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Influence of the 5-HT_{2C} receptor antagonist SB242,084 on behaviour produced by the 5-HT_2 agonist Ro60-0175 and the indirect 5-HT agonist dexfenfluramine

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1 Ro60-0175 has been described as a selective agonist at the 5-HT_{2C} receptor, yet it has only 10-fold higher affinity at the 5-HT_{2C} compared to the 5-HT_{2A} subtype, and equivalent affinity for the 5-HT_{2B} receptor.

2 The selective 5-HT_{2C} receptor antagonist SB242,084 (0.5 mg kg^{-1} i.p.), blocked the hypoactivity and penile grooming induced by Ro60-0175 (1 mg kg^{-1} s.c.). The combination of SB242,084 (0.5 mg kg^{-1} i.p.) and Ro60-0175 (3–10 mg kg^{-1}) produced a completely different pattern of behaviours including wet-dog shakes, hyperactivity and back muscle contractions. These latter effects were blocked by the selective 5-HT_{2A} receptor antagonist MDL100,907 (0.5 mg kg^{-1} i.p.), but not the 5-HT_{2B} receptor antagonist SB215,505 (3 mg kg^{-1} p.o.).

3 The indirect 5-HT releaser/reuptake inhibitor dexfenfluramine (1–10 mg kg^{-1} i.p.) produced a mild increase in locomotor activity, penile grooming, and occasional back muscle contractions and wet-dog shakes. Pre-treatment with SB242,084 (0.5 mg kg^{-1}), blocked the incidence of penile grooming, and markedly potentiated both the dexfenfluramine-induced hyperactivity, the incidence of back muscle contractions, and to a lesser extent wet-dog shakes. Some toxicity was also evident in animals treated with dexfenfluramine (10 mg kg^{-1})/SB242,084 (0.5 mg kg^{-1}), but not in any other treatment groups.

4 The hyperactivity and toxicity produced by the dexfenfluramine (10 mg kg^{-1})/SB242,084 (0.5 mg kg^{-1}) combination was replicated in a further study, and hyperthermia was also recorded. Both hyperthermia and toxicity were blocked by MDL100,907 (0.5 mg kg^{-1}) but not SB215,505 (3 mg kg^{-1}). An attenuation of the hyperlocomotor response was also observed following MDL100,907.

5 These findings suggest that 5-HT_{2C} receptor activation can inhibit the expression of behaviours mediated through other 5-HT receptor subtypes.

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Abbreviations: ANOVA, analysis of variance; DOI, (±)-2-dimethoxy-4-iodoamphetamine hydrochloride; LMA, locomotor activity; LSD, lysergic acid diethylamide; mCPP, *m*-(chlorophenyl)piperazine; Ro60-0175, (S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methlethylamine); SB242,084, (6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbamoyl] indoline)

Introduction

There are presently at least 14 distinct 5-HT receptor subtypes which are encoded by distinct genes, and which appear to have functional roles (Boess & Martin, 1994; Hoyer & Martin, 1997; Barnes & Sharp, 1999). The 5-HT_2 receptor is comprised of 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors. They are grouped according to similar sequence homology and second messenger system, each being positively coupled to phospholipase C (Hoyer & Martin, 1997; Baxter *et al*., 1995).

Activation of the 5-HT_{2A} receptor has been associated with the hallucinogenic properties of the phenylisopropylamines such as DOI, and the indoleamine, LSD (Aghajanian & Marek, 1999). Upon systemic administration, these drugs also produce specific behaviours which comprise part of the 5-HT syndrome, including wet-dog shakes, back muscle contractions and forepaw treading (Fone *et al*., 1989; Pranzatelli, 1990; Wettstein *et al*., 1999; Ouagazzal *et al*., 2001). In contrast, hypoactivity and penile grooming/erections are characteristic behavioural signs following activation of 5-HT_{2C} receptors by agents such as mCPP and (S)-2-(6-chloro-5-fluoroindol-1-yl)-1-methlethylamine) (Ro60-0175, Millan *et al*., 1997; Martin *et al*., 1998). However, neither mCPP nor Ro60-0175 show more than 10 fold selectivity for the 5-HT_{2C} receptor compared to other 5-HT receptor subtypes (Porter *et al*., 1999), yet surprisingly neither drug seems to elicit other 5-HT receptor-mediated behavioural signs (Millan *et al*., 1997; Martin *et al*., 1998).

In a recent study, Tecott and coworkers (Heiser *et al*., 1999; Heiser & Tecott, 2000) described a paradoxical effect of mCPP in mice deficient in the 5-HT_{2C} receptor (Tecott *et al*., 1995). More specifically, in 5-HT_{2C} receptor knock-out mice, a hyperactivity was unmasked following mCPP treatment, which was in stark contrast to the hypoactivity typically seen

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with this drug in non-genetically manipulated rats and mice (Kennett & Curzon, 1988). Furthermore, this hyperactivity was blocked by the 5-HT$_{1B/1D}$ receptor antagonist GR127935 (Clitheroe *et al*., 1994). These findings are of interest in that they highlight interactions between the 5-HT receptor family, and suggest that 5-HT$_{2C}$ receptor activation can elicit a profound effect on behaviour, which can mask that mediated through other 5-HT receptor subtypes.

