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Serotonin 2C receptor agonists and the behavioural satiety sequence in mice

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Abstract

The studies reported here examined the role of the 5-hydroxytryptamine (5-HT)$_{2C}$ receptor subtype in the control of ingestive behaviour in mice. Behavioural satiety sequence (BSS) and food intake measurements were taken, comparing the selective 5-HT$_{2C}$ receptor agonist (*S*)-2-(6-chloro-5-fluoro-indol-l-yl)-l-methylethylamine hydrochloride (Ro 60-0175; 1.0, 3.0 and 10.0 mg/kg) and D-fenfluramine (3.0 mg/kg). Ro 60-0175 produced a dose-dependent decrease in food intake. The effects of Ro 60-0175 (3.0 mg/kg) on the BSS were similar to the hypophagic effects of D-fenfluramine (3.0 mg/kg). In a second experiment, the specific effects on feeding produced by Ro 60-0175 (5.6 mg/kg) were attenuated by pretreatment with the selective 5-HT$_{2C}$ receptor antagonist 6-chloro-5-methyl-1-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242084; 0.5 mg/kg). The 5-HT$_{1B/2C}$ receptor agonist 1-(*m*-chlorophenyl)piperazine (*m*CPP; 3 mg/kg) also produced a substantial decrease in food intake, which was attenuated by SB 242084 (0.5 mg/kg). A dose of the selective 5-HT$_{1B/1D}$ antagonist 2′-methyl-4′(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-(5-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide (GR 127935; 3.0 mg/kg) that successfully attenuated the action of the 5-HT$_{1B}$ agonist 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (RU 24969; 5.0 mg/kg) failed to attenuate *m*CPP-induced hypophagia. These data suggest that Ro 60-0175- and *m*CPP-induced hypophagia in mice are mediated via activation of 5-HT$_{2C}$ receptors and that stimulation of 5-HT$_{1B}$ receptors plays only a minor role in *m*CPP-induced hypophagia. © 2002 Elsevier Science Inc. All rights reserved.

Keywords: Ro 60-0175; GR 127935; SB 242084; *m*CPP; RU 24969; 5-HT$_{2C}$ receptors; 5-HT$_{1B}$ receptors; Satiety; Feeding

1. Introduction

Manipulations of brain serotonergic systems have suggested a modulatory role for the neurotransmitter 5-hydroxytryptamine (5-HT) in feeding behaviour (Dourish, 1992; Simansky, 1996). A variety of approaches have suggested that 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor subtypes are strong candidates for mediating the inhibitory action of serotonin on feeding behaviour (Kennett and Curzon, 1988; Dourish et al., 1989). Studies using the 5-HT$_{1B/2C}$ receptor agonist 1-(*m*-chlorophenyl)piperazine (*m*CPP) indicate an effect on feeding consistent with enhanced satiety (Kitchener and Dourish, 1994). However the 5-HT$_{2A/2C}$ agonist DOI tends to fragment the organisation of feeding-related behaviour in addition to reducing food intake (Simansky and Vaidya, 1990) and the 5-HT$_{1A/1B}$ receptor agonist 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole (RU 24969) increases activity in addition to reducing feeding (Kitchener and Dourish, 1994). Antagonist studies challenging the hypophagic action of the 5-HT releaser fenfluramine have also implicated both 5-HT$_{1B}$ and 5-HT$_{2C}$ receptor subtypes (Neill and Cooper, 1989; Grignaschi and Samanin, 1992; Vickers et al., 1996). However, the interpretation of some of these studies is constrained by the relative lack of selective ligands that were available at the time.

More recently, the role of the 5-HT$_{1B}$ receptor subtype in ingestive behaviour in the rat has been characterised using the selective agonist CP-94,253. This agent produced a reduction in food intake and advanced the behavioural satiety sequence (BSS) in rats (Halford and Blundell, 1996). In addition, Lee and Simansky (1997) showed that

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CP-94,253 produced a dose-related reduction of intake of both food pellets and sucrose solution in rats. This hypophagic action appeared to be mediated by a direct effect on meal termination.

Recent investigations using the selective $5HT_{2C}$ receptor agonist (*S*)-2-(6-chloro-5-fluoro-indol-l-yl)-l-methylethylamine hydrochloride (Ro 60-0175; Martin et al., 1995) have supported a role for the $5\text{-}HT_{2C}$ receptor in the modulation of feeding. Rats consumed significantly less palatable boiled potato when administered Ro 60-0175 (0.3, 1.0, 3.0 and 10.0 mg/kg po). The minimal effective dose to produce a significant reduction (40%) in food intake was 10 mg/kg (Martin et al., 1998). A previous report from our laboratory has described a microstructural analysis of meal patterning in rats administered Ro 60-0175 (3 mg/kg ip). Ro 60-0175 induced a reduction in rate of feeding, a decrease in meal size and an increase in latency to feed (Clifton et al., 2000a). These changes were similar to those observed followed administration of D-fenfluramine (3 mg/kg) in the same study and are consistent with enhancement of satiety. Ro 60-0175 was also shown to reduce the volume of a palatable glucose/saccharin solution consumed in a short-term intake test (Clifton et al., 2000a), where the reduction in drinking resulted from a decrease in the number of clusters of licking rather than a decrease in cluster size. A change of this nature has been associated in previous studies with enhanced satiety rather than decreased palatability (Davis and Smith, 1992). Furthermore, continuous administration of Ro 60-0175 via osmotic pumps over 14 days produced a marked and sustained decrease in both body weight and food intake in rats (Vickers et al., 2000).

