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Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule

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Abstract

Brain serotonin interacts with dopamine function in a complex fashion. Previous work from our laboratory showed that activation of 5-HT_{1B} receptors within the nucleus accumbens attenuates the ability of amphetamine to increase responding for conditioned reinforcement. The primary purpose of these experiments was to determine the impact of 5-HT receptor stimulation, with particular focus on 5-HT_{1B} receptors in the nucleus accumbens on the reinforcing effect of amphetamine. To this end several experiments determined the effects of injecting 5-HT, and various 5-HT agonists, into the nucleus accumbens on responding for intravenous infusions of amphetamine (60 μg/kg) delivered according to a progressive ratio schedule of reinforcement. Both 5-HT (2.5, 5 and 10 μg) and the selective 5-HT_{1B} receptor agonist CP93,129 (0.625, 1.25 and 2.5 μg) dose-dependently reduced responding for amphetamine. Injections of 5-HT but not CP93,129 also reduced responding for food under a similar PR schedule. The 5-HT_{1A} agonist 8-OH-DPAT (5 μg) and the nonselective 5-HT_2 agonist DOI (10 μg) failed to alter amphetamine self-administration. Pretreatment with the selective 5-$HT_{1B/1D}$ receptor antagonist GR127935 (3 mg/kg) attenuated the ability of 5-HT and CP93,129 to reduce amphetamine self-administration following their injection into the nucleus accumbens. These results extend our previous findings that increasing 5-HT activity in the nucleus accumbens inhibits dopamine-dependent behaviour, and further indicate that activation of 5-HT_{1B} receptors is particularly important in this regard.

Keywords: 5-HT_{1B} receptor amphetamine self-administration; Nucleus accumbens; CP93,129; GR127935; Dopamine

1. Introduction

The behavioural effects of psychomotor stimulants such as amphetamine include increased locomotor activity and potentiated responding for conditioned reinforcers. Such drugs are also self-administered indicating that they possess primary reinforcing, or rewarding, effects. Experiments involving intracranial microinjections of amphetamine, or dopamine-depleting lesions have demonstrated that these behavioural effects of amphetamine are mediated in large part by increased dopamine neurotransmission in the nucleus accumbens. Infusion of amphetamine into the nucleus accumbens stimulates locomotor activity (e.g., Pijnenburg et al., 1976) and increases responding for conditioned reinforcement (e.g., Taylor and Robbins, 1986; Fletcher, 1995, 1996; Fletcher and Korth, 1999a). Animals will also self-administer amphetamine directly into the nucleus accumbens (Hoebel et al., 1983). All of these behaviours are also blocked by 6-hydroxydopamine-induced depletion of dopamine in the nucleus accumbens (Kelly and Iversen, 1976; Lyness et al., 1979; Taylor and Robbins, 1986). While the mesolimbic dopamine system represents the primary neurochemical substrate for mediating the behavioural effects of amphetamine, other neurotransmitters systems interact with dopamine. One such neurotransmitter is 5-hydroxtryptamine (5-HT; serotonin), and a large body of evidence indicates that, in general, 5-HT modulates the activity of dopaminergic systems (see reviews by Kelland and Chiodo, 1996; Saito et al., 1996), although the nature of this modulation is complex depending upon the 5-HT receptor subtype involved (Barnes and Sharp, 1999). Not surprisingly then, the beha-

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vioural effects of amphetamine can be altered by treatments which alter 5-HT function.

Amphetamine-stimulated locomotion (Hollister et al., 1976), responding for conditioned reinforcement (Fletcher, 1995) and intravenous self-administration of amphetamine (Porrino et al., 1989; Lyness et al., 1980; Smith et al., 1986) are reduced by systemic administration of indirect agonists of 5-HT neurotransmission including tryptophan, 5-hydroxtryptophan, fenfluramine and fluoxetine. Cocaine self-administration is also reduced by such indirect 5-HT agonists (Carroll et al., 1990; McGregor et al., 1993; Richardson and Roberts, 1991). These findings are indicative of a general inhibitory role of 5-HT systems on psychomotor stimulant-induced behavioural effects. However, because of the use of systemic injection of nonselective and indirect 5-HT agonists they do not shed any light on either the neuroanatomical locus of 5-HT modulation of amphetamine effects or the identity of the 5-HT receptor subtypes that may be involved in mediating these effects. With regard to the issue of where in the brain 5-HT may modulate the effects of psychomotor stimulants evidence suggests that the nucleus accumbens may be one important site. Several studies have shown that infusion of 5-HT into this site inhibits the locomotor stimulant effect of amphetamine (Carter and Pycock, 1978; Costall et al., 1979), or of dopamine itself (Jones et al., 1981). The ability of amphetamine to potentiate responding for conditioned reinforcement is also attenuated by 5-HT infusions into the nucleus accumbens (Fletcher, 1996). Data obtained from experiments involving microdialysis have shown that 5-HT acting through multiple receptor subtypes can influence DA levels in the nucleus accumbens and/or dorsal striatum (Benloucif and Galloway, 1991; Benloucif et al., 1993; Parsons and Justice, 1993; Saito et al., 1996). However, very few experiments have documented the effects of specific 5-HT receptor agonists injected into the nucleus accumbens on amphetamine-mediated behaviours.