It was the purpose of the present series of studies to follow up on these findings. Specifically, we examined the overt behaviours produced by various doses of Ro60-0175 in the presence and absence of the selective 5-HT$_{2C}$ receptor antagonist (6-chloro-5-methyl-1-[6-(2-methylpyridin-3-yloxy) pyridin-3-yl carbamoyl] indoline) (SB242,084; Kennett *et al*., 1997). In common with Heiser & Tecott (2000), following pre-treatment with SB242,084, we observed a paradoxical hyperactivity to Ro60-0175, and an unmasking of hitherto unseen behaviours including wet-dog shakes and back muscle contractions. These were shown in a subsequent experiment to be completely blocked by the 5-HT$_{2A}$ receptor antagonist MDL100,907 (Kehne *et al*., 1996). Following this observation, we then studied the indirect 5-HT agonist dexfenfluramine, both in the presence and absence of SB242,084. Again, co-treatment with SB242,084 resulted in a marked change in the behavioural profile of dexfenfluramine, including the emergence of toxicity.

Methods

Animals and housing

Male Sprague-Dawley rats (BRL, Fullinsdorf, body weight 230–270 g) were used throughout. Animals were housed in groups of four within a holding room controlled for temperature (22±2°C), and lighting (lights on: 06:00–18:00 h). All experiments were conducted during the light phase. Rats received food and water *ad-libitum* except during periods of behavioural observation. All experiments were conducted in accordance with the relevant local and national guidelines regarding animal experimentation.

Experimental design

In each study a crossover design was used, in that the same rats were run in a locomotor activity (LMA) and visual observation test. Seven days separated each test and the sequence of each was counterbalanced across animals. Treatment designation for individual rats was counterbalanced across the LMA and visual observation experiments.

For the measurement of LMA, the animals were singly placed into activity chambers (36×24×19 cm, L×W×H; Benwick Electronics, U.K.) containing sawdust bedding for a period of 60 min. Locomotor activity was automatically recorded by photocell interruption. The animals were naïve to the test apparatus at the time of the LMA experiment.

For the measurement of behavioural signs, subjects were treated with the appropriate treatment and individually placed in observation chambers (26×10×30 cm, L×W×H), with a mirror placed behind the chamber to allow an all round view of behaviour. Over a 60 min observation period, the following behaviours were scored: penile grooming (number of distinct penile grooming bouts accompanied by an erection), wet-dog shakes (total number) and back muscle contractions (scored as present or absent within 5 min time bins, i.e total possible score = 12). The experimenter was unaware of drug treatment at the time of testing.

Experiment 1: Ro60-0175

A total of eight treatment groups were used in the initial part of this study ($n=8$ rats per group). Ro60-0175 was tested at four doses (vehicle, 1, 3, 10 mg kg^{-1}) in the presence or absence of the 5-HT$_{2C}$ antagonist SB242,084 (0.5 mg kg^{-1}). This dose of SB242,084 was selected on both published and in-house data, confirming 5-HT$_{2C}$ receptor blockade *in vivo* (Kennett *et al*., 1997; Grottick *et al*., 2000). Ro60-0175 was tested at doses previously reported as 5-HT$_{2C}$ selective, and at higher doses likely to be less selective (Millan *et al*., 1997; Martin *et al*., 1998; Dekeyne *et al*., 1999; Kennett *et al*., 2000).

In a subsequent experiment utilizing a further four groups of experimentally naïve rats ($n=8$ per group), the effect of the 5-HT$_{2A}$ antagonist MDL100,907 (0.5 mg kg^{-1}) was tested against the hyperlocomotion, wet-dog shakes, and back muscle contractions induced by the Ro60-0175 (10)/ SB242,084 (0.5) combination.

A further experiment evaluated the effect of the 5-HT$_{2B}$ preferring antagonist SB215,505 (3 mg kg^{-1} p.o.) against the hyperlocomotion, wet-dog shakes, and back muscle contractions induced by the Ro60-0175 (10)/SB242,084 (0.5) combination. The dose, route and pre-treatment time was based on published work on this compound (Kennett *et al*., 1998; Reavill *et al*., 1999).

Experiment 2: Dexfenfluramine

A total of eight treatment groups were used in this study. Dexfenfluramine was tested at four doses (vehicle, 1, 3, 10 mg kg^{-1}) in the presence or absence of the 5-HT$_{2C}$ antagonist SB242,084 (0.5 mg kg^{-1}). Initial group sizes were $n=8$, however during the course of this study, some toxicity was evident in the dex (10)/ SB242,084 pre-treated group. Final group sizes reflect this adjustment.

In a separate group of rats, a study was designed to examine the effect of MDL100,907 (0.5 mg kg^{-1} i.p.) and SB215,505 (3 mg kg^{-1} p.o.) against the behavioural changes and toxicity seen following dex (10)/SB242,085 (0.5) treatment. For this particular study the animals rectal body temperature was measured immediately pre and 60 min post treatment. During the intervening 60 min period the animals were tested in activity chambers but were not formally assessed for signs of 5-HT behavioural syndrome. A total of 10 rats per group were used for this study.