Results from feeding studies using transgenic mice lacking either functional $5\text{-}HT_{2C}$ or $5\text{-}HT_{1B}$ receptors have also recently been reported. Thus, Tecott et al. (1995) demonstrated that $5\text{-}HT_{2C}$ receptor knockout mice were insensitive to the hypophagic effect of *m*CPP. More recently, Vickers et al. (1999) showed that these mice are less sensitive to the hypophagic effect of D-fenfluramine when tested in the BSS paradigm. In addition, Lucas et al. (1998) showed that 18-h food-deprived $5\text{-}HT_{1B}$ receptor knockout mice were less sensitive to the hypophagic action of racemic fenfluramine. These mice show a similar blunted response to the hypophagic action of D-fenfluramine when tested in the BSS paradigm (Clifton et al., 2000b).

The aim of the present study was to characterise the effect of the $5\text{-}HT_{2C}$ receptor agonist Ro 60-0175 and the $5\text{-}HT_{1B/2C}$ receptor agonist *m*CPP in the mouse BSS paradigm. This approach is advantageous in that induces a relatively large meal without using food deprivation. In the absence of drug treatment, ingestion of food is followed by a clear sequence of behaviour that progresses towards rest. Drug-related reductions in food intake associated with advancement of the behavioural sequence suggest enhancement of satiety. However, drug-induced reductions of food intake associated with disruption of the sequence suggest an action through nonspecific behavioural mechanisms. We anticipated that our studies would be of value in two ways. First, the BSS has only recently been characterised in mice (Vickers et al., 1999), and there are no published studies using 5-HT receptor agonists in this species, despite the increasing importance of studies using genetically manipulated mice. In addition, the published results from rat and mouse studies using fenfluramine already suggest a different relationship of 5-HT receptor subtypes to feeding behaviour in these two species. Antagonist studies in the rat suggest that activation of $5\text{-}HT_{2C}$ receptors are critical to the effects of D-fenfluramine and that $5\text{-}HT_{1B}$ receptor activation appears to play no significant role (Vickers et al., 2001), whereas the transgenic studies cited above suggest that activation of both $5\text{-}HT_{1B}$ and $5\text{-}HT_{2C}$ receptors underlies the action of D-fenfluramine in mice. Studies of the effects of 5-HT receptor agonists in the mouse should allow us to determine if a species difference underlies these apparent differences.

We initially determined a dose–effect curve for the $5\text{-}HT_{2C}$ receptor agonist Ro 60-0175 using D-fenfluramine (3 mg/kg) as a reference standard. In a second experiment, we challenged the behavioural action of Ro 60-0175 with the selective $5\text{-}HT_{2C}$ receptor antagonist 6-chloro-5-methyl-1-[2(2-methylpyridyl-3-oxy)-pyrid-5-yl carbamoyl] indoline (SB 242084; Kennett et al., 1997b). Further support for a role of the $5\text{-}HT_{2C}$ receptor in modulating feeding behaviour in the mouse was sought through a study of the effect of *m*CPP, again challenged by SB 242084. The role of $5\text{-}HT_{1B}$ receptor activation in the effects of *m*CPP on feeding behaviour in the mouse was examined using the $5\text{-}HT_{1B}$ receptor antagonist 2′-methyl-4′(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-(5-methoxy-3-(4-methyl-piperazin-1-yl)-phenyl]amide (GR 127935; Skingle et al., 1995).

2. Materials and methods

2.1. Animals

Separate naive groups ($n = 12$) of male C57/Bl6 mice (University of Sussex colony) with initial body weights in the range of 25–35 g were used in each experiment. Animals were housed singly and maintained on a 12/12-h light/dark cycle (lights on 05:30 h), with a red light (15-W fluorescent tube and red filter) providing minimal illumination during the dark period. Temperature was maintained at 21 ± 1 °C and humidity at $50 \pm 15\%$. Animals had free access to chow (standard rat and mouse expanded diet, B&K, Hull, UK) and tap water.

2.2. Drugs

D-Fenfluramine hydrochloride (Institut de Recherches Internationales Servier, Neuilly-sur-Seine, France), *m*CPP (Sigma, Poole, UK), GR 127935 (kindly donated by Glaxo-Wellcome, Stevenage, UK) and RU 24969 hemisuccinate

(Tocris, Bristol, UK) were dissolved in 0.9% (w/v) saline solution. Ro 60-0175 (synthesised at Vernalis Research, UK) was dissolved in 10% (w/v) polyethylene glycol (PEG; Sigma). SB 242084 (synthesised at Vernalis Research) was dissolved in PEG at 20% of the final required volume, which was then made up with 8% (w/v) hydroxypropyl-β-cyclodextrin (Tocris) and 25-mM citric acid (Sigma) in 0.9% saline. All drugs were administered by the intraperitoneal (ip) route in an injection volume of 10 ml/kg.

2.3. Test diet

During the experiments, mice were presented for 40 min each day with a palatable wet mash consisting of 1 part powdered chow to 2.5 parts tap water. The test diet was presented in clear plastic Petri dishes (5 cm diameter) attached to rectangular pieces of aluminum (3 × 12 cm). Food intake was corrected for spillage and recorded to 0.1 g. Before testing began, the test diet was presented daily until intake was stable.

2.4. Experimental procedure

2.4.1. BSS

A preweighed dish of test diet was presented and the BSS was observed for a period of 40 min beginning at 14:30–15:30 h. The sequence was recorded using the method described by Vickers et al. (1999). Behavioural categories were *feed*, holding or ingesting of food and drinking; *active*, moving, rearing and other behaviour patterns not defined elsewhere; *groom*, body care movements using mouth or forelimbs and *inactive*, characterised as an absence of movement with a resting posture with or without eye closure.