Recently we reported that a selective 5-HT_{1B} receptor agonist CP93,129 (Macor et al., 1990) reduced the effects of amphetamine on responding for conditioned reinforcement (Fletcher and Korth, 1999a). A specific action involving 5-HT_{1B} receptors was further demonstrated by the ability of the 5-$HT_{1B/1D}$ antagonist GR127935 (Skingle et al., 1996) to block the action of CP93,129. Additionally the nonselective 5-HT_2 agonist DOI and the selective 5-HT_{1A} agonist 8-OH-DPAT did not alter the effect of amphetamine. All of these results point to an important role for 5-HT_{1B} receptors in the nucleus accumbens in modulating the activity of amphetamine on responding for conditioned reinforcement.

Recent experiments have documented changes in drug reinforcement, primarily involving cocaine, following genetic or pharmacological manipulation of $5HT_{1B}$ receptor function. Mice lacking the 5-HT_{1B} receptor gene show enhanced self-administration of cocaine (Rocha et al., 1997, 1998), a finding that is broadly in keeping with the ability of 5-HT_{1B} agonists injected into the nucleus accumbens to reduce the behavioural effect of amphetamine. In contrast self-administration of cocaine (Parsons et al., 1998), though not amphetamine (Fletcher and Korth, 1999b), is enhanced by systemic injections of 5-HT_{1B} receptor agonists in a manner consistent with an increase in the reinforcing efficacy of cocaine. Given our findings that 5-HT and 5-HT_{1B} agonists infused into the nucleus accumbens block the ability of amphetamine to potentiate responding for conditioned reinforcement the aim of the work described here was to determine the effects of these manipulations on amphetamine self-administration. Specifically, we examined the effects of 5-HT, and of CP93,129 in rats self-administering amphetamine on a progressive ratio schedule. The effects of these manipulations on responding for food were also examined in an attempt to define the behavioural specificity of these manipulations.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats weighing 280–340 g at the time of surgery were housed individually in polycarbonate cages. Water was freely available, food availability varied as described below. The housing room was maintained at a constant temperature of 22 ± 2 °C, under a 12 h light–dark cycle with lights on at 8 a.m.

2.2. Surgery

In a single session each rat underwent surgery to implant guide cannulae in the vicinity of the nucleus accumbens, and a catheter in the right jugular vein. Rats were anaesthetised with 50–60 mg/kg ip sodium pentobarbital (Somnotol) and placed in a stereotaxic frame. Bilateral stainless steel guide cannulae (22 ga) were implanted aimed at the nucleus accumbens. The cannulae were positioned 2.5 mm above the intended injection site, according to the coordinates: AP +3.2, L +1.1, D/V −5.9 mm relative to bregma, with the incisor bar set at +5 mm. Cannulae were anchored to the skull using jeweller's screws and dental cement. Stainless steel (28 ga) wire obdurators were used to keep the cannulae clear. On completion of this surgery rats were removed from the stereotaxic frame and a catheter constructed of silastic tubing (0.025 in outer diameter) was inserted into the right jugular vein. The terminal end of the catheter was a length of 23 ga stainless steel tubing, which was cemented inside a nylon bolt. The catheter exited between the scapulae, and could be quickly attached and detached from the drug delivery line by means of a small plastic nut cemented to the end of a stainless steel spring protecting the line.

2.3. Apparatus

Testing was conducted in 16 operant chambers measuring 28 cm long, 21 cm wide and 21 cm high (Med.

Associates, St. Albans, VT). Each chamber contained a food pellet dispenser, two response levers 4.5 cm wide and 7 cm above the floor of the chamber and a stimulus light located 6 cm above each lever. A counterbalanced arm held a fluid swivel above the ceiling of the chamber. This swivel was attached at one end by Tygon tubing to a syringe mounted on a motor driven syringe pump (Razel) located outside the chamber. At the other end of the swivel a length of Tygon tubing, encased in a stainless steel tether connected the animal's catheter to the syringe via the swivel. Each chamber was illuminated by a house-light and housed in a sound-attenuating box equipped with a ventilating fan. The apparatus was controlled, and the data collected, by a 386-SX IBM-type computer.

