Evidence that the unilateral activation of 5-HT_{1D} receptors in the substantia nigra of the guinea-pig elicits contralateral rotation

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1 The effects of various 5-hydroxytryptamine (5-HT) receptor agonists were examined following unilateral infusion into the substantia nigra (SN) of the guinea-pig.

2 The 5-HT₁ receptor agonists, 5-carboxamidotryptamine (5-CT) (2–25 µg), sumatriptan (10–25 µg) and RU24969 (25 µg) all induced a marked contralateral rotation. In contrast, the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT, 10–25 µg) produced only a very small response, whilst the selective 5-HT_{1C}/5-HT₂ receptor agonist (±)-1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride ((±)-DOI) (25 µg) and the 5-HT₃ receptor agonist, 2-methyl 5-HT (2-Me5-HT, 25 µg) were without effect.

3 The contralateral rotation induced by 5-CT (10 µg) was attenuated following pretreatment with the non-selective 5-HT₁/5-HT₂ receptor antagonists methiothepin (1 mg kg⁻¹, s.c.) and metergoline (5–10 mg kg⁻¹, s.c.) but not the 5-HT_{1C}/5-HT₂ antagonist ritanserin (1 mg kg⁻¹, s.c.) or the 5-HT₃ antagonist, ondansetron (0.5 mg kg⁻¹, s.c.). An involvement of dopaminergic systems in the rotational response to 5-CT was implied by the antagonism of 5-CT-induced rotation by haloperidol (0.3 mg kg⁻¹, s.c.).

4 At doses lower than those required to produce contralateral rotation, 5-CT (0.08–0.4 µg) and sumatriptan (2 µg) induced a small, but nonetheless consistent, ipsilateral rotation.

5 The data with agonists and antagonists taken together suggest that 5-CT-induced contralateral rotation may be mediated by 5-HT_{1D} receptor activation but definitive classification of the receptor will not be possible until selective 5-HT_{1D}-antagonists become available. This may therefore represent the first model to study this receptor subtype *in vivo*.

Introduction

Central 5-hydroxytryptamine (5-HT) receptor subtypes have been classified into three main categories, namely 5-HT₁-like, 5-HT₂ and 5-HT₃ (Bradley *et al.*, 1986). Furthermore, the 5-HT₁-like receptor appears to consist of a heterogeneous pool of which at least four subtypes, termed 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D}, have been described (see Peroutka, 1988 for review). The 5-HT_{1D} receptor has recently attracted wide interest, in particular for its demonstration in human brain (Waeber *et al.*, 1988) and possible involvement in movement disorders such as Huntington's chorea (Waeber & Palacios, 1989). In addition, sumatriptan (GR43175) (Humphrey *et al.*, 1988), which has been shown to be effective in the treatment of migraine, (Doenicke *et al.*, 1988) also shows some selectivity for the 5-HT_{1D} binding site (Schoeffter & Hoyer, 1989).

In addition to man, central 5-HT_{1D} receptors have been found in calf, pig, dog, pigeon, monkey and guinea-pig (Heuring & Peroutka, 1987; Waeber *et al.*, 1989a,b). In each species studied to date, the distribution of this receptor subtype is similar with the highest density in regions associated with the dopamine nigrostriatal system e.g. the substantia nigra (SN), globus pallidus and striatum (Waeber *et al.*, 1989a). Functionally, 5-HT_{1D} receptors have been shown to be negatively coupled to adenylate cyclase (Schoeffter *et al.*, 1988) and they may serve as the terminal 5-HT inhibitory autoreceptor (Schlicker *et al.*, 1989). Indeed, with respect to central distribution and function, the 5-HT_{1D} receptor is very similar to the 5-HT_{1B} subtype, which appears confined to the rat, mouse and opossum (see Hoyer & Middlemiss, 1989). Thus, the 5-HT_{1D} receptor may represent an evolutionary development of the 5-HT_{1B} subtype.