2.4.2. Experiment 1: D-fenfluramine and Ro 60-0175 and the BSS

Animals were presented with the test diet until consumption reached an asymptotic level (approximately 14 days). During this time, the mice were given two habituation satiety sequence sessions separated by 72 h. Animals were given an injection of 0.9% saline (10 ml/kg ip) 30 min before the second of these sessions. In the test phase of the experiment, animals were treated with either vehicle, D-fenfluramine (3.0 mg/kg) or doses of Ro 60-0175 (1.0, 3.0, 10.0 mg/kg) in a Latin square design. After 30 min, normal lab chow was removed and they were presented with the test diet. Animals were observed for the 40-min experimental period. After the test session, the dish containing wet mash was removed and reweighed. Standard lab chow was replaced into the animal's food hopper. Test sessions were separated by at least 72 h.

2.4.3. Experiment 2a: antagonism of Ro 60-0175-induced hypophagia by SB 242084

Using a similar within-subject design to Experiment 1, mice were treated with either vehicle or SB 242084 (0.5 mg/kg ip), and 20 min later, they were treated with either vehicle or Ro 60-0175 (5.6 mg/kg ip). After a further 30 min, they were tested as in Experiment 1. The intermediate dose of Ro 60-0175 was chosen because in the first experiment 3-mg/kg Ro 60-0175 did not produce a significant reduction in food intake, whereas motor stereotypy that might have interfered with feeding behaviour was evident after administration of 10-mg/kg Ro 60-0175. This dose of SB 242084 was chosen because a similar dose potently inhibited *m*CPP-induced hypolocomotion in the rat (Kennett et al., 1997b).

2.4.4. Experiment 2b: antagonism of mCPP-induced hypophagia by SB 242084

This experiment was performed using an identical method to that described for Experiment 2a. Mice were treated prior to the test session with either vehicle (8% hydroxy-β-cyclodextrin) or SB 242084 (0.5 mg/kg). After 20 min, they were treated with either vehicle (0.9% saline) or *m*CPP (3.0 mg/kg). After a further 30 min, animals were tested as in Experiment 1. This dose of *m*CPP was chosen because it produced a significant reduction in food intake in an earlier study using the same mouse strain (Tecott et al., 1995).

2.4.5. Experiment 3a: antagonism of mCPP-induced hypophagia by GR 127935

This experiment was performed using an identical method to that described for Experiment 2a. Mice were treated prior to the test session with either GR 127935 (3.0 mg/kg) or 0.9% saline. After 20 min, they were administered with either vehicle (0.9% saline) or *m*CPP (3.0 mg/kg). After a further 30 min, animals were tested as in Experiment 1. This dose of GR 127935 was chosen because similar doses block the behavioural syndrome induced by the 5-$HT_{1A/1B}$ agonist RU 24969 in the mouse (O'Neill et al., 1996).

2.4.6. Experiment 3b: antagonism of RU 24969-induced hypophagia by GR 127935

This experiment was performed using an identical method to that described for Experiment 2a. Mice ($n = 12$) were treated prior to the test session with either vehicle (0.9% saline) or GR 127935 (3.0 mg/kg). After 20 min, they were administered with either vehicle (0.9% saline) or RU 24969 (5.0 mg/kg). After a further 30 min, animals were tested as in Experiment 1.

2.5. Statistics

Food intake was expressed as mean (± S.E.M.) intake of wet mash (g) and analysed using repeated-measures ANOVA, with dose as the repeated-measures factor. Each of the four mutually exclusive behaviour patterns associated with the BSS were treated separately. They were summed into 5-min bins (maximum score 10), plotted as proportions

Table 1
Food intake in a 40-min test session

Drug treatment	Food intake (g) ± S.E.M.
Vehicle	1.82 (0.18)
1-mg/kg Ro 60-0175	1.91 (0.21)
3-mg/kg Ro 60-0175	1.55 (0.31)
10-mg/kg Ro 60-0175	1.18 (0.23)*
3-mg/kg D-fenfluramine	1.00 (0.19)*

* $P < .05$ (Dunnett's test by comparison with vehicle).

and analysed using repeated-measures ANOVA with time and drug treatment(s) as the repeated-measures factors. Subsequent paired comparisons between control and experimental groups were made using Dunnett's test. Statistical analysis was carried out using the Genstat computer statistical package (Genstat 5 Committee, 1987).

3. Results

3.1. Experiment 1: D-fenfluramine or Ro 60-0175 and the BSS

Drug treatment produced a reduction in food intake [$F(4,40) = 3.36$, $P < .05$] that was dose related for Ro 60-0175 (Table 1). Ro 60-0175 10 mg/kg reduced wet mash intake by 35% ($P < .05$) and treatment with D-fenfluramine resulted in a 45% decrease in wet mash intake ($P < .05$).

Vehicle-treated animals showed the expected sequence of behaviour, with relatively high frequencies of feeding early in the session being replaced by activity, grooming and rest as the observations continued (Fig. 1). Ro 60-0175 and D-fenfluramine reduced feeding behaviour [Time × Drug Interaction: $F(28,280) = 3.52$, $P < .001$]. This interaction arose from a marked reduction in feeding following treatment with Ro 60-0175 (3.10 mg/kg) or D-fenfluramine in early time periods (Fig. 1). Active behaviour was also reduced by Ro 60-0175 (3.10 mg/kg) or D-fenfluramine (Fig. 1). Again, the effects were time dependent [Time × Drug interaction: $F(28,280) = 1.57$, $P < .05$], being most noticeable at intermediate time periods (Fig. 1). Grooming was unaffected by either drug treatment. Inactive behaviour was enhanced by D-fenfluramine and Ro 60-0175 across all time bins [main effect: $F(4,40) = 6.80$, $P < .001$; Time × Drug interaction: NS].

3.2. Experiment 2a: antagonism of Ro 60-0175-induced hypophagia by SB 242084

Treatment with Ro 60-0175 5.6 mg/kg reduced food intake (Table 2). Pretreatment with SB 242084 had no intrinsic effect on food intake but strongly attenuated the hypophagic effect of Ro 60-0175, giving a significant Treatment × Pretreatment interaction [$F(1,11) = 13.85$, $P < .05$].

