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5-HT_4 receptor antagonism does not affect motor and reward mechanisms in the rat

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Abstract

5-HT_4 receptors are concentrated in areas of the brain which are rich in dopamine neuronal markers, which may suggest that they influence motor and reward processes. We tested this hypothesis by examining the effects of a 5-HT_4 receptor antagonist, 8-amino-7-chloro-(*N*-butyl-4-piperidyl)methylbenzo-1,4-dioxan-5-carboxylate hydrochloride (SB-204070-A) on amphetamine- and nicotine-induced locomotor stimulation in intact rats. In rats with unilateral 6-hydroxydopamine-induced lesions of the ascending nigrostriatal dopaminergic projection, SB-204070-A was tested for its effects on amphetamine-induced rotation. SB-204070-A was also tested for its effects on rewarded behaviour maintained by intracranial self-stimulation. SB-204070-A did not alter behaviour under any of these conditions, suggesting a lack of involvement of the 5-HT_4 receptor in motor and reward processes.

Keywords: SB-204070-A; 5-HT_4 receptor; Locomotor activity; Circling; Self-stimulation, intracranial

1. Introduction

The role of 5-HT_4 receptors in the central nervous system is unclear. Recent evidence suggests that this receptor may be involved in mild anxiety in rodents (Costall and Naylor, 1993; Silvestre et al., 1996; Kennett et al., 1997) and whilst no effects of 5-HT_4 receptor antagonists on learning have been observed (Eglen et al., 1995), activation of this receptor using exogenous ligands has been reported to improve this behaviour in rodents (Eglen et al., 1995; Meneses and Hong, 1997). Anatomical studies in rodent brain have shown 5-HT_4 binding sites to be concentrated in extrapyramidal forebrain areas (Grossman et al., 1993; Waeber et al., 1993, 1994; Jakeman et al., 1994; Domenech et al., 1994). These studies have recently been extended to show that 5-HT_4 receptors are probably colocalised with γ-aminobutyric acid (GABA) projections from the striatum to the substantia nigra, and are highly concentrated in the shell region of the nucleus accumbens (Patel et al., 1995). This localisation pattern may suggest the involvement of 5-HT_4 receptors in mechanisms of reward and motor behaviour. We have investigated whether antagonism of 5-HT_4 receptors by the potent and highly selective 5-HT_4 receptor antagonist, SB-204070-A, (Wardle et al., 1994) modulates locomotor behaviour and reward mechanisms.

2. Materials and methods

2.1. Subjects

Procedures were performed in accordance with the Animals (Scientific Procedures) Act of 1986 and SmithKline Beecham ethical guidelines. Male Sprague–Dawley and Lister Hooded rats were obtained from Charles River, UK, and maintained on a 12-h light/dark cycle (lights on: 0700–1900 h) at 20 ± 1°C.

2.2. Drugs

SB-204070-A (8-amino-7-chloro-(*N*-butyl-4-piperidyl)-methylbenzo-1,4-dioxan-5-carboxylate hydrochloride) was synthesised at SmithKline Beecham Pharmaceuticals; apomorphine hydrochloride, dexamphetamine sulphate and co-

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caine hydrochloride were supplied by Sigma; nicotine bitartrate was supplied by BDH. Doses of SB-204070-A were selected on the basis of previous in vivo studies (Kennett et al., 1997).

2.3. Surgery

2.3.1. 6-Hydroxydopamine lesion

Male Sprague–Dawley rats (225–250 g) were anaesthetised with fentanyl (Sublimaze®; Janssen Pharmaceutica; 45 μg in 0.9 ml/100 g i.p.) and medetomidine hydrochloride (Domitor™; Farmos; 40 μg in 0.04 ml/100 g i.m.). Rats were then positioned in a Kopf stereotaxic frame with the incisor bar raised 5 mm. An incision was made in the scalp and a small burr hole was made in the calvarium. Coordinates were 4.3 mm anterior from the interaural line and 1.9 mm lateral from the midline (De Groot, 1967). A 10-μl Hamilton syringe was lowered to 8.2 mm below the dura into the left medial forebrain bundle, and 6-hydroxydopamine hydrobromide solution (6-OHDA; 8 μg in 3 μl saline containing 2 μg ascorbic acid) was infused over 3 min. The needle was left in situ for a further 3 min and then slowly withdrawn. The incision was sutured and the rats treated with atipamezole hydrochloride (Antisedan®; Farmos; 0.1 mg in 0.02 ml/100 g i.p.) to reverse anaesthesia, and with nalbuphine hydrochloride (Nubain®; Farmos; 0.2 mg in 0.02 ml/100 g i.p.) as analgesic.