Drugs and injections

SB242,084 (6-chloro-5-methyl-1-[2-(2-methylpyridyl-3-oxy)-pyrid-5-yl carbomyl] indoline), MDL100,907 ((±)2,3-dimethoxyphenyl-1-[2-4-(piperidine)-methanol]), Ro60-0175 ((S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$) and dexfenfluramine were synthesized within the

Chemistry Department at Hoffmann-La Roche Ltd., Basel, Switzerland. SB215,505 (6-chloro-5-methyl-1-(5-quinolylcarbamoyl) indoline) was generously provided by SmithKline Beecham Pharmaceuticals, Harlow, U.K. Ro60-0175 was dissolved in 0.9% saline before subcutaneous injection immediately prior to test onset. Dexfenfluramine was dissolved in 0.9% saline solution and injected 10 min prior to test *via* the intraperitoneal route. SB242,084 was prepared in 0.9% saline solution containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid. SB242,084 was injected by the intraperitoneal route with a pre-treatment time of 30 min. MDL100,907 was dissolved in 0.9% saline solution containing 0.3% Tween and injected s.c. 30 min before test onset. SB215,505 was dissolved in a solution of 0.3% Tween, and injected p.o. 60 min before test at a volume of 5 ml kg^{-1}. All drug doses are expressed as that of the base.

Data analysis

Activity data and scores derived from penile grooming and wet-dog shakes were analysed by one or two way ANOVA for independent groups using Statistica software. Data for back muscle contractions first underwent square root transformation before a similar ANOVA procedure was applied. Tabulated data for back muscle contractions reflect scores prior to transformation. Following a significant main effect or interaction, post-hoc comparisons were carried out using the Neuman-Keuls test. In all cases the accepted level of significance was taken at $P<0.05$.

Results

Experiment 1: Ro60-0175

In vehicle pre-treated rats, Ro60-0175 (1–10 mg kg^{-1}) produced a dose related hypoactivity [$F(3,28)=5.5$, $P<0.01$], with a significant decrease compared to controls at 3–10 mg kg^{-1}. In contrast, following pre-treatment with SB242,084 (0.5 mg kg^{-1}) a significant hyperactivity emerged [$F(3,28)=3.1$, $P<0.05$]. Consequently 2-way ANOVA revealed a significant interaction between Ro60-0175 and SB242,084 pre-treatment [$F(3,56)=6.8$, $P<0.01$]. SB242,084 alone did not significantly affect activity, although there was a trend toward an increase ($P=0.08$) (Figure 1A).

SB242,084 (0.5 mg kg^{-1}) pre-treatment alone did not produce any overt behavioural change (Figure 1), but blocked the incidence of penile grooming induced by the lowest dose of Ro60-0175 (1 mg kg^{-1}). Thus a main effect of Ro60-0175 on penile grooming was observed [$F(3,56)=10.4$, $P<0.01$] and a significant Ro60-0175 × SB242,084 interaction [$F(3,56)=17.2$, $P<0.01$] (Figure 1B). Incidence of wet-dog shakes following treatment with Ro60-0175 alone (1–10 mg kg^{-1}) did not differ significantly at any dose from controls. However, SB242,084 pre-treatment potentiated Ro60-0175-induced wet-dog shakes [Ro60-0175 × SB242,084 interaction: $F(3,56)=6.2$, $P<0.01$]. Ro60-0175 alone did not produce a significant number of back muscle contractions [$F(3,28)=1.0$, NS) however these were increased by SB242,084 pre-treatment [$F(3,56)=5.2$, $P<0.01$] (e.g. vehicle = 0, Ro60-0175 10 mg kg^{-1} = 0, SB242,084 = 0, Ro60-0175 (10)/SB242,084 = 3 ± 1).

The effect of MDL100,907 (0.5 mg kg^{-1}) (MDL) and SB215,505 (3 mg kg^{-1} p.o.) on the hyperactivity, wet-dog shakes and back muscle contractions produced by the Ro60-0175 (10 mg kg^{-1})/SB242,084 (0.5 mg kg^{-1}) combination was examined. In this study, the Ro60-0175/SB242,084 combination resulted in a significant hyperactivity and an increased incidence of wet-dog shakes and back muscle contractions compared to vehicle controls. MDL pre-treatment alone did not affect either measure, yet completely prevented the Ro60-0175/SB242,084-induced behavioural response (Figure 2A,B). [Ro60-0175/SB242,084 × MDL interaction – LMA: $F(1,32)=18.4$, $P<0.01$; wet-dog shakes: $F(1,28)=29.6$, $P<$

