ELSEVIER

European Journal of Pharmacology 370 (1999) 85–92

ejp

GR46611 potentiates 5-HT$_{1A}$ receptor-mediated locomotor activity in the guinea pig

Michael F. O'Neill *, Graham J. Sanger

Lilly Research Centre, Eli Lilly and Company, Erl Wood Manor, Sunninghill Rd., Windlesham, Surrey, GU20 6PH, UK

Received 27 October 1998; received in revised form 4 February 1999; accepted 9 February 1999

Abstract

5-HT$_{1B/D}$ receptor agonists such as GR46611 (3-[3-(2-Dimethylaminoethyl)-*H*-indol-5-yl]-*N*-(4-methoxybenzyl)acrylamide) are known to lower body temperature in guinea pigs. Although stimulation of their functional analogs in rats, the 5-HT$_{1B}$ receptor induces hyperlocomotion, this effect has yet to be demonstrated with 5-HT$_{1B/D}$ receptor agonists in the guinea pig. Previous studies have shown that 5-HT$_{1A}$ agonists increase locomotor activity in guinea pigs. The current study set out to examine the effects of 5-HT$_{1B/D}$ receptor stimulation on locomotor activity in the guinea pig and to examine the interaction between 5-HT$_{1A}$ and 5-HT$_{1B/D}$ receptor stimulation on locomotor activity in that species. The full agonist at 5-HT$_{1A}$ receptors, 8-OH-DPAT (*R*(+)-8-Hydroxy-dipropylaminotetralin HBr) dose-dependently increased locomotor activity in guinea pigs (0.3–1.25 mg kg^{-1} s.c.), as to a lesser extent, did the partial agonist, buspirone (8-[4-[4-(2-Pyramidinyl)-1-piperazinyl]butyl]-8-azaspiro[4.5]decane-7,9-dione HCl) (5.0–20.0 mg kg^{-1} s.c.). The 5-HT$_{1B/D}$ receptor agonist GR46611 had no effect on locomotor activity in guinea pigs at doses up to 40 mg kg^{-1} s.c. 8-OH-DPAT-induced behavioural activation was reversed by the selective 5-HT$_{1A}$ receptor antagonist WAY100635 (*N*-[-2-[4-(-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyrinidyl) cyclo hexanocarboxamide trihydro-chloride), with a minimum effective dose of 0.006 mg kg^{-1}, but not by the 5-HT$_{1B/D}$ receptor antagonist GR127935 (2′-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)phenyl]-amide) (0.25–1.0 mg kg^{-1}). GR46611, at doses that were without effect given alone (0.5–2.5 mg kg^{-1}), significantly enhanced the locomotor response to subthreshold doses of 8-OH-DPAT (0.5 mg kg^{-1}) and buspirone (10 mg kg^{-1}). The effect of GR46611 on 8-OH-DPAT-induced hyperactivity was reversed by pretreatment with GR127935 and with WAY 100635 indicating that activation of both receptors was required for the expression of locomotor hyperactivity. These findings suggest that activation of 5-HT$_{1B/D}$ receptors alone may not stimulate locomotor activity but it does potentiate the locomotion induced by 5-HT$_{1A}$ receptor stimulation in guinea pigs.

Keywords: 5-HT$_{1B/D}$ receptor; Locomotor activity; (Guinea pig); GR46611; 8-OH-DPAT (*R*(+)-8-Hydroxy-dipropylaminotetralin HBr)

1. Introduction

The classification of the 5-HT$_{1B}$ receptor has undergone some revision and what was originally identified as the 5-HT$_{1B}$ receptor has now been re-designated the r5-HT$_{1B}$ receptor to underline its status as a uniquely rodent (rat and mouse) receptor while its close structural analog, formerly described as the 5-HT$_{1D\beta}$ in other species (guinea pig, monkey and human) is now the 5-HT$_{1B}$ receptor. The 5-HT$_{1D\alpha}$ receptor is now simply the 5-HT$_{1D}$ receptor (for a review see Hartig et al., 1996). In addition to their structural similarities, these receptors also perform similar functional roles, acting as terminal and cell body autoreceptors regulating release of 5-HT (Starkey and Skingle, 1994; Davidson and Stamford, 1995).

Activation of central rat type 5-HT$_{1B}$ receptors with agonist such as RU24969 (5-methoxy-3-(1,2,5,6-tetrahydro-4-pyridinyl)-1*H*-indole) and anpirtoline, increases locomotor activity in mice (Cheetham and Heal, 1993; O'Neill et al., 1996, 1997c) and in rats (O'Neill and Parameswaran, 1997). Furthermore, this increase in locomotion is reversed by pretreatment with the 5-HT$_{1B/D}$/receptor antagonist GR127935 (O'Neill et al., 1996; O'Neill and Parameswaran, 1997). GR127935 (2′-methyl-4-(5-methyl-[1,2,4]oxadiazol-3-yl)-biphenyl-4-carboxylic acid [4-methoxy-3-(4-methyl-piperazin-1-yl)phenyl]-amide) is

* Corresponding author. Tel.: +44-1276-853139; Fax: +44-01276-853525; E-mail: oneill_michael_f@lilly.com

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00090-4

an antagonist selective for the 5-$HT_{1B/D}$ family of receptors over other 5-HT receptor subtypes (Starkey and Skingle, 1994) further implicating these receptors in the mediation of the locomotor response to the agonists. Consequences of activation of central 5-HT_{1D} or human-type 5-HT_{1B} receptors is less clearly understood. The species differences outlined above mean that the mouse or rat are unsuitable models for investigating the functional role of these receptors. As yet, the behavioural effects of 5-$HT_{1B/D}$ receptor selective agents have yet to be fully characterised in a species endowed with these receptors such as the guinea pig.

