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ROLE OF 5-HYDROXYTRYPTAMINE_{1B} RECEPTORS AND 5-HYDROXYTRYPTAMINE UPTAKE INHIBITION IN THE COCAINE-EVOKED DISCRIMINATIVE STIMULUS EFFECTS IN RATS

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Mesolimbic dopamine pathways play a critical role in the behavioural effects of cocaine in rodents. Nonetheless, research has also demonstrated involvement of 5-hydroxytryptamine (5-HT; serotonin) transmission in these effects. The present study investigated the ability of selective 5-HT_{1B} receptor ligands and a 5-HT reuptake inhibitor to substitute for or to alter (enhance or antagonise) the discriminative stimulus effects of cocaine. Male Wistar rats were trained to discriminate cocaine (10 mg/kg, i.p.) from saline (i.p.) in a two-choice, water-reinforced fixed ratio (FR) 20 drug discrimination paradigm. In substitution tests, the selective 5-HT_{1B} receptor agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxy-pyrrolo[3,2-b]pyridine (CP 94253; 2.5–5 mg/kg, i.p.) and the 5-HT reuptake inhibitor fluoxetine (5–10 mg/kg, i.p.) elicited ca. 40 and 0% drug-lever responding, respectively. In combination experiments, CP 94253 (2.5–5 mg/kg) given with submaximal doses of cocaine (0.3–2.5 mg/kg) produced a leftward shift in the cocaine dose-response curve; pretreatment with CP 94253 (5 mg/kg) prior to a dose of cocaine (2.5 mg/kg) which elicited lower than 40% drug-lever responding, caused full substitution. Fluoxetine (5 and 10 mg/kg) given in combination with a submaximal dose of cocaine (2.5 mg/kg) produced a 100% drug-lever responding. Pretreatment with the 5-HT_{1B} receptor antagonists N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide (GR 127935; 0.5–5 mg/kg, s.c.) and 3-(3-dimethylamino-propyl)-4-hydroxy-N-[4-(4-pyridinyl)-phenyl]benzamide (GR 55562; 1 mg/kg, s.c.) failed to modulate the dose-effect curve for cocaine (0.6–5 mg/kg). On the other hand, GR 127935 (5 mg/kg) and GR 55562 (1 mg/kg) significantly attenuated the enhancement of cocaine discrimination evoked by a combination of CP 94253 (5 mg/kg) or fluoxetine (5 mg/kg) and cocaine (2.5 mg/kg). These results indicate that 5-HT_{1B} receptors are not directly involved in the cocaine-induced discriminative stimuli in rats. On the other hand, they indicate that pharmacological stimulation of 5-HT_{1B} receptors — that also seem to be a target for fluoxetine-mediated increase in 5-HT neurotransmission — can enhance the overall effects of cocaine.

Key words: cocaine, CP 94253, fluoxetine, GR 127935, GR 55562, discriminative stimulus effects, rats.

INTRODUCTION

It is well established that cocaine preferentially affects brain dopamine systems. In fact, cocaine elevates extracellular concentrations of the neurotransmitter via inhibition of dopamine reuptake (1) and such increases appear mainly in a terminal region of the mesolimbic dopamine pathway, i.e. the nucleus accumbens (2). A vast body of evidence reveals that the latter brain structure is closely related to locomotor, sensitising, reinforcing and discriminative stimulus properties of cocaine (3, 4, 5).

Apart from its role in dopamine neurotransmission, cocaine also inhibits the reuptake of 5-hydroxytryptamine (5-HT; serotonin) (6). Recent findings indicate an involvement of 5-HT neurotransmission in the behavioural effects induced by cocaine. Thus, 5-HT neurotoxin or 5-HT synthesis inhibitors have been demonstrated to potentiate the hyperactivity produced by cocaine (7, 8). Similarly, in cocaine self-administration procedures lesions of the 5-HT system and drugs increasing 5-HT neurotransmission (e.g. tryptophan, fluoxetine) have been shown to enhance and decrease, respectively, the drug-induced reinforcing effects (9, 10, 11). Interestingly, selective 5-HT reuptake inhibitors can either enhance or antagonise the interoceptive effects in cocaine discrimination (12, 13, 14, 15, 16).

