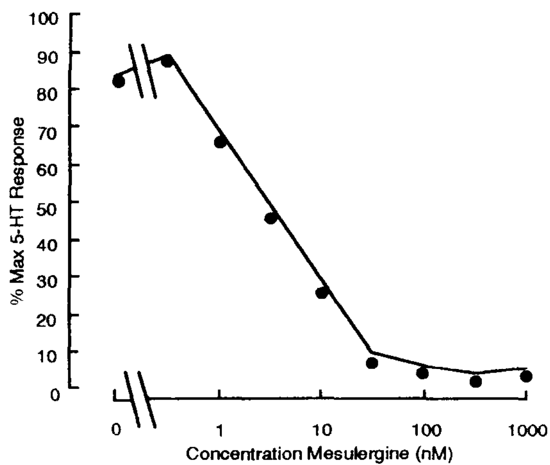
Antagonism studies, however, do not support the role of either $5\text{-}HT_{1B}$ or $5\text{-}HT_{2C}$ receptors in the discriminative cue of mCPP. They suggest that the effect of mCPP is mediated by an unclassified 5-HT receptor subtype. None of the antagonists tested were able to block the effect of mCPP in the present experiments. It is unlikely that the $5\text{-}HT_{1B}$ receptor is involved in this response, because pindolol failed to antagonise the effect of mCPP. The $5\text{-}HT_2$ antagonist ritanserin, which should antagonise effects mediated via either $5\text{-}HT_{2A}$, $5\text{-}HT_{2B}$, or $5\text{-}HT_{2C}$ receptors, was also inactive. Indeed, although mianserin, methiothepin, ritanserin, mesulergine and SB 200646 all antagonised the effect of 5-HT on IP_3 turnover in the choroid plexus, a classical $5\text{-}HT_{2C}$–mediated response (9), they all failed to antagonise the mCPP response in the drug discrimination studies. All five antagonists appeared to be silent antagonists at the $5\text{-}HT_{2C}$ receptor in the rat choroid plexus (i.e., they antagonised the effect of 5-HT but did not themselves stimulate IP_3 production), and therefore, even allowing for the possibility that some of these compounds may have different absorption and distribution properties *in vivo*, the fact that none of them was able to antagonise the mCPP cue suggests that it is not mediated by $5\text{-}HT_{2C}$ receptors. This conclusion is at variance with previously published studies











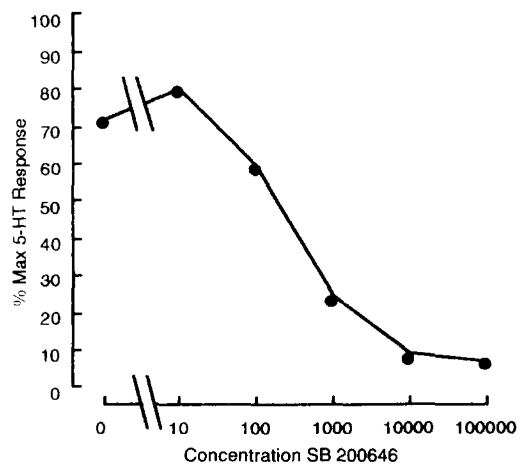


FIG. 1. Effect of 5-HT on IP_3 formation in the rat choroid plexus (A) and the effect of mianserin (B), ritanserin (C), methiothepin (D), mesulergine (E), and SB 200646 (F) on a submaximal stimulation of IP_3 production produced by 100 nM 5-HT.

(6,24) that claim that the discriminative cue of mCPP is mediated by the 5-HT_{2C} receptor. This is clearly not the case, because SB 200646 failed to antagonise the response.

