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Modulation of the effects of cocaine by 5-HT1B receptors: a comparison of knockouts and antagonists

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Abstract

Serotonergic transmission has been suggested to modulate the effects of cocaine. However, the specific receptors underlying this phenomenon have not been identified. To evaluate the role of the 5-HT1B receptor in mediating the actions of cocaine, we used two model systems: knockout (KO) mice lacking the 5-HT1B receptor and an acute treatment with the 5-HT1B/1D antagonist GR127935. GR127935 attenuated the ability of cocaine to stimulate locomotion and induce c-*fos* expression in the striatum. However, GR127935 had no apparent effect on the rewarding or sensitizing effects of cocaine. In contrast, as demonstrated previously, the 5-HT1B receptor KO mice showed a heightened locomotor response to cocaine, as well as an increased propensity to self-administer cocaine. Thus, an acute pharmacological blockade of the 5-HT1B receptor decreases some effects of cocaine, while a constitutive genetic KO of the same receptor has opposite effects. These results suggest that compensatory changes have taken place during the development of the 5-HT1B KO mice, which may have rendered these mice more vulnerable to cocaine. The 5-HT1B KO mice should therefore be considered as a genetic model of vulnerability to drug abuse rather than a classic pharmacological tool.

Keywords: 5-HT1B receptors; Cocaine; GR127935; Locomotor activity; Behavioral sensitization; c-*fos* induction; C57Bl/6J mice; 129/Sv mice

1. Introduction

Cocaine has been suggested to derive a portion of its abuse potential from direct neuropharmacological action on brain reward circuits, specifically the mesostriatal and mesolimbic dopamine pathways. Indeed, the locomotor-activating and -reinforcing effects of cocaine are generally ascribed to its ability to augment dopamine-mediated neurotransmission by inhibiting dopamine uptake [20,33,34]. However, there is evidence that some of the other pharmacological properties of cocaine, especially its inhibition of serotonin (5-HT) and norepinephrine reuptake [32], may in fact play a crucial role in both its actions and abuse potential. Agents that only block dopamine and norepinephrine reuptake, but lack strong serotonergic activity, are less intensely abused than cocaine by humans and less avidly self-administered by animals [11]. Knockout (KO) mice lacking the reuptake transporter for dopamine still self-administer cocaine [36]. This suggests that in some situations, the actions of cocaine at the 5-HT transporter and/or the norepinephrine transporter may mediate its reinforcing effects.

Other research, however, indicates that 5-HT antagonizes the rewarding properties of cocaine. Enhancement of 5-HT levels via dietary tryptophan or 5-HT reuptake inhibitors like fluoxetine significantly reduces self-administration of cocaine [6,29,31]. Conversely, decreased 5-HT levels via 5,7-dihydroxytryptamine lesions or depletion of the 5-HT precursor tryptophan appear to increase the reinforcing effects of cocaine [21,22]. In those studies, however, 5-HT levels were manipulated as a whole, so these effects might reflect the net activation of several of the 14 different

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serotonin receptor subtypes and/or the net activation of several anatomically and functionally distinct 5-HT circuits [18,23,39]. The in vivo effects of cocaine are likely mediated by several serotonergic and dopaminergic receptors. The net effect of activating all these receptors might be very complex, explaining some of the controversy as to whether 5-HT exerts a stimulatory or an inhibitory effect on dopamine-mediated behaviors. More specific studies using selective agonists of the different 5-HT receptors, microinjected in selected brain structures, have shown a positive modulating effect of the serotonergic system on the functional activity of the mesotelencephalic dopaminergic system [2,10,26,46]. Although a variety of 5-HT receptors might be involved in this phenomenon [7], evidence points toward a prominent role of the 5-HT1B receptor [16]. Its abundance in motor control centers (the globus pallidus, substantia nigra (SN), and deep cerebellar nuclei) [3,25], has suggested its involvement in the control of locomotor activity. 5-HT1B receptor activation with either systemic or central administration of RU24969 (a 5-HT1A/1B agonist) results in a dose-dependent increase in locomotor activity [8,13]. In contrast, the locomotor-stimulating effect of RU24969 is completely absent in mice lacking the 5-HT1B receptor [38]. Furthermore, in vitro pharmacological studies have indicated that direct or indirect (i.e., with cocaine) 5-HT1B receptor stimulation enhances dopamine efflux in the caudate putamen (CP) and nucleus accumbens (NAcc) [14,17]. Likewise, recent behavioral studies have suggested the involvement of 5-HT1B receptors in modulating the effects of cocaine, since 5-HT1B receptor agonists (1) substitute nearly fully for cocaine in drug discrimination studies [4] and (2) enhance some of the reinforcing properties of cocaine [27,28] while decreasing others [9,15]. KO mice have also been used to investigate the roles of 5-HT1B receptors [38,43]. We have demonstrated that 5-HT1B KO mice display a markedly reduced effect of cocaine on c-*fos* induction in different brain structures, most notably in the striatum [24]. We have also shown that 5-HT1B KO mice have an enhanced locomotor response to cocaine, as well as an increased propensity to self-administer cocaine [36]. It appears from these various studies that 5-HT1B receptors have different effects depending on the specific responses to cocaine that are measured and depending on the tools that are used (KOs vs. antagonists) [1,43]. We decided therefore to compare directly the effects of KOs and antagonists on a series of responses to cocaine. We find that while some responses to cocaine are similarly decreased in KOs as by antagonists (c-*fos* induction), other responses are markedly different. The locomotor response to cocaine is increased in KOs while it is reduced in the presence of an antagonist. The rewarding effect of cocaine as measured in a progressive ratio (PR) schedule of self-administration is increased in the KOs and unaffected by the antagonist. A possible rationale for such differences comes from the increasing evidence that KO mice undergo compensatory changes during development. As a result, the phenotype of such KOs may not mirror the effects of antagonists; but may be more indicative of what can happen in genetic disorders [12].