2.3.2. Electrode implantation for intracranial self-stimulation

Male Lister Hooded rats (200–225 g) were prepared for surgery in a similar manner to that described above, and placed into the stereotaxic frame. After exposure of the calvarium, the skull surface was kept level with lambda and bregma in the same horizontal plane, and a burr hole introduced at the coordinates 3.0 mm posterior to bregma and 1.8 mm lateral to the midline according to the atlas of Paxinos and Watson (1986). A bipolar twisted stainless steel electrode (0.25 mm diameter, Plastic Products, USA) was lowered 8.2 mm below the dura into the lateral hypothalamus. This was secured in position with four stainless steel screws and zinc polycarbonate cement (Poly-F-Plus®, Dentsply). Anaesthesia was then reversed as described above. The rats were placed on shredded paper under artificial heat during recovery.

2.4. Behavioural assessment

2.4.1. Locomotor activity

Locomotor activity was measured by recording the number of occasions that horizontal infra red light beams were interrupted in activity monitors (model AM1052 Benwick Electronics) controlled by a Compaq 386 microcomputer.

The effect of SB-204070 on locomotor activity was studied by administering the drug at doses of 0.03–3 mg/kg i.p. and placing the rats directly into the test boxes. Locomotor activity was monitored for 1 h. In the case of the amphetamine locomotor activity test, rats received SB-204070-A (0.03–3 mg/kg i.p.) and were habituated to the test boxes for 30 min prior to injection of amphetamine (0.55 mg/kg s.c.).

Acute nicotine administration causes locomotor depression, and only after repeated exposure does nicotine increase locomotor activity (Morrison and Stephenson, 1972; Clarke and Kumar, 1983). For this reason, in nicotine locomotor activity experiments, a similar protocol to that for amphetamine was used except that rats were previously made tolerant to nicotine by eight daily injections (0.4 mg/kg s.c.) on the days prior to tests.

Locomotor activity was monitored for 1 h after amphetamine and nicotine. After $\log_{10}$ transformation, data were analysed by one-factor analysis of variance (ANOVA) and Dunnett's *t*-test.

2.4.2. Circling

During the second and third postoperative week, rats received apomorphine hydrochloride (0.5 mg/kg s.c.) in order to assess their circling response. Those rats which were observed to display pronounced contralateral asymmetry with contraversive circling were selected for experiments as it has been demonstrated previously that more than 90% destruction of the nigrostriatal pathway is necessary in order for apomorphine to produce contraversive circling (Hefti et al., 1980). Circling experiments commenced 3 weeks after surgery. Circling rates were monitored using automated rotation meters (model RM1057, Benwick Electronics) controlled by a Compaq 386 microcomputer. Rats received SB-204070-A (3 or 10 mg/kg i.p.) followed 30 min later by amphetamine (1.2 mg/kg s.c.) or saline (1 ml/kg s.c.) and ipsiversive circles were counted for 1 h. Data were analysed by one-way ANOVA and Dunnett's *t*-test after $\log_{10}$ transformation.

2.4.3. Intracranial self-stimulation

For intracranial self-stimulation (ICSS) experiments, training and testing took place in operant chambers (Camden Instruments). Initially, rats were trained to press a lever on a continuous reinforcement schedule to deliver positively reinforcing stimuli of 0.5-s trains of cathodal current (400 μA) consisting of 0.1-ms pulses at 100 Hz (Hatcher et al., 1995). Animals which sustained robust responding on this schedule (approximately 85%) were selected for further study. After three sessions, responders were then trained to lever-press on the continuous reinforcement schedule in fifteen 2-min trials under the same stimulation parameters as above with the exception that the frequency was randomly switched between 10, 100 and 300 Hz between trials. During the third and final stage of

training, rats were stabilised on test sessions which began with three warm-up trials (2 min at 100 Hz) and three extinction trials. These were followed by two blocks of 10 (2 min) trials in which stimulation trains varied pseudorandomly between 30 and 300 Hz. After stabilisation of responding, drug treatments commenced using a randomised Latin Square design. Rats were then tested immediately for cocaine (20 mg/kg i.p.) or SB-204070-A (1–10 mg/kg i.p., 10 min pretreatment)-induced shifts of reward threshold in 55 min sessions. The frequency at which responding was 50% of maximum (M_{50}) was calculated and expressed as the percentage of baseline. Data were analysed using ANOVA and post hoc pairwise comparisons in Research System/Explorer (RS/E; Bolt, Beranek and Newman©, 1992).