2.4. Behavioural testing

Prior to surgery rats were trained to lever press for food pellets. Rats were food restricted (15 g per day), placed in the operant chambers and trained to press the left lever for food (45 mg Noyes pellets) according to a fixed ratio (FR) 1 schedule. Rats were allowed a maximum of 100 pellets during daily 30 min sessions. Any rats failing to obtain 100 pellets by the third day of training were placed in the operant boxes overnight and allowed 300 food pellets delivered according to the FR1 schedule. A water dish was also placed inside the operant chamber during this session. Thereafter, rats were placed in the chamber only during the 30 min session during the day-time. Once rats had earned 100 pellets on each of 3 consecutive days they were considered lever-trained, and were subsequently maintained on 20 g of lab chow per day. One week after catheters were implanted rats were allowed to respond for infusions (0.1 ml delivered over 4 s) of D-amphetamine (60 μg/kg) on a FR1 schedule. Each infusion was accompanied by illumination of a stimulus light which remained on for a 20 s time-out period after the infusion. Once responding was stable, a progressive ratio (PR) schedule was implemented in which the number of responses required to obtain an infusion increased for successive infusions. The progression was derived from the equation: response ratio=$[5 \times e^{(0.2 \times \text{infusion no.})} - 5]$, and yielded response ratios of 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, etc. (Richardson and Roberts, 1996). Sessions lasted until a period of 1 h without an infusion had elapsed, or were a maximum of 5 h in length. The number of infusions earned before this breaking point was recorded. The infusion dose was held constant at 60 μg/kg throughout. All self-administration sessions began with a non-contingent infusion of amphetamine.

In some rats the effects of injecting 5-HT ligands on responding for food were examined. These rats were restricted to 15 g food per day and trained to lever press for food pellets under a FR1 schedule as described above. The same progressive ratio schedule as used for amphetamine self-administration was implemented. Sessions terminated after a 20-min period had elapsed without a reinforcer delivery. This criterion was adopted to produce approximately similar break-points to those found in rats responding for amphetamine.

2.5. Experiments

A series of five experiments was conducted with a separate group of animals used in each case. In all experiments repeated measures designs were used such that each animals was tested under every dose condition, or every agonist plus antagonist combination. Test days were separated by a minimum period of 72 h, and rats were run under their normal schedule on drug-free days. On any given test day approximately equal numbers of animals were run in each treatment condition. Care was taken to ensure that there was no orderly sequence to the treatments.

Experiment 1 investigated the effects of injecting saline, 2.5, 5 and 10 μg of 5-HT on responding for amphetamine ($n = 10$) or food ($n = 6$). Experiment 2 determined the effects of saline, 0.625, 1.25 and 2.5 μg CP93,129 on responding for amphetamine ($n = 12$) and food ($n = 10$). Experiment 3 determined the effects of saline, 5 μg 8-OH-DPAT and 10 μg DOI on responding for amphetamine ($n = 11$). In Experiment 4, the effects of pretreatment with 3 mg/kg GR127935 on the reduction in responding for amphetamine induced by 5 μg 5-HT was examined ($n = 10$). Experiment 5 examined the effect of pretreatment with 3 mg/kg GR127935 on the reduction in responding for amphetamine induced by 1.25 μg CP93,129 ($n = 12$). In each experiment rats received four intracerebral drug injections, with the exception of Experiment 3 where only three microinjections were given.

2.6. Drugs and drug delivery

5-hydroxtryptamine (5-HT) bimaleate (Sigma), CP93,129 (3-(1,2,5,6-tetrahydropyrid-4-yl)pyrrolol[3,2-*b*]pyrid-5-one; Pfizer), 8-hydroxy-di-*n*-propylamino-tetralin hydrobromide (8-OH-DPAT, RBI), ±-2,5-dimethoxy-4-iodoamphetamine hydrobromide (DOI; RBI) were injected into the nucleus accumbens using a fine glass microinjector (0.1–0.2 mm tip diameter), extending 2.5 mm beyond the guide cannula tip, attached to a Hamilton syringe via a length of Tygon tubing. These drugs were dissolved in sterile 0.9% saline, and infused in a volume of 1 μl over a 1-min period with the needle left in place for a further 1 min to minimise reflux up the cannula shaft. GR127935 hydrochloride (Glaxo) was dissolved in distilled water, with the aid of heating up to 70 °C and injected SC in a volume of 1 ml/kg.