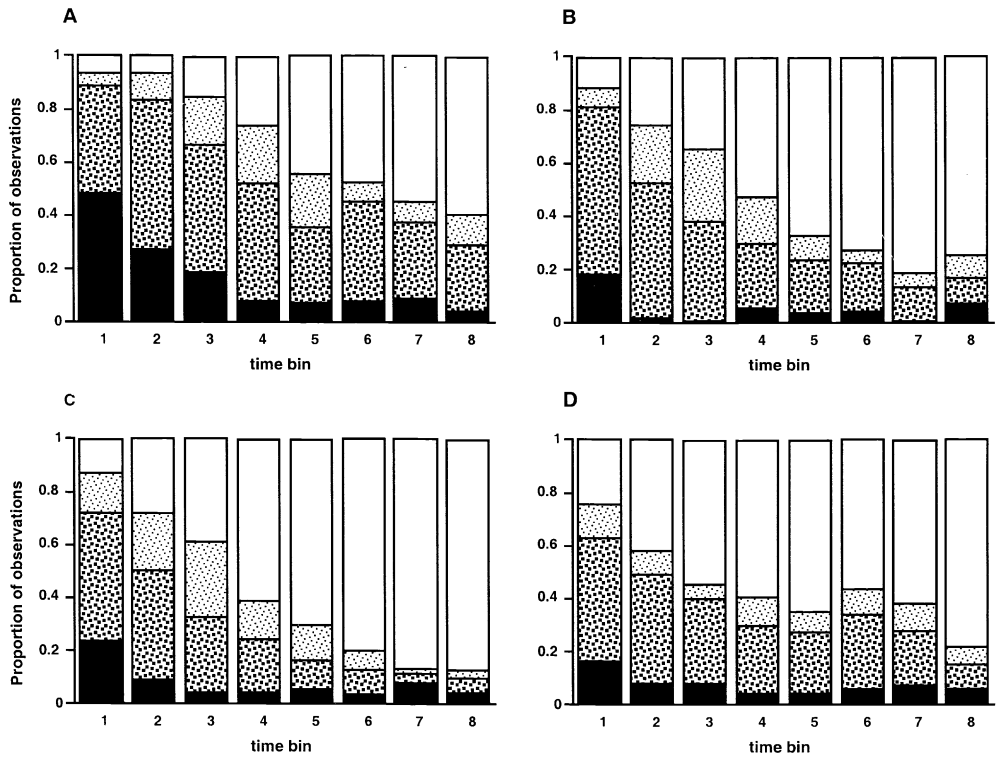

Fig. 1. The effects of vehicle (A), 3-mg/kg D-fenfluramine (B), 3-mg/kg Ro 60-0175 (C) or 10-mg/kg Ro 60-0175 (D) on the temporal organisation of the BSS. Results are expressed as the proportion of behavioural observations in 5-min bins that were classified as either *feed*, *active*, *groom* or *inactive*. Each animal ($n = 12$) was observed on 10 occasions (i.e. every 30 s) during each time bin.

Table 2
Food intake in 40-min test session

Antagonist treatment	Drug treatment	
Experiment 2a	Vehicle	5.6-mg/kg Ro 60-0175
Vehicle	0.99 (0.08)	0.53 (0.08)
0.5-mg/kg SB 242084	0.96 (0.05)	0.91 (0.05)*,†
Experiment 2b	Vehicle	3-mg/kg *m*CPP
Vehicle	1.05 (0.11)	0.66 (0.09)
0.5-mg/kg SB 242084	0.97 (0.11)	0.90 (0.06)†
Experiment 3a	Vehicle	5.6-mg/kg Ro 60-0175
Vehicle	1.38 (0.14)	0.56 (0.15)
3-mg/kg GR 127935	1.50 (0.14)	0.82 (0.15)
Experiment 3b	Vehicle	5-mg/kg RU 24969
Vehicle	1.86 (0.11)	1.07 (0.14)
3-mg/kg GR 127935	1.88 (0.12)	1.51 (0.16)*,†

* Significant ($P<.05$) Treatment × Pretreatment interaction.
† Significant difference ($P<.05$) between drug groups pretreated with vehicle and antagonist.

Feeding behaviour was suppressed by Ro 60-0175 but increased slightly above control levels when Ro 60-0175 was preceded by treatment with SB 242084 (Fig. 2), leading to a significant Treatment × Pretreatment interaction [$F(1,11)=8.82$, $P<.02$]. Records of active behaviour showed a different pattern of response to feeding, with clear enhancement associated with the SB 242084 condition, regardless of treatment with Ro 60-0175 [main effect: $F(1,11)=17.28$, $P<.01$]. There were no effects of Ro 60-0175 alone on active behaviour at this dose (all F's involving drug NS). As in Experiment 1, grooming behaviour was unaffected by drug treatment and there was also no effect of pretreatment with SB 242084. Inactive behaviour was decreased following treatment with SB 242084 [main effect: $F(1,11)=21.72$, $P<.001$; Fig. 2].

3.3. Experiment 2b: antagonism of mCPP-induced hypophagia by SB 242084

Treatment with *m*CPP caused a substantial reduction in food intake, which was absent following pretreatment with SB 242084 (Table 2). ANOVA gave a significant effect of treatment [$F(1,11)=13.94$, $P<.005$] and a marginally nonsignificant Treatment × Pretreatment interaction [$F(1,11)=4.21$, $P=.065$]. However, the predicted difference between *m*CPP-treated groups given pretreatment with either vehicle or SB 242084 was highly significant ($t_d=3.74$, $P<.01$).