GR46611 (3-[3-(2-Dimethylaminoethyl)-*H*-indol-5-yl]-*N*-(4-methoxybenzyl)acrylamide) is an agonist with equal

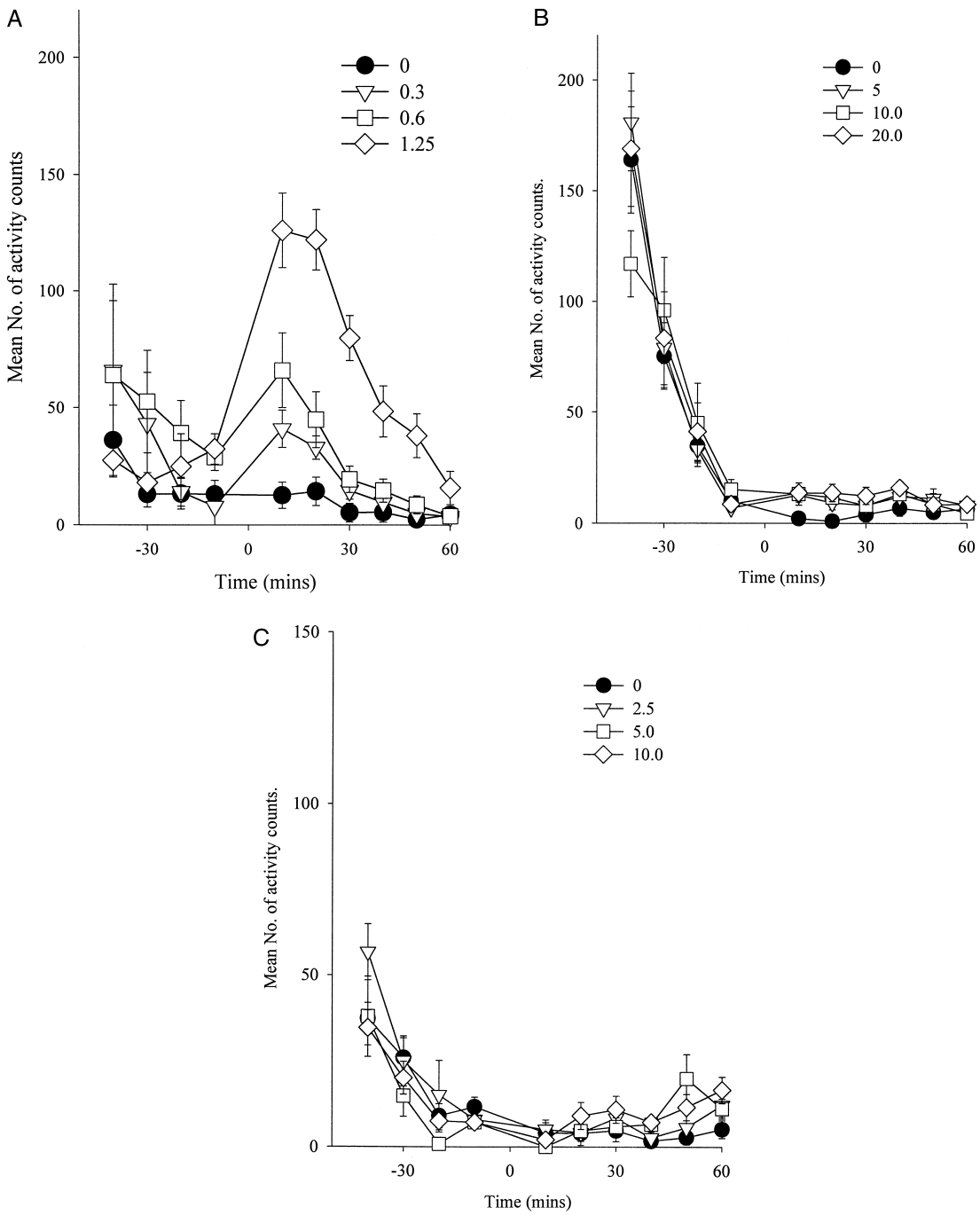

Fig. 1. (a) Effect of 8-OH-DPAT on locomotor activity in guinea pigs. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$). (b) Effect of buspirone on locomotor activity in guinea pigs. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$). (c) Effect of GR46611 on locomotor activity in guinea pigs. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$).

Table 1
Effect of 5-HT1 receptor agonists on locomotor activity in the Guinea pig

Compound (mg kg^{-1})	Locomotor Activity	sig
8-OH-DPAT		
0	44 ± 12	
0.3	106 ± 21	
0.6	157 ± 38	
1.25	429 ± 57	c
Buspirone		
Vehicle	25 ± 14	
5	61 ± 9	a
10	58 ± 9	a
20	72 ± 8	b
GR46611	Habituated	
0	22 ± 12	
2.5	38 ± 8	
5	48 ± 14	
10	57 ± 8	
	Unhabituated	
0	60 ± 27	
5	43 ± 11	
10	31 ± 12	
20	68 ± 17	
40	102 ± 20	

Data are means of total activity over 60 min following injection ± S.E.M. for each group ($n \geq 5$).
Significance vs. vehicle (0 mg kg^{-1}) calculated by LSM test following significant ANOVA.
a: $P < 0.05$.
b: $P < 0.01$.
c: $P < 0.001$.

activity at human-type 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors and also activity at 5-HT$_{1A}$ receptors (Skingle et al., 1995). This compound reduces body temperature in guinea pigs when given systemically (Skingle et al., 1994). Another agonist has also been described which also induces hypothermia in guinea pigs, SKF99101H, (3-(2-dimethylaminoethyl)-4-chloro-5-propoxyindole hemifumarate) further implicating 5-HT$_{1B/D}$ receptors in the mediation of this effect (Hatcher et al., 1995). This is further supported by the fact that the hypothermia induced by both compounds is reversed by GR127935 (Skingle et al., 1995; Hatcher et al., 1995).