The failure of the nonselective 5-HT antagonist methiothepin to antagonise the mCPP cue would suggest that neither the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5}, 5-HT_{6}, nor 5-HT_{7} receptors are involved. mCPP has been shown to be a 5-HT_{3} antagonist (21); however, in the present study, MDL 72222 neither generalised to nor antagonised the mCPP cue, suggesting that the cue is not mediated via a stimulation or an antagonism of 5-HT_{3} receptors. Consequently, the only known 5-HT receptors that are not covered by the antagonists used in the present study are 5-HT_{1E}, 5-HT_{1F}, and 5-HT_{4} receptors, and to our knowledge the affinity of mCPP for these receptors is unknown. Although mCPP has been shown to have reasonable affinity for α-adrenoceptors (14) the α-adrenoceptor antagonist yohimbine neither antagonised nor generalised to the mCPP cue.

These data also suggest that the mCPP cue cannot be explained in terms of mediation by multiple receptor subtypes. For example, Callahan and Cunningham (6) suggested that both 5-HT_{1B} and 5-HT_{2C} receptors could be involved in the response. This, however, cannot be the case, as methiothepin has high affinity for both receptors (15) but was unable to antagonise the effect of mCPP. However, the data do not exclude the possibility that the response could be mediated by a simultaneous action at 5-HT_{2C} and either 5-HT_{1E}, 5-HT_{1F}, or 5-HT_{4} receptors.

Mesulergine partially generalised to the mCPP discriminative cue. This effect was also observed by Callahan and Cunningham (6), who suggested that if the discriminative cue were mediated through a 5-HT_{2C} receptor, mesulergine may display some partial agonism at this receptor. In the rat choroid plexus, however, mesulergine was a silent antagonist in that it antagonised the stimulation caused by 5-HT but had no intrinsic activity itself. Mesulergine, however, does have high affinity for DA receptors labelled with $[^3\text{H}]$spiperone (7). Furthermore, the DA antagonist haloperidol, which has no known affinity for 5-HT receptor subtypes, was able to generalise to the mCPP discriminative cue. This could suggest that the cue is either mediated via an unknown 5-HT receptor that has high affinity for haloperidol, mesulergine, and mCPP, and/or that the discriminative cue to mCPP is mediated by a DA synapse that is distal to the postsynaptic 5-HT receptor through which mCPP mediates its effects. The latter possibility is supported by the fact that low doses of the DA agonist apomorphine that are usually thought to be selective for presynaptic autoreceptors on DA neurones in behavioural studies (13) also partially generalises to the mCPP cue. At low doses, apomorphine may inhibit DA release by stimulating presynaptic DA autoreceptors and reducing DA neurotransmission. This would have a similar effect to the DA antagonist haloperidol, which blocks postsynaptic DA receptors. We therefore suggest that the 5-HT receptor that mediates the effects of mCPP in the present model also inhibits DA neurotransmission. These conclusions are supported by the fact that amphetamine, a compound normally thought to increase DA neurotransmission by causing the release of DA from DA nerve terminals, partially antagonises the discrimination cue of mCPP. The DA synapse is probably distal to that of the 5-HT, because although amphetamine partially antagonises the mCPP cue, mCPP has no effect on the amphetamine cue. This explanation would therefore account for the generalisation of the 5-HT releasing agent fenfluramine. It would also account for the partial generalisation of the DA autoreceptor agonist apomorphine and the DA antagonist haloperidol to the mCPP cue and its partial antagonism by amphetamine.

With some drugs, as the dose increases to greater than the training dose, drug lever responding and response rates fall. Thus, if an antagonist is given at varying doses while keeping the dose of the training drug constant, when response rates are severely disrupted it is impossible to determine which way a dose–response curve is being shifted. However, our explanation of the data that dopaminergic mechanisms may underlie the mCPP discriminative stimulus could be tested in future experiments in which systematic shifts of the mCPP dose-response curves are carried out.

In summary, the discriminative cue of mCPP appears to be mediated by an unknown postsynaptic 5-HT receptor. Although other authors have claimed that the response is mediated by 5-HT_{2C} receptors, the present study, which used a larger number of antagonists and the selective $5\text{-HT}_{2B}/5\text{-HT}_{2C}$ antagonist SB 200646, does not suggest the involvement of 5-HT_{2C} receptors. Furthermore, it appears that the response is mediated by a decrease in dopaminergic neurotransmission.

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