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Effects of 5-HT_{1B} receptor ligands microinjected into the ventral tegmental area on cocaine discrimination in rats

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Abstract

Some recent data indicate a significant interaction between serotonin (5-hydroxytryptamine; 5-HT) and dopamine in mesolimbic brain structures (e.g. the ventral tegmental area) which modulate the behavioral effects of cocaine in rats. The present study investigated the role of 5-HT_{1B} receptors in the ventral tegmental area in the discriminative stimulus effects of cocaine in rats. Male Wistar rats were trained to discriminate cocaine (10 mg/kg, intraperitoneally (i.p.)) from saline (i.p.) in a two-choice, water-reinforced fixed-ratio 20 procedure. After reaching the cocaine–saline discrimination criterion, the rats were stereotaxically implanted with bilateral cannulae in the ventral tegmental area and were then microinjected with selective 5-HT_{1B} receptor ligands. In substitution studies, microinjections of the 5-HT_{1B} receptor antagonist, 3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride (GR 55562; 0.1–1 μg/side), did not evoke cocaine-lever responding, whereas the 5-HT_{1B} receptor agonist, 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one (CP 93129; 0.3–1 μg/side), induced partial substitution for cocaine. Intra-tegmental microinjections with the 5-HT_{1B} receptor antagonist, GR 55562 (0.1–1 μg/side), before cocaine (5 mg/kg), which alone produced 98% cocaine-lever responses, decreased in a dose-dependent manner the discriminative stimulus effects of the psychostimulant. On the other hand, combination tests using a fixed dose of CP 93129 (0.3 or 1 μg/side), given into the ventral tegmental area prior to low systemic doses of cocaine (1.25–2.5 mg/kg), increased cocaine discrimination. These results seem to indicate that tegmental 5-HT_{1B} receptors are necessary to express the discriminative stimulus effects of cocaine.

Keywords: 5-HT_{1B} receptor; Cocaine; CP 93129; GR 55562; Discriminative stimulus effect; Ventral tegmental area

1. Introduction

Cocaine inhibits the dopamine reuptake mechanism (Koe, 1976) and indirectly activates dopamine receptors (Kelly and Iversen, 1976); consequently, behavioral responses to cocaine (locomotor, sensitizing, rewarding and discriminative stimulus effects) depend on dopamine neurotransmission (Kelly and Iversen, 1976). In this respect, a crucial role is played by the dopamine mesoaccumbal pathway from the ventral tegmental area to the nucleus accumbens (Kelly and Iversen, 1976; Callahan et al., 1994; Callahan and Cunningham, 1997; McBride et al., 1999).

Some evidence indicates that not only dopamine but also serotonin (5-hydroxytryptamine; 5-HT) neurotransmission mediate some behavioral effects of cocaine (Parsons et al., 1995; Walsh and Cunningham, 1997). Among several 5-HT receptors, 5-HT_{1B} ones were found to be an important substrate for the locomotor stimulant effects of cocaine (McCreary et al., 1997; Przegaliński et al., 2001). Furthermore, pharmacological stimulation of these receptors enhanced the locomotor (Przegaliński et al., 2001), sensitizing (Przegaliński et al., 2001), rewarding (Parsons et al., 1998) and discriminative stimulus effects of cocaine (Callahan and Cunningham, 1995, 1997; Filip et al., 2001). Regarding the latter effect, we found previously that activation of 5-HT_{1B} receptors in the nucleus accumbens core (but not the shell) enhanced the cocaine-induced discriminative stimulus effect (Filip et al., 2002). The above behavioral observations are supported by the following data: (1) cocaine inhibits not only dopamine reuptake but also the reuptake of other neurotransmitters including 5-HT (Koe, 1976); (2) 5-HT_{1B} receptors act not only as autoreceptors regulating 5-HT release (Sharp et al., 1989; Hjorth and Tao, 1991), but also as

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heteroreceptors regulating the release of other neurotransmitters, of which dopamine is an example (Sarhan et al., 1999, 2000); (3) 5-HT_{1B} receptors and their mRNA are distributed in several brain areas including the mesoaccumbens dopamine pathway (Bruinvels et al., 1993; Bonaventure et al., 1998).