Evidence that the activation of these receptors in the guinea pig has any functional equivalent to the hyperactivity induced by the 5-HT$_{1B}$ receptor agonists in rats and mice has yet to be determined although some preliminary evidence suggests that this may be the case. The striatum is one of the brain areas with the densest population of 5-HT$_{1B/D}$ receptors (Maroteaux et al., 1992; Bruinvels et al., 1993). Unilateral intrastriatal administration of the non-selective 5-CT receptor agonist and sumatriptan, a 5-HT$_{1D}$ receptor agonist, induced contralateral rotation in guinea pigs (Higgins et al., 1991).

These studies suggest that activation of central 5-HT$_{1B/D}$ receptors may be involved in the mediation of locomotor activation in the guinea pig. To test this hypothesis, we compared the effects of the 5-HT$_{1D}$ receptor agonist GR46611 to the locomotor activation induced by 8-OH-DPAT (*R*(+)-8-Hydroxy-dipropylaminotetralin), the 5-HT$_{1A}$ receptor selective agonist, previously shown to increase locomotor activity in guinea pigs (Evenden, 1994).

2. Materials and methods

2.1. Animals

Female Dunkin Hartley guinea pigs (250–350 g, Harlan, UK) were housed in groups of five under standard conditions with normal light cycle (light 0700 to 1400 h). Animals were allowed food and water ad libitum. Experiments were performed between 1000 and 1700 h. Each guinea pig was used once only. Animals were housed and all experiments were conducted in compliance with and under the guidance of UK Home Office Animals (Scientific procedures) Act (1986).

2.2. Drugs

8-OH-DPAT (Research Biochemicals) and buspirone (Sigma) were dissolved in distilled water. GR127935, GR46611, RU24969 and WAY100635 (*N*-[-2-[4-(methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyrinidyl) cyclo hex-

Table 2
Effect of 5-HT1 receptor antagonists on locomotor activity induced by 8-OH-DPAT (1 mg kg^{-1}) in the guinea pig

Treatment	Locomotor activity	sig
GR127935		
Vehicle/Vehicle	105 ± 37	
Vehicle/8-OH-DPAT (1.0 mg kg^{-1})	374 ± 77	b
GR 0.25/8-OH-DPAT	305 ± 25	b
GR 0.5/8-OH-DPAT	315 ± 61	b
GR 1.0/8-OH-DPAT	266 ± 57	a
WAY100635		
8-OH-DPAT (1 mg kg^{-1})		
Vehicle/Vehicle	57 ± 9	
Vehicle/8-OH-DPAT	382 ± 52	c
WAY 0.001/8-OH-DPAT	313 ± 23	b
WAY 0.003/8-OH-DPAT	280 ± 52	a
WAY 0.006/8-OH-DPAT	236 ± 37	a,f

Data are means of total activity over 60 min following injection ± S.E.M. for each group ($n \geq 5$).
Significance calculated by LSM test following significant ANOVA.
a: $P < 0.05$.
b: $P < 0.01$.
c: $P < 0.001$ vs. vehicle/vehicle.
f: $P < 0.001$ vs. vehicle/8-OH-DPAT.

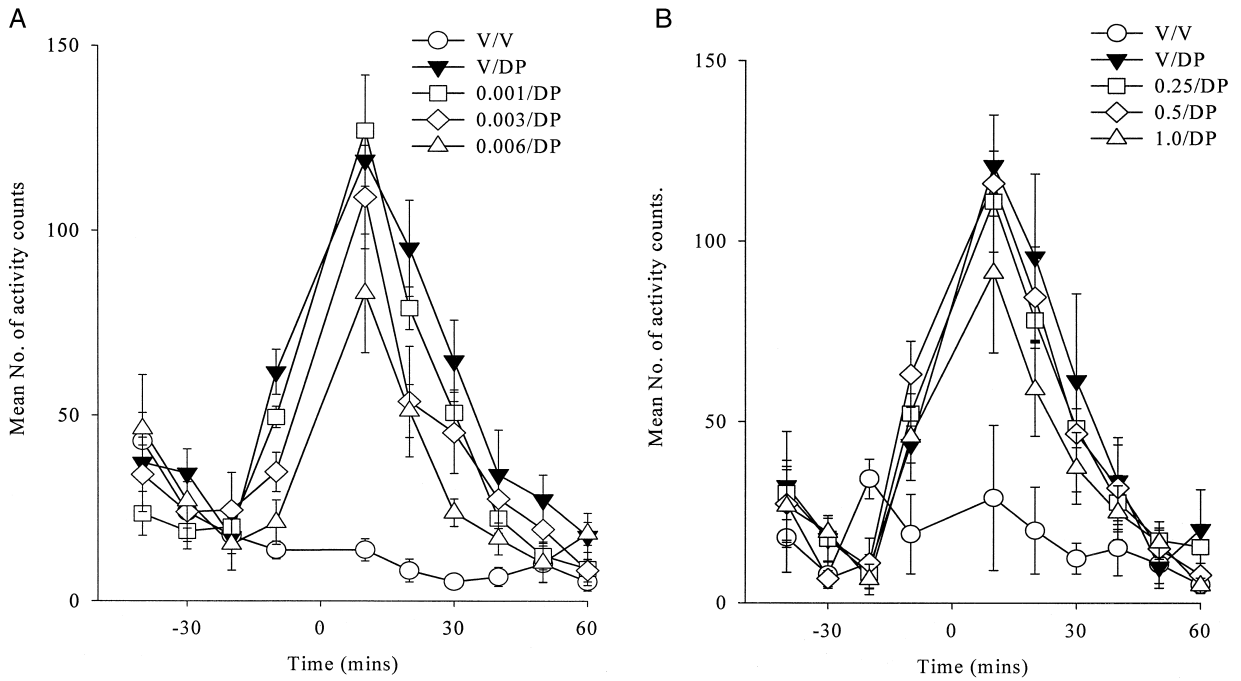

Fig. 2. (a) Effect of WAY100635 on hyperactivity induced by 8-OH-DPAT (1.0 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$). (b) Effect of GR127935 on hyperactivity induced by 8-OH-DPAT (1.0 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$).