Regarding the 14 recently described 5-HT receptors (17, 18), several findings suggest a role for 5-HT_{1B} receptors in the effects of cocaine: 1) the transcript and protein for 5-HT_{1B} receptors are located in different brain structures including the dopamine mesolimbic system (19, 20); 2) activation of 5-HT_{1B} receptors increases basal (21, 22) and cocaine-stimulated (23) extracellular dopamine concentrations in the nucleus accumbens; 3) agonists of 5-HT_{1B} receptors enhance locomotor (24), sensitising (24) and reinforcing effects of cocaine (25, 26); they also engender a dose-dependent leftward shift in the cocaine dose-response curve in a drug discrimination model (13, 16). On the other hand, antagonists of 5-HT_{1B} receptors inhibit cocaine-induced locomotor hyperactivity (24, 27).

The present study focussed on a potential interaction between 5-HT_{1B} receptors and the 5-HT reuptake mechanism in the discriminative stimulus effects of cocaine. In this rat operant behavioural model we tested the hypothesis about whether new and more selective 5-HT_{1B} receptor ligands, i.e. the agonist 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxy-pyrrolo[3,2-b]pyridine (CP 94253; (28)), the antagonists N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide (GR 127935; (29) or 3-(3-dimethylamino)propyl)-4-hydroxy-N-[4-(4-pyridinyl)-phenyl]benzamide (GR 55562; (30), and the 5-HT reuptake inhibitor fluoxetine (31) affected the discriminative stimulus effects of cocaine.

MATERIALS AND METHODS

Animals

The experiment was performed on male Wistar rats (280–300 g). The animals were housed in groups of two to a cage at a room temperature of $20 \pm 1^\circ\text{C}$ on a 12 h light/dark cycle (the light on between 6.00–18.00 h). Although food (Bacutil pellets) was always available, the water that each animal received was restricted to the amount given during training sessions in the operant chambers, after test sessions (15 min), and at weekends. All the experiments were carried out in compliance with the Polish Animal Protection Bill of April 21, 1997, and with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Drugs

The following drugs were used (in parentheses: pre-session injection times, route of injection, suppliers): cocaine HCl (–15 min; i.p.; Merck, Germany), 3-(1,2,5,6-tetrahydro-4-pyridyl)-5-propoxypyrrolo[3,2-b]pyridine (CP 94253; –30 min; i.p.; Pfizer, USA), fluoxetine HCl (–45 min; i.p.; Eli Lilly & Co., USA), N-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2'-methyl-4'-(5-methyl-[1,2,4]oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide HCl (GR 127935; –60 min; s.c.; Glaxo Wellcome, UK) and 3-(3-dimethylamino)propyl-4-hydroxy-N-[4-(4-pyridinyl)phenyl]-benzamide HCl (GR 55562; –60 min; s.c.; Tocris, UK). Cocaine, CP 94253, fluoxetine and GR 55562 were dissolved in saline, while GR 127935 was suspended in a 20% β -cyclodextrin (RBI, USA). All the drugs were injected in a volume of 1 ml/kg.

Apparatus

Commercially available, two-lever operant chambers (Coulbourn Instruments, model E10-10; USA) were used. Each chamber was equipped with a water dispenser mounted equidistant between two response levers on one wall and contained in a light- and sound-attenuating shell. Illumination was provided by a 28-V house light; ventilation and masking noise were supplied by a blower. A computer was used to programme and record all the experimental events.

Discrimination procedure

Rats were trained to discriminate cocaine (10 mg/kg) from saline (0.9% NaCl). The drug or saline were administered i.p. 15 min before daily (Monday-Friday) sessions (30 min). The initial training ("errorless" training) began under a fixed ratio (FR) 1 schedule of continuous water reinforcement with only a stimulus-appropriate (drug or saline) lever presented. The ratio schedule was increased until all the animals responded reliably under a FR 20 schedule for each experimental condition. Half the animals were reinforced for left-lever responses after drug administration and for right-lever responses after saline. The conditions were reversed for the remaining animals. To control possible development of position cues on the basis of olfactory stimuli, a pseudorandom relationship was maintained between the lever programmed to deliver reinforcement for each consecutive subject, run in the same experimental chamber (32). During that phase of training, cocaine and saline were administered in nonsystematic order, and neither training condition prevailed for more than three consecutive sessions.

When the responding stabilized on an FR 20 schedule, discrimination training started with both levers presented simultaneously. The rats were required to respond on the

stimulus-appropriate (correct) lever to obtain reinforcement (water); there were no programmed consequences of responding on the incorrect lever. That phase of training continued until all the animals fulfilled the criterion (an individual mean accuracy of at least 80% correct responses before the first reinforcer during 10 consecutive sessions). After the rats acquired the cocaine-saline discrimination, the training sessions were shortened from 30 to 15 min.