*m*CPP led to an early termination of feeding behaviour, which was partially reversed by pretreatment with SB 242084 (Fig. 3), leading to a significant Time- × Treatment × Pretreatment interaction [$F(7,77)=2.4$, $P<.05$] in addition to a main effect of treatment [$F(1,11)=7.81$, $P<.02$]. As in Experiment 2, active behaviour was increased following administration of the antagonist SB 242084 [$F(1,11)=37.82$, $P<.001$]. This effect was independent of treatment with *m*CPP since there was no significant Treatment × Pretreatment inter-

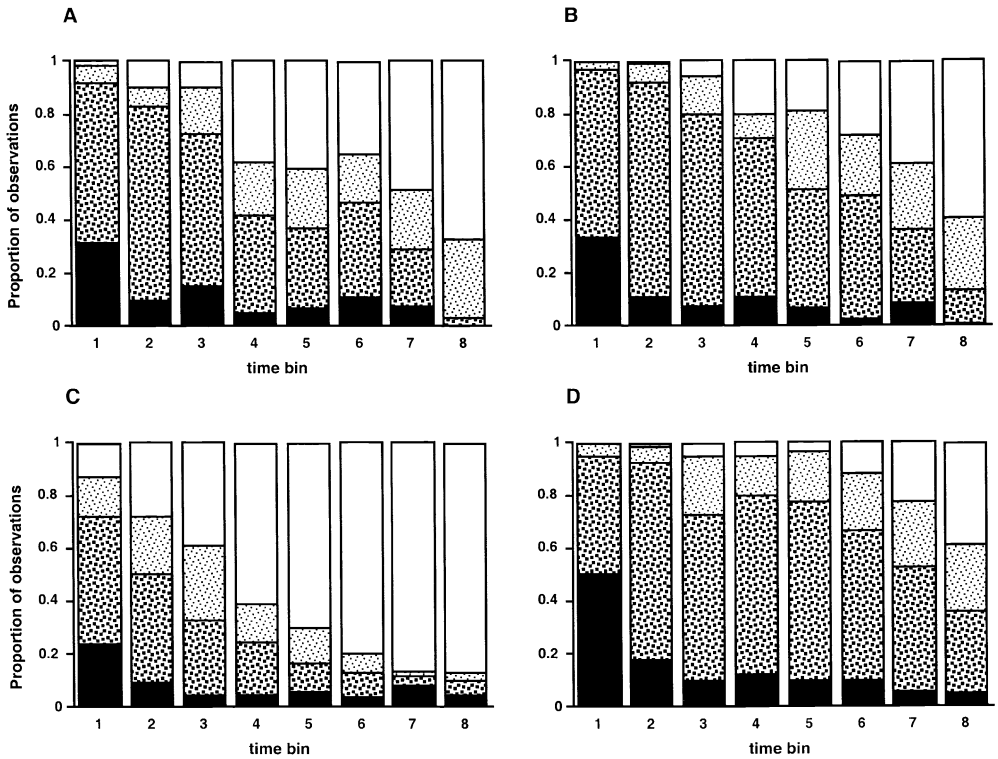

Fig. 2. The effects of vehicle (A), 0.5-mg/kg SB 242084 (B), 5.6-mg/kg Ro 60-0175 (C) or the combined drug treatments (D) on the temporal organisation of the BSS. Data expressed as in Fig. 1.

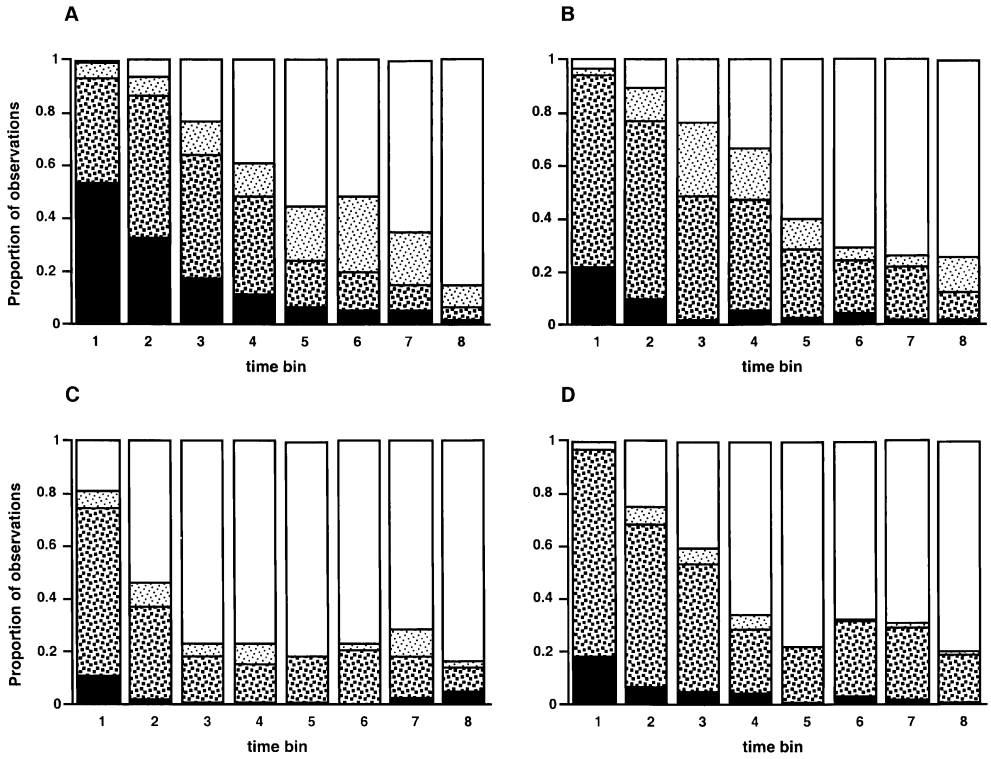

Fig. 3. The effects of vehicle (A), 0.5-mg/kg SB 242084 (B), 3-mg/kg *m*CPP (C) or the combined drug treatments (D) on the temporal organisation of the BSS. Data expressed as in Fig. 1.

action [$F(1,11) = 0.07$, NS]. *m*CPP alone also had no effect on active behaviour [$F(1,11) = 0.04$, NS].

