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Stimulation of 5-HT_{1B} receptors causes hypothermia in the guinea pig

Jim J. Hagan [a,*], Paula D. Slade [a], Laramie Gaster [b], Phillip Jeffrey [c], Jonathan P. Hatcher [a], D.N. Middlemiss [a]

[a] *Neuroscience Research, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AW, UK*
[b] *Medicinal Chemistry, SmithKline Beecham Pharmaceuticals, New Frontiers Science Park North, Third Avenue, Harlow, Essex CM19 5AW, UK*
[c] *Drug Metabolism and Pharmacokinetics, SmithKline Beecham, Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, UK*

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Abstract

The selective, brain penetrant, 5-$HT_{1B/D}$ (formerly 5-$HT_{1D\beta/\alpha}$) receptor agonist SKF-99101H (3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate) (30 mg/kg i.p.) causes a dose related fall in rectal temperature in guinea pigs which previous studies have shown to be blocked by the non-selective 5-$HT_{1B/D}$ receptor antagonist GR-127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1′biphenyl]-4-carboxamide oxalate). The present study shows that the hypothermic response to SKF-99101H is dose-dependently blocked by SB-224289G (1′-methyl-5-(2′-methyl-4′-[(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidone] hemioxalate) (0.3–10.0 mg/kg p.o.) (ED_{50} 3.62 mg/kg), which is the first compound to be described which is more than 60 fold selective for the 5-HT_{1B} receptor over the 5-HT_{1D} receptor. SB-216641A (*N*-[3-(2-dimethylamino) ethoxy-4-methoxy-phenyl] 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl)-4-carboxamide hydrochloride) (0.6–20.0 mg/kg i.p.), which is somewhat less selective (30 fold) for the 5-HT_{1B} receptor over the 5-HT_{1D} receptor had a similar effect (ED_{50} 4.43 mg/kg). The brain penetrant 5-HT_{1D} selective receptor antagonist, BRL-15572 (4-(3-chlorophenyl)-α-(diphenylmethyl)-1-piperazineethanol dihydrochloride) (0.3–100.0 mg/kg i.p.) was inactive. When administered alone neither BRL-15572 (0.1–10 mg/kg i.p.) nor SB-224289G (2.2–22 mg/kg p.o.) had an effect on body temperature. These data demonstrate that 5-HT_{1B} (formerly 5-$HT_{1D\beta}$) and not 5-HT_{1D} (formerly 5-$HT_{1D\alpha}$) receptors mediate the hypothermic response to SKF-99101H (30 mg/kg i.p.) in guinea pigs. The compounds described are useful pharmacological tools for distinguishing responses to 5-HT_{1B} and 5-HT_{1D} receptors. © 1997 Elsevier Science B.V.

Keywords: Hypothermia; 5-HT; (Guinea pig); 5-HT_{1D} receptor; 5-HT_{1B} receptor; SKF-99101H; SB-216641A; BRL-15572; SB-224289G

1. Introduction

Receptors of the 5-HT_{1D} class, incorporating the 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ subtypes (Hartig et al., 1993) were originally distinguished, on pharmacological grounds, from the 5-HT_{1B} receptors found in rodents (Humphrey et al., 1993). However, in the light of recent advances in the molecular biology of 5-HT receptors, this classification system is no longer appropriate. In order to align the nomenclature with the human genome, the receptor classification has recently been modified (Hartig et al., 1996). The 5-HT_{1B} receptor subclass now incorporates the human (h)5-HT_{1B} receptor (previously 5-$HT_{1D\beta}$ and found also in guinea pig) and other species homologues, including rat (r5-HT_{1B}). The 5-$HT_{1D\alpha}$ receptor reverts to the appellation 5-HT_{1D} and it too incorporates species homologues (Hartig et al., 1996).

Previous pharmacological studies in vivo have shown that stimulation of 5-$HT_{1B/D}$ (formerly 5$HT_{1D\beta/\alpha}$) receptors mediate hypothermia in the guinea pig. Hatcher et al. (1995) reported that the 5-$HT_{1B/D}$ selective, brain penetrant receptor agonist SKF-99101H (3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate)-induced hypothermia in guinea pigs which was blocked by the 5-$HT_{1B/D}$ selective receptor antagonists GR-125743 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl)phenyl]-3-methyl-4-(4-pyridinyl) benzamide) and GR-127935 (*N*-[4-methoxy-3-(4-methyl-1-piperazinyl) phenyl]-2′-methyl-4′-

* Corresponding author.