2. Methods

2.1. Subjects

Male 129/Sv wild-type (WT, 27–30 g) inbred mice and male 129/Sv KO mice lacking 5-HT1B receptors (27–30 g) (generated as described previously in Ref. [38], see also Ref. [30] for a description of the genetic background) were bred at Columbia University (Center for Neurobiology and Behavior, New York, NY, USA). They were 14 weeks old at the beginning of the experiments. For the self-administration studies, 129/Sv WT and KO were housed individually and maintained at 28 g (±5 g) by restricting their access to food. C57Bl/6J mice, aged 4 weeks on arrival (19–23 g), were obtained from Jackson Laboratories (Bar Harbor, ME). They were 12 weeks old at the beginning of the experiments. Mice were housed by strain in communal cages (five mice per cage), under conditions of constant temperature (22±1°C) and a 12:12-h cycle of illumination (lights on at 0600 h). Food and water were accessible ad libitum unless otherwise indicated.

2.2. Drugs

All drugs were administered intraperitoneally (1.0 ml/kg) and were dissolved in isotonic saline just prior to use. Cocaine was purchased from Sigma (St Louis, MO); GR127935 was provided by Glaxo Group Research (United Kingdom).

2.3. Immunohistochemistry

Two hours after the last cocaine injection, mice were anesthetized with a ketamine–rompum mixture and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer for 10 min. The brains were post-fixed in 4% paraformaldehyde for 2 h at room temperature and placed in 30% sucrose in PBS for 48 h at 4°C. Sections (30 μm) were cut in a freezing microtome and collected in PBS. Free-floating sections were pre-treated with 3% H_2O_2 in PBS and incubated overnight at 4°C in affinity-purified primary antibody raised against the c-Fos M-peptide (kindly provided by Dr. M. Iadarola) diluted 1:5000 in PBS containing 0.3% Triton X-100, 10% N goat serum (Gibco) and 1% BSA (Boehringer-Mannheim; Indianapolis, IN). Following three PBS washes, sections were carried through standard avidin–biotin immunohistochemical protocols using an Elite Vectastain kit (Vector Laboratories, Burlingame, CA). Chromogen reaction was performed with diaminobenzidine (Sigma) and 0.003% H_2O_2 for 10 min. The sections were mounted on chromalum-coated slides and coverslipped with Aqua-PolyMount (Polysciences, Warrington,

PA). Omission of the primary antibody or pre-adsorption with the M-peptide resulted in absence of labeling.

2.4. Cell counting

Counting of immunopositive nuclei was performed using a computerized image analysis system (MCID, Imaging Research) attached to a microscope (Leica Diaplan). The counting was performed in a semi-automated fashion with shading error acquired in a nonsample-containing part of the preparation to correct for uneven distribution of light, and form and shape factors suitable for the used magnification ($\times$ 100). The number of Fos-like immunoreactive cells in the CP is presented as the mean number of cells counted in anterior, middle, and posterior CP in three 30-μm coronal sections ($n = 4-6$ animals per treatment). The three different antero-posterior levels correspond to the F 1.0, F 0.4, and F $-$0.2 levels of Slotnick and Leonard's Atlas of Mouse Forebrain.