It has been established that the nucleus accumbens (Wood and Emmett-Oglesby, 1989; Callahan et al., 1994), but not the ventral tegmental area (De La Garza et al., 1998), are one of the key anatomical brain substrates underlying discriminative stimulus effects of cocaine. However, it has been reported that pharmacological manipulations of some neurotransmitter systems, e.g. 5-HT, γ-aminobutyric acid (GABA), in the ventral tegmental area affected accumbens-dependent locomotor (McMahon et al., 2001) and reinforcing (Brebner et al., 2000) effects of cocaine. In the present study, we investigated the role of 5-HT_{1B} receptors in the ventral tegmental area in the discriminative stimulus effects of cocaine to verify the hypothesis that 5-HT_{1B} receptor ligands in dopamine cell bodies indirectly affected the above-described behavioral response to cocaine. To this end, we tested the selective 5-HT_{1B} receptor ligands, 3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride (GR 55562) and 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one (CP 93129), an antagonist and an agonist, respectively, (Macor et al., 1990; Chopin et al., 1994; Lamothe et al., 1997) following their direct microinjection into the ventral tegmental area.

2. Materials and methods

2.1. Animals

Male Wistar rats (280–300 g; $N=20$) were housed two to a cage at a room temperature of 20 ± 1 °C, on a 12-h light/dark cycle (the light on between 0600 and 1800 h). Although food (Labofeed pellets) was always available, the water received by each animal was restricted to the amount given during training sessions in operant chambers, after test sessions (15 min) and at weekends. All the experiments were approved by the Institutional Committee for Laboratory Animal Welfare and Ethics, and they followed the International Animal Guide for the Care and Use of Laboratory Animals.

2.2. Drugs

The following drugs were used (in parentheses: presession injection times, suppliers): cocaine hydrochloride (−15 min; Merck, Germany), 1,4-dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5*H*-pyrrolo[3,2-b]pyridin-5-one (CP 93129; −15 min; Pfizer, USA) and 3-[3-(dimethylamino)-propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl]benzamide dihydrochloride (GR 55562; −20 min; Tocris, UK). All the drugs were dissolved in sterile saline (0.9% NaCl). Cocaine was injected intraperitoneally (i.p.) in a volume of 1 ml/kg. CP 93129 or GR 55562 was injected intracranially in a volume of 0.2 μl/side.

2.3. Discrimination procedure

The rats were trained to discriminate cocaine (10 mg/kg) from saline (0.9% NaCl), as described previously (Filip et al., 2001). Briefly, the drug or saline was administered i.p. 15 min before daily (Monday–Friday) sessions (30 min), in two-lever operant chambers (Med-Associates; USA), on a schedule (fixed ratio, FR 1) of continuous water reinforcement (0.05 ml of water per reinforcement). The reinforcement schedule was increased until all the animals responded reliably to each experimental condition under an FR 20 schedule. This phase of training continued until all the animals met the criterion (an individual mean accuracy of at least 80% of correct responses, before the first reinforcer during 10 consecutive sessions). After the rats reached 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 alternated with test sessions to maintain discrimination accuracy. Only the rats that had met an 80% performance criterion during the preceding cocaine and saline sessions were used in the tests. Upon completion of 20 responses to either lever, or after the session time elapsed, a single reinforcer was delivered and the animals were removed from the chamber. Once in their home cages, all the rats were allowed 10–15 min of free access to water.