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(5-methyl-1,2,4-oxadiazol-3-yl) [1,1′biphenyl]-4-carboxamide oxalate) (Skingle et al., 1993, 1996). Skingle et al. (1994) also reported a hypothermic response in guinea pigs following administration of GR-46611 (3-[3-(2-dimethylamino-ethyl)-1H-indol-6-yl]-*N*-(4-methoxy-benzyl) acrylamide), a brain penetrant 5-HT receptor agonist which is equipotent on central 5-HT_{1D}, 5-HT_{1A} and 5-HT_{1B} receptors. This hypothermic response was also blocked by the 5-$HT_{1B/D}$ receptor antagonist GR-127935.

Until recently it has been impossible to distinguish pharmacologically between 5-HT_{1B} and 5-HT_{1D} receptors, but compounds have now been identified which show selectivity for both receptor subtypes (Price et al., 1996, 1997; Roberts et al., 1997: see Table 1). SB-216641A (*N*-[3-(2-dimethylamino) ethoxy-4-methoxy-phenyl] 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl)-4-carboxamide hydrochloride) is the first partial agonist/antagonist to demonstrate high affinity and selectivity for h5-HT_{1B} receptors over h5-HT_{1D} receptors (Price et al., 1996, 1997). SB-224289G (1′-methyl-5-(2′-methyl-4′-[(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidone] hemioxalate) is an inverse agonist at both receptors (Roberts et al., 1997) and is 60 fold selective for h5-HT_{1B} over h5-HT_{1D} receptors in radioligand binding and functional assays (see Table 1). BRL-15572 (4-(3-chlorophenyl)-α-(diphenylmethyl)-1-piperazineethanol dihydrochloride) is more than 60 fold selective for h5-HT_{1D} over h5-HT_{1B} receptors (Price et al., 1996, 1997). It is a partial agonist at both receptors in GTPγS assays in vitro (Price et al., 1996, 1997) but in native tissues it is a competitive antagonist (Schlicker et al., 1997).

These compounds provide ideal tools for the receptor subtype characterisation of 5-$HT_{1B/1D}$ responses and the aim of the present experiments was to determine whether the hypothermic response to SKF-99101H in guinea pigs (Hatcher et al., 1995) is mediated by 5-HT_{1B} or 5-HT_{1D} receptors.

2. Materials and methods

2.1. Subjects

Male Dunkin-Hartley guinea pigs (Charles River UK) weighing between 270 and 400 g were used (except where noted). Animals were housed in groups of five or six, in a temperature controlled environment (20°C ± 1°C) and maintained on a 12 h light/dark cycle (lights on 7.00–19.00) for at least five days prior to use with ad libitum access to food and water. On test days animals were either housed, in pairs, in opaque observation cages (24 cm × 45 cm × 20 cm) at least two hours prior to the start of the experiment (for intraperitoneal dosing) or starved overnight in pairs in clear, grid-bottomed starving cages (24 cm × 45 cm × 20 cm) (for perioral dosing).

Experiments were carried out in compliance with the Animals (Scientific Procedures) Act 1986 and conformed to SmithKline Beecham ethical guidelines.

2.2. Temperature monitoring

Rectal temperature was monitored using an electronic thermometer (Comark, model 9001) coupled to a rectal probe (Comark, model BS4937K). Animals were manually restrained and temperature measured by inserting the probe approximately 4 to 5 cm into the rectum for a period of 15 s or until a stable reading was obtained. Antagonist or vehicle was administered intraperitoneally (i.p.) at 15 min (−15) prior to injection of SKF-99101H or periorally (p.o.) at 45 min (−45) prior to injection of SKF-99101H. SKF-99101H or vehicle was administered i.p. at time 0. For experiments in which an antagonist was administered i.p., temperature measurements were taken 30 min (−30), 15 min (−15) and immediately prior to (0) administration of SKF-99101H. Measurements were repeated at 15, 30, 45, 60, 90 and 120 min after the injection of SKF-99101H. For experiments in which the antagonist was administered