Test sessions were initiated once all the animals met the above-mentioned criterion, and were conducted once or twice a week. Cocaine and saline sessions intervened between test sessions to maintain discrimination accuracy. Only rats that met an 80% performance criterion during the preceding cocaine and saline sessions were used in the tests. During the test sessions, the animals were placed in chambers in the same manner as during training sessions. Upon completion of 20 responses on either lever, or after a session time (15 min) elapsed, a single reinforcer was delivered, and the animals were removed from the chamber. In the home cages all the rats were allowed 15 min of free access to water.

Two pharmacological test manipulations were performed during the test sessions. In substitution tests, the animals were tested for lever selection after administration of various doses of the training drug or novel compounds. In combination tests, the rats were given a fixed dose of a test drug before different doses of cocaine (0.6–10 mg/kg).

Data analysis

During training sessions, the accuracy (mean \pm S.E.M.) was defined as a ratio of correct responses to total responses before the delivery of the first reinforcer; during the test sessions, the performance (mean \pm S.E.M.) was expressed as a ratio of drug-lever responses to total responses before the delivery of the first reinforcer. Response rates (responses per second), regarded as a measure of behavioural disruption, were evaluated during the training and test sessions. For the training sessions, the response rate (mean \pm S.E.M.) was calculated as a total number of responses to either lever before completion of the first FR 20, divided by the number of minutes taken to complete the FR. During the test sessions, the response rate (mean \pm S.E.M.) was calculated as a total number of responses before completion of 20 responses on either lever, divided by the number of minutes taken to complete the FR 20. Only the data from animals that completed the FR 20 during the test sessions were used. Student's *t*-test for repeated measurements was used to compare the percentage of cocaine-lever responding and response rates during the test sessions with the corresponding values of either the previous cocaine sessions (substitution tests) or the test dose of cocaine (combination tests). A two-way analysis of variance for repeated measurements was used to determine whether the percentage of cocaine-lever responding and the response rates observed during tests with several doses of cocaine in combination with either saline or the tested drugs differed; to analyze each dose of cocaine separately, *post-hoc* comparisons were made with Student's *t*-test. A drug was considered to substitute fully for cocaine if at least 80% of the responses were made to the cocaine-appropriate lever after a dose of that drug; similarly, complete antagonism was said to occur when at most 20% of the responses were made on the drug lever after pretreatment with a dose of a potential antagonist given in combination with cocaine, 10 mg/kg. The dose predicted to elicit a 50% drug-appropriate responding (ED_{50}) was calculated using Litchfield and Wilcoxon's methods (33).

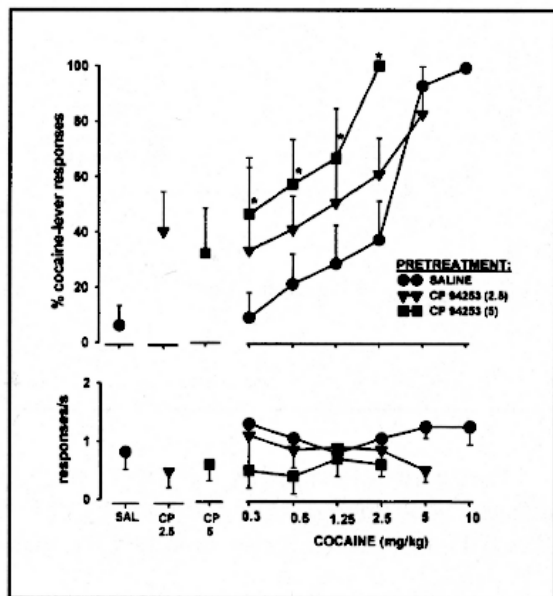
RESULTS

All the rats ($N = 26$) used in the experiment developed the ability to discriminate between cocaine and saline. The stimulus control of both the training drug and saline injections was stable throughout the experiment.

Cocaine (10 mg/kg) and saline produced approximately 99 and 6% drug-lever responding, respectively. The training dose of cocaine did not affect the response rates (cocaine 1.0 ± 0.2 responses/s, saline: 0.85 ± 0.25 responses/s; results not shown; $N = 26$).

In substitution studies, cocaine (0.3–10 mg/kg) induced a dose-dependent increase in the drug-lever responding (13–99%; *Figs. 1, 2, 3*).