During test sessions, a systemic dose–response curve for cocaine was made before and after surgical implantation of cannulae; the rats were tested 15 min after cocaine injection (0.6–10 mg/kg, i.p.). In subsequent substitution tests, the lever selection was assessed after bilateral intracranial injection of sterile saline (0.9% NaCl; 0.2 μl/side), CP 93129 (0.3, 1 and 3 μg/side; −15 min) or GR 55562 (0.1, 0.3 and 1 μg/side; −20 min) combined with systemic injection of saline (1 ml/kg, i.p.). Control tests were also carried out on rats that had been tested for lever selection 15 min following administration of either saline or cocaine (10 mg/kg, i.p.), preceded by intracranial injection of saline (0.2 μl/side). In combination tests, intracranial administration of CP 93129 (0.3 and 1 μg/side; −15 min) or GR 55562 (0.1, 0.3 and 1 μg/side; −20 min) preceded the injection of systemic cocaine, which produced either less (in potentiation tests) or more (in antagonism tests) than 80% of the cocaine-lever responding when given alone; the rats were tested for lever selection 15 min later.

2.4. Implantation of cannulae

The rats were anesthetized with intramuscular (i.m.) ketamine, 100 mg/kg, xylazine, 65 mg/kg, in 0.9% NaCl.

Having the tooth bar of the Kopf stereotaxic instrument positioned at −3.3 mm below the interaural line and using the intersections of the bregma and longitudinal sutures as origins, the rats were implanted bilaterally with stainless steel guide cannulae (9 mm long, 0.4 mm o.d., 0.3 mm i.d.) 2 mm above the ventral tegmental area (AP = −5.8 mm from the bregma, ML = ±0.5 mm, DV = −6 mm) (Paxinos and Watson, 1998). The guide cannulae were fastened to the skull with stainless steel screws and cranioplastic cement. The rats were allowed a 1-week recovery during which they were handled and weighed daily.

Following recovery, discrimination training was resumed. After 3–4 weeks, the systemic dose–response curve for cocaine was repeated and was found not to differ from that made prior to surgery (data not shown). During this period, the rats were habituated to a short-term confinement associated with the intracranial microinjection technique by removing the internal cannulae, gently restraining the rats for ca. 2–3 min, and replacing the obturators. For each microinjection, the bilateral obturators were removed and replaced with two stainless steel bilateral internal cannulae (11 mm long, 0.3 mm o.d.) positioned 2 mm below the tips of bilateral guide cannulae. The bilateral internal cannulae were attached to two 5-μl Hamilton syringes via PE-50 tubing (Small Parts, USA). A microsyringe drive (BAS, West Lafayette, USA), operated with a programmable controller (Bee Hive Controller, BAS), delivered a volume of 0.2 μl/side at a rate of 0.1 μl/min. A diffusion time of 1 min was allowed before the removal of the injection cannulae and replacement of the obturators. Each rat received 10 intracranial microinjections.

2.5. Histology

After completion of the experiment, the rats were overdosed with chloral hydrate (800 mg/kg, i.p.) and their brains were removed and stored for 3 days in a 20% sucrose–10% formalin solution. Then the brains were cut into 50-μm sections, which were later mounted onto gelatin-coated glass slides. The brain sections were defatted, stained with cresyl violet, cleared with xylene and cover-slipped. The sections were analyzed using a light microscope to determine the location of the cannulae. Only the animals whose cannulae were within the ventral tegmental area were used for statistical analysis.

2.6. Statistical analyses

During training and test sessions, accuracy (mean ± S.E.M.) was defined as the ratio of correct responses to the total number of responses before delivery of the first reinforcer. Response rates (responses per second; mean ± S.E.M.), regarded as a measure of behavioral disruption, were calculated as the total number of responses to either lever before completion of the first FR 20, divided by the number of seconds required to complete the FR. 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 drug-lever responding and response rates during the test sessions with the corresponding values from the preceding drug session (substitution tests) or the test dose given alone (combination tests). An analysis of variance for repeated measures was used to determine whether the percentage of cocaine-appropriate responses and response rates observed at a fixed dose of cocaine differed in the presence or absence of a dose of the 5-HT_{1B} receptor agonist and antagonist (combination tests); post hoc comparisons at a dose of cocaine with or without the test drug were made with the Student's *t*-test.

3. Results

Fig. 1 shows a representative distribution of injection sites in the ventral tegmental area (the cannula placements extended between 5.8 and 6.3 mm to the bregma). The rats whose cannula placements were outside this brain area were discarded. No significant tissue damage was observed during histological examination of sections.