2.7. Histology

At the completion of the experiments rats were deeply anaesthetised with Somnotol and a volume of 0.5 μl fast-green dye was injected into each brain site to aid in the

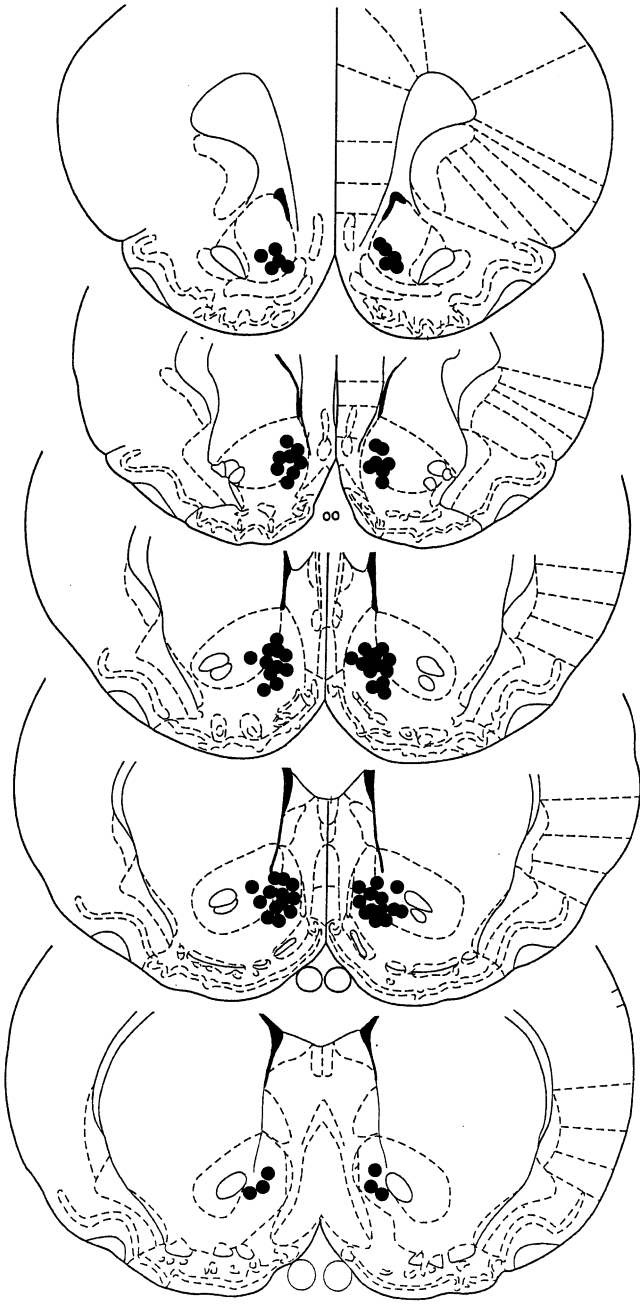

Fig. 1. Schematic diagram showing the location of injection sites within the nucleus accumbens. The number of sites shown is fewer than the number of animals used because of overlap of injection sites. Sections are adapted from Paxinos and Watson (1998) and range from +2.7 mm (top) to +0.7 mm (bottom) at 0.5 mm intervals.

localisation of injection sites. The brains were removed and stored in formaldehyde for at least 7 days, and then stored in 30% sucrose solution. Brains were then frozen, cut in a cryostat in 40 μm sections and stained with cresyl violet. Approximately 10% of the total number of animals used were found to have one or both injection sites located outside the nucleus accumbens (Paxinos and Watson, 1998). These animals were not used in the data analysis, and group sizes given above reflect this adjustment.

2.8. Statistical analysis

The primary dependent variable measured was the number of amphetamine infusions, or the number of food pellets, earned. This value was used as it represents a natural log transformation of the final ratio to which animals responded. Compared to data derived from ratios completed the variance associated with infusion number is less likely to violate the assumptions of homogeneity of variance for analysis of variance (Richardson and Roberts, 1996). For the first three experiments, the data were analysed using a one-way analysis of variance with repeated measures. Following a significant main effect, post hoc comparisons were made using Dunnett's test for comparisons against a control mean. For Experiments 4 and 5, data were analysed using a two way analysis of variance with dose of 5-HT, or CP93,129 as one factor and GR127935 as the other factor. Post hoc comparisons were made using Tukey's test.