Fig. 1. Dose-response curves for cocaine following pretreatment with saline or CP 94253 (2.5–5 mg/kg) in rats trained to discriminate cocaine (10 mg/kg) from saline. For comparison, effects of saline (SAL) or CP 94253 (CP; 2.5 and 5 mg/kg), given alone, are shown. The upper panel shows a percentage (mean \pm S.E.M.) of the cocaine-lever responding during the test session; the lower panel illustrates response rate/s (mean \pm S.E.M.). The number of animals tested in each treatment regimen: $n = 7$ –14. Asterisks indicate significant differences in the cocaine-lever responses or response rates in rats pretreated with the same dose of cocaine in combination with saline or the drug ($P < 0.05$).



Given alone, CP 94253, 2.5 and 5 mg/kg, produced 40 and 36% of the cocaine-lever responding, respectively. CP 94253 did not change the rats' response rates (*Fig. 1*). In combination tests, pretreatment with a fixed dose of CP 94253 (2.5 or 5 mg/kg) enhanced cocaine discriminability ($F_{4,78} = 3.93$, $P < 0.01$ or $F_{3,64} = 5.62$, $P < 0.01$, respectively), with a leftward shift of its dose-response curve (*Fig. 1*, the upper panel) and a reduction in the ED_{50} value for cocaine (*Table 1*). The response rates were not altered after a combination of CP 94253 (2.5 mg/kg) + cocaine ($F_{4,78} = 2.63$, $P > 0.05$), or CP 94253 (5 mg/kg) + cocaine ($F_{3,64} = 2.02$, $P > 0.05$) (*Fig. 1*, the lower panel).

Fluoxetine, 5 and 10 mg/kg, neither substituted for cocaine nor affected the response rate of animals (*Fig. 2*, the upper panel). Given in combination with cocaine (0.6–2.5 mg/kg), fluoxetine, 5 or 10 mg/kg, enhanced cocaine discriminability ($F_{2,30} = 7.37$, $P < 0.001$ or $F_{2,30} = 3.37$, $P < 0.05$, respectively), with a leftward shift of its dose-response curve (*Fig. 2*, the upper panel) and a reduction in the ED_{50} value for cocaine (*Table 1*). The response rates were not changed following combined administration of fluoxetine,

5 mg/kg + cocaine ($F_{2,30} = 1.01$, $P > 0.05$) or of fluoxetine, 10 mg/kg + cocaine ($F_{2,30} = 0.79$, $P > 0.05$) (Fig. 2, the lower panel).

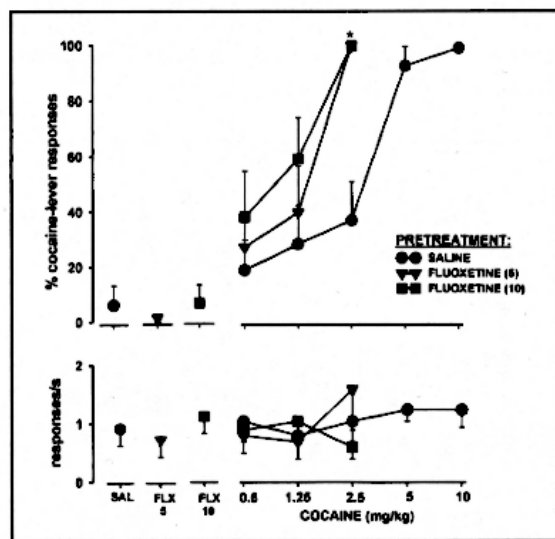
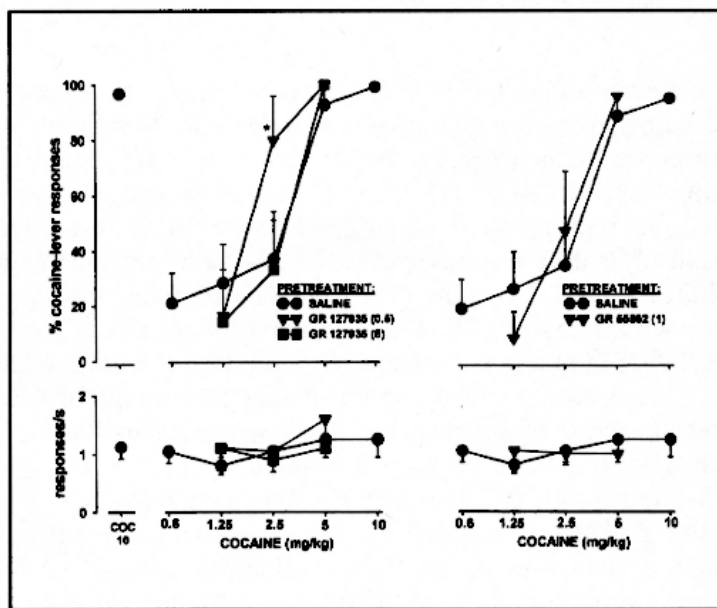


Fig. 2. Dose-response curves for cocaine following saline or fluoxetine (5–10 mg/kg) in rats trained to discriminate cocaine (10 mg/kg) from saline. For comparison, effects of saline (SAL) or fluoxetine (FLX; 5 and 10 mg/kg), given alone, are shown. See Fig. 1 for further explanation. The number of animals tested in each treatment regimen: $n = 6-14$.