2.5. Self-administration

Self-administration experiments took place in mouse operant chambers (model ENV-300; Med Associates, Georgia, VT), as previously described [35]. Initially, mice were trained to press a lever under fixed ratio (FR, 1:2) schedule using sweetened condensed milk solution as reinforcer (one part of sweetened condensed milk: two parts of water); after responding was stable on both levers, each subject was implanted with a permanent indwelling jugular catheter as previously described [35,37]. Two days following surgery, mice started training under FR 1 schedule of reinforcement, with cocaine, 2.0-mg/kg/0.02 ml injection as reinforcer. Sessions lasted either until 20 injections were self-administered or until approximately 3 h had elapsed. At the start of each session, a priming injection of cocaine (2.0 mg/kg in 0.02 ml) was given. Priming and subsequent injections were accompanied by a flashing of the stimulus light and followed by a 30-s timeout in the dark when pressing a lever had no consequences. Once mice met acquisition criteria for self-administration (at least 75% of active lever pressings and at least 20 injections within 3 h for three consecutive sessions), they were switched to a PR schedule. Daily PR sessions started with a 2.0-mg/kg per injection priming infusion, and mice were permitted to self-administer cocaine each day during 3-h session. Each subsequent cocaine injection (2.0 mg/kg per injection) occurred upon completion of each of the ratios in the exponential sequence previously described [37]. There was a 1-h time limit to obtain each reinforcer, and failure to obtain the reinforcer terminated the session. Acquisition of the PR schedule is defined as at least three consecutive sessions in which the number of infusions earned (i.e., breaking point) did not differ by more than three injections.

Once such a stable baseline was acquired under PR, mice were injected intraperitoneally with either saline or GR127935 (10 mg/kg), 10 min before the self-administration session, during three consecutive sessions. The dependent variable was the total number of cocaine infusions earned in the third day.

2.6. Locomotor monitoring

All experiments were performed during the illuminated part of the cycle, under conditions of dim light and low noise. Activity in the testing cages was monitored using a videotracking system (Poly-Track, San Diego Instruments, CA) that records an animal's location and path (expressed in centimeters), as well as the number of rearings and exploratory nose-pokes. The testing cages were 40×40 cm^2 white Plexiglas boxes with eight holes in the wall (each equipped with an infrared photocell for the detection of nose-pokes, and an array of infrared beams to detect rearings). The boxes were cleaned thoroughly between testing sessions.

In order to observe the qualitative behavioral effects of cocaine, animals were also videotaped and their behavior was evaluated later by two trained observers, who were blind to both mouse genotype and drug treatment. Each animal was observed for 1 min at five different time points during the test session: 10, 20, 30, 40, and 50 min post-injection. Each mouse was assigned a single numerical score for each time period: 0: normal, quiet behavior; 1: normal, exploratory behavior; 2: rapid locomotion; 3: repetitive movement in a restricted area of the cage; 4: intense stereotypy (head weaving and circling) in one area of the cage [44]. For analysis, the number of times each animal was assigned each score was summed and then averaged for each condition.

Each mouse was handled daily for 5 days before the first exposure to the testing cage. On the day before testing, all animals were injected with isotonic saline and placed in the open field for 1 h. This was intended to reduce the effects of novelty on the day drug testing began. Each test session began with 30 min in the testing box after a saline injection, but before the administration of any drug. The antagonist (GR127935) was injected 30 min before cocaine. Behavioral activity was recorded from the first saline injection to 1 h after the last drug injection.

3. Results

3.1. Experiment 1: Increased locomotor response to cocaine in 5-HT1B KO mice

The administration of cocaine only slightly increased locomotor activity in 129/Sv WT mice, although robust locomotor stimulation was observed in 5-HT1B KO mice (Fig. 1A), as previously described in our laboratory [37]. For acute cocaine administration, ANOVA revealed a main effect of treatment [$F(2,48) = 3.42$, $P < .05$] and a main effect of genotype [$F(1,48) = 8.06$, $P < .01$], with no interaction between treatment and genotype. Analysis of behavioral scores confirmed that after a 40-mg/kg injec-