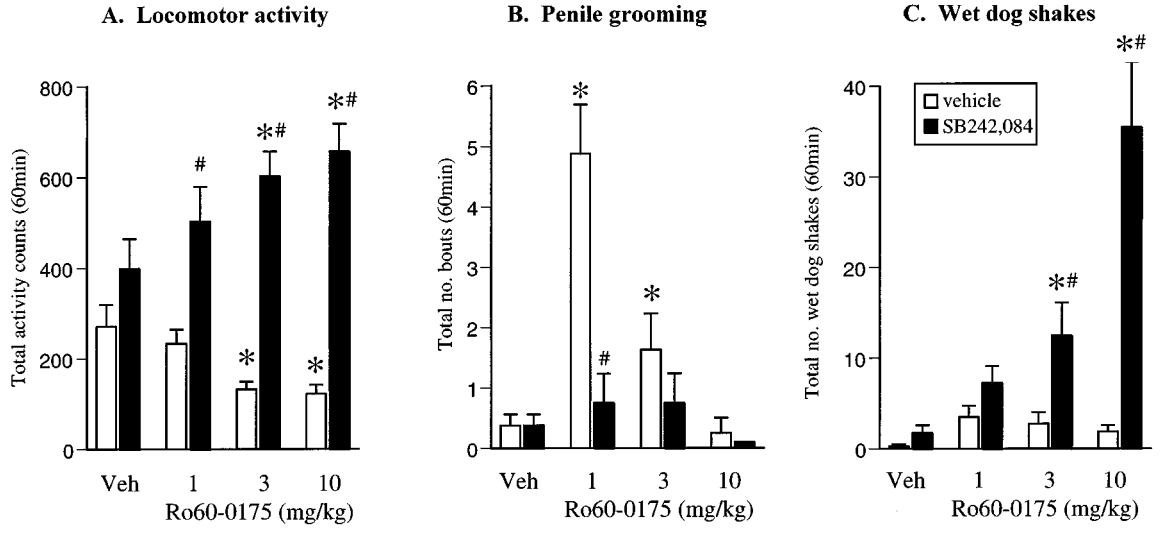

Figure 1 Effect of Ro60-0175 (1–10 mg kg^{-1}) alone and in combination with the selective 5-HT$_{2C}$ receptor antagonist SB242,084 (0.5 mg kg^{-1}) on locomotor activity (A), number of bouts of penile grooming (B) and number of wet-dog shakes (C) recorded over a 60 min test session. $n=8$ rats per group. *$P<0.05$ vs respective vehicle group. #$P<0.05$ vs respective Ro60-0175/veh group (Newman-Keuls test).

0.01; back muscle contractions: $F(1,28)=185.6$, $P<0.01$]. In contrast SB215,505 failed to significantly influence the Ro60-0175/SB242,084 response as evidenced by no significant Ro60-0175/SB242,084 × SB215,505 interaction for the main parameters measured [LMA: $F(1,20)=1.0$, NS; wet-dog shakes: $F(1,20)=1.9$, NS; back muscle contractions: $F(1,20)=0.6$, NS] (Figure 2C,D).

Experiment 2: Dexfenfluramine

Dexfenfluramine (1–10 mg kg^{-1}) pre-treatment produced a modest hyperactivity [$F(3,28)=9.1$, $P<0.01$], that was significantly different from controls at 10 mg kg^{-1}. In combination with SB242,084 (0.5 mg kg^{-1}) a marked increase in this measure was observed [DEX × SB242,084 interaction: $F(3,56)=28.9$, $P<0.01$], with SB242,084 potentiating the locomotor change produced by each dose of dexfenfluramine (Figure 3A). In this experiment, SB242,084 pre-treatment alone did not affect activity.

Dexfenfluramine induced penile grooming [$F(3,27)=4.7$, $P<0.01$] and a dose related incidence of wet-dog shakes [$F(3,27)=11.7$, $P<0.01$]. Similar to Experiment 1, SB242,084 (0.5 mg kg^{-1}) pre-treatment inhibited the incidence of penile grooming [DEX × SB242,084 interaction – penile grooming