Pretreatment with GR 127935 (5 mg/kg) or GR 55562 (1 mg/kg) neither affected cocaine discrimination ($F_{2,40} = 1.07$, $P > 0.05$ or $F_{2,42} = 2.03$, $P > 0.05$, respectively), nor modified its dose-effect curve (Fig. 3) and the ED_{50}

Fig. 3. Dose-response curves for cocaine following saline, GR 127935 (0.5–5 mg/kg) or GR 55562 (1 mg/kg) in rats trained to discriminate cocaine (10 mg/kg) from saline. For comparison, effects of cocaine (COC; 10 mg/kg) given alone, are shown. See Fig. 1 for further explanation. The number of animals tested in each treatment regimen: $n = 6-12$.



value for cocaine (Table 1). A combination of GR 127935 (0.5 mg/kg) with cocaine enhanced cocaine discrimination ($F_{2,40} = 8.30$, $P < 0.001$); however, that pretreatment did not modify the ED_{50} value for cocaine (Table 1). The response rates of animals were not affected after combined administration of cocaine with GR 127935, 0.5 mg/kg ($F_{2,40} = 0.77$, $P > 0.05$), GR 127935, 5 mg/kg ($F_{2,40} = 0.52$, $P > 0.05$) or GR 55562, 1 mg/kg ($F_{2,40} = 0.34$, $P > 0.05$) (Fig. 3).

Following exposure to GR 127935 (5 mg/kg) or GR 55562 (1 mg/kg), a significant reduction in the discriminative stimulus effects of combined treatment with CP 94253 (5 mg/kg)+cocaine (1.25 mg/kg) or CP 94253 (5 mg/kg)+cocaine (2.5 mg/kg) — which evoked approximately 63 and 90% drug-lever responses, respectively, — was observed (Fig. 4). Pre-exposure to GR 127935 or GR 55562 did not affect the response rates (results not shown).

GR 127935 (5 mg/kg) or GR 55562 (1 mg/kg) — when used prior to combined administration of fluoxetine (5 mg/kg)+cocaine (1.25 mg/kg) or fluoxetine (5 mg/kg)+cocaine (2.5 mg/kg), which induced approximately 40 and 100% drug-lever responses, respectively, — significantly decreased the cocaine discriminative effects (Fig. 5). Pre-exposure to GR 127935 or GR 55562 did not affect the response rates (results not shown).