anocarboxamide trihydro-chloride), were all synthesized at Lilly Research Centre. GR127935 and GR46611 were dissolved in 20 μl of concentrated lactic acid and subsequently dissolved in distilled water. pH of solutions was readjusted to pH 4–5 using 1 M NaOH. The remaining compounds were dissolved in 25% β-cyclodextrin in water. All drugs were administered subcutaneously (s.c.) in the scruff of the neck in a volume of 1 ml/kg.

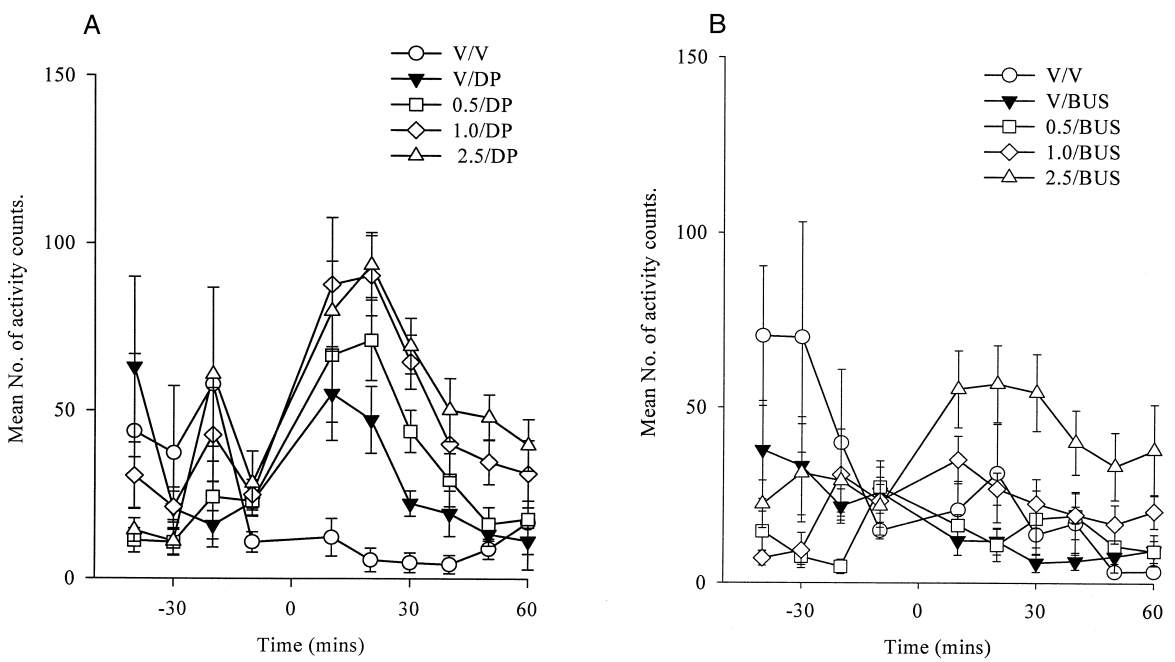

Fig. 3. (a) Effect of GR46611 on hyperactivity induced by 8-OH-DPAT (0.5 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$). (b) Effect of GR46611 on hyperactivity induced by buspirone (10 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$).

Table 3
Effect of GR46611 pretreatment on locomotor response to 8-OH-DPAT and buspirone in the guinea pig

Treatment	Locomotor activity	sig
8-OH-DPAT (0.5 mg kg^{-1})		
Vehicle/Vehicle	84 ± 29	
Vehicle/8-OH-DPAT	202 + 42	
GR46611 0.5/8-OH-DPAT	274 ± 27	a
GR46611 1.0/8-OH-DPAT	425 ± 67	c,f
GR 46611 2.5/8-OH-DPAT	464 ± 66	c,f
Buspirone (10 mg kg^{-1})		
Vehicle/Vehicle	119 ± 27	
Vehicle/Buspirone	69 ± 14	
GR46611 0.5/Buspirone	126 ± 25	
GR46611 1.0/Buspirone	185 ± 39	
GR46611 2.5/Buspirone	389 ± 68	c,f

Data are means of total activity over 60 min following injection ± S.E.M. for each group ($n \geq 5$).
Significance calculated by LSM test following significant ANOVA.
a: $P < 0.05$.
c: $P < 0.001$ vs. vehicle/vehicle.
f: $P < 0.001$ vs. vehicle/8-OH-DPAT or vehicle/Buspirone.

2.3. Apparatus

Activity was measured in opaque Perspex cylinders 40 cm in diameter with three equally spaced photocells and a central light source. An activity count was measured when the animal moved between the light source and the detector photocell. The locomotor apparatus was connected to a BBC microcomputer which simultaneously recorded activity.

2.4. Locomotor activity

Guinea pigs were individually placed into the apparatus for a 40 min habituation period. They were then removed from the apparatus and injected subcutaneously (s.c.) with the appropriate dose of test compound or vehicle ($n = 6$ per group). The animals were then immediately returned to the test apparatus and their activity monitored for a further 60 min.

2.5. Effect of compounds on agonist-induced hyperactivity

The guinea pigs were injected s.c. with pretreatment (GR127935, WAY100635) or vehicle control and were placed immediately in the photocell cages where activity was measured for 40 min. When 40 min elapsed, they were then injected with the appropriate agonist or vehicle and then returned to the activity cages and their activity was measured for a further 60 min.