*m*CPP reduced grooming behaviour throughout the BSS [main effect: $F(1,11) = 42.83$, $P < .001$; Fig. 3]. Pretreatment with SB 242084 had no independent effect on grooming, but the combination of *m*CPP and SB 242084 led to a further reduction in grooming [$F(1,11) = 5.12$, $P < .05$; Fig. 3b]. *m*CPP significantly enhanced the level of inactive behaviour as well as bringing forward the onset of this behaviour compared to vehicle [Treatment × Time interaction: $F(1,11) = 10.17$, $P < .01$; Fig. 3]. Pretreatment with the 5-HT_{2C} receptor antagonist SB 242084 decreased the overall level of resting [$F(1,11) = 18.21$, $P < .001$] as well as delaying the increase in inactive behaviour that occurred following *m*CPP [$F(7,77) = 2.30$, $P < .05$].

3.4. Experiment 3a: antagonism of mCPP-induced hypophagia by GR 127935

Whilst food intake was decreased by *m*CPP [$F(1,11) = 41.87$, $P < .001$], GR 127935 had no significant impact on this reduction [Treatment × Pretreatment interaction: $F(1,11) = 0.83$, NS; Table 2 and Fig. 4].

The effects of *m*CPP on the satiety sequence were very similar to those observed in the previous experiment (Fig. 5). Thus, *m*CPP significantly reduced feeding behaviour [$F(1,11) = 69.82$, $P < .001$]. However, GR 127935 alone had no significant effect on feeding behaviour [$F(1,11) = 2.55$, NS] and did not attenuate the decrease in feeding behaviour elicited by *m*CPP [Treatment × Pretreatment interaction: $F(1,11) = 0.03$, NS]. *m*CPP produced a significant decrease in active behaviour [$F(1,11) = 12.88$, $P < .005$]. Pretreatment with GR 127935 had no intrinsic effect on active behaviour nor did it modify the effects of *m*CPP on this behaviour (all *F*'s involving drug NS). Grooming behaviour was also decreased following *m*CPP treatment [$F(1,11) = 23.46$, $P < .001$]. This reduction was unaffected by pretreatment with GR 127935 [$F(1,11) = 0.07$, NS]. *m*CPP increased the amount of resting behaviour [$F(1,11) = 61.03$, $P < .001$], and pretreatment with GR 127935 failed to attenuate this effect of *m*CPP [Treatment × Pretreatment interaction: $F(1,11) = 0.15$, NS].

3.5. Experiment 3b: antagonism of RU 24969-induced hypophagia by GR 127935

RU 24969 produced a suppression of food intake, which was attenuated by GR 127935 leading to a significant Treatment × Pretreatment interaction [$F(1,11) = 6.48$, $P < .05$; Table 2].

Consistent with the hypophagic effect, RU 24969 also reduced the proportion of time spent feeding (Fig. 5), and this reduction was attenuated by GR 127935 leading to a Treatment × Pretreatment interaction [$F(1,11) = 16.53$, $P < .002$]. RU 24969 also reduced active behaviour, but for this behaviour, the effect was enhanced in the presence

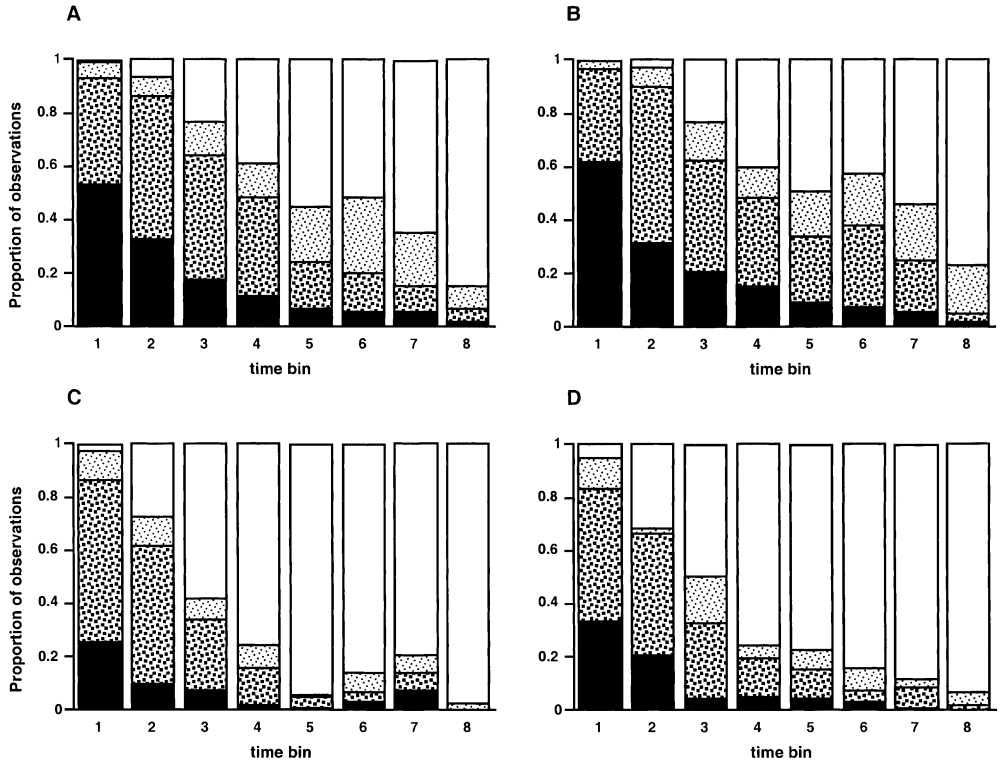

Fig. 4. The effects of vehicle (A), 3-mg/kg GR 127935 (B), 3-mg/kg *m*CPP (C) or the combined drug treatments (D) on the temporal organisation of the BSS. Data expressed as in Fig. 1.