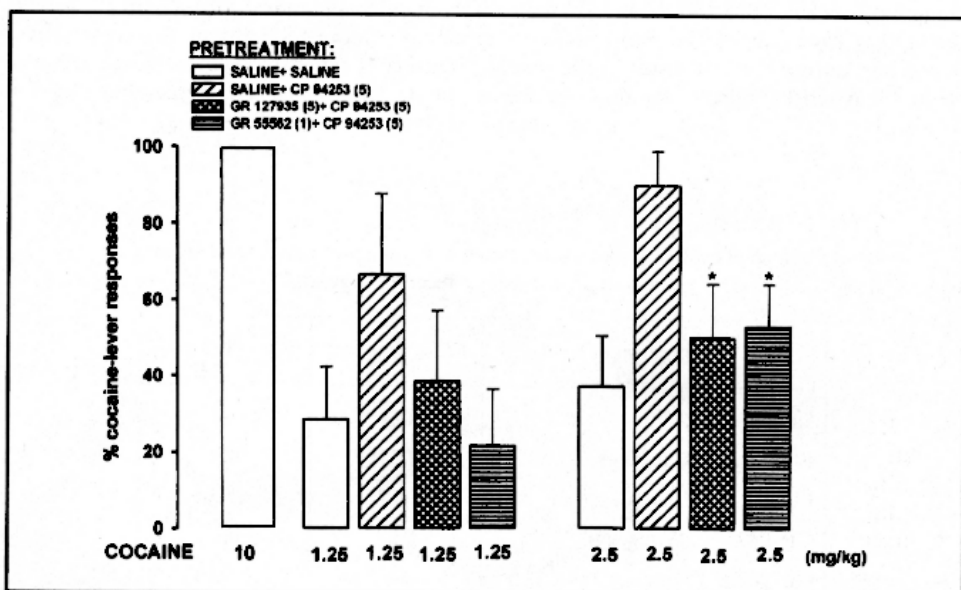


Fig. 4. Effects of GR 127935 (5 mg/kg) or GR 55562 (1 mg/kg) on the CP 94253 (5 mg/kg)+cocaine (1.25 and 2.5 mg/kg)-lever responding in rats trained to discriminate cocaine (10 mg/kg) from saline. The panel shows a percentage (mean \pm S.E.M.) of the cocaine-lever responding during the test session. The number of animals tested in each treatment regimen: $n = 6-8$. Asterisks indicate significant difference in the % cocaine-lever responding between a 5-HT_{1B} receptor antagonist and saline ($P < 0.05$).

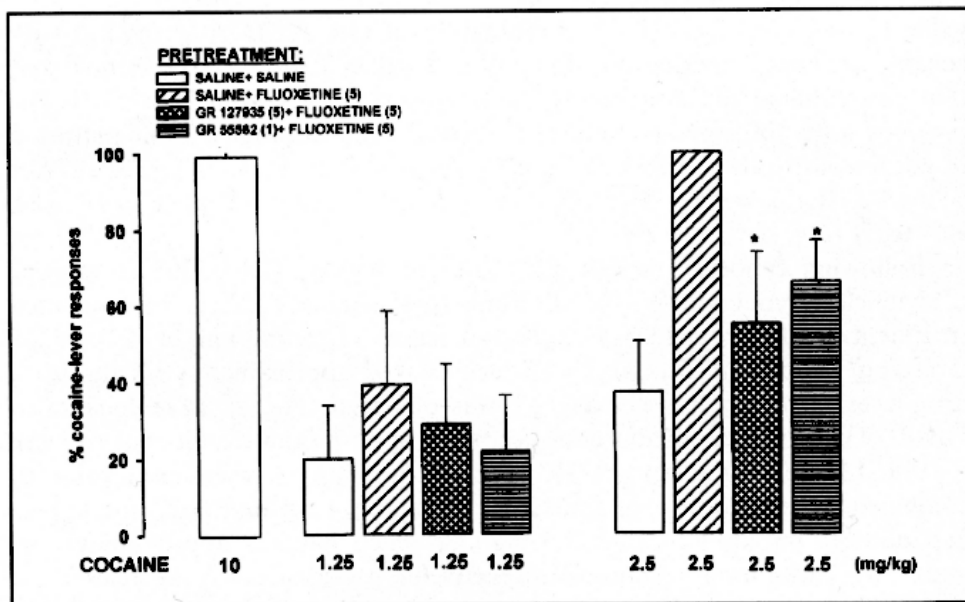


Fig. 5. Effects of GR 127935 (5 mg/kg) or GR 55562 (1 mg/kg) on the fluoxetine (5 mg/kg)+ cocaine (1.25 and 2.5 mg/kg)-lever responding in rats trained to discriminate cocaine (10 mg/kg) from saline. The panel shows a percentage (mean \pm S.E.M.) of the cocaine-lever responding during the test session. The number of animals tested in each treatment regimen: $n = 6-9$. Asterisks indicate significant difference in the % cocaine-lever responding between a 5-HT_{1B} receptor antagonist and saline ($P < 0.05$).

Table 1. The ED₅₀ values for cocaine in rats pretreated with saline, fluoxetine or 5-HT_{1B} receptor ligands.

Pretreatment	ED ₅₀ (mg/kg)	P
Saline	1.64	---
Fluoxetine (5 mg/kg)	1.31	n.s.
Fluoxetine (10 mg/kg)	0.87	< 0.05
CP 94253 (2.5 mg/kg)	0.98	n.s.
CP 94253 (5 mg/kg)	0.42	< 0.01
GR 127935 (0.5 mg/kg)	1.38	n.s.
GR 127935 (5 mg/kg)	2.17	n.s.
GR 55562 (1 mg/kg)	1.70	n.s.