In the experiments where the effect of GR127935 of WAY100635 pretreatment on GR46611-induced potentiation of 8-OH-DPAT-induced activity was tested, the antagonist was given immediately before the animals were placed in the test apparatus. Then, 10 min later, the animals received a dose of GR46611 or vehicle. After a further 20 min, the animals received the dose of 8-OH-DPAT. Activity was again measured for 60 min after 8-OH-DPAT administration.

2.6. Measurements and data analysis

The activity counts were accumulated into 10 min bins. Data from these bins were analysed by one-way analysis of variance ANOVA (SAS) for total activity over the test period. When the ANOVA was significant, the means were compared by a least square means test (LSM).

3. Results

3.1. Effects of 5-HT$_{1A}$ and 5-HT$_{1B/D}$ receptor agonists on locomotor activity in the guinea pig

8-OH-DPAT dose-dependently increased locomotor activity [$F(3,26) = 19.46$, $P < 0.0001$] in guinea pigs (Fig. 1a; Table 1). Post hoc LSM tests indicated that the minimum effective dose (min ED) was 1.25 mg kg^{-1}. Post hoc LSM tests for significant ANOVAs for each individual time point showed that the highest dose tested (1.25 mg kg^{-1}) induced levels of activity significantly increased compared to controls up to 50 min post-injection. Buspirone (5.0–20 mg kg^{-1}) induced a small but significant increase in the total number of activity counts [$F(3,26) = 3.99$, $P < 0.05$]. The effect was not of the same magnitude as that produced by 8-OH-DPAT (Table 1) and did not increase locomotor activity at any individual time point in the 60 min test period (Fig. 1b). GR46611 (2.5–10 mg kg^{-1}) had no effect on locomotor activity in the guinea pig

Table 4
Effect of GR127935 and WAY100635 on enhancement of locomotor effect of 8-OH-DPAT (0.5 mg kg^{-1}) by GR46611 (5 mg kg^{-1}) in the guinea pig

Treatment	Locomotor activity	sig
GR127935		
Vehicle/Vehicle/Vehicle	99 ± 40	
Vehicle/Vehicle/8-OH-DPAT	295 ± 100	
Vehicle/GR4/8-OH-DPAT	608 ± 87	c
GR127935/GR46611/8-OH-DPAT	230 ± 45	d
WAY100635		
Vehicle/Vehicle/Vehicle	103 ± 26	
Vehicle/Vehicle/8-OH-DPAT	178 ± 53	
Vehicle/GR46611/8-OH-DPAT	512 ± 54	c
WAY/GR46611/8-OH-DPAT	176 ± 40	f

Data are means of total activity over 60 min following injection ± S.E.M. for each group ($n \geq 5$).
Significance calculated by LSM test following significant ANOVA.
c: $P < 0.001$ vs. vehicle/vehicle/vehicle.
e: $P < 0.01$ vs. vehicle/vehicle/8-OH-DPAT.

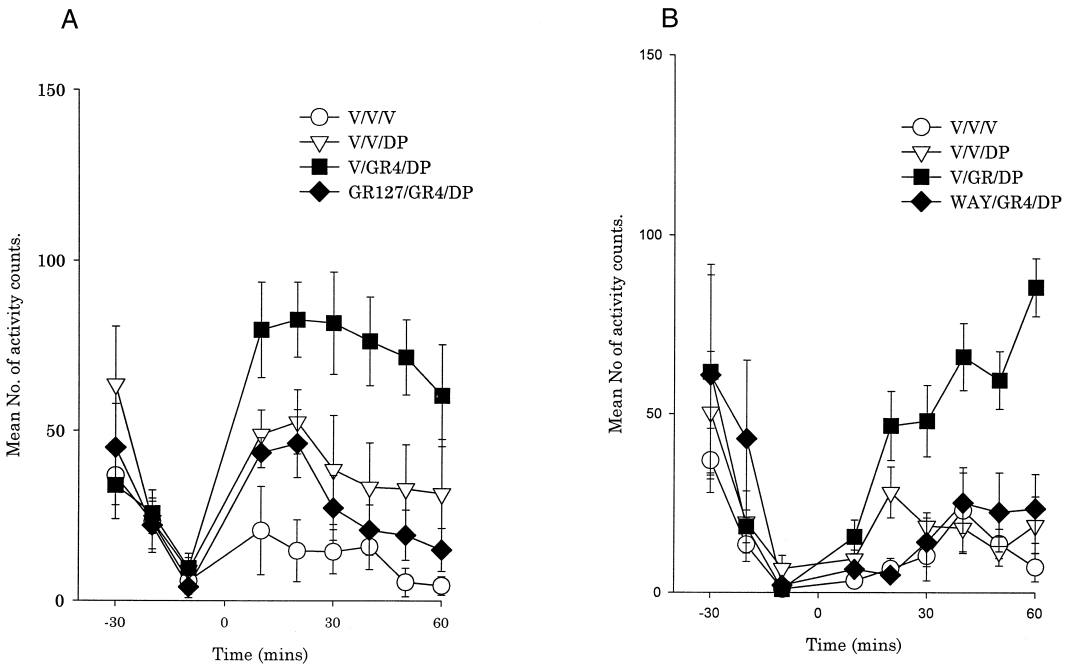

Fig. 4. (a) Effect of GR127935 on hyperactivity induced by a combination of GR46611 (5.0 mg kg^{-1}) and 8-OH-DPAT (0.5 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$). (b) Effect of WAY100635 on hyperactivity induced by a combination of GR46611 (5.0 mg kg^{-1}) and 8-OH-DPAT (0.5 mg kg^{-1}) in the guinea pig. *X*-axis shows time post injection, *Y*-axis shows mean no. of activity counts for each 10 min time bin. Data are means ± S.E.M. for each group ($n \geq 5$).

at doses up to 10 mg kg^{-1} (Fig. 1c; Table 1). As it was possible that some degree of tonic activation may be required to elicit any stimulant effect of GR46611, higher doses (10–40 mg kg^{-1}) were also tested in unhabituated animals but again no increase in activity was found [$F(4,25) = 2.08$ $P = 0.11$] (Table 1).