3. Results

3.1. Effect of SB-204070-A on spontaneous hyperactivity

In the study of the unhabituated locomotor activity, there was no overall effect of treatment ($F(5,42) = 1.51$; $P > 0.2$). Post hoc analysis showed that no single treatment affected locomotor activity compared to saline treatment (highest $t(5) = 1.86$; $P > 0.05$).

3.2. Effect of SB-204070-A on amphetamine-induced hyperactivity

In the study of the effects of SB-204070-A on amphetamine-induced hyperactivity (Fig. 1, middle panel), ANOVA showed a significant overall effect of treatment ($F(5,42) = 12.9$; $P < 0.001$). Thus, amphetamine increased the number of beam breaks to 2030 ± 244 compared to 328 ± 81 after saline injection ($P < 0.01$). SB-204070-A (0.1–3 mg/kg i.p.) did not influence the amphetamine response.

3.3. Effect of SB-204070-A on nicotine-induced hyperactivity

In the nicotine-induced hyperactivity experiment (Fig. 1, bottom panel), nicotine (0.4 mg/kg s.c.) increased the total number of beam breaks to 985 ± 210 compared to 212 ± 38 after saline treatment ($P < 0.01$). SB-204070-A (0.1–3 mg/kg i.p.) did not have any effect on the nicotine response.

3.4. Effect of SB-204070-A on amphetamine-induced circling

In the test of SB-204070-A effects on amphetamine-induced rotation (Fig. 2), there was an overall effect of treatment on circling rate ($F(5,42) = 55.35$; $P < 0.001$).

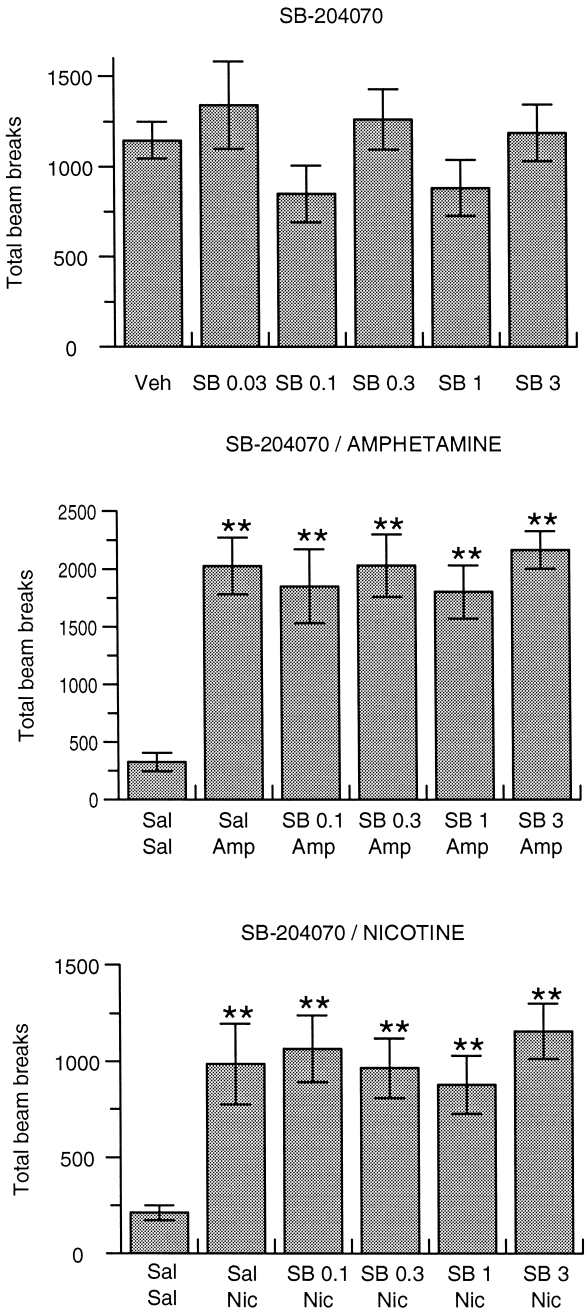

Fig. 1. (Top) Effect of SB-204070-A on spontaneous hyperactivity; (middle) amphetamine hyperactivity; (bottom) nicotine hyperactivity. Untransformed data are presented as means ± 1 S.E.M. Amphetamine and nicotine increased locomotor activity above saline controls (** $P < 0.01$). Amphetamine- and nicotine-induced hyperactivity after SB-204070-A (0.1–3 mg/kg i.p.) pretreatment remained significantly higher than the saline control value (** $P < 0.01$, in each case; $n = 8$ per treatment group).