In rats, both electrophysiological and behavioural studies suggest that 5-HT has an inhibitory effect on nigrostriatal

dopamine function (Dray *et al.*, 1976; Pycoc, 1980). Consistent with these findings, James & Starr (1980) reported that unilateral injection of 5-HT into the SN of the rat elicited ipsilateral rotation with a corresponding decrease in striatal dopamine metabolism on the injected side. Paradoxically, however, Oberlander *et al.* (1981) observed contralateral rotation to this same treatment and the intra-nigral injection of the 5-HT₁ receptor agonist, RU24969, produced behaviours consistent with an activation of the nigrostriatal dopamine pathway (Oberlander, 1983). Based on our current understanding of central 5-HT function, an involvement of 5-HT_{1B} receptors in at least some of these responses seems likely, since nigral 5-HT receptors in the rat appear to be primarily of this subtype (Pazos *et al.*, 1985; Verge *et al.*, 1986). In the search for a model in which to examine central 5-HT_{1D} receptor function *in vivo*, we therefore decided to study the effects of various 5-HT agonists following unilateral injection into the SN of the guinea-pig. In a preliminary account (Higgins *et al.*, 1990) we described how the selective 5-HT₁-like receptor agonists 5-carboxamidotryptamine (5-CT) and sumatriptan, but not 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT), induced a marked contralateral rotation when injected into the substantia nigra. Furthermore, this response may be antagonized by the non-selective 5-HT₁/5-HT₂ receptor antagonist metergoline. We now describe a more extensive analysis of this behavioural response. Based upon our current understanding of 5-HT receptor subtypes, this model may thus represent the first demonstration of central 5-HT_{1D} receptor function *in vivo*.

Methods

Animals and housing

Male, Dunkin-Hartley guinea-pigs (300–500 g, Interfauna) were used throughout. The animals were housed in groups of 5 at a temperature of 20 ± 1°C on a 12 h light/dark cycle (lights on 6 h 00 min–18 h 00 min). Animals were allowed free

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access to food (SDS FD1 guinea-pig diet) and water except during behavioural testing.

Stereotaxic surgery

Guinea-pigs were anaesthetized with chloral hydrate (400 mg kg⁻¹, i.p.) and ketamine (40 mg kg⁻¹, i.p.) and placed in a Kopf stereotaxic frame. Guide cannulae consisting of a stainless steel tube (23 gauge) in a perspex holder, were implanted unilaterally, 8 mm above the left substantia nigra (SN) (Anterior +6.0 mm (interaural line), Lateral +2.2 mm (midline), Ventral -4.0 mm (skull surface)) with the mouth-piece set 3.2 mm below zero (Luparello, 1967). Stainless steel stylets (30 gauge) were used to keep the guide cannula patent during the 7 day recovery period. These extended 1 mm beyond the cannula tip.

Central microinjections

Seven days after surgery, the guinea-pigs were lightly restrained, the stylets removed and a microinjection needle (30 gauge) was inserted into the guide cannula such that it terminated 8 mm below the cannula tip. Drug solutions or saline vehicle were slowly infused over a 120 s period in a dose volume of 2 µl. A further 30 s was allowed for diffusion away from the cannula tip. The injection units were then removed and the stylets replaced. Unless otherwise stated, the guinea-pigs were used in one experiment only.

Testing for rotational behaviour

Immediately after the intra-nigral injection of test compound or vehicle, the guinea-pigs were placed individually in hemispherical bowls (33 cm diameter; 30 cm high) typically for a 3 h period unless otherwise stated. Rotational behaviour was automatically measured connecting the animals via a harness to a transducer which, in turn, was linked to an on-line computer (Olivetti M28) for the automatic recording of both ipsilateral (I) and contralateral (C) rotations.

For antagonist studies, the test compound or vehicle was administered subcutaneously in the nape of the neck, 30 min before the nigral injection of 5-CT (10 µg).

Testing for locomotor activity

To determine whether antagonism of 5-CT-induced rotation was due to sedation rather than pharmacological antagonism, each effective compound was assessed for overt signs of behavioural depression and locomotor activity at doses which antagonized 5-CT. All treatments were administered subcutaneously, 30 min before placing (otherwise untreated) guinea-pigs singly in activity monitors for 3 h. The transparent locomotor activity cages (length 40 cm × width 25 cm × height 30 cm) had eight infra-red photocells on each of three levels; five along the length and three across the width of the cage with a 7.5 cm space between each. The vertical distance from the floor of the cage was adjustable for the 3 levels of photocells but was set so that both normal ambulation and rearing behaviour could be monitored. Beam crossings were counted by a microcomputer in 15 min epochs over the 3 h test period.