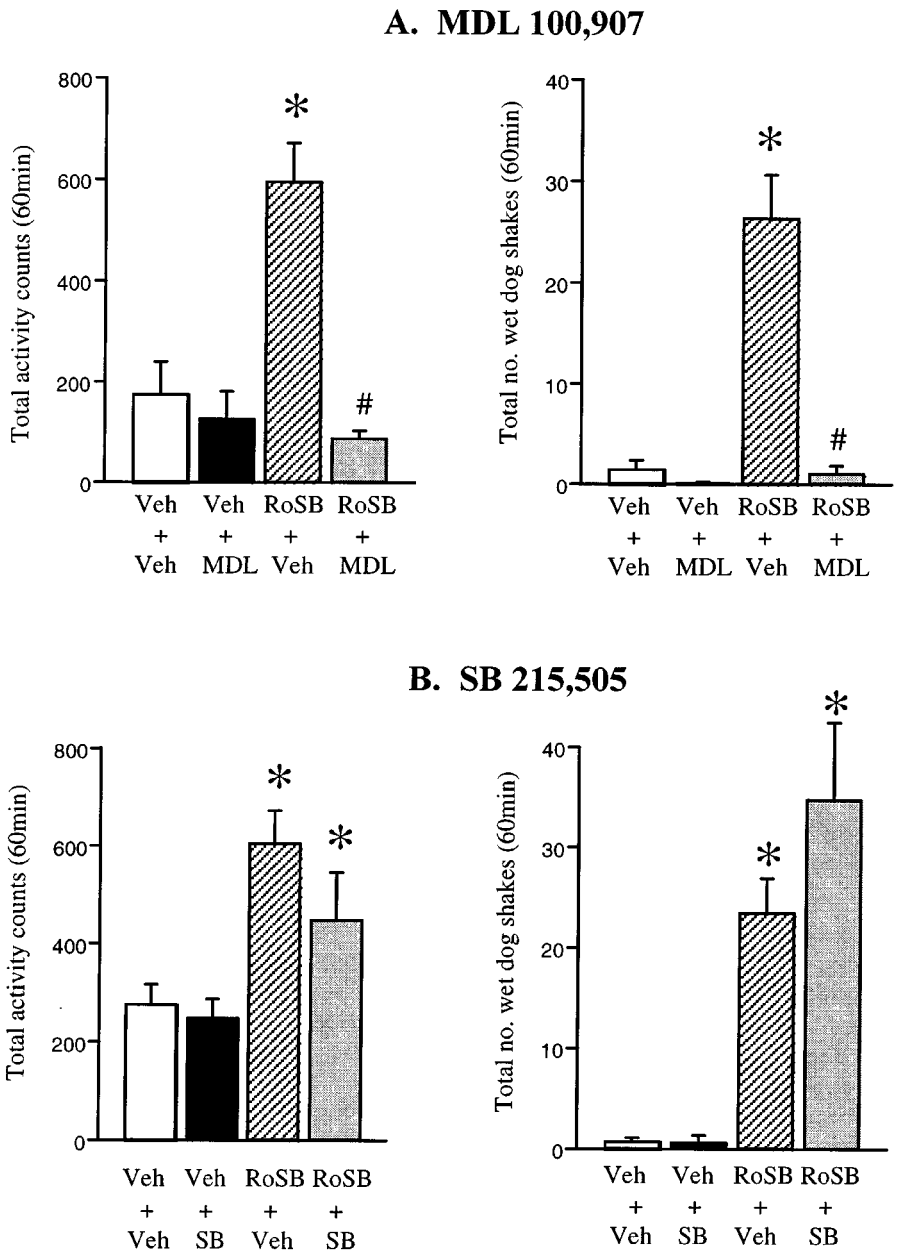

Figure 2 Effect of the 5-HT$_{2A}$ receptor antagonist MDL100,907 (0.5 mg kg^{-1}; A) and the 5-HT$_{2B}$ receptor antagonist SB215,505 (3 mg kg^{-1}; B) against the hyperactivity and wet-dog shakes produced by the combination of Ro60-0175 (10 mg kg^{-1}) and SB242,084 (0.5 mg kg^{-1}) (RoSB group). $n=6-8$ rats per group. *$P<0.05$ vs vehicle/vehicle group. #$P<0.05$ vs RoSB/veh group (Newman-Keuls test).

($F(3,53)=5.9$, $P<0.01$)]. A significant interaction term was not observed for wet-dog shakes [$F(3,53)=1.9$, NS], although *post-hoc* analyses revealed a significant increase in the dexfenfluramine (10 mg kg^{-1}) group following SB242,084 pre-treatment (Figure 3B,C). Dexfenfluramine also induced back muscle contractions at the 10 mg kg^{-1} dose [$F(3,27)=13.5$, $P<0.01$], whilst SB242,084 treatment alone did not influence this parameter. However, the combination of dexfenfluramine and SB242,084 produced a marked increase in the incidence of back muscle contractions [DEX × SB242,084 interaction: $F(3,53)=3.3$, $P<0.05$] (Table 1).

A notable finding from this study was the toxicity that emerged following the combination of dexfenfluramine 10 mg kg^{-1} and SB242,084 0.5 mg kg^{-1}. Six animals out of 16 treated with this combination (38%), died within 24 h of treatment. Animals who survived this treatment appeared normal at the time of retest 7 days later. No toxicity was evident in any of the other treatment groups.

In a final study, the effect of MDL100,907 and SB215,505 against the hyperactivity and toxicity produced by the dexfenfluramine (10 mg kg^{-1}) and SB242,084 (0.5 mg kg^{-1}) combination (Dex/SB242,084) was examined. Body temperature assessment was also included in this study (see Discussion). Again the combination of Dex/SB242,084 resulted in a significant hyperactivity (Dex/SB242,084 main effect $F(1,54)=426.1$; $P<0.01$), which was attenuated by MDL100,907 (0.5 mg kg^{-1} i.p.) but not SB215,505 (3 mg kg^{-1} p.o.) (Figure 4A). Dex/SB242,084 also produced a hyperthermic response (+3°C) (Dex/SB242,084 main effect: $F(1,54)=8.8$, $P<0.01$), which was completely reversed by MDL100,907 but not SB215,505 (Figure 4B). MDL100,907 also appeared to prevent the toxicity produced by the Dex/SB242,084 combination recorded up to 24 h post dosing (Dex/SB242,084 + vehicle: 3/10 deaths; Dex/SB242,084 + SB215,505: 4/10 deaths; Dex/SB242,084 + MDL100,907: 0/10 deaths).