Table 1
Receptor binding affinity of SB-216641A, SB-224289G and BRL-15572 at 5-HT receptors

Compound	5-HT Receptor subtype (pK_i)										
	5-HT_{1A}	5-HT_{1B}	5-HT_{1D}	5-HT_{1E}	5-HT_{1F}	5-HT_{2A}	5-HT_{2B}	5-HT_{2C}	5-HT_{6}	5-HT_{7}	5-HT_{1B} (G. pig str) [a]
SB-216641A [b]	6.3	9.0	7.6	< 6	< 6	7.3	5.8	6.8	< 5.5	< 5	8.8
SB-224289G	5.5 [d]	8.0 [c]	6.2 [c]	< 5.0 [d]	< 5.0 [d]	5.8 [c]	NT	6.2 [c]	NT	NT	NT
BRL-15572 [b]	7.7	6.1	7.9	5.2	6.0	6.6	7.4	6.2	5.9	6.3	5.8

Affinities of compounds at human cloned 5-HT receptors (5-HT_{1E}, 5-HT_{1B} and 5-HT_{1D} receptors were expressed in CHO cells while 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_{7} receptors were expressed in 293 HEK cells). Affinity was measured as the displacement of [^{3}H]5-HT from 5-HT_1 receptors, displacement of [^{3}H]-8-OHDPAT from 5-HT_{1A} receptors, [^{3}H]-ketanserin from 5-HT_{2A} receptors, [^{3}H]-mesulergine from 5-HT_{2C} receptors and [^{3}H]5-CT from 5-HT_{7} receptors.

NT: not tested.

[a] Inhibition of [^{3}H]5-CT binding to membranes from guinea-pig striatum. Data are means.

[b] Data taken from Price et al. (1997).

[c] Data from Roberts et al. (1997).

[d] Data taken from Gaster (1996).

p.o. temperature measurements were taken at 60 min (−60), 45 min (−45) and immediately prior to (0) administration of SKF 99101H. Further measurements were taken 45, 60 and 90 min after the injection of SKF-99101H.

2.3. Pharmacokinetics of BRL-15572

The relative CNS penetration of BRL-15572 was investigated following intravenous infusion to steady -state in the conscious guinea pig. Following chronic cannulation of the jugular vein (Griffiths et al., 1996) and a suitable recovery period (3 days), two male guinea pigs (approx. 500 g body weight) were infused with BRL-15572 (0.123 mM dihydrochlride salt) dissolved in normal saline containing 2% (v/v) dimethylsulfoxide (DMSO) at a target dose of 4.92 μmol/kg (5 mg free base/kg) over 8 h.

At the end of the infusion, the animals were killed, exsanguinated and the brains removed. Brain tissues were weighed and homogenised with an equal volume of water. To samples of blood and brain homogenate (approx. 100 μl), 0.5 ml of ammonium acetate (0.1 M, pH 5) containing an appropriate internal standard was added and mixed thoroughly. Samples were then extracted with 5 ml of toluene/isoamyl alcohol (98:2 v/v), mixed (mechanical shaking for 20 min) and then centrifuged ($1614 \times g$ for 5 min at room temperature). The organic layer was transferred, evaporated to dryness under nitrogen and reconstituted with 100 μl mobile phase (60:40 (v/v) 0.01 M ammonium acetate and acetonitrile). Samples (90 μl) were assayed for BRL-15572 (free base) concentrations using positive ion electrospray high performance liquid chromatography with tandem mass spectrometry (HPLC-MS-MS) (Quattro 4000) with a flow rate of 1.0 ml/min and a Hypersil BDS C18 column (5 μm; Shandon Scientific). The lower limit of the assay was 20 ng/ml and the assay was linear up to 500 ng/ml