The acquisition of cocaine (10 mg/kg) vs. saline discrimination was reached in an average of 29 sessions (ranging between 20 and 37). After surgery, the criterion was met in 15 sessions by rats with the cannulae implanted in the ventral tegmental area. Administration of systemic cocaine produced a dose-dependent increase in the cocaine-lever responding prior to (data not shown) and after surgical

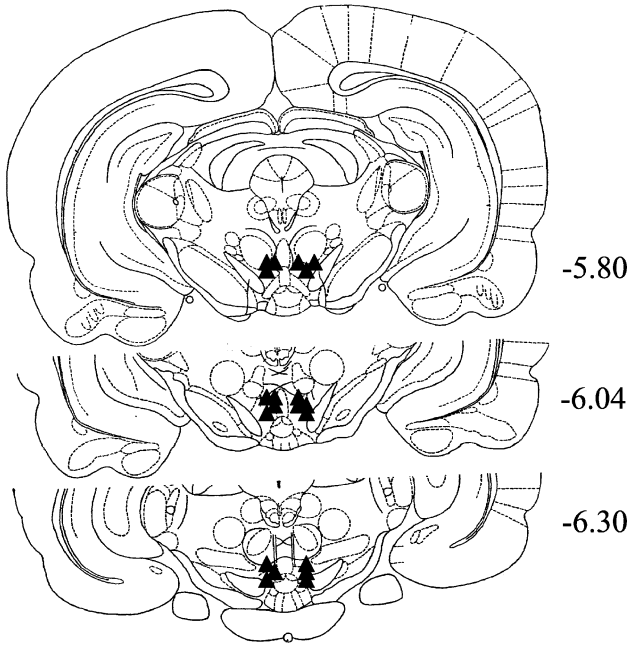

Fig. 1. Schematic diagram showing the representative injection sites (the numbers beside the plates denote the distance from the bregma [in mm]).

implantation of the cannulae (Fig. 2, top); drug-lever responding after cocaine, 0.6–10 mg/kg, ranged from 5% to 100%. No differences between the pre- (data not shown) and the post-surgery cocaine (Fig. 2, top) dose–response curves were observed.

Systemic administration of saline evoked less than 10% drug-lever responding (data not shown), as did intra-ventral tegmental area microinjection of saline prior to systemic injection of saline (Fig. 2, top).

The response rates after saline or after all the test doses of cocaine did not differ significantly from those recorded during the preceding cocaine session (Fig. 2, bottom; $P>0.05$).

3.1. Substitution studies with 5-HT$_{1b}$ receptor ligands

Intra-ventral tegmental area microinjection of GR 55562 (0.1, 0.3 or 1 μg/side) evoked no substitution for the drug, the drug-lever responding values being 10%, 8% and 10%, respectively (Fig. 2, top). The response rates were unaltered (Fig. 2, bottom).

Following intra-ventral tegmental area administration of CP 93129 (0.3, 1 or 3 μg/side), partial substitution (ca. 53% of the drug-lever responding after 1 μg/side of CP 93129) for cocaine was observed (Fig. 2, top). CP 93129 (0.3 or 1 μg/side) did not alter the response rates (Fig. 2, bottom). None of the seven animals completed the FR20 (behavioral disruption) when given CP 93129 in a dose of 3 μg/side (Fig. 2, bottom).