Histology

Upon completion of testing, the guinea-pigs were killed by cervical dislocation and then injected with 2 µl of India ink into the left SN. The brains were then removed and sectioned on a freezing microtome for histological placement of injection sites. Animals in which India ink was sited outside the SN were excluded from the study.

Drugs

5-Carboxamidotryptamine (5-CT), 5-methoxy-3(1,2,3,6-tetrahydropyridin-4-yl)1H indole (RU24969), 2-methyl 5-HT,

ondansetron (1,2,3,9-tetrahydro-9-methyl-3-[2-methyl-1H-imidazol-1-yl]-methyl]-4H carbazol-4-one) and sumatriptan (3-(2-dimethylamino)ethyl-N-methyl-1H-indole-5 methane sulphonamide) were all synthesized in the Neurochemistry Department, Glaxo Group Research Ltd., Ware. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH DPAT), (±)1-(4-iodo-2,5-dimethoxyphenyl)-2-aminopropane hydrochloride (DOI) and haloperidol were obtained from Research Biochemicals Inc. The suppliers of the remaining drugs were as follows: methiothepin maleate (Roche), metergoline (Farmitalia) and ritanerlin (Janssen).

All drugs, unless otherwise stated, were dissolved or suspended in 0.9% sodium chloride solution (Steriflex). Methiothepin maleate was dissolved in 1 ml ethanol containing a few drops of 1 M HCl and made up to final volume in saline. Both ritanerlin and haloperidol were dissolved in 0.1 M tartaric acid and metergoline in 1 M ascorbate before addition of saline. For each treatment, the control group received identical vehicle alone. All drug concentrations are expressed as free base.

Drugs for subcutaneous or intraperitoneal injection were administered in a dose volume of 2 ml kg⁻¹, except chloral hydrate which was injected in a volume of 10 ml kg⁻¹. The pH of all solutions was in the range 4–7. For intra-nigral injections, a dose volume of 2 µl was used.

Results

Effect of 5-carboxamidotryptamine following unilateral injection into the substantia nigra in the guinea-pig

Microinfusion of 5-CT (2–25 µg) into the left SN of the guinea-pig induced a strong contralateral (C) rotation (Figure 1). Although this response was almost immediate in onset, at higher doses (10–25 µg) the most intense period of activity was 1–4 h post injection (Figure 2). This apparent delay may be due to the induction of ataxia which impaired locomotor performance. During this time period, the animals showed a clear asymmetrical posture with the head directed away from the injected side.

At lower doses of 5-CT (0.08–2 µg), a small but nonetheless reproducible, ipsilateral (I) rotation was observed (Figure 1). The magnitude of this effect was much smaller than the contralateral response (e.g. 5-CT 0.4 µg I = 39 ± 16 rotations 3 h⁻¹; 5-CT 25 µg C = 436 ± 109 rotations 3 h⁻¹).

In order to determine the possibility of re-testing guinea-pigs, the effect of repeated nigral injections of 5-CT (10 µg) was examined. All drug injections were made into the left SN and the results are shown in Table 1. When a recovery period of 7 days was allowed between consecutive microinfusions of 5-CT (10 µg), the contralateral rotation induced by the second dose

Figure 1 Rotational behaviour elicited by 5-HT₁ receptor agonists following unilateral intra-nigral injection in the guinea-pig. Contralateral (hatched columns) and ipsilateral (solid columns) rotations were recorded for 3 h. Significant differences (**P* < 0.05; ***P* < 0.01) compared to vehicle were calculated by the paired *t* test. Figures in parentheses above each column refer to the number of animals used in each treatment group; vertical bars show s.e.