3. Results

Fig. 1 shows the distribution of injection sites within the nucleus accumbens. As shown in Fig. 2 injection of 5-HT

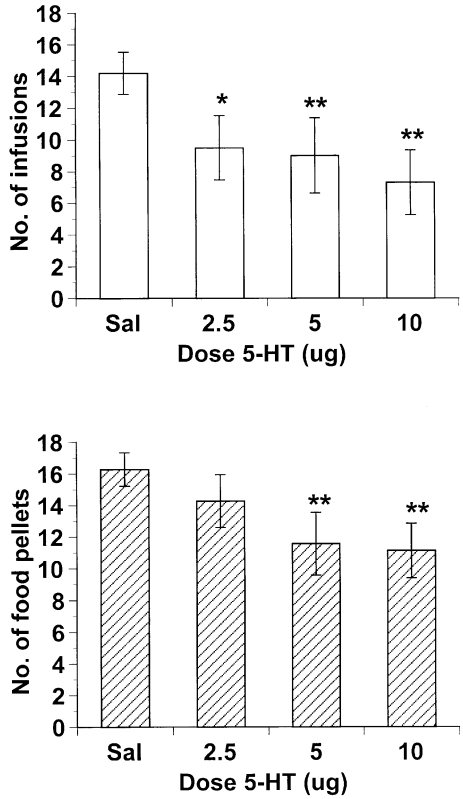

Fig. 2. The effects of injections of different doses of 5-HT and saline (Sal) into the nucleus accumbens on the number of amphetamine infusions (upper panel) and number of food pellets (lower panel) earned under a progressive ratio schedule. Bars represent mean (S.E.M.) number of infusions or food pellets earned by separate groups of rats ($n = 10$ and 6, respectively). * $P < .05$, ** $P < .01$ compared to Sal treatment.

Table 1
Effects of 5-HT and CP93,129 on latency to begin self-administration and on mean inter-infusion intervals

Drug	Dose (μg)				*F* ratio (*df*)
5-HT	Sal	2.5	5	10	
Latency to first infusion	93.4 ± 28.1	137.6 ± 29.2	128.4 ± 37.1	212.8 ± 43.1*	2.97 (3,27), $P < .05$
Mean inter-infusion interval[a]	579.3 ± 78.1	544.0 ± 98.1	623 ± 67.9	698.2 ± 118.2	1.03 (3,27), NS
CP93,129	Sal	0.0625	1.25	2.5	
Latency to first infusion	106.3 ± 29.1	132.4 ± 36.5	117.2 ± 28.9	178.4 ± 43.2	2.51 (3,33), NS
Mean inter-infusion interval[a]	612.5 ± 65.3	671.2 ± 56.3	583.2 ± 68.7	654.2 ± 78.9	1.11 (3,33), NS

Values represent mean ± S.E.M. values from 10 (5-HT) and 12 (CP93,129) rats.
* $P < .05$ compared to saline.
[a] Mean inter-infusion interval is the average interval calculated after excluding the first and last inter-infusion intervals.

into the nucleus accumbens dose-dependently reduced the number of amphetamine infusions [$F(3,27) = 4.78$, $P < .01$] and the number of food pellets [$F(3,27) = 3.94$, $P < .025$] earned. All doses of 5-HT significantly reduced amphetamine intake, whereas only the 5 and 10 μg doses significantly reduced responding for food. The mean inter-infusion interval was not significantly altered by 5-HT, but the highest dose moderately increased the latency to obtain the first infusion of the session (Table 1). In these experiments as in all subsequent experiments behaviour observed following saline injection did not differ from baseline behaviour. Thus, the injection procedure itself did not adversely affect responding in these cannulated rats.

As illustrated in Fig. 3 injections of CP93,129 into the nucleus accumbens dose-dependently reduced responding for amphetamine [$F(3,33) = 4.62$, $P < .01$] but not food [$F(3,27) = 2.64$, $P = .69$]. Responding for amphetamine was significantly reduced by 1.25 and 2.5 μg CP93,129. CP93,129 did not alter the latency to begin self-administering amphetamine, nor the mean inter-infusion interval (Table 1).

Fig. 4 shows that neither 8-OH-DPAT nor DOI altered self-administration of amphetamine [$F(2,20) = 1.02$, $P > .3$].

The upper panel of Fig. 5 shows the effects of pretreatment with 3 mg/kg GR127935 on the reduction in amphetamine self-administration induced by 5 μg 5-HT. Analysis of variance revealed significant main effects of both 5-HT [$F(1,9) = 47.37$, $P < .001$] and the antagonist treatment [$F(1,9) = 10.49$, $P < .001$]. The interaction between these two treatments was not significant [$F(1,9) = 2.65$, $P = .08$]. Post hoc tests confirmed that the number of amphetamine infusions earned following the combination of GR127935 plus 5-HT was significantly higher than following saline plus 5-HT. However, a complete blockade of the effect was