3.2. Effect of antagonists on agonist-induced locomotion

WAY100635 (0.001–0.006 mg kg^{-1}) dose-dependently reversed the hyperactivating effects of 8-OH-DPAT (1.0 mg kg^{-1}). Post hoc LSM tests showed that the min ED was 0.006 mg kg^{-1} (Table 2) (P vs. Vehicle/8-OH-DPAT < 0.05) with lower doses having no significant effect. WAY100635 had no effect on spontaneous locomotor activity in the habituation period (Fig. 2a). GR127935 at even higher doses (0.25–1.0 mg kg^{-1}) had no significant effect on the hyperactivity induced by 8-OH-DPAT (1.0 mg kg^{-1}) (Fig. 2b) or on spontaneous activity measured in the habituation period.

3.3. Effect of combined 5-HT_{1A} and 5-$HT_{1B/D}$ receptor agonists on locomotor activity in the guinea pig

GR46611 (0.5–2.5 mg kg^{-1}) dose-dependently enhanced the locomotor response to a dose of 8-OH-DPAT (0.5 mg kg^{-1}) that did not significantly increase activity alone. Post hoc LSM tests showed that all doses of GR46611 were significantly different from both the Vehicle/Vehicle and Vehicle/8-OH-DPAT-treated groups (Fig. 3a). Similarly, the same doses of GR46611 (0.5–2.5 mg kg^{-1}) significantly enhanced the locomotor response to a dose of buspirone (10 mg kg^{-1}) that did not significantly increase locomotor activity when given alone (Fig. 3b; Table 3).

GR127935 (0.25 mg kg^{-1}) significantly reversed the potentiation of the subthreshold dose of 8-OH-DPAT (0.5 mg kg^{-1}) induced by GR46611 (5 mg kg^{-1}) (Table 4a). Similarly, WAY100635 (0.5 mg kg^{-1}) also reversed the effects of the combination of 8-OH-DPAT (0.5 mg kg^{-1}) and GR46611 (5 mg kg^{-1}) (Fig. 4b).

4. Discussion

As previously described, the selective 5-HT_{1A} receptor agonist, 8-OH-DPAT, significantly increased locomotor activity in guinea pigs (Evenden, 1994). This effect was reversed by low doses of WAY100635, the selective 5-HT_{1A} receptor antagonist (Fletcher et al., 1994; Khawaja et al., 1995) but not by the 5-$HT_{1B/D}$ receptor antagonist GR127935 at doses that have previously been shown to reverse the effects of GR46611 in vivo (Skingle et al., 1994). A much smaller but significant effect on total levels of locomotor activity was seen following administration of buspirone (5–20 mg kg^{-1}). This also concurs with the proposition that potency in stimulating locomotor activity in guinea pigs correlated with the agonist efficacy of the 5-HT_{1A} ligands (Evenden, 1994). Somewhat surprisingly, no evidence of elements of 5-HT syndrome such as flat-

tened body posture or lower lip retraction were evident in the guinea pigs treated with the agonists even at the relatively high doses used in the present study.

The 5-$HT_{1B/D}$ receptor agonist GR46611 had no effect on locomotor activity in guinea pigs, even at doses up to 40 mg kg^{-1}. One possible explanation for the lack of effect of GR46611 may have been that the compound did not cross the blood–brain barrier. However, it has previously been shown that GR46611 (10 mg kg^{-1} s.c.) significantly reduced extracellular concentrations of serotonin as measured by in vivo microdialysis (Skingle et al., 1995; O'Neill et al., 1997b). This suggests that GR46611 clearly effects central 5-HT function and that any difference in functional effects cannot be ascribed to differential penetration of the blood–brain barrier.

To examine the consequences of simultaneous activation of both 5-$HT_{1B/D}$ and 5-HT_{1A} receptors, we pretreated guinea pigs with GR46611 and subsequently dosed them with a subthreshold dose of 8-OH-DPAT that was also without a significant effect on locomotor activity. In this experiment, we saw a significant increase in the locomotor response to 8-OH-DPAT. The doses of GR46611 used (0.5–2.5 mg kg^{-1}) had no effect when given alone in the previously outlined experiments. To help discount the possibility of a pharmacokinetic interaction, we also examined the effects of the same doses of GR46611 on the locomotor activity induced by the azapirone, buspirone, which was also significantly enhanced. Furthermore the fact that the antagonist GR127935 significantly reversed by enhancement of 8-OH-DPAT by GR46611 suggested that the effect was indeed 5-$HT_{1B/D}$ receptor mediated. The blockade of the enhancing effect by WAY100635 also helped support the view that both 5-HT_{1A} and 5-$HT_{1B/D}$ receptors were involved in the mediation of the locomotor activity observed while also helping to rule out any pharmacodynamic interaction as a possible explanation of the effect.