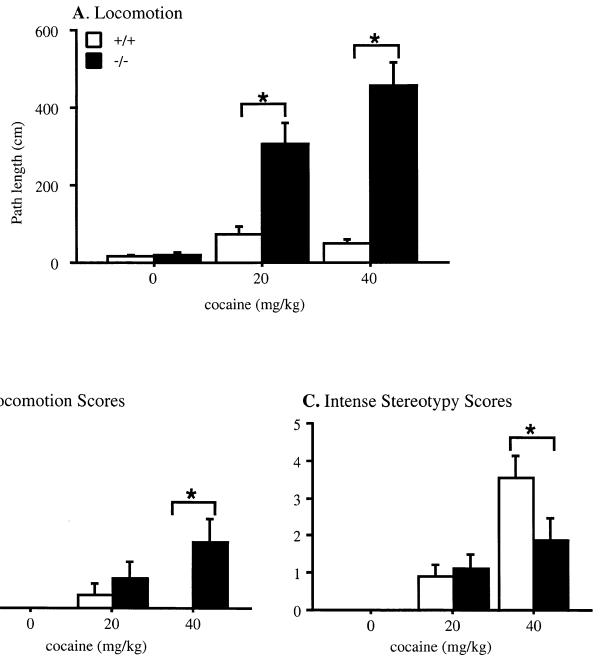

Fig. 1. (A) Effect of cocaine (20–40 mg/kg, ip) on locomotion in 129/Sv WT (+/+) and 5-HT1B KO (−/−) mice. Each bar represents mean path length (in centimeters) + S.E.M. covered in 5 min and averaged over 1 h for 10–12 mice per group. * Significant difference between KO and WT mice according to post-hoc Scheffé, $P<.05$. (B) Average number of times + S.E.M. each animal was scored as engaging in locomotor behavior. At 40 mg/kg cocaine, KO mice show significantly more locomotor behavior than WT (*$P<.05$, post-hoc Scheffé test). (C) Average number of times + S.E.M. each animal was scored as engaging in intense stereotypy. At 40 mg/kg cocaine, WT mice showed significantly more stereotypy (*$P<.05$, post-hoc Scheffé test).

tion of cocaine, KO mice spent more time engaged in locomotor behavior (Fig. 1B), while WT mice spent more time engaged in stereotypy (Fig. 1C). For intense stereotypy, ANOVA showed a main effect of cocaine dose [$F(1,33)=13.20$, $P<.001$] and an interaction of genotype and dose [$F(1,33)=3.90$, $P<.05$]. Each treatment group used in this experiment contained 10–12 mice.

3.2. Experiment 2: GR127935 attenuates the locomotor effects of cocaine

We used C57Bl/6J mice in this experiment because the locomotor effects of acute and repeated injections of cocaine are much more dramatic in this strain than in 129/Sv mice (compare Figs. 1 and 2). A single administration of cocaine (20 mg/kg) induced a sixfold increase of locomotion in WT C57Bl/6J mice (16 animals) compared to the saline control group (10 animals). There was a main effect of treatment [$F(5,89)=33.29$, $P<.001$], with Scheffé comparisons indicating a significant difference between cocaine- and saline-treated animals ($P<.0001$; Fig. 2A). Mice receiving a single GR127935 injection of either 3 mg/kg (10 mice) or 10 mg/kg (10 mice) exhibited the same locomotor activity as those from the control group ($P>.99$). Nevertheless, GR127935 reduced significantly the hyperlocomotion elicited by acute cocaine injection (14 mice, cocaine + 10 mg/kg GR127935 vs. cocaine alone: $P<.01$). Similarly, the number of rearings was significantly increased by 20 mg/kg cocaine (Fig. 2B). ANOVA showed a significant main effect of treatment [$F(5,86)=8.36$, $P<.0001$]. The increase in rearings elicited by cocaine was blocked by GR127935 pre-treatment (Scheffé comparison of cocaine alone vs. cocaine + 10 mg/kg GR127935: $P<.01$). GR127935 had a similar inhibitory effect on cocaine-elicited locomotion and rearing in 129/Sv mice and no effect in 5-HT1B KO mice (not shown).

3.3. Experiment 3: GR127935 has no effect on behavioral sensitization to cocaine

To assess the effects of GR127935 on cocaine-induced behavioral sensitization, we extended Experiment 2 by