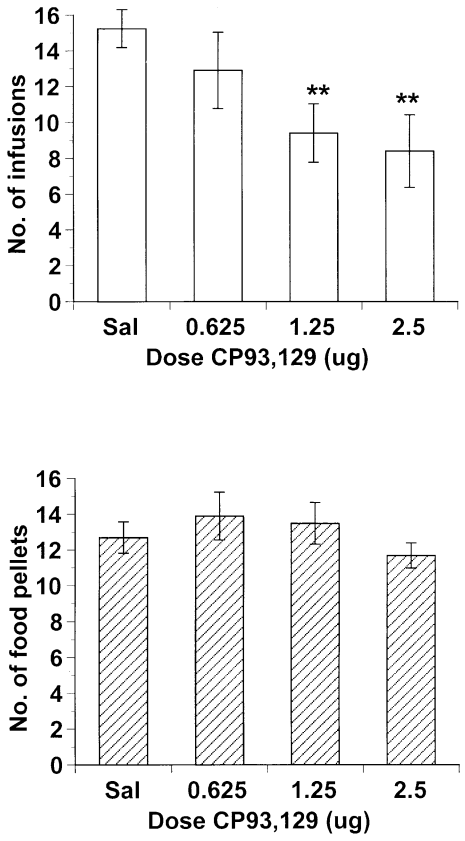

Fig. 3. The effects of injections of different doses of CP93,129 and Sal into the nucleus accumbens on the number of amphetamine infusions (upper panel) and number of food pellets (lower panel) earned under a progressive ratio schedule. Bars represent mean (S.E.M.) number of infusions or food pellets earned by separate groups of rats ($n = 12$ and 10, respectively). * $P < .05$, ** $P < .01$ compared to Sal treatment.

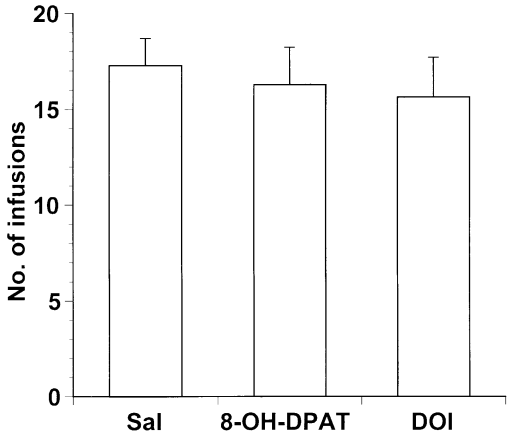

Fig. 4. The effects of Sal, 5 μg 8-OH-DPAT and 10 μg DOI on amphetamine self-administration. Bars represent mean (S.E.M.) number of infusions earned by 11 rats.

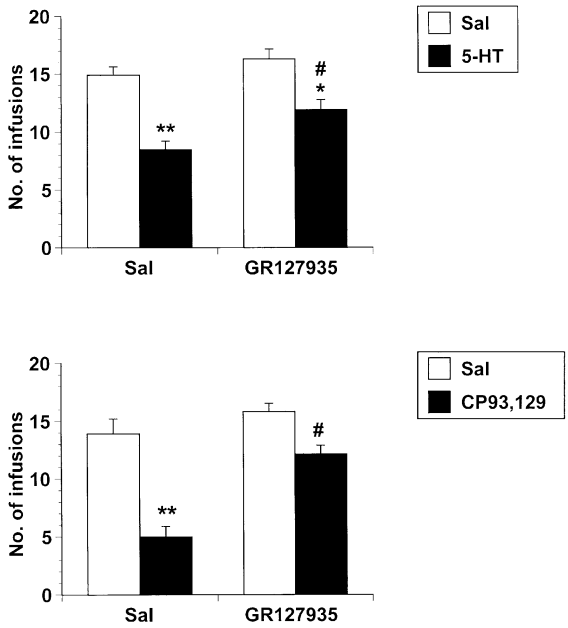

Fig. 5. The upper panel shows the effects of pretreatment with 3 mg/kg GR127935 on the suppressant effect of 5 μg 5-HT injected into the nucleus accumbens on amphetamine self-administration. The lower panel shows the effects of pretreatment with 3 mg/kg GR127935 on the suppressant effect of 1.25 μg CP93129 injected into the nucleus accumbens on amphetamine self-administration. The bars represent the mean (S.E.M.) number of infusions earned by separate groups of rats ($n = 10$ and 12, respectively, for the 5-HT and CP93,129 experiments). * $P < .05$, ** $P < .01$ compared to Sal–Sal treatment; # $P < .01$ compared to Sal–5-HT or Sal–CP93,129 treatment.

not observed because the number of infusions earned under the GR127935 plus 5-HT condition was significantly lower than under the saline alone condition. GR127935 alone did not significantly alter amphetamine self-administration. The lower panel of Fig. 5 illustrates the effect of GR127935 on the reduction in responding induced by 1.25 μg CP93,129. Analysis of variance revealed significant main effects of the agonist [$F(1,11) = 39.96$, $P < .001$] and of the antagonist [$F(1,11) = 56.45$, $P < .001$]. The interaction term was also significant [$F(1,11) = 8.33$, $P = .02$]. Post hoc tests revealed that the number of amphetamine infusions earned following GR127935 plus CP93,129 was significantly increased above the number earned following treatment with CP93,129 alone, and was not different from saline-treatment alone.