Discussion

Ro60-0175 has been described in the literature as a 5-HT$_{2C}$ receptor preferring agonist (Millan *et al*., 1997). When tested *in vivo* this compound seems to satisfy this claim, producing behaviours characteristic of 5-HT$_{2C}$ receptor activation including hypoactivity, penile grooming and hypophagia (Millan *et al*., 1997; Martin *et al*., 1998; Kennett *et al*., 2000), but not behaviours mediated through other 5-HT (notably 5-HT$_{2A}$) receptors. Ro60-0175 also produces an interoceptive cue that is most likely 5-HT$_{2C}$ receptor mediated (Dekeyne *et al*., 1999). However, *in vitro* Ro60-0175 has reasonable affinity and efficacy at human 5-HT$_{2A}$ and particularly human (and rat) 5-HT$_{2B}$ receptors (Martin *et al*., 1998; Porter *et al*., 1999). The findings from Experiment 1 suggest that the apparent *in vivo* selectivity of this drug is because its 5-HT$_{2C}$ agonist properties inhibit the

Table 1 Effect of dexfenfluramine and SB242,084 on the incidence of back muscle contractions

	Dexfenfluramine (mg kg^{-1})			
	Veh	*1*	*3*	*10*
Vehicle	0	0	0	5±2*
SB242,084 (0.5 mg kg^{-1})	0	2±1	6±2*#	10±1*#

Back muscle contractions were scored as present or absent within 5-min time-bins measured over a 60-min test session, i.e. a total possible score of 12. *$P<0.05$ vs respective vehicle group. #$P<0.05$ vs respective dexfenfluramine/veh group (Neuman-Keuls test).

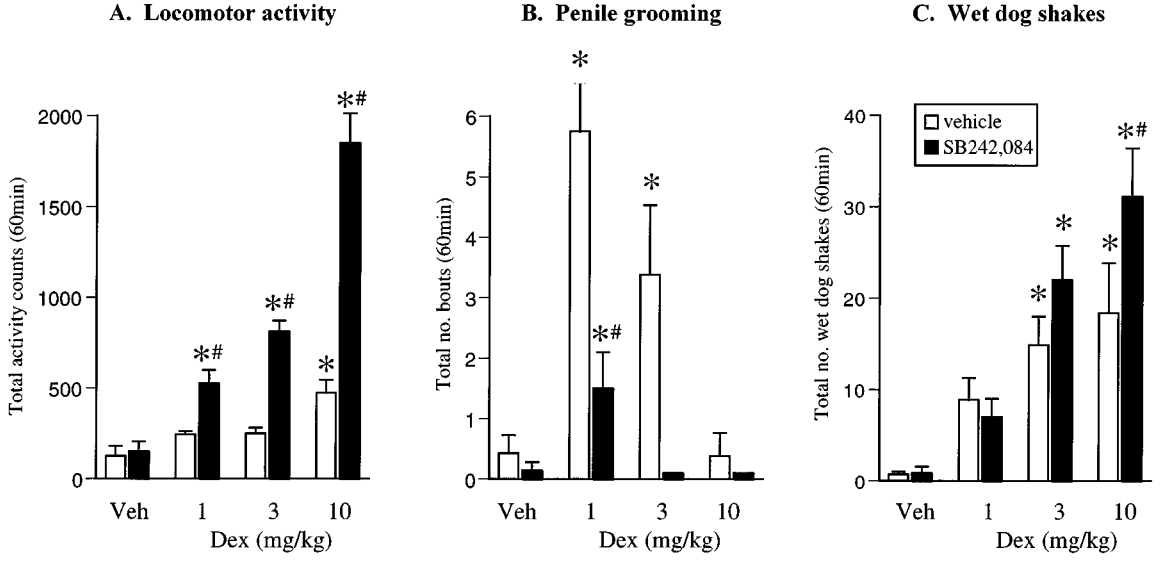

Figure 3 Effect of dexfenfluramine (1–10 mg kg^{-1}) alone and in combination with the selective 5-HT$_{2C}$ receptor antagonist SB242,084 (0.5 mg kg^{-1}) on locomotor activity (A), number of bouts of penile grooming (B) and number of wet-dog shakes (C) recorded over a 60-min test session. *$P<0.05$ vs respective vehicle group. #$P<0.05$ vs respective dexfenfluramine/veh group (Newman-Keuls test).