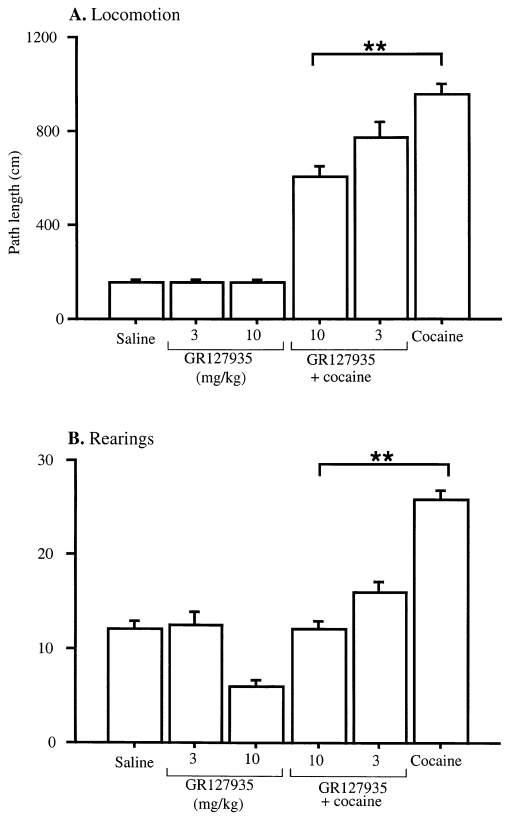

Fig. 2. Effect of GR127935 (3 or 10 mg/kg, ip) on cocaine (20 mg/kg, ip)-elicited locomotor hyperactivity (A) or rearings (B) in C57Bl/6J mice. Columns represent mean path length (in centimeters) or mean number of rearings + S.E.M. covered in 5 min and averaged over 1 h for 10 mice per group. GR127935 was administered 30 min before cocaine. Post-hoc Scheffé tests show that mice given 20 mg/kg cocaine (**) are significantly more active than mice given saline ($P<.001$), 3 mg/kg GR127935 alone ($P<.0001$), 10 mg/kg GR127935 alone ($P<.0001$), or 20 mg/kg cocaine with 10 mg/kg GR127935 ($P<.01$). For rearings, post-hoc Scheffé tests show that mice given 20 mg/kg cocaine (**) are significantly more active than mice given saline ($P<.005$), 3 mg/kg GR127935 alone ($P<.05$), 10 mg/kg GR127935 alone ($P<.0005$), or 20 mg/kg cocaine with 10 mg/kg GR127935 ($P<.01$).

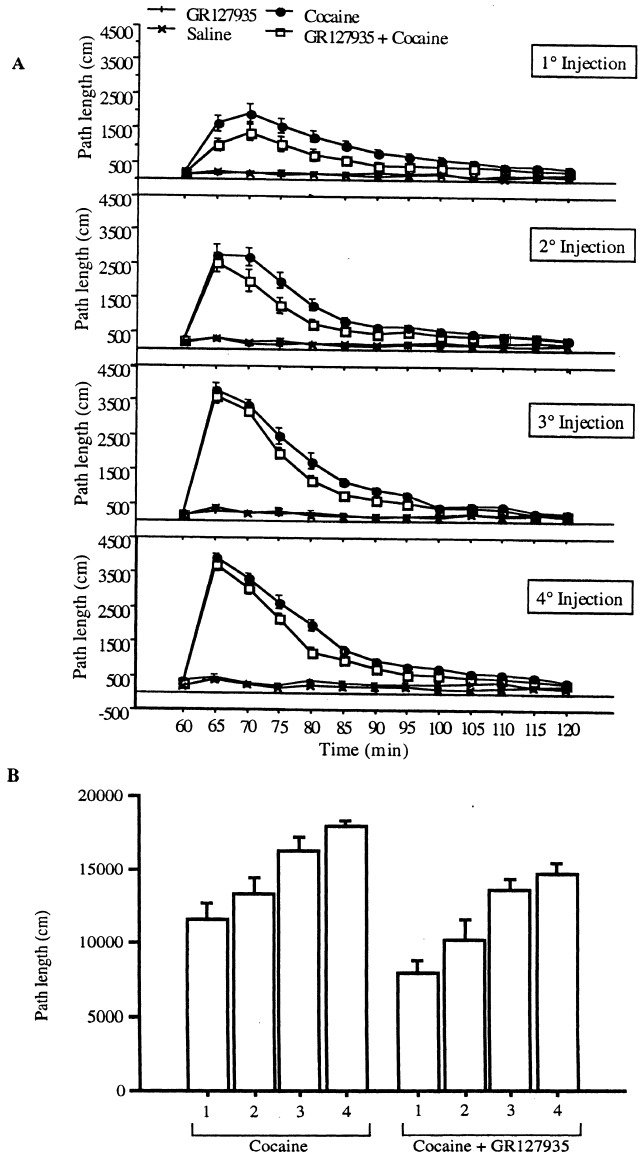

Fig. 3. (A) Effect of GR127935 on the sensitization of locomotion elicited by four repeated injections of cocaine (20 mg/kg, 1 week apart) in C57Bl/6J mice. Each bar represents the mean path length (in centimeters) + S.E.M. covered in 5 min. Each mouse was injected intraperitoneally, once a week, with saline ($n = 10$), GR127935 (3 mg/kg, $n = 10$), cocaine ($n = 13$), or both GR127935 + cocaine ($n = 13$). GR127935 was always administered 30 min before cocaine. (B) Progressive increase in locomotor activity after repeated injections of cocaine (20 mg/kg), or cocaine + GR127935 (3 mg/kg). The sessions are numbered 1–4; bars reflect total locomotion for each day (5 min averaged over 60 min).