4. Discussion

Injecting 5-HT into the nucleus accumbens reduced amphetamine self-administration maintained on a progressive ratio schedule. This inhibitory effect of 5-HT is consistent with previous observations that 5-HT injected into the nucleus accumbens inhibits other behavioural effects of amphetamine including locomotor activity (Carter and Pycock, 1978) and responding for a conditioned reinforcer (Fletcher, 1996). This finding is also consistent with the observation that 5-HT infused into the nucleus accumbens reduces responding for brain stimulation reward derived from the lateral hypothalamus (Redgrave, 1978), a behaviour that is thought to indirectly activate the mesolimbic dopamine system. Responding maintained by food reinforcement was also attenuated following 5-HT injected into the nucleus accumbens. This latter result indicates that the ability of 5-HT to suppress operant responding for intravenous amphetamine infusions is not particularly specific to the drug reinforcer. However, there was some evidence that these two effects could be dissociated in terms of the lowest doses of 5-HT that reduced responding. Thus, amphetamine-maintained responding was significantly reduced at a dose of 2.5 μg, whereas food-maintained responding was not affected at this dose.

Responding for amphetamine was also reduced by the selective 5-HT_{1B} agonist CP93,129. The magnitude of the reduction in self-administration was comparable to that induced by 5-HT. Over the dose range tested CP93,129 did not alter responding for food indicating a more behaviourally specific effect of this drug compared to 5-HT. However, it is important to note that in a previous study higher doses of CP93,129 injected into the nucleus accumbens reduced responding for water reinforcement, and this effect was particularly pronounced at doses of 10 μg (Fletcher and Korth, 1999a). Thus, the apparent behavioural specificity of CP93,129 is also dose-dependent. The ability of both 5-HT and CP93,129 to reduce responding for amphetamine was reversed by the 5-$HT_{1B/1D}$ receptor antagonist GR127935 implicating the 5-HT_{1B} receptor in mediating the effects of 5-HT and CP93,129. Further evidence of the involvement of 5-HT_{1B} receptors in altering amphetamine self-administration derives from the fact that the selective 5-HT_{1A} agonist 8-OH-DPAT and the non-selective 5-HT_2 agonist DOI both failed to affect self-administration. Although both drugs were tested at only one dose these doses have been shown to induce significant behavioural effects when injected into other brain regions (e.g., Fletcher, 1993; Sipes and Geyer, 1997). The lack of effect of DOI or 8-OH-DPAT on amphetamine self-administration is consistent with the report that these drugs do not modify the ability of amphetamine to potentiate responding for a conditioned reinforcer (Fletcher and Korth, 1999a). While GR127935 attenuated the effect of 5-HT this reversal was not complete. At the present time it is unclear whether this indicates that actions at additional 5-HT receptor subtypes, or even a nonspecific effect of 5-HT, may contribute to the suppression of amphetamine self-administration.

Systemic administration of RU24969 reduces amphetamine self-administration and this effect is blocked by GR127935 (Fletcher and Korth, 1999b) indicating the involvement of 5-HT_{1B} receptors in mediating the effect of RU24969. However, the behavioural specificity of this effect is questionable since RU24969 appeared to disrupt other operant behaviours including responding for water

and conditioned reinforcers (Fletcher and Korth, 1999b) and in a drug discrimination procedure (Callahan and Cunningham, 1997). The present results indicate that specific activation of 5-HT_{1B} receptors in the nucleus accumbens exerts a reasonably selective effect in that responding for amphetamine but not food can be altered by this manipulation at least at low doses. Comparing the effects of systemically injected RU24969 with those of intra-accumbens CP93,129 it is likely that RU24969 disrupts operant responding by acting at sites other than the nucleus accumbens.