Amphetamine (1.2 mg/kg s.c.) increased ipsiversive circling to 254 ± 14 rotations compared to 23 ± 7 after saline injection ($t(5) = 9.4$; $P < 0.01$). The ipsiversive circling rate to amphetamine in the presence of SB-204070-A (3 and 10 mg/kg i.p.) was 293 ± 29 and 256 ± 30 rotations

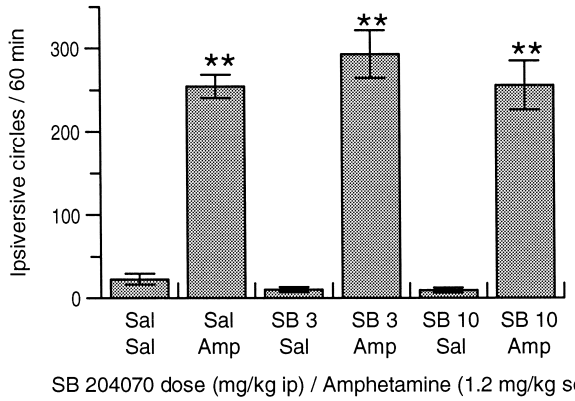

Fig. 2. Effect of SB-204070-A (30 min pretreatment; 3 or 10 mg/kg i.p.) on the circling response produced by amphetamine (1.2 mg/kg s.c.). Untransformed data are presented as means ± 1 S.E.M. Amphetamine (1.2 mg/kg s.c.) increased ipsiversive circling above control value (** $P < 0.01$). After SB-204070-A (3 or 10 mg/kg i.p.) pretreatment, amphetamine (1.2 mg/kg s.c.)-induced ipsiversive circling remained significantly higher than the saline control ($n = 8$ per treatment group).

per hour, respectively, and remained significantly higher than after saline treatment ($t(5) = 9.8$ and 9.3; $P < 0.01$).

3.5. Effect of SB-204070-A and cocaine on intracranial self-stimulation

In ICSS tests (Fig. 3), the reward threshold (M_{50}) value after saline was 88.4% of nontreated baseline. After administration of the positive control, cocaine (20 mg/kg i.p.), the M_{50} value was reduced to 53.8% of baseline, and this was significantly different from saline ($P < 0.05$). SB-204070-A (1–10 mg/kg i.p.) neither increased nor decreased reward threshold compared to saline.

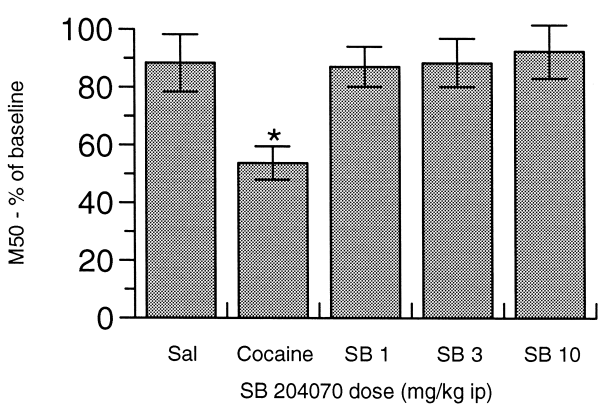

Fig. 3. Effect of SB-204070-A (30 min pretreatment; 1–10 mg/kg i.p.) and cocaine (20 mg/kg i.p.) on intracranial self-stimulation reward threshold (M_{50}). Data are presented as means ± 1 S.E.M. Cocaine significantly reduced M_{50} compared to saline control value (* $P < 0.05$). SB-204070-A (1–10 mg/kg i.p.) had no effect on reward threshold ($n = 8$ per treatment group).