repeating it four times with 1 week between each open-field exposure. C57Bl/6J mice were divided in four groups that received before each experiment either saline (10 mice), GR127935 (3 mg/kg, 10 mice), cocaine (20 mg/kg, 16 mice), or cocaine + GR127935 (13 mice). For all animals, there was a main effect of GR127935 treatment [$F(1,34) = 12.46$, $P < .005$], a main effect of cocaine treatment [$F(1,34) = 431.27$, $P < .0001$], and an interaction of cocaine and GR127935 treatment [$F(1,34) = 20.96$, $P < .0001$]. This confirms the results of our acute experiment. There was a main effect of the number of injections [$F(3,102) = 14.84$, $P < .0001$] and an interaction of cocaine treatment with the number of injections [$F(3,102) = 9.79$, $P < .0001$]. There was no significant interaction of GR127935 treatment with the number of injections. This suggests that the effect of cocaine did increase with the number of injections, with or without GR127935. To further examine the effect of GR127935 on locomotor sensitization, we analyzed the effect of GR127935 on cocaine-treated and saline-treated mice separately. The locomotor activity of saline-treated [$F(3,21) = 0.02$, $P > .9$] mice did not significantly change over the course of the four injections (Fig. 3A). However, for cocaine-treated animals, the ANOVA showed a significant effect of the number of cocaine injections on locomotor activity [$F(3,57) = 15.6$; $P < .0001$] (Fig. 3B). These results clearly showed that the locomotor activity of C57Bl/6J mice in the open-field sensitized to repeated cocaine injections. Mice repeatedly injected with both cocaine and GR127935 also showed significant sensitization of their locomotor response to cocaine injections [$F(3,33) = 11.69$; $P < .0001$] (Fig. 3B). These results suggest that activation of the 5-HT1B receptor is not required for the development of locomotor sensitization to cocaine.

3.4. Experiment 4: GR127935 has no effect on self-administration of cocaine in a PR schedule

Pre-treatment with GR127935 (10 mg/kg) did not influence cocaine self-administration (2.0 mg/kg per injection) under PR in either WT or KO mice (Fig. 4). A two-way ANOVA [Genotype (WT, KO) × Treatment (baseline, saline, GR127935)] showed a nonsignificant effect of treat-

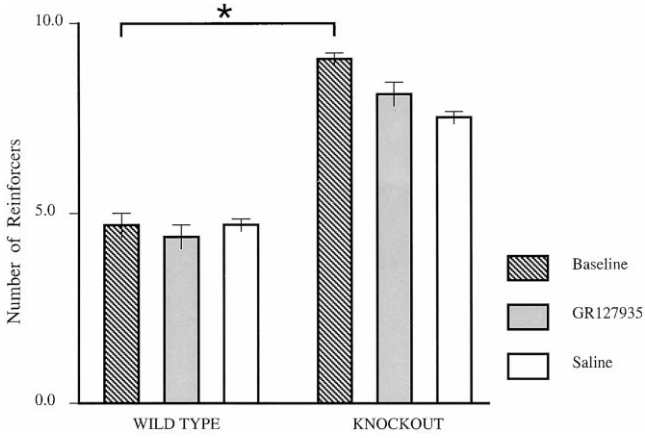

Fig. 4. Effect of GR127935 (10 mg/kg) on self-administration of cocaine (2 mg/kg per injection) in 129/Sv WT (+/+) and 5-HT1B KO (−/−) mice. Columns represent mean + S.E.M. of number of reinforcements obtained on a PR schedule (breaking point). There is a significant difference between the genotypes in baseline conditions ($P < .05$). There are no significant differences between the saline and GR127935 pre-treatment in either genotype.