In contrast to our results showing a suppression of amphetamine self-administration following intra-accumbens injections of CP93,129 injections of CP93,129 or CP94,253 by the intracerebroventricular route increased the number of cocaine infusions earned under a PR schedule (Parsons et al., 1998). These results have led Parsons et al. (1998) to suggest that 5-HT_{1B} receptor agonists increase the reinforcing efficacy of cocaine. The finding that amphetamine self-administration is reduced by specific activation of nucleus accumbens 5-HT_{1B} receptors appears to be at odds with this conclusion. A number of explanations can be offered to account for these discrepancies. The first is that CP93,129 does indeed enhance the reinforcing effects of amphetamine, but that the 1-h cut-off period used in these experiments for defining the breaking point is too short. Previous work has shown that increasing the infusion dose of amphetamine from 60 μg/kg to 120 μg/kg leads to a significant lengthening of the mean interval between infusions (Fletcher, 1998). Richardson and Roberts (1996) have reported that at a dose of 750 μg/kg rats will respond for up to 18 h with mean inter-infusion intervals of about 1 h. For this reason, mean inter infusion intervals were calculated for rats receiving 5-HT and CP93,129 injections in the nucleus accumbens. These intervals were not significantly lengthened compared to those obtained under saline injection. Thus, it is highly unlikely that the reduction in breaking points for amphetamine self-administration induced by 5-HT and CP93,129 is an artifact of the definition of the breaking point.

A more likely possibility to account for the differing effects of nucleus accumbens versus ICV injections of 5-HT_{1B} agonists on psychomotor stimulant reinforcement could relate to differences in the neuroanatomical sites of action of these manipulations. Systemically injected RU24969 potentiates cocaine-induced increases of dopamine in the nucleus accumbens, possibly via inhibition of GABA neurons in the ventral tegmental area (Parsons et al., 1999). Local perfusion of the VTA with RU24969 (O'Dell et al., 2000), 5-HT and TFMPP (Guan and McBride, 1989) which also activates 5-HT_{1B} receptors, increases extracellular levels of DA in the nucleus accumbens. The ability of ICV injections of 5-HT_{1B} agonists to increase cocaine reinforcement may reflect an action mediated at the level of the VTA. When injected via the ICV route CP93,129 enhanced cocaine self-administration at a dose of 3 μg (Parsons et al., 1998), which is only marginally higher than the doses that suppressed responding for amphetamine when injected into the nucleus accumbens. Depending on the degree of receptor reserve, and degree of receptor occupancy necessary to evoke 5-HT_{1B} receptor-mediated changes in the activity of neurons within the VTA or nucleus accumbens it is possible that low doses of 5-HT_{1B} agonists injected ICV may preferentially alter neuronal function in the VTA versus the N.Acc. One implication of this suggestion is that the modulation of psychomotor stimulant drug reinforcement may be modulated differentially by 5-HT_{1B} receptor stimulation depending upon its anatomical location. It is interesting to note then that 5-HT infused into the nucleus accumbens reduces responding for brain stimulation reward (Redgrave, 1978), whereas infusions in the vicinity of the VTA increase this behaviour (Redgrave and Horrell, 1976).

The nucleus accumbens, and its efferents to the ventral pallidum are critical components of the reward circuitry of the brain (Bardo, 1998; Kalivas et al., 1993). Dopamine appears to be the most important neurotransmitter involved in mediating the rewarding effects of psychomotor stimulants (see Section 1). The ability of 5-HT and CP93,129 to reduce the reinforcing effects of amphetamine is unlikely to involve a direct interaction with DA systems since both 5-HT and CP93,129 elevate DA levels in DA-rich terminal regions such as the nucleus accumbens and dorsal striatum (Benloucif et al., 1993; Galloway et al., 1993; Parsons and Justice, 1993), an effect which might be expected to increase the reinforcing efficacy of amphetamine. In addition to dopamine, a number of other neurotransmitters within the nucleus accumbens contribute to the flow of reward-related information through this circuitry including acetylcholine, GABA and glutamate (Bardo, 1998; Kalivas et al., 1993; Mark et al., 1999). Stimulation of 5-HT_{1B} receptors modulates the release of all three of these neurotransmitters (Bobker and Williams, 1989; Boeijinga and Boddeke, 1993; Cassel et al., 1995; Johnson et al., 1992; Rada et al., 1993) and so it is possible that 5-HT_{1B} receptor stimulation in the nucleus accumbens could act via complex mechanisms on multiple systems to disrupt this circuitry leading to attenuation of reward-related behaviours.

In summary, amphetamine self-administration is reduced by 5-HT and by a selective 5-HT_{1B} agonist infused into the nucleus accumbens. Responding for other reinforcers is also attenuated though at higher doses than required for reducing amphetamine self-administration. These results point to a somewhat selective role for 5-HT_{1B} receptors in the nucleus accumbens in modulating the reinforcing effects of intravenous amphetamine. The precise mechanism responsible for this action is unclear. Evidence that 5-HT_{1B} receptors function as heteroreceptors (Barnes and Sharp, 1999) modulating terminal release of multiple neurotransmitters systems indicates that 5-HT_{1B} receptor stimulation could alter motivated behaviour by disrupting the flow of reward-related neural activity in mesolimbic circuitry.

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