n.s. — not significant

DISCUSSION

In line with some earlier findings, our results show that 5-HT potentiates the dopamine-mediated discriminative stimulus effects of cocaine. In fact, we found that fluoxetine, a drug which inhibits 5-HT uptake and elevates dialysate 5-HT levels in the brain (31), significantly enhanced the discriminative stimulus effects of submaximal doses of cocaine and produced a leftward shift in its dose-response curve, while when administered alone, fluoxetine did not substitute for cocaine. These data are in agreement with the earlier studies of Callahan and Cunningham (14, 16) and Herges and Taylor (34) who found that fluoxetine in a dose range (4–10 mg/kg) similar to that used in our study enhanced cocaine discrimination and locomotor activation, respectively. Such potentiation was also observed in a drug discrimination model for another selective 5-HT reuptake inhibitor, sertraline (13), but not for citalopram (13, 15). The latter drug was much less effective in rats (13), and even attenuated the cocaine dose-response function in squirrel monkeys (15). The reason for such a discrepancy in the effects of selective 5-HT reuptake inhibitors on cocaine discrimination is not clear, but it is noteworthy that there exist some important differences in the affinity for 5-HT and other monoamines uptake sites of these drugs. Thus, in contrast to citalopram, fluoxetine and sertraline are less selective for 5-HT neurotransmission since they show a moderate affinity for the norepinephrine transporter site (35). Moreover, though none of these selective 5-HT reuptake inhibitors binds to the dopamine transporter site, fluoxetine and sertraline do enhance extracellular dopamine release (36). Both these findings seem to be in agreement with some recent data showing full substitution of selective norepinephrine reuptake inhibitors (37), as well as of a dopamine releaser or a dopamine uptake inhibitor (12, 38) for cocaine discrimination.

Both the selective 5-HT reuptake inhibitor fluoxetine and the selective 5-HT_{1B} receptor agonist CP 94253 enhanced the discriminative stimulus effects of cocaine. In fact, after pretreatment with 2.5 and 5 mg/kg of CP 94253, a leftward shift in the cocaine dose-response curve and a significant reduction (i.e. 1.67 and 3.9 times, respectively) in the ED₅₀ values for cocaine were observed. However, in contrast to fluoxetine, CP 94253 alone weakly substituted for cocaine. Both the fluoxetine- and the CP 94253-mediated enhancement of cocaine discriminative stimulus effects seems to be specific, since neither the inhibitor of 5-HT uptake nor the 5-HT_{1B} receptor agonist evoked any behavioural disruption in rats; on the other hand, a decrease — not related to the dose used — in the rate of responding was observed.

The enhancement of cocaine discrimination by CP 94253 is also in line with some other data on the influence of 5-HT_{1B} receptor agonists on behavioural effects of the psychostimulant. Actually, Callahan and Cunningham (14, 16)

reported that RU 24969 and TFMPP, agonists of 5-HT_{1B/1A} and 5-HT_{1B/2C} receptors, respectively, enhanced the discriminative stimulus effects of cocaine and partly substituted (ca. 50–70%) for the psychostimulant in a drug discrimination task. Furthermore, the latter agonists were found to enhance the reinforcing effects of self-administered cocaine (26). A recent study from our laboratory shows that both the locomotor activity effects and sensitisation to cocaine were augmented by the 5-HT_{1B} receptor agonist CP 94253 (24).

The potentiating effects of both CP 94253 and fluoxetine on cocaine discrimination were attenuated by GR 127935 and GR 55562, 5-HT_{1B} receptor antagonists (29, 30, 39, 40). Although GR 127935 fails to discriminate between 5-HT_{1B} and 5-HT_{1D} receptors in binding studies (39), the 5-HT_{1B/1D} selectivity of GR 55562 (30) suggests that activation of 5-HT_{1B} receptors underlies our above-mentioned effects of CP 94253 and fluoxetine. In line with the present drug discrimination data, it was demonstrated that GR 127935 reversed the effects of a 5-HT_{1B} agonist on cocaine locomotor behaviours (24). Interestingly, the latter antagonist also attenuated the 5-HT_{1B} receptor agonist-mediated enhancement of the amphetamine (41) or ethanol self-administration (42) and discrimination (43), both those behavioural effects depending on an increased dopamine neurotransmission.

Our findings on CP 94253 and fluoxetine in the discriminative stimulus effects of cocaine showed an interaction between 5-HT and dopamine pathways, with a direct and indirect, respectively, stimulation of 5-HT_{1B} receptors. Actually, it has been found that activation of 5-HT_{1B} receptors leads to an increase in basal (21, 22) and cocaine-stimulated (23) extracellular dopamine concentrations in the nucleus accumbens, a brain area involved in cocaine discrimination (44). Similarly, fluoxetine has been found to stimulate basal dopamine release; however, such an effect appears in the prefrontal cortex only (36). Interestingly, recent microdialysis studies showed that the 5-HT-, fluoxetine- or 5-HT_{1B} receptor agonist-mediated increases in dopamine extracellular release were reduced by co-perfusion with GR 127935 (45, 46) which *per-se* did not affect brain dopamine metabolism (47). Since fluoxetine and CP 94253 have no affinity for dopamine receptors or the dopamine transporter site (29, 35), direct stimulation of dopamine neurotransmission by these drugs can be excluded. In line with the above observation, neither SCH 23390 nor haloperidol, dopamine D₁-like and D₂-like receptor antagonists, respectively, blocked the partial substitution evoked by a 5-HT_{1B} receptor agonist for cocaine discrimination (14).