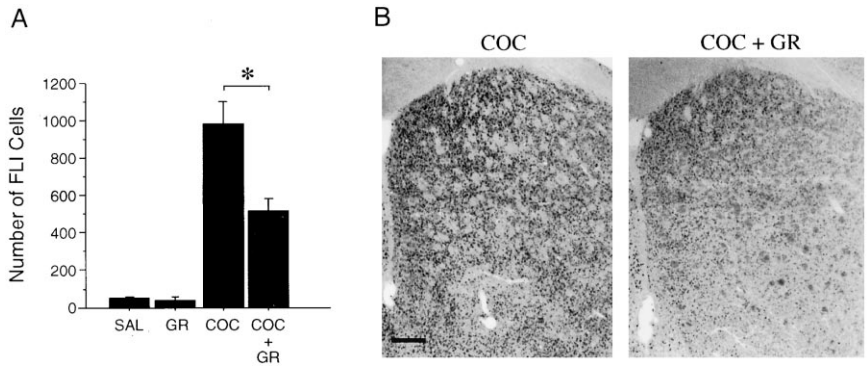

Fig. 5. Effect of GR127935 on cocaine-elicited Fos-like immunoreactivity in the striatum of C57Bl/6J mice. Each animal received two intraperitoneal injections at a 30-min interval. Control animals received two saline injections (Sal). Cocaine was given as second injection (Coc, 30 mg/kg) preceded by either saline or GR127935 (GR, 10 mg/kg). (A) Values represent the mean number of Fos-like immunoreactive cells counted in anterior, middle, and posterior CP in three 30-μm coronal sections of four to six animals per group. Pre-treatment with GR127935 significantly decreased the number of Fos immunoreactive cells induced by cocaine in WT mice (* $P < .01$). (B) Photomicrographs showing the effect of GR127935 on the increased Fos-like immunoreactivity elicited by cocaine in the anterior CP of C57Bl/6J mice. Scale bar indicates 200 μm for both photographs.

ment [$F(2,22) = 2.18$; $P < .05$], and a nonsignificant interaction [$F(2,22) = 2$; $P > .05$]. However, a highly significant effect of genotype [$F(1,22) = 63.88$; $P < .0001$] confirmed that KO mice responded to a higher breakpoint to cocaine when compared to WT (KO baseline = 8.92 ± 1.1; WT baseline = 4.75 ± 1.3) as previously reported [37].

3.5. Experiment 5: GR127935 decreases cocaine-induced Fos expression

We had already shown that GR127935 attenuates the effects of cocaine on striatal Fos-like immunoreactivity in 129/Sv mice [24]. We show here that the same results are obtained in C57Bl/6J mice. The number of Fos-positive cells observed in three coronal sections of four to five animals per group was counted and analyzed. As shown in Fig. 5, a 10-mg/kg, ip, dose of GR127935 alone had no significant effect on the level of Fos-like immunoreactivity in the striatum of C57Bl/6J mice. However, when co-administered with a 30-mg/kg, ip, dose of cocaine, GR127935 produced a significant reduction (45%, $P < .01$ by t test) in the number of Fos-like immunoreactive cells induced by cocaine alone.

4. Discussion

Using the 5-HT1B antagonist GR127935, we have shown that the 5-HT1B receptor influences some cocaine-related responses but not others. Specifically, activation of the 5-HT1B receptor appears to contribute to both the induction of striatal c-*fos* and the increase in locomotor activity elicited by cocaine, since GR127935 attenuates these effects. These results are in agreement with pharmacological data suggesting that cocaine can act as an indirect agonist of the 5-HT1B receptor and that 5-HT1B receptors can modulate dopamine neurotransmission. Stimulation of 5-HT1B receptors localized on the terminals of GABAergic afferents emanating from the striatum and NAcc [3] has been suggested to result in a decrease in GABA release, which may lead to a disinhibition of dopaminergic neurons in the SN and the ventral tegmental area (VTA) [5,19] and to a facilitation of dopamine release in the striatum and NAcc [14,17].

We also investigated the role of the 5-HT1B receptor in the reinforcing effects of cocaine. Mounting evidence suggests the importance of serotonin in reward pathways. Mice lacking the dopamine transporter, the direct target of cocaine, still self-administer the drug, suggesting that cocaine's actions on the serotonin system can modulate its reinforcing effects [36]. In rats, RU24969 substituted nearly fully for cocaine in drug discrimination tests [4]. Likewise, RU24969 induced a leftward shift in doses of cocaine in an intravenous self-administration paradigm, suggesting that 5-HT1B agonists might potentiate the effects of cocaine [27,28]. However, we show here that acute blockade of the 5-HT1B receptor does not reduce the reinforcing properties of cocaine as measured in a PR self-administration paradigm. This agrees well with recent data showing that while GR127935 can prevent RU24969 from enhancing cocaine-mediated reward, GR127935 alone does not affect self-administration of cocaine in rats [28]. This suggests that while stimulation of the 5-HT1B receptor can enhance cocaine-mediated reward, tonic activation of the receptor is not required for the rewarding effects of cocaine.