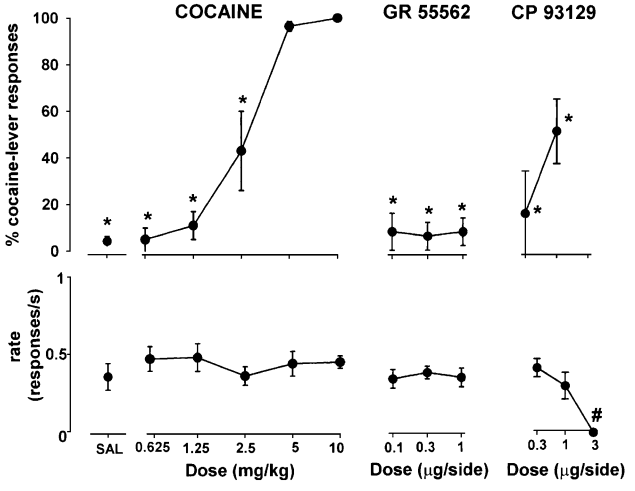

Fig. 2. Results of substitution tests with systemic saline or cocaine and with intra-ventral tegmental area microinjections of either saline or 5-HT$_{1B}$ receptor ligands in rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline. Symbols represent the performance after saline (SAL; 0.2 μl/side), cocaine (i.p.), GR 55562 (0.1–1 μg/side) or CP 93129 (0.3–3 μg/side) expressed as the mean percentage of cocaine-lever responses (± S.E.M.; top panels) or as response rates (responses/s ± S.E.M.; bottom panels). All the data show the mean values from six rats. Asterisks indicate differences in the percentage of drug-lever responding between test sessions and the corresponding values for preceding injections of cocaine ($P<0.05$). The animals' responding was disrupted after CP 93129, 3 μg/side (#; see bottom right panel).

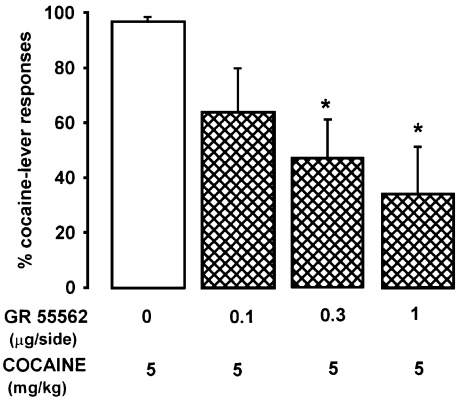

Fig. 3. Results of combination tests with intra-ventral tegmental area microinjections of the 5-HT$_{1B}$ receptor antagonist, GR 55562, to rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline. Bars indicate the performance of a group of animals injected with a fixed dose of cocaine (5 mg/kg, i.p.), following intra-ventral tegmental area saline or GR 55562 (0.1–1 μg/side). All the data show the mean values from seven rats. Asterisks indicate differences in the percentage of drug-lever responding between the 5-HT$_{1B}$ receptor antagonist + cocaine and the corresponding values for saline + cocaine ($P<0.05$). The responding rates were not affected by any drug pretreatment (see Results).

3.2. Combination studies with 5-HT$_{1b}$ receptor ligands

Combined administration of GR 55562 (0.1, 0.3 or 1 μg/side) into the ventral tegmental area decreased in a dose-dependent manner the discriminative stimulus effects produced by cocaine, 5 mg/kg, which per se induced a 98% drug-lever responding ($F(3,27)=3.14$, $P<0.05$; Fig. 3);

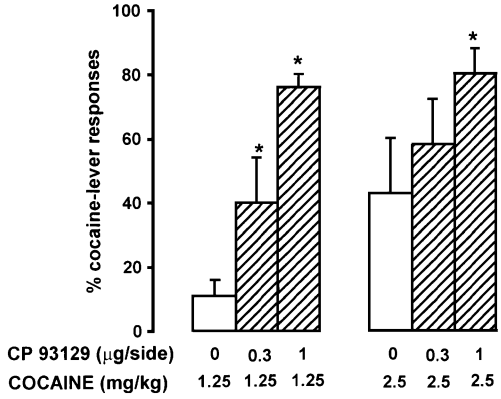

Fig. 4. Results of combination tests with intra-ventral tegmental area microinjections of the 5-HT$_{1B}$ receptor agonist, CP 93129, to rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline. Bars indicate the performance after saline (0.2 μl/side), CP 93129 (0.3 μg/side) or CP 93129 (1 μg/side) injected with different doses of cocaine (1.25–2.5 mg/kg, i.p.). All the data show the mean values from seven rats. Asterisks indicate differences in the percentage of drug-lever responding between the 5-HT$_{1B}$ receptor agonist + a dose of cocaine and the corresponding values for saline + a dose of cocaine ($P<0.05$). The responding rates were not affected by any drug treatment (see Results).

significant attenuation was observed following GR 55562, 0.3 or 1 μg/side ($P<0.05$). The response rates were unaltered after the above treatment combinations ($F(3,27)=0.18$, $P>0.05$).