The explanation which anatomical substrates and neuronal mechanisms are responsible for the enhancement of cocaine discrimination by fluoxetine or CP 94253 is not an easy task. It has been demonstrated that acute systemic administration of fluoxetine or other selective 5-HT uptake inhibitors elicits only a small elevation or even no change in the extracellular neurotransmitter

level in the NAc or frontal cortex, terminal areas of the 5-HT system (48, 49, 50). In contrast to the above-described findings on 5-HT uptake inhibitors, a reduction in the extracellular output of 5-HT has been found after the agonist-evoked activation of 5-HT_{1B} receptors (51, 52). Although some recent findings postulate that blockade rather than activation of 5-HT_{1B} receptors at 5-HT terminals enhances the action of 5-HT uptake inhibitors on 5-HT neurotransmission (53, 54), our findings seem to exclude a role of presynaptically located 5-HT_{1B} receptors in the observed behavioural effects, since fluoxetine and CP 94253 acted in the same direction on cocaine discrimination and since enhancing effect of fluoxetine was reduced by the 5-HT_{1B} receptor antagonist. In partial support our data, O'Neill and co-workers (55) demonstrated that GR 127935 blocked behavioural effects of a 5-HT uptake inhibitor.

Since 5-HT_{1B} receptors have also been reported to function as inhibitory heteroreceptors modulating the release of other neurotransmitters (56, 57, 58), another possible mechanism whereby 5-HT_{1B} receptor agonists could enhance cocaine behaviours following an increase in dopamine release would be the diminishing release of a neurotransmitter (such as, a γ -aminobutyric acid, GABA) which exerts an inhibitory effect on dopamine release (59, 60). Indeed, a high density of 5-HT_{1B} receptors is found in the mesolimbic dopamine pathway (20), and the 5-HT_{1B} receptor-mediated enhancement (due to the reduction of GABA release in the ventral tegmental area) of the cocaine-stimulated accumbal dopamine release (23) following disinhibition of dopamine cell bodies (58) has been reported. The observed potentiation of cocaine effects may also be mediated by stimulation of 5-HT_{1B} heteroreceptors located in other brain areas, such as, e.g. the subicular area of the ventral hippocampus or the NAc. It has been observed that intra-subicular stimulation of 5-HT_{1B} receptors, which are located on the glutamatergic neurons projecting to the NAc, reproduces the enhancing effects on DA levels evoked by systemic administration of 5-HT_{1B} receptor agonists (22). Interestingly enough, in the NAc it was found that 5-HT through 5-HT_{1B} receptors inhibited the synaptic potentials on GABA-ergic medium-spiny neurons by decreasing the excitatory input from ascending glutamate-containing neurons (61). The latter effect of 5-HT has been potentiated by pretreatment with cocaine or citalopram (61). However, since in our study drugs were administered systemically, it may only be speculated on which brain area(s) is (are) responsible for the 5-HT_{1B} receptor-mediated enhancement of cocaine discrimination. To approach this issue, further experiments using a microinjection technique are necessary.

In our study we also observed that 5-HT_{1B} receptors are not directly involved in the discriminative stimulus effects of cocaine. In fact, cocaine discrimination was not affected by the 5-HT_{1B} receptor antagonists administered in doses which had been reported to antagonize a number of

responses to 5-HT_{1B} receptor agonists (27, 55, 62). The negative results obtained with GR 127935 or GR 55562 regarding cocaine discrimination are somewhat in line with our recent data that show that the former antagonist did not affect cocaine sensitisation (24); on the other hand, however, it almost totally blocked the locomotor hyperactivity induced by acute treatment with cocaine (24, 27).

In conclusion, our results indicate that 5-HT_{1B} receptors are not involved in the ability of cocaine to produce a discriminative stimulus effects in rats. They indicate, however, that pharmacological stimulation of 5-HT_{1B} receptors — which also seem to be a target for the fluoxetine-mediated increase in 5-HT neurotransmission — can enhance the overall effects of cocaine.

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