Since the addictive properties of cocaine may be associated with its ability to induce behavioral sensitization, we also investigated whether some of the behavioral effects of chronic cocaine administration may require 5-HT1B receptor stimulation. We observed clear behavioral sensitization in C57Bl/6J mice, i.e., an increased responsiveness to cocaine following repeated injections. Interestingly, 5-HT1B receptor blockade with GR127935 did not prevent the establishment of this sensitization to cocaine even

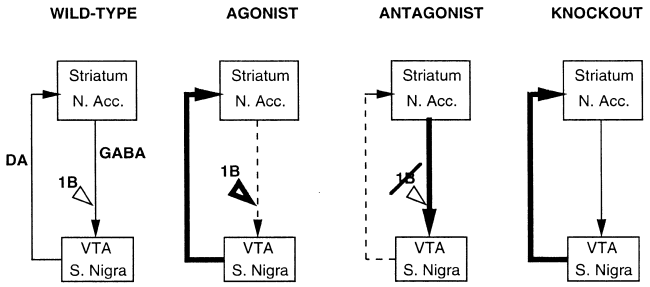

Fig. 6. Proposed model of 5-HT1B modulation of GABA and DA neurotransmission, comparing the acute effects of pharmacological manipulations and the compensations that may take place after genetic manipulations. DA dopaminergic neurons coming from the SN and the VTA and innervating the striatum and the NAcc. The thick DA line corresponds to increased activity of DA neurons while the dashed line corresponds to decreased activity. The 1B corresponds to 5-HT1B receptors located on GABAergic terminals.

though the amplitude of the locomotor response to cocaine was lower in the presence of the antagonist. This result may be related to reports indicating that the initial response to cocaine and the development of sensitization are controlled by different factors [42,45].

In summary, we have shown, using pharmacological methods, that acute blockade of the 5-HT1B receptor reduces the stimulatory effects of acute cocaine administration, as measured by both neuronal activation (c-*fos* induction) and locomotor activation. However, the antagonist did not seem to affect more long-term effects of cocaine, like reinforcement or sensitization. In contrast, when the 5-HT1B receptor is chronically absent, as in the gene KO, nearly every measure of cocaine response is altered: KO mice show less striatal c-*fos* induction; more locomotion after both acute and chronic cocaine; higher break points for cocaine self-administration; and an altered pattern of behavioral sensitization [37]. In other words, except for the c-*fos* response, there is little resemblance between the consequences of the KO and the effects of the antagonist on the responses to cocaine. This type of discrepancy is not restricted to the effects of cocaine, since we have also found differences in other behavioral responses. The KOs are more aggressive while the antagonist has no effect on aggressive behavior (our unpublished results). These differences bring into light a fundamental difference between KOs and antagonists; KOs develop without the 5-HT1B receptor and may therefore undergo plastic changes to compensate for the missing protein. We have in fact identified a number of changes in the basal ganglia circuitry of the 5-HT1B KO mice, which may be responsible for their altered responses to cocaine. These changes include increased levels of the transcription factor ΔFosB [37], the dopamine D1 receptor, as well as increased dopamine release in the NAcc [40,41].

A schematic of the relevant neural circuitry may help clarify the key differences between the pharmacological and genetic models (Fig. 6). In the normal circuitry, activation of 5-HT1B receptors located on the terminals of striatal GABAergic neurons inhibits GABA release in VTA and SN, which results in increased activity of DA neurons and therefore increased DA release in NAcc and striatum (assuming that tonic serotonergic activity, mediated via the 5-HT1B receptor, is the basal functional state of this circuit). When an antagonist acutely blocks the 5-HT1B receptor, the GABA input is enhanced, and, consequently, the DA output is reduced. Initially, the KO may have had a similar neurochemical effect. But it is possible that a chronic reduction in DA neurotransmission is deleterious, so the organism may compensate by boosting DA transmission. Evidence for such compensatory changes comes from a recent study showing increased DA levels in the NAcc of 5-HT1B KO mice [41]. Such an increased DA function may maintain homeostasis in normal baseline conditions, but when drugs such as cocaine further heighten DA levels, behavioral changes may be observed.

Thus, the pharmacological and genetic approaches can play complementary roles in dissecting the function of a particular receptor in a complex behavioral response. Pharmacology can demonstrate the function of the receptor when the relevant circuitry is acutely stimulated; while the gene KO may illustrate the impact of the receptor on neural plasticity, homeostasis, and animal models of genetic diseases.

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