2.4. Drugs

The following drugs were used and were prepared at SmithKline Beecham: BRL-15572 (4-(3-chlorophenyl)-α-(diphenylmethyl)-1-piperazineethanol dihydrochloride), SB-216641A (*N*-[3-(2-dimethylamino) ethoxy-4-methoxy-phenyl] 2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl)-(1,1′-biphenyl)-4-carboxamide hydrochloride), SB-224289G (1′-methyl-5-(2′-methyl-4′-[(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-yl]carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-f]indole-3,4′-piperidone] hemioxalate) and SKF-99101H (3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate), BRL-15572 and SB-216641A were dissolved in distilled water. SB-224289G and SKF-99101H were suspended in 1% methyl cellulose water. For each treatment the control group received the relevant vehicle. BRL-15572, SB-216641A and SKF-99101H were administered i.p. at a dose volume of 5 ml/kg body weight. SB-224289G was administered p.o. at a dose volume of 1 ml/kg body weight. The dose of SKF-99101H (30 mg/kg i.p.) was chosen on the basis of previous studies (Hatcher et al., 1995). Routes of administration were chosen on the basis of pharmacokinetic data (data not shown) and doses are quoted as mg/kg of the salt form.

2.5. Data analysis

Temperature at each time point was expressed as a function of the temperature at time 0. A time course for each experiment was plotted but there was no evidence that the antagonists altered the time course of SKF-99101H induced hypothermia. Therefore to simplify data analysis the peak temperature drop was calculated for each animal. These peak drops provide a simple measure of the maximal drug effect. Changes in temperature were assessed using a one way analysis of variance (ANOVA) with post

Table 2
Effects of SB-224289G and SB-216641A on hypothermia induced by the 5-$HT_{1B/D}$ agonist SKF-99101H (30 mg/kg i.p.) in guinea pigs

SB-224289G [a]		SB-216641A [b]	
treatment/dose (mg/kg)	body temp. drop (°C)	treatment/dose (mg/kg)	body temp. drop (°C)
Veh/Veh	−0.12 ± 0.07	Veh/Veh	−0.03 ± 0.04
Veh/SKF99101	−1.92 ± 0.23 (100) [c,d]	Veh/SKF99101	−2.78 ± 0.22 (100) [d,e]
SB 0.3 mg/kg/SKF99101	−1.77 ± 0.27 (93)	SB 0.6 mg/kg/SKF99101	−2.33 ± 0.23 (84)
SB 1.0 mg/kg/SKF99101	−1.63 ± 0.18 (85)	SB 2.0 mg/kg/SKF99101	−1.47 ± 0.47 (53) [f]
SB 3.0 mg/kg/SKF99101	−1.00 ± 0.38 (52) [f]	SB 6.0 mg/kg/SKF99101	−1.15 ± 0.38 (41) [f]
SB 10.0/SKF99101	−0.67 ± 0.19 (35) [g]	SB 20.0 mg/kg/SKF99101	−1.17 ± 0.36 (42) [f]
ED_{50} (95% confidence limits)	3.62 (1.62–19.88)	ED_{50} (95% confidence limits)	4.43 (1.62–18.23)

Figures in parentheses are % of vehicle/SKF-99101H control values. $n = 6$ per group.
[a] Antagonist dosed p.o. 45 min prior to SKF-99101H.
[b] Antagonist dosed i.p. 15 min prior to SKF-99101H.
[c] $P < 0.01$ vs. veh/veh controls.
[d] Data are the means of the maximum temperature drops recorded after treatment.
[e] $P < 0.05$ vs. veh/veh controls.
[f] $P < 0.05$ vs. veh/SKF-99101H controls
[g] $P < 0.01$ vs. veh/SKF-99101H controls.

Table 3
Lack of effects of SB-224289G and BRL-15572 alone on body temperature in guinea pigs

SB-224289G [a]		BRL-15572 [b]	
treatment/dose (mg/kg)	body temp. drop (°C)	treatment/dose (mg/kg)	body temp. drop (°C)
Veh	−0.07 ± 0.08 [c]	Veh	0.06 ± 0.05 [c]
SKF99101 (30 mg/kg) [d]	−2.00 ± 0.44 [e]	SKF99101	NT
SB-224289G 2.2 mg/kg	−0.23 ± 0.09	BRL-15572 0.1 mg/kg	0.11 ± 0.09
SB-224289G 4.6 mg/kg	−0.05 ± 0.11	BRL-15572 0.3 mg/kg	0.00 ± 0.06
SB-224289G 10.0 mg/kg	−0.03 ± 0.07	BRL-15572 1.0 mg/kg	0.00 ± 0.06
SB-224289G 22.0 mg/kg	−0.03 ± 0.11	BRL-15572 3.0 mg/kg	0.00 ± 0.06
		BRL-15572 10 mg/kg	−0.1 ± 0.04