Pretreatment with CP 93129, 0.3 or 1 μg/side, induced a significant effect on the percentage of cocaine-appropriate responding evoked by cocaine in a dose of 1.25 mg/kg ($F(2,20)=6.13$, $P<0.01$) and of 2.5 mg/kg ($F(2,20)=3.89$, $P<0.05$). Intra-ventral tegmental area microinjection of CP 93129 (0.3 or 1 μg/side) in combination with systemic cocaine (1.25 mg/kg), which induced ca. 11% drug-lever responses, significantly increased cocaine discrimination (Fig. 4; $P<0.05$). Significant increase of cocaine discrimination was also found following intra-ventral tegmental area administration of CP 93129 (1, but not 0.3, μg/side) in combination with systemic cocaine (2.5 mg/kg), which induced ca. 43% drug-lever responses (Fig. 4; $P<0.05$). The response rates were unaltered after CP 93129 + cocaine (1.25 mg/kg), $F(2,20)=1.59$, $P>0.05$, and after CP 93129 + cocaine (2.5 mg/kg), $F(2,20)=1.64$, $P>0.05$.

Intra-ventral tegmental area microinjection of GR 55562 (0.1 μg/side) reduced the CP 93129 (1 μg/side)-induced increase in the discriminative stimulus effects of cocaine (1.25 mg/kg) (Fig. 5, $P<0.05$). The response rates were unaltered after the above treatment combinations: cocaine (1.25 mg/kg) = 0.45 responses/s (± 0.08), CP 93129 (1 μg/side) + cocaine (1.25 mg/kg) = 0.37 responses/s (± 0.06), and GR 55562 (0.1 μg/side) + CP 93129 (1 μg/side) + cocaine (1.25 mg/kg) = 0.36 responses/s (± 0.08).

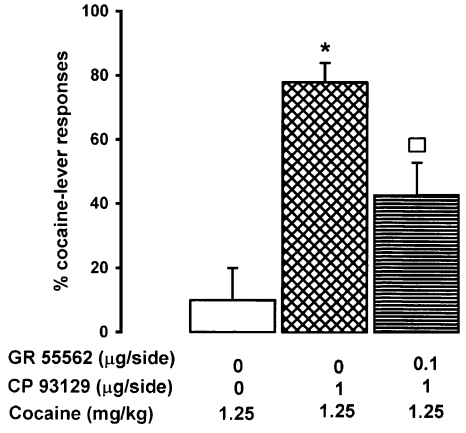

Fig. 5. Results of combination tests with intra-ventral tegmental area microinjections of the 5-HT_{1B} receptor antagonist, GR 55562, on the CP 93129-mediated increase of cocaine discrimination in rats trained to discriminate cocaine (10 mg/kg, i.p.) from saline. Bars indicate the performance after saline (0.2 μl/side), CP 93129 (1 μg/side) or GR 55562 (0.1 μg/side) + CP 93129 (1 μg/side) injected with cocaine (1.25 mg/kg, i.p.). All the data show the mean values from seven rats. An asterisk indicates differences in the percentage of drug-lever responding between saline + the 5-HT_{1B} receptor agonist + cocaine and the corresponding values for saline + saline + cocaine ($P<0.05$), a square indicates differences in the percentage of drug-lever responding between GR 55562 + CP 93129 + cocaine and the corresponding value for saline + CP 93129 + cocaine ($P<0.05$). The responding rates were not affected by any drug treatment (see Results).