4. Discussion

From our current knowledge of the function of basal forebrain areas (Mogenson and Yang, 1991; Blackburn et al., 1992) which have been shown to contain 5-HT_4 receptors, we hypothesised that 5-HT_4 receptor function may be associated with motor, motivational and reward processes. The present study set out to investigate whether blockade of 5-HT_4 receptors with the antagonist SB-204070-A would modulate behavioural effects caused by stimulating dopamine mechanisms in the rat with particular reference to locomotor activity and circling behaviour, or whether SB-204070 would influence reward mechanisms as measured by lever pressing for ICSS. Locomotor activation was achieved by using unhabituated rats or by treating habituated rats with two pharmacologically distinct agents, amphetamine or nicotine. Amphetamine acts by releasing dopamine into the synaptic cleft to cause stimulation of postsynaptic dopamine receptors (Azzaro and Rutledge, 1973). Nicotine's locomotor stimulant activity is thought to derive from its agonist action on nicotinic cholinergic receptors in the ventral tegmental area (Grenhoff et al., 1986; Reavill and Stolerman, 1990; Museo and Wise, 1995). A common feature of these agents is to increase synaptic dopamine levels in terminal areas of the striatum and nucleus accumbens, although in the case of nicotine, acute treatment has a locomotor depressant action and at least five occasions of nicotine administration are required before locomotor activation is seen (Morrison and Stephenson, 1972; Clarke and Kumar, 1983). In agreement, in the present study, nicotine increased locomotor activity in tolerant rats. However, SB-204070-A failed to alter nicotine-induced hyperactivity. Amphetamine caused greater locomotor stimulation than nicotine, and this was not altered by SB-204070-A. Furthermore, in the lesion model, where amphetamine produces postural asymmetry through hemispheric imbalance of striatal dopamine release (Ungerstedt, 1971), SB-204070-A had no influence on circling. In the ICSS procedure, cocaine has been reported to have rewarding properties (Crow, 1970) and this was confirmed in the present study. Cocaine caused a significant reduction of the reward threshold (M_{50}), thus demonstrating that the procedure was sensitive to pharmacological intervention. However, SB-204070-A did not significantly alter M_{50}, indicating a lack of interaction with reward pathways. Whether SB-204070-A would potentiate or attenuate the cocaine-induced shift in M_{50} requires further study.

The lack of positive effects observed in these experiments is unlikely to reflect inadequate dosing. The dose range of SB-204070-A included a dose previously shown to be active in a test of anxiolytic-like activity; thus, a dose of 1 mg/kg of SB-204070-A increased the percentage time that rats spent on the open arms of the elevated *x*-maze (Kennett et al., 1997). Furthermore, although Silvestre et al. (1996) showed that SB-204070-A produced an

anxiolytic response 10 min after administration but not after 30 min, pharmacokinetic constraints are unlikely to have been important. Doses of SB-204070-A used here were higher than those previously shown to be effective in the peripheral nervous system (Banner et al., 1993, 1996; Bingham et al., 1995) and SB-204070 has been shown to attain a 7:1 brain/blood ratio after i.v. infusion (V.A. Lewis, SB Pharmaceuticals, unpublished). When taken together with the observation that SB-204070-A exhibits anxiolytic-like activity after 30 min pretreatment (Kennett et al., 1997), it is reasonable to suppose that SB-204070-A should have achieved brain concentrations adequate to affect behaviour.

On the basis of the known location of 5-HT_4 receptors in areas rich in dopamine neurons and terminals (Grossman et al., 1993; Waeber et al., 1994; Jakeman et al., 1994; Domenech et al., 1994) and the high concentrations of 5-HT_4 receptors in the shell region of the nucleus accumbens (Patel et al., 1995), 5-HT_4 receptors might be predicted to influence dopamine neurochemical functions. Indeed in vitro and in vivo evidence suggests that 5-HT_4 receptor agonists increase dopamine release (Steward and Barnes, 1994; Steward et al., 1995; Bonhomme et al., 1995). More recently, in vivo microdialysis studies have shown that direct stimulation of striatal 5-HT_4 receptors increases dopamine efflux and this effect could be partially reversed by application of the 5-HT_4 receptor antagonist, GR-125,283, through the dialysis probe (De Deurwaerdère et al., 1997). However, others have failed to show a similar effect with drugs administered systemically rather than through the dialysis probe (Taylor and Routledge, 1996).

To summarise, published data show that 5-HT_4 receptor blockade alters behaviour in the elevated *x*-maze (Kennett et al., 1997; Silvestre et al., 1996) and it has been reported that alcohol intake in alcohol preferring rats is reduced by the 5-HT_4 receptor antagonist, GR-113808 (Panocka et al., 1995). However, our studies failed to find any evidence for 5-HT_4 receptor involvement in behaviours thought to involve forebrain dopaminergic neurons. The lack of behavioural effects in these experiments may reflect a lack of endogenous 5-HT tone on 5-HT_4 receptors and it may be necessary to raise 5-HT tone at 5-HT_4 receptors to produce a response which is amenable to blockade by 5-HT_4 receptor antagonists, though to date this approach has not demonstrated 5-HT_4 receptor involvement in 5-HT driven behavioural changes (Artaiz et al., 1998). Behavioural changes have been rarely detected using 5-HT_4 receptor agonists, such as BIMU-1 and BIMU-8 although these agents are not selective (Meneses and Hong, 1997. However, problems associated with brain penetrance, poor selectivity and partial agonist activity may obscure the detection of such activity and it is worth noting a clinical report that the partial 5-HT_4 receptor agonist cisapride, aggravated parkinsonian tremor (Sempere et al., 1995). Selective, CNS penetrant 5-HT_4 receptor agonists are needed to pursue this approach further.

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