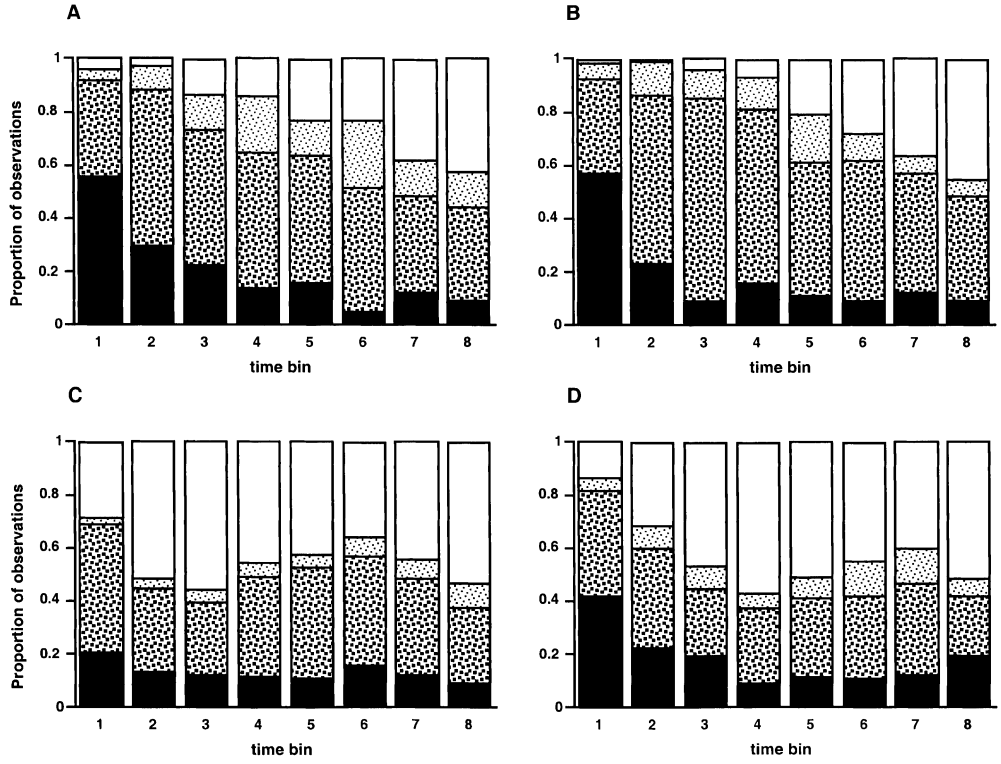

Fig. 5. The effects of vehicle (A), 3-mg/kg GR 127935 (B), 5-mg/kg RU 24969 (C) or the combined drug treatments (D) on the temporal organisation of the BSS. Data expressed as in Fig. 1.

of GR 127935 [$F(1,11) = 5.06$, $P < .05$]. Grooming behaviour was reduced by RU 24969, and this decrease was attenuated by GR 127935 [$F(1,11) = 6.76$, $P < .05$]. Finally, resting behaviour was enhanced by RU 24969 [$F(1,11) = 21.07$, $P < .001$], but GR 127935 had no intrinsic effect or interaction with RU 24969 (all F's involving antagonist, NS).

4. Discussion

Our data demonstrate that mice habituated to a palatable mash meal exhibited a clear satiety sequence comparable to that described in rats (e.g. Antin et al., 1975) and other mammalian species (Clifton, 1994). The selective 5-HT_{2C} agonist Ro 60-0175 produced a substantial dose-dependent hypophagic response consistent with data from rat studies (Clifton et al., 2000a,b). D-Fenfluramine, as expected from our earlier studies (Vickers et al., 1999), decreased food intake and led to an advancement in the satiety sequence. The 5-HT_{2C} receptor agonist Ro 60-0175 (3 mg/kg) and D-fenfluramine (3 mg/kg) produced a similar profile of change in the BSS. In a subsequent experiment, the hypophagic effects of either Ro 60-0175 (5.6 mg/kg) or *m*CPP (3 mg/kg) were attenuated by the selective 5-HT_{2C} receptor antagonist SB 242084. By contrast, in the final experiment, the 5-HT_{1B} receptor antagonist GR 127935 had no significant impact on *m*CPP-induced hypophagia at a dose that was successful in attenuating the hypophagic effect of the 5-$HT_{1A/1B}$ receptor agonist RU 24969.

Ro 60-0175 was originally described as a highly selective 5-HT_{2C} agonist (Martin et al., 1998). However, more recent reports have suggested that this drug also has high agonist efficacy at 5-HT_{2A} and 5-HT_{2B} receptors (Porter et al., 1999). Stimulation of 5-HT_{2B} receptors leads to an enhancement of food intake (Kennett et al., 1997a), whereas stimulation of 5-HT_{2A} receptors is associated with decreased food intake, although perhaps not through a behaviourally selective route. This conclusion is supported by the disruption to feeding behaviour that resulted after administration of the 5-$HT_{2A/2C}$ agonist DOI (Simansky and Vaidya, 1990; Kitchener and Dourish, 1994). Thus, the action of Ro 60-0175 at 5-HT_{2A} receptors is potentially relevant to its hypophagic action. However, SB 242084 has negligible affinity for 5-HT_{2A} receptors (Kennett et al., 1997a,b) so it appears likely that the hypophagic action of Ro 60-0175 involves a substantial 5-HT_{2C}-mediated component.