NT: Not tested; $n = 6$ per group.
[a] Antagonist dosed p.o. 45 min prior to testing.
[b] Antagonist dosed i.p. 15 min prior to testing.
[c] Data are the means of the maximum temperature drops recorded after treatment.
[d] In this study SKF-99101H was used as a positive control.
[e] $P < 0.01$ vs. veh/veh controls.

hoc analysis carried out using Dunnett's *t*-tests. ED_{50} values and 95% confidence limits, where appropriate, were calculated using a linear fit procedure in RS1™ (Draper and Smith, 1966).

3. Results

Mean baseline temperatures were typically between 37.8 and 38.6°C. SKF-99101H (30 mg/kg i.p.) caused a drop in guinea pig body temperature in the range between 1.5 and 3°C which peaked at 90 min and lasted for longer than 2 h. This confirmed previous data (Hatcher et al., 1995).

The selective 5-HT_{1B} receptor antagonist SB-216641A (0.6–20 mg/kg) caused a dose related blockade of the hypothermia induced by SKF-99101H ($F(5,30) = 9.44$ $P < 0.001$) which reached significance at 2.0, 6.0 and 20 mg/kg ($P < 0.05$) (Table 2). SB-224289G (0.3–10.0 mg/kg) also caused a dose related blockade of the hypothermic effect of SKF-99101H ($F(5,30) = 8.71$: $P < 0.001$) which reached significance at 3.0 and 10.0 mg/kg ($P < 0.01$) (Table 2). SB-224289G (2.2–22 mg/kg) did not significantly affect body temperature at any dose tested when given alone (Table 3). A statistically significant effect in this experiment ($F(5,30) = 15.5$: $P < 0.001$) was due to the hypothermia induced by SKF-99101H ($P < 0.01$) which was used as a positive control. The ED50's and 95% confidence limits for SB-216641A and SB-224289G were calculated and are shown in Table 2.

BRL-15572 was found to readily penetrate the guinea-pig brain, the terminal blood:brain ratio was estimated to be approx. 7:1 following an 8 h intravenous infusion. BRL-15572 (0.1–10 mg/kg) did not significantly affect body temperature when administered alone (Table 3). BRL-15572 (1.0–10 mg/kg) also failed to antagonise the hypothermic effect of SKF-99101H ($F(4,27) = 15.1$: $P < 0.001$: SKF-99101H > vehicle/vehicle controls $P < 0.01$). Higher doses of BRL-15572 (10–100 mg/kg) were also inactive ($F(3,20) = 24.19$; $P < 0.001$) SKF-99101H > vehicle/vehicle controls $P < 0.01$) (Table 4).

4. Discussion

The brain penetrant 5-$HT_{1B/D}$ receptor agonist SKF-99101H caused a significant hypothermic response in guinea pigs, confirming previous findings (Hatcher et al., 1995). The average temperature drop varied between ex-

Table 4
Lack of effect of BRL-15572 on hypothermia induced by the 5-$HT_{1B/D}$ agonist SKF-99101H (30 mg/kg i.p.) in guinea pigs

Treatment/dose (mg/kg)	Body temp. drop (°C)	Treatment/dose (mg/kg)	Body temp. drop (°C)
Veh/Veh	0.00 ± 0.03 [a]	Veh/Veh	−0.03 ± 0.03
Veh/SKF99101	−1.65 ± 0.17 (100) [b]	Veh/SKF99101	−2.18 ± 0.15 (100) [b]
BRL 1.0 mg/kg/SKF99101 [c]	−1.73 ± 0.12 (105)	BRL 10 mg/kg/SKF99101	−2.33 ± 0.29 (107)
BRL 3.0 mg/kg/SKF99101	−1.82 ± 0.17 (111)	BRL 100 mg/kg/SKF99101	−2.21 ± 0.29 (101)
BRL 10 mg/kg/SKF99101	−1.64 ± 0.30 (99)		