Figure 2 The duration of action of the contralateral rotation induced by unilateral intra-nigral injection of 5-carboxamido-tryptamine (5-CT) in guinea-pigs. Contralateral rotations were recorded every 15 min for up to 5 h following unilateral injection of 5-CT. The following doses refer to the total dose given to each guinea-pig: (■) 0.4 µg, (▲) 2 µg, (●) 10 µg and (○) 25 µg.

was significantly lower ($P < 0.05$, paired t test) than the response to the initial infusion. If the recovery period was extended to 14 or 21 days, the second response was still reduced, although not significantly. Because of this apparent desensitisation, all other experiments were carried out using naive animals.

The relationship between the rotational response to a 10 µg dose of 5-CT and the histological location of the injection site is shown by Figure 3. These data show that microinfusions made directly into the SN were associated with high (>150 rotations 3 h^{-1}) contralateral rotation scores to 5-CT. Animals which displayed little or no rotation to 5-CT tended to have infusion sites on the borders of, or outside, the SN.

Effect of other 5-hydroxytryptamine agonists on turning behaviour in the guinea-pig following unilateral injection into the substantia nigra

Significant contralateral rotation was induced by the infusion of sumatriptan (10–25 µg), RU24969 (25 µg) or 8-OH DPAT (10–25 µg) into the SN, although the response to 8-OH DPAT was much smaller than that elicited by the other compounds (Figure 1). In common with 5-CT, sumatriptan at a dose (2 µg) subthreshold for inducing contralateral rotation, produced a small ipsilateral response as did 8-OH DPAT (25 µg).

In contrast to the effects of the 5-HT₁ receptor agonists, the 5-HT₃ receptor agonist, 2-Me 5-HT, failed to induce any significant rotational behaviour following intra-nigral infusion (25 µg = $I = 7 \pm 3$ rotations 3 h^{-1} ; $C = 11 \pm 1$ rotations 3 h^{-1} , $n = 4$). The selective 5-HT_{1C/2} receptor agonist ($\pm$)-DOI (25 µg) was relatively ineffective although some rotational behaviour was observed ($I = 58 \pm 28$ rotations 3 h^{-1} ; $C = 32 \pm 30$ rotations 3 h^{-1} , $n = 4$). However, there was no obvious preference for either direction and these scores may therefore reflect the behavioural hyperactivity which became apparent approximately 60 min after DOI infusion.

Table 1 Desensitization of 5-carboxamidotryptamine (5-CT)-induced contralateral rotation following intra-nigral injection in the guinea-pig

Interval between doses of 5-CT	Contralateral rotations per 3 h induced by 5-CT	
	First Dose	Second Dose
7 days	350 $\pm$ 56	212 $\pm$ 74*
14 days	374 $\pm$ 107	156 $\pm$ 36
21 days	308 $\pm$ 101	194 $\pm$ 62

Five guinea-pigs were used for each treatment group. Animals were dosed with 5-CT (10 µg) intra-nigraly on day 1 and then again on day 7, 14 or 21. Values are mean $\pm$ s.e.mean.

*Indicates a significant difference ($P < 0.05$) between response to first and second dose (paired t test).

Figure 3 Correlation of the contralateral rotational behaviour induced by intra-nigral injection of 5-carboxamidotryptamine (5-CT, 10 µg) with site of injection in guinea-pig brain. Figure modified from the stereotaxic atlas of Luparello (1967). Symbols denote site of injection placement. Injections into the centre of the substantia nigra (SN) induced >150 rotations in 3 h (■). Injections on the periphery of the SN induced between 100 and 150 rotations in 3 h (●) whereas placements outside the SN caused less than 100 rotations (○). Anterior co-ordinates measured from the interaural line.

Effect of various antagonists on the contralateral rotation response produced by 5-carboxamidotryptamine (10 µg)

The contralateral rotation induced by intra-nigral infusion of 5-CT (10 µg) was dose-dependently blocked after pretreatment with methiothepin (1 mg kg^{-1} , s.c.) or metergoline (5–10 mg kg^{-1} , s.c.) (Figure 4). Following saline pretreatment, the

Figure 4 Antagonism of 5-carboxamidotryptamine (5-CT)