Although *m*CPP is widely used as a probe of 5-HT_{2C} receptor activation in both the laboratory (e.g. Kennett and Curzon, 1988) and the clinic (e.g. Sargent et al., 1997), it has substantial affinity for other receptor subtypes, including the 5-HT_{1B} receptor (Barnes and Sharp, 1999). By contrast with Ro 60-0175, *m*CPP has only a weak partial agonist action at 5-HT_{2A} receptors (Barnes and Sharp, 1999). Studies using selective 5-HT_{1B} agonists, such CP 94-253, suggest an important role for this receptor in the modulation of food intake (Halford and Blundell, 1996; Lee and Simansky, 1997). In addition, a recent report suggests that it is a 5-HT releasing agent with a similar potency to D-fenfluramine after systemic administration in the rat (Baumann et al., 2001). For all these reasons, it was of considerable interest to challenge *m*CPP-induced hypophagia with either a selective 5-HT_{2C} or a selective 5-HT_{1B} receptor antagonist. These results were relatively straightforward since a selective 5-HT_{2C} antagonist (SB 242084) attenuated the *m*CPP-induced hypophagia, whereas a selective 5-HT_{1B} antagonist (GR 127935) had a negligible effect. This conclusion held for both food intake and for the proportion of time spent feeding. Since the same dose of GR 127935 attenuated the hypophagic effect of RU 24969, which has a well established action at 5-HT_{1B} receptors (Barnes and Sharp, 1999), we can be confident that GR 127935 had been administered at an appropriate dose. Thus, it appears that 5-HT_{2C} receptor activation makes the major contribution to the hypophagic actions of both *m*CPP and Ro 60-0175 in the mouse.

*m*CPP, in contrast to Ro 60-0175, reduced the incidence of grooming behaviour. This, in itself, suggests that the effect was unlikely to have been mediated by 5-HT_{2C} receptor activation. The failure of SB 242084 to attenuate the effect is consistent with this interpretation. However, it also seems unlikely that the effect is mediated by 5-HT_{1B} receptor stimulation since GR 127935 failed to attenuate the *m*CPP-induced reduction in grooming.

Heisler and Tecott (2000) recently reported that *m*CPP produced a decrease in activity in wild type mice but an increase in activity in 5-HT_{2C} knockout mice. They postulated that stimulation of 5-HT_{2C} receptors results in hypoactivity, whereas 5-HT_{1B} receptor stimulation results in hyperactivity. Thus, loss of 5-HT_{2C} receptor function in their mutant mice would have led to a stimulation of activity after treatment with a mixed 5-$HT_{1B/2C}$ receptor agonist such as *m*CPP. Our data are consistent with this interpretation. When *m*CPP was combined with SB 242084, then activity levels tended to rise, whereas the combination of *m*CPP and GR 127935 tended to lead to hypoactivity. These effects were relatively small by comparison with those reported by Heisler and Tecott (2000). This difference might easily be attributed to variations in the way in which activity was recorded in our own study and that of Heisler and Tecott (2000). However, we have also examined the effect of *m*CPP in the satiety sequence paradigm using wild type and 5-HT_{2C} receptor knockout mice (Hewitt and Clifton, unpublished data) and found very much greater hyperactivity in the mutant mice than that observed here after SB 242084 pretreatment.

Treatment with the 5-$HT_{1A/1B}$ agonist RU 24969 was associated with decreased activity. This result was unexpected both in relation to the data for *m*CPP and GR 127935 discussed above and to previous reports. Thus, Kennett et al. (1987) and Kitchener and Dourish (1994) both reported that RU 24969 elevated locomotor activity in rats, and Cheetham and Heal (1993) and O'Neill et al. (1996) reported similar effects in mice. However, in both of the latter reports, the

animals were tested only once in a novel environment and with lower doses of RU 24969. It may be that one of these parameters is critical. In particular, there are no data comparing the effect of 5-HT_{1B} agonists on locomotor activity in mice tested either in novel or familiar environments.

SB 242084 alone had no effect on food intake. Such an effect might be predicted on the basis of an inhibitory effect of serotonin on feeding. Nonselective serotonin antagonists, such as metergoline and methysergide, have been reported to stimulate food intake in rats (Fletcher, 1998; Dourish et al., 1989). The selective 5-HT_{2C} antagonist RS 10221 has also been reported to increase food intake (Bonhaus et al., 1997) although this study had a puzzling feature in that the antagonist failed to reverse 5-HT_{2C}-mediated effects on activity. However, in an extensive series of studies, Kennett et al. (1997a,b) failed to find any evidence of hyperphagia following treatment with SB 242084. One hypothesis that might explain the lack of action of SB 242084 on food intake is that 5-HT_{2C}-mediated effects on feeding occur relatively late in the sequence of events leading from ingestion of food to behavioural satiety and that hyperphagia, especially in already sated animals, can only be obtained when both early and late events in this sequence are inhibited. For example, 5-HT_{1B} receptor stimulation may modulate the processing of interoceptive and exteroceptive stimuli at a brainstem level and represent one example of an early effect (Lee and Simansky, 1997). 5-HT_{2C}-mediated effects might, by contrast, act on motivational or incentive-like variables. Thus, a drug that acts as an antagonist at both 5-HT_{2C} and 5-HT_{1B} receptors may impair the development of satiety and enhance intake, whereas a drug having a more selective action at 5-HT_{2C} receptors may be without effect.

In summary, therefore, our data suggest that Ro 60-0175 and *m*CPP reduce food intake through behaviourally selective mechanisms in the mouse. Ro 60-0175 and *m*CPP are both 5-HT_{2C} agonists but differ substantially in their action at other 5-HT subtypes relevant to the modulation of food intake. However, we have shown that the effects of these drugs are only substantially attenuated by antagonists at the 5-HT_{2C} receptor. Our data therefore strengthen the argument that 5-HT_{2C} receptor stimulation is one important component of the sequence of events that lead to satiety in several rodent species.

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