Figures in parentheses are % of vehicle/SKF-99101H control values. $n = 6$ per group.
[a] Data are the means of the maximum temperature drops recorded after treatment.
[b] $P < 0.05$ vs. veh/veh controls.
[c] Antagonist dosed i.p. 15 min prior to SKF-99101H.

periments (range approximately 1.5–3.0°C) but variation within experiments was small. SKF-99101H is selective for the 5-$HT_{1B/D}$ receptor in radioligand binding studies (Hatcher et al., 1995) and the evidence suggests that hypothermia is mediated centrally (Komaromi, 1976; Humphrey et al., 1990; Skingle et al., 1994; Hatcher et al., 1995) through post-synaptic 5-$HT_{1B/D}$ receptors (Hatcher et al., 1995). Previous studies have shown that the response to SKF-99101H does not involve 5-$HT_{2A/2C}$, 5-HT_3, α_1 or α_2 adrenoceptors or muscarinic receptors but is blocked by the 5-$HT_{1B/D}$ receptor antagonists GR-127935 and GR-125743, suggesting that either the 5-HT_{1B} or the 5-HT_{1D} receptor mediates the response (Hatcher et al., 1995).

The 5-HT_{1B} antagonist SB-224289G, which is 60 fold selective over 5-HT_{1D} receptors (Roberts et al., 1997), dose dependently inhibited the hypothermic effect of SKF-99101H. This suggests that it is 5-HT_{1B} receptors and not 5-HT_{1D} receptors which mediate the effect of the agonist. This conclusion is supported by the data obtained with the less selective SB-216641A, which also blocked the effects of SKF-99101H. Unlike SB-224289G the effects of SB-216641A appeared to plateau at the highest dose. Further work would be required to determine if this is a reliable effect but it might be caused by pharmacokinetic differences between the compounds or differences in their receptor selectivity profile (see Table 1).

In addition, BRL-15572, which is 60 fold selective for the 5-HT_{1D} over the 5-HT_{1B} receptor was inactive, even at very high doses and despite evidence that it readily penetrates the brain. Although BRL-15572 has affinity for the human 5-HT_{1A} receptor our previous studies (Hatcher et al., 1995) have excluded a key role for 5-HT_{1A} receptor stimulation in mediating the hypothermic response to SKF-99101H. Neither SB-224289G nor BRL-15572 caused hypothermia when given alone. Thus, use of these novel and selective compounds has allowed the functions of the 5-HT_{1B} receptors and 5-HT_{1D} receptors to be dissociated in vivo.

In functional studies with recombinant systems in vitro both SB-216641A and BRL-15572 display partial agonist activity at the 5-HT_{1B} and 5-HT_{1D} receptors in [^{35}S]GTPγS binding and cAMP accumulation assays (Price et al., 1996, 1997). GR-127935 has also been reported to display intrinsic activity in high receptor expression systems (Watson et al., 1995, 1996; Zgombick et al., 1996) but no agonist activity has been reported with this compound in vivo (Skingle et al., 1996; Hatcher et al., 1995). Similarly, studies in native tissues with SB-216641A and BRL-15572 fail to demonstrate intrinsic activity (Schlicker et al., 1997). Thus, unless very high levels of receptor reserve exist in native tissues, both SB-216641A and BRL-15572 behave as antagonists. SB-224289G is an inverse agonist at both receptors in recombinant systems (Roberts et al., 1997). However, the relevance of inverse agonism at G-protein coupled receptors has been a subject for debate in the literature (Baxter and Tilford, 1995). Both SB-216641A and SB-224289G dose-dependently block the effects of SKF-99101H in vivo, despite their different pharmacological profiles in functional tests in vitro. Furthermore, SB-224289G had no effect on body temperature when given alone. Therefore all the data are consistent with these compounds being antagonists in vivo.

In conclusion, the data suggest that the hypothermic effects of SKF-99101H in guinea pigs is mediated through 5-HT_{1B} receptors. SKF-99101H, SB-216641A, SB-224289G and BRL-15572 will be important tools for the further exploration of the biological roles of 5-HT_{1B} and 5-HT_{1D} receptors.

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