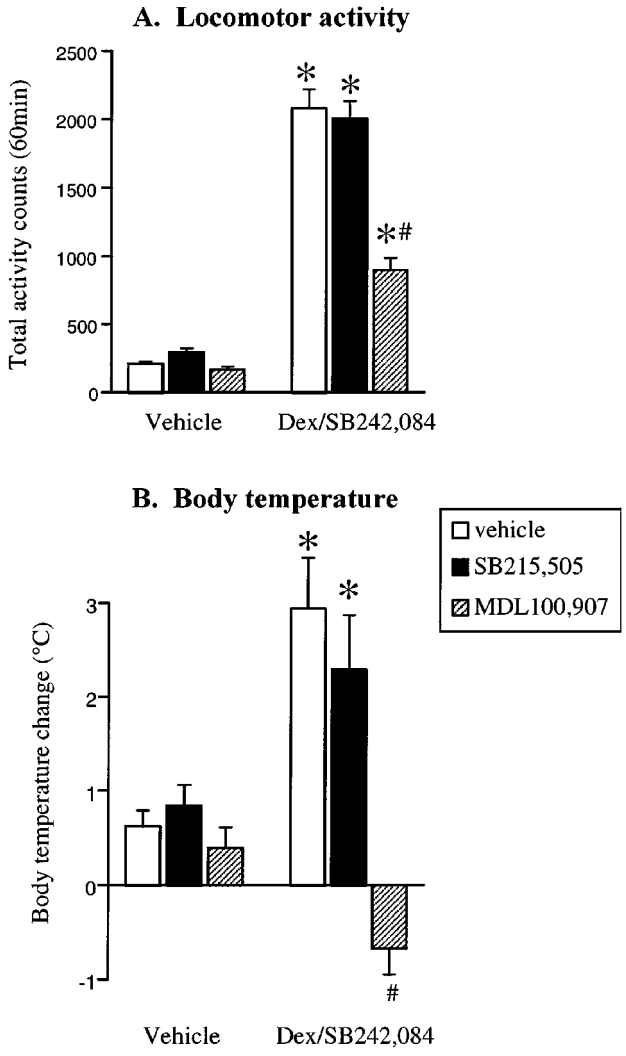

Figure 4 Effect of vehicle, SB215,505 (3 mg kg^{-1}), MDL100,907 (0.5 mg kg^{-1}) against the hyperactivity (A) and hyperthermia (B) produced the combination of dexfenfluramine (10 mg kg^{-1}) and SB242,084 (0.5 mg kg^{-1}). *$P<0.05$ vs respective vehicle group. #$P<0.05$ vs dexfenfluramine/SB242,084/veh group (Newman-Keuls test).

expression of 5-HT$_{2A}$ receptor-mediated behaviours, most notably wet-dog shakes, back muscle contractions and hyperactivity. The doses of Ro60-0175 producing these effects (3–10 mg kg^{-1}) were approximately 10 fold higher than the doses required to elicit 5-HT$_{2C}$ related behaviours (0.3–1 mg kg^{-1}; Millan *et al*., 1997; Martin *et al*., 1998; Kennett *et al*., 2000), consistent with the relative potencies of Ro60-0175 at human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (Porter *et al*., 1999).

Therefore the results from Experiment 1 are similar to the observations of Heiser & Tecott (2000), who described a paradoxical hyperactivity following another 5-HT$_{2C}$ preferring agonist mCPP in a line of 5-HT$_{2C}$ receptor k.o. mice (Tecott *et al*., 1995). Pre-treatment with the selective 5-HT$_{2B/2C}$ receptor antagonist, SB206,553 (Kennett *et al*., 1996), resulted in a similar hyperactivity manifest in wild type mice following mCPP treatment, confirming that these effects were not simply a non-specific consequence of changes brought about by the gene targeting technique (Heiser & Tecott, 2000). Furthermore, during the course of this work we became aware of similar findings reported in preliminary form by Vickers *et al*. (2000). Taken together with the present data, these findings demonstrate that 5-HT$_{2C}$ receptor activation can markedly influence behaviour, and inhibit the expression of behaviours mediated through other 5-HT receptor subtypes.

Ro60-0175 has affinity and efficacy at 5-HT$_{2B}$ receptors seemingly equivalent to that at the 5-HT$_{2C}$ receptor (Martin *et al*., 1998; Porter *et al*., 1999). However the involvement of this receptor subtype on the repertoire of behaviours produced by this drug seems minimal given the lack of effect of SB215,505 against the Ro60-0175/SB242,084 treatment combination. SB215,505 has recently been described in the literature as a 5-HT$_{2B}$ receptor antagonist with moderate selectivity over 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors (pK$_i$ h5-HT$_{2B}$ 8.3±0.1, h5-HT$_{2A}$ 6.8±0.2, h5-HT$_{2C}$ 7.7±0.1; see Table 1 from Reavill *et al*., 1999). Indeed Kennett *et al*. (1998) reported antagonism of an anticonflict effect of BW-723C86 following SB215,505 (3 mg kg^{-1} p.o.) pre-treatment, suggesting the dose and route of administration used in the present study to be pharmacologically relevant.