It has been shown previously that 5-HT_{1A} and rat-type 5-HT_{1B} receptors interact synergistically to increase locomotor activity in rats. RU24969 is an agonist with affinity for both 5-HT_{1A} and 5-HT_{1B} receptors (Hoyer, 1991). RU24969 induces hyperactivity in rats (see Introduction). This hyperactivity is attenuated by antagonists acting at both 5-HT_{1A} and 5-$HT_{1B/D}$ receptors (Kalkman, 1995; O'Neill and Parameswaran, 1997). The hyperactivity induced by RU24969 in the rat is quantitatively and qualitatively different from that induced by the more selective 5-HT_{1B} receptor agonist anpirtoline (Schlicker et al., 1992; O'Neill and Parameswaran, 1997). RU24969 induces a large increase in locomotion, with rats showing a stereotyped circling of the activity cage producing counts in the 1000s per 10 min bin while anpirtoline only produces activity in the 100s even at higher doses and little other overt behavioural change. RU24969 induces other overt behavioural changes such as flat body posture and lower lip retraction clearly indicating 5-HT_{1A} receptor activation. Furthermore, O'Neill and Parameswaran (1997) showed that an RU24969-like behavioural profile could be produced by co-administration of anpirtoline and 8-OH-DPAT.

The lack of an intrinsic locomotor effect of GR46611 may reflect a lack of functional activity at 5-HT_{1A} receptors in vivo, although it has been reported to have activity at these receptors in vitro (Skingle et al., 1995). As further evidence of this possibility we have failed to observe an antidepressant-like effect of GR46611 in the mouse forced swim test (O'Neill, unpublished results). This is in spite of the fact that other agonists at 5-HT_{1A} receptors show antidepressant-like effects in this test (Wieland and Lucki, 1990; O'Neill et al., 1997a).

The results presented here parallel the effects observed in rats with the combination of 8-OH-DPAT and anpirtoline implies that the locomotor response to 5-HT_{1A} receptor agonism is greatly increased by simultaneous stimulation of 5-$HT_{1B/D}$ receptors. This suggests that synergistic effects of simultaneous activation of both 5-HT_{1A} and 5-$HT_{1B/D}$ receptors can be observed in both in the rat and the guinea pig. It would also appear that the 5-HT_{1B} and 5-$HT_{1B/D}$ receptors have homologous functions in both species.

The high levels of 5-$HT_{1B/D}$ receptor binding in the basal ganglia as stated in the Introduction implies a role for these receptors in the control of movement. Although 5-$HT_{1B/D}$ receptors are primarily located on axon terminals as evidenced by the differential location of 5-$HT_{1B/D}$ binding sites and 5-$HT_{1B/D}$ mRNAs in these areas (Maroteaux et al., 1992; Boschert et al., 1994), 5,7,-DHT lesions did not attenuate RU24969-induced locomotor activity in the mouse (Cheetham and Heal, 1993) suggesting that the 5-HT_{1B} receptors mediating this response were not located on 5-HT axon terminals. This suggests that 5-$HT_{1B/D}$ receptor agonists may increase locomotor activity via modulating the release of transmitters other than serotonin. 5-$HT_{1B/D}$ receptors have also been shown to exist on terminals of descending GABAergic neurons, for example, which project from the striatum to the substantia nigra (Waeber and Palacios, 1989) and may be part of a negative feedback loop controlling activity in the nigrostriatal pathway. In vivo microdialysis experiments in rats have shown that 5-HT facilitates dopamine release in the anterior striatum (Benloucif and Galloway, 1991). Interestingly, these authors also compared the effects of 8-OH-DPAT and RU24969 on synaptic concentrations of dopamine and found that the mixed 5-$HT_{1A/B}$ agonist induced a 300% increase in dopamine levels while 8-OH-DPAT induced only a 40% increase. Unfortunately, a selective 5-HT_{1B} receptor agonist such as anpirtoline was not tested so it is not possible to determine if the larger effect of RU24969 was due to its activity on 5-HT_{1B} receptors alone or due to its combined activity on 5-HT_{1A} and 5-HT_{1B} receptors as the locomotor data discussed above would suggest. 5-HT has also been shown to facilitate dopamine release as measured in the nucleus accumbens by in vivo microdialy-

sis in guinea pigs (Hallbus et al., 1997). This effect was blocked by GR127935 again implicating 5-$HT_{1B/D}$ mechanisms in the mediation of 5-HT facilitation of dopamine release. This would suggest that the increase in locomotor activity may be due to 5-$HT_{1B/D}$ mediated facilitation of dopamine release in the basal ganglia. Further studies to elucidate the interaction with other neuronal systems will increase our understanding of this area and possibly indicate the therapeutic relevance of these finding to conditions involving an impairment of motor control such as Parkinson's Disease or Huntingdon's Chorea.

GR46611 shows no selectivity between 5-HT_{1B} and 5-HT_{1D} receptors (Starkey and Skingle, 1994; Hatcher et al., 1995). Neither does the antagonist GR127935 distinguish between these subtypes. Thus, as yet, it is not possible to say which receptor subtype or subtypes may mediate the effects described here. Further studies with selective agents as they become available will clarify this issue but it is unlikely that differential effects at either 5-HT_{1B} or 5-HT_{1D} receptor subtypes is responsible for the differing behavioural profiles of the agonists.

In conclusion, the 5-$HT_{1B/D}$ receptor agonist GR46611 did not induce hyperactivity alone but did significantly enhance the locomotor response to a subthreshold dose of 8-OH-DPAT. The results presented here indicate that 5-$HT_{1B/D}$ and 5-HT_{1A} receptors synergistically promote locomotor activity in the guinea pig.

References

Benloucif, S., Galloway, M.P., 1991. Facilitation of dopamine release in vivo by serotonin agonists: studies with microdialysis. Eur. J. Pharmacol. 200, 1–8.

Boschert, U., Ait Amara, D., Segu, L., Hen, R., 1994. The mouse 5-hydroxytryptamine$_{1B}$ receptor is localized primarily on axon terminals. Neuroscience 58, 167–182.

Bruinvels, A.T., Palacios, J.M., Hoyer, D., 1993. Autoradiographic characterisation and localisation of 5-HT_{1D} compared to 5-HT_{1B} binding sites in rat brain. Naunyn-Schmiedeberg's Arch. Pharmacol. 347, 569–582.

Cheetham, S.C., Heal, D.J., 1993. Evidence that RU 24969-induced locomotor activity in C57/B1/6 mice is specifically mediated by the 5-HT_{1B} receptor. Br. J. Pharmacol. 110, 1621–1629.

Davidson, C., Stamford, J.A., 1995. Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} autoreceptors. Br. J. Pharmacol. 114, 1107–1109.

Evenden, J.L., 1994. The effect of 5-HT_{1A} receptor agonists on locomotor activity in the guinea pig. Br. J. Pharmacol 112, 861–866.

Fletcher, A., Bill, D.J., Cliffe, I.A., Forster, E.A., Jones, D., Reilly, Y., 1994. Pharmacological profile of WAY-100635, a potent and selective 5-HT_{1A} receptor antagonist. Br. J. Pharmacol. 112, 91.

Hallbus, M., Magnusson, T., Magnusson, O., 1997. Influence of 5-$HT_{1B/D}$ receptors on dopamine release in the guinea pig nucleus accumbens: a microdialysis study. Neurosci. Lett. 225, 57–60.

Hartig, P., Hoyer, D., Humphrey, P.P.A., Martin, G., 1996. Alignment of receptor classification with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. Trends Pharmacol. Sci. 17, 103–105.

Hatcher, J.P., Slade, P.D., Roberts, C., Hagan, J.J., 1995. 5-HT_{1D} receptors mediate SKF99101H-induced hypothermia in the guinea pig. J. Psychopharmacol. 9, 234–241.

Higgins, G.A., Jordan, C.C., Skingle, M., 1991. Evidence that the unilateral activation of 5-HT_{1D} receptors in the substantia nigra of the guinea pig elicits contralateral rotation. Br. J. Pharmacol. 102, 305–310.

Hoyer, D., 1991. 5-Hydroxytrytophan receptors and effector coupling mechanisms in peripheral tissues. In: Fozard, J. (Ed.), The peripheral actions of 5-hydroxytryptamine. Oxford Univ. Press, Oxford, 1991, pp. 72–99.

Kalkman, H.O., 1995. RU24969-induced locomotion in rats is mediated by 5-HT_{1A} receptors. Naunyn-Schmiedeberg's Arch. Pharmacol. 352, 583–584.

Khawaja, X., Evans, N., Reilly, Y., Ennis, C., Minchin, M., 1995. Characterisation of the binding of [^{3}H] WAY-100635, a novel 5-Hydroxytryptamine$_{1A}$ receptor antagonist, to rat brain. J Neurochem. 64, 2716–2726.

Maroteaux, L., Saudau, F., Amlaiky, N., Boschert, U., Plassat, J.L., Hen, R., 1992. Mouse 5-HT_{1B} serotonin receptor: cloning, functional expression and localization in motor centers. Proc. Natl. Acad. Sci. U.S.A. 89, 3020–3024.

O'Neill, M.F., Parameswaran, T., 1997. RU24969-induced behavioural syndrome requires both 5-HT_{1A} and 5-HT_{1B} receptor stimulation. Psychopharmacology 132, 255–260.

O'Neill, M.F., Fernandez, A.G., Palacios, J.M., 1996. GR127935 blocks the locomotor and antidepressant-like effects of RU 24969 and the action of antidepressants in the mouse tail suspension test. Pharmacol. Biochem. Behav. 53 (3), 535–539.

O'Neill, M.F., Moore, N.A., Dobson, D.R., Steggles, D., Conway, M.W., 1997a. Effect of antagonism of 5-HT_{1A} and 5-HT_{1B} receptors on response to 5-HT1 agonists and imipramine in the forced swim test in mice. Br. J. Pharmacol. (Abs) (in press.)

O'Neill, M.F., Mitchell, S.N., Sanger, G.J., Gurling, J., Moore, N.A., Dobson, D.R., 1997b. 5-HT_{1D} agonist SKF99101H increases locomotor activity and suppresses basal 5-HT in the hypothalamus of the Guinea Pig. Soc. Neurosci. Abstr. 387.5.

O'Neill, M.F., Fernández, A.G., Palacios, J.M., 1997c. Activation of central 5-HT_{1B} receptors increases locomotor activity in mice. Human Psychopharmacol. 12, 431–435.

Schlicker, E., Werner, U., Hamon, M., Gozlan, H., Nickel, B., Szelenyi, I., Gothert, M., 1992. Anpirtoline, a novel, highly potent 5-HT_{1B} receptor agonist with antinociceptive/antidepressant-like actions in rodents. Br. J. Pharmacol. 105, 732–738.

Skingle, M., Higgins, G.A., Feniuk, W., 1994. Stimulation of central 5-HT_{1D} receptors causes hypothermia in the guinea-pig. J. Psychopharmacol. 8, 14–21.

Skingle, M., Sleight, A.J., Feniuk, W., 1995. Effects of the 5-HT_{1D} receptor antagonist GR127935 on extracellular levels of 5-HT in the guinea pig frontal cortex as measured by microdialysis. Neuropharmacology 34, 377–382.

Starkey, S.J., Skingle, M., 1994. 5-HT_{1D} as well as 5-HT_{1A} autoreceptors modulate 5-HT release in the guinea pig dorsal raphe nucleus. Neuropharmacology 33, 393–402.

Waeber, C., Palacios, J.M., 1989. Serotonin-1 receptor binding sites in the human basal ganglia are decreased in Huntingdon's chorea but not in Parkinson's disease: a quantitative in vitro autoradiographic study. Neuroscience 32, 337–347.

Wieland, S., Lucki, I., 1990. Antidepressant-like activity of 5-HT_{1A} agonists measured in the forced swim test. Psychopharmacology 101, 497–504.