4. Discussion

In the present study, we found that 5-HT_{1B} receptors in the ventral tegmental area modulate the discriminative stimulus effects of cocaine in rats, since the 5-HT_{1B} receptor antagonist, GR 55562 (Lamothe et al., 1997), microinjected locally to this brain structure, reduced the behavioral response to the psychostimulant. Our findings also indicate that pharmacological stimulation of 5-HT_{1B} receptors following intra-tegmental administration of their selective agonist, CP 93129 (Macor et al., 1990; Chopin et al., 1994), produces a significant increase in the recognition of cocaine stimulus effects in rats. Taking into account that the 5-HT_{1B} receptor agonist per se induces partial substitution for cocaine (i.e. 20–53% of drug-lever responses) in a drug discrimination model, the observed increase following CP 93129 and low doses of cocaine might be an additive effect.

It is noteworthy that the 5-HT_{1B} receptor antagonist, GR 55562, was used in doses of 0.1–1 μg/side, doses that in the nucleus accumbens shell or core exerted a protective effect against the cocaine-induced hyperactivaton and development of sensitization in rats (Przegaliński et al., 2002), or against the 5-HT_{1B} receptor agonist-mediated enhancement of cocaine discrimination (Filip et al., 2002), respectively. On the other hand, CP 93129 was administered in a dose range capable of inducing some 5-HT_{1B} receptor-mediated functional responses in rats. Thus, when injected at a dose range of 1–10 μg into the paraventricular nucleus of the hypothalamus or into the ventral tegmental area, the latter 5-HT_{1B} receptor agonist was found either to decrease food intake (Macor et al., 1990) or to enhance basal locomotor activity (Przegaliński et al., unpublished data), respectively. Other experiments showed that intra-cerebroventricular injections of CP 93129 in doses of 1–10 μg potentiated cocaine reinforcement in a self-administration model (Parsons et al., 1998), whereas intra-accumbens core administration of this agonist in a dose of 1 μg increased the discriminative stimulus effects of cocaine (Filip et al., 2002).

Our present findings demonstrating that the ventral tegmental area is a site of action of 5-HT_{1B} receptors to control the discriminative stimulus effect of cocaine corroborate the results of some experiments on the blockade of development of cocaine sensitization following local microinjection of GR 55562 (Przegaliński et al., unpublished data). On the other hand, our data and other authors' showed that systemic administration of 5-HT_{1B} receptor antagonists, e.g. GR 127935 (McCreary et al., 1997, Filip et al., 2001), GR 55562 (Filip et al., 2001) or SB 216641 (Filip and Nowak, unpublished data), did not affect the discriminative stimulus effects of cocaine and amphetamine in rats. Lack of modulation of cocaine discrimination was also reported after intra-accumbens shell or core microinjection of GR 55562 (Filip et al., 2002). Both the previous (Filip et al., 2002) and the present data suggest that the 5-HT_{1B} receptor population present in the ventral tegmental area controls the behavioral effects of cocaine, whereas the same receptors located in nucleus

accumbens subregions seem not to be involved in the modulation of discrimination to cocaine. Further studies are necessary to answer the question: why did systemic administration of 5-HT_{1B} receptor antagonists not decrease the discriminative stimulus effects of cocaine, while intra-tegmental injections of the antagonist did attenuate the latter responses. Regarding such differences, it should be emphasized that the most critical factor could be the local concentration of 5-HT_{1B} receptor antagonists. It is very likely that intra-tegmental administration of 5-HT_{1B} receptor antagonists results in a higher local concentration and greater activation of 5-HT_{1B} receptors than can be achieved by systemic administration of these drugs. Alternatively, systemically administered 5-HT_{1B} receptor antagonists might modulate brain structures other than the ventral tegmental area with an opposite action on cocaine-induced discrimination.

Another finding now reported is that the ventral tegmental area is an important site for 5-HT_{1B} receptor agonists to increase the discriminative stimulus effects of cocaine. Actually, intra-tegmental bilateral administration of CP 93129 together with low doses of systemically injected cocaine produced a significant increase in the recognition of cocaine stimulus effects in rats; however, when either drug was given separately, it produced weak, partial substitution for cocaine. Such an increase in the drug-lever responding seen after CP 93129 plus the moderate dose of cocaine seems to be an additive effect, composed of the 5-HT_{1B} receptor agonist's effect and of the psychostimulant's own effects, similarly recognized by the animals. However, when CP 93129 was given concurrently with the ineffective dose of cocaine, its effects were greater than additive. The above results are consistent with some earlier findings for the effect of systemic administration of 5-HT_{1B} receptor agonists on psychostimulant-induced discrimination. Actually, the systemically injected 5-HT_{1B} receptor agonists, RU 24969 (Callahan and Cunningham, 1995) and CP 94253 (Filip et al., 2001), enhanced the cocaine-induced effects and shifted to the left its dose–response curves in a drug discrimination model. By enhancing the stimulus effect of cocaine, the 5-HT_{1B} receptors located in the ventral tegmental area share the properties of 5-HT_{1B} receptors located in the nucleus accumbens core, but not in the shell (Filip et al., 2002).

As to the mechanism underlying the 5-HT_{1B}–cocaine interaction in the ventral tegmental area, the following issues should be considered: (1) the ventral tegmental area receives dense 5-HT innervation from the raphe nuclei (Conrad et al., 1974; Azmitia and Segal, 1978) and possesses both the transcript and protein for 5-HT_{1B} receptors (Bruinvels et al., 1993; Pazos and Palacios, 1985); (2) 5-HT_{1B} receptors act not only as autoreceptors inhibiting 5-HT release (Hjorth and Tao, 1991; Sharp et al., 1989), but also as heteroreceptors present on dopamine (Sarhan et al., 1999), glutamate (Boeijinga and Boddeke, 1996) or GABA (Chadha et al., 2000) nerve terminals. Regarding the latter neurotransmitter, it has been found that the 5-HT_{1B} receptors located in the ventral tegmental area can function as heteroreceptors inhibiting GABA release from descending GABA projection neurons and/or GABA interneurons (Yan and Yan, 2001b); (3) besides that of dopamine, cocaine also promotes 5-HT release via a direct effect on the 5-HT transporter (Ritz et al., 1990; Andrews and Lucki, 2001). Interestingly, it has been reported that the cocaine-induced inhibition of GABA release in the ventral tegmental area is due to the release of 5-HT acting on 5-HT_{1B} receptors (Cameron and Williams, 1994; Parsons et al., 1999); (4) in vivo studies indicate that 5-HT_{1B} receptor agonists injected either systemically or locally (intra-accumbally or intra-tegmentally) increase basal and cocaine-stimulated extracellular dopamine concentrations in the nucleus accumbens (Guan and McBride, 1989; Parsons et al., 1999; Yan and Yan, 2001a).

In the light of the above and some other data, it has recently been hypothesized that the 5-HT_{1B} receptor–dopamine interaction in the ventral tegmental area is indirect, that it may be connected with GABA release. In fact, activation of 5-HT_{1B} receptors inhibits GABA release, which in consequence disinhibits dopamine neurons, whereas blockade of 5-HT_{1B} receptors enhances the GABA input followed by reduction of dopamine neurotransmission (Castanon et al., 2000). In the present study, such a mechanism may have been responsible for the increase or inhibition by the 5-HT_{1B} receptor agonist or antagonist administered to the ventral tegmental area, respectively, on cocaine discrimination. It should be stressed that, although the nucleus accumbens (Callahan et al., 1994), but not the ventral tegmental area (De La Garza et al., 1998), has been found to be the anatomical substrate underlying discriminative stimulus effects of cocaine, the present results indicate that this behavioral response to cocaine may be regulated "upstream" by 5-HT_{1B} receptors in the dopamine cell bodies.

Summing up, the present report shows that intra-tegmental microinjections of the 5-HT_{1B} receptor antagonist, GR 55562, decrease in a dose-dependent manner the discriminative stimulus effects of cocaine. On the other hand, the 5-HT_{1B} receptor agonist, CP 93129, given into the ventral tegmental area prior to systemic cocaine increases cocaine discrimination. These results seem to indicate that the tegmental 5-HT_{1B} receptors are necessary for full expression of the discriminative stimulus effects of cocaine.

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