In the second part of this study, we examined the 5-HT reuptake inhibitor/releaser dexfenfluramine, both alone, and in combination with SB242,084. Despite producing a generalized facilitation of 5-HT function, dexfenfluramine produces some behavioural effects indicative of 5-HT$_{2C}$ activation (e.g. hypophagia and penile grooming) (Berendsen & Broekkamp, 1987; Bickerdike *et al*., 1999). Indeed, each of these effects are blocked by antagonists at the 5-HT$_{2C}$ receptor, confirming that this receptor is a principal mediator of at least some of dexfenfluramine's effects on behaviour (Hartley *et al*., 1995; Bickerdike *et al*., 1999; present study). Again, the combination of dexfenfluramine with SB242,084 resulted in a change in behavioural profile, including potentiation of both an existing hyperactivity and of 5-HT-mediated behavioural signs. The most striking observation from this experiment was the emergence of toxicity in animals treated with the dexfenfluramine (10 mg kg^{-1})/SB242,084 combination. We speculated that this toxicity may be associated with the hyperthermia which has been noted following central 5-HT$_{2A}$ receptor agonist treatment (Gudelsky *et al*., 1986), exacerbated perhaps by the intense hyperactivity seen in this treatment group. Accordingly, in a follow-up study we confirmed that the Dex/SB242,084 treatment combination resulted in a marked hyperthermic response that was blocked by MDL100,907. Toxicity was also eliminated by this pre-treatment combination. These studies identify a striking adverse interaction between dexfenfluramine and a 5-HT$_{2C}$ receptor antagonist that should be recognised for its potential clinical implication.

In some respects, the behavioural profile of dexfenfluramine and Ro60-0175 were similar, most notably in the induction of penile grooming, with a maximal incidence at the lowest dose tested. At higher doses the frequency of this behaviour was reduced. A similar biphasic effect of 5-HT$_{2C}$ agonists on penile grooming/erection has been reported by others, and the decline may relate to the induction of alternative and competing behaviours (e.g. wet-dog shakes/hypolocomotion), probably in some cases related to interactions with other 5-HT receptor subtypes (Berendsen &

Broekkamp, 1990; Millan *et al*., 1997; Martin *et al*., 1998). In contrast to the hypolocomotor effects of Ro60-0175, dexfenfluramine produced a mild increase in activity, which was greatly potentiated by SB242,084 – considerably more so than the Ro60-0175/SB242,084 combination. This hyperactivity was only partially blocked by MDL100,907 pretreatment. Notwithstanding the limited dose ranges studied, this difference may relate to additional 5-HT receptors activated by dexfenfluramine, such as 5-HT$_{1B/1D}$ which additionally influence locomotor activity in the rodent (Oberlander *et al*., 1983; Cheetham & Heal, 1993). Selective antagonists at the various 5-HT receptors would be useful tools for the pharmacological dissection of this effect.

A final consideration is that the interactions identified in these experiments do not reflect receptor mediated events, but rather a pharmacodynamic or pharmacokinetic interaction between SB242,084 and Ro60-0175 or dexfenfluramine. We feel that this is unlikely for the following reasons. Firstly, in the Ro60-0175 study, the treatment combination of Ro60-0175 with SB242,084 resulted in a completely distinct pattern of behaviour; whereas a metabolic interaction might be expected to potentiate an existing behavioural pattern. Secondly these behavioural effects were completely blocked by pre-treatment with MDL100,907, consistent with it being a 5-HT$_{2A}$ receptor mediated response (Martin *et al*., 1998; Porter *et al*., 1999). Thirdly, SB242,084 has been reported to have extremely low inhibitory activity at cytochrome P450 enzymes (IC_{50}'s >100 μM; Bromidge *et al*., 1997) making it unlikely to interact with the metabolism of either dexfenfluramine or Ro60-0175, at least at the relatively low doses used in the present study. However, the availability of plasma levels of Ro60-0175 and dexfenfluramine with and without SB242,084 pretreatment, would have been a useful addition to supplement these conclusions.

The present studies therefore provide some explanation for the apparent paradox that while Ro60-0175 (and mCPP; see Kennett & Curzon, 1988) seem to have little selectivity for the 5-HT$_{2C}$ receptor *in vitro* (Porter *et al*., 1999), *in vivo* both drugs seem to function as selective agonists at this receptor. Preferential activation of this receptor would appear to inhibit the expression of other non-5-HT$_{2C}$ receptor mediated effects. The converse may also be true for 5-HT$_{2A}$ receptor agonists, such as LSD and DOI, which again appear to have little selectivity over other members of the 5-HT$_2$ receptor subclass *in vitro* (Egan *et al*., 1998; Porter *et al*., 1999). *In vivo* both LSD and DOI induce wet-dog shakes, back muscle contractions and hyperactivity, effects likely attributable to 5-HT$_{2A}$ receptor agonism (Wettstein *et al*., 1999; Ouagazzal *et al*., 2001). However, at doses which should achieve appreciable activation of 5-HT$_{2C}$ receptors, no signs of 5-HT$_{2C}$-mediated behaviour are apparent. It is quite possible that these interactions extend to higher CNS functions such as perception and mood, and perhaps explain why drugs such as LSD are hallucinogenic while pharmacologically similar (but not identical) drugs such as lisuride are not (Egan *et al*., 1998). The present studies also indicate that the combination of dexfenfluramine and a 5-HT$_{2C}$ antagonist can elicit a severe 5-HT syndrome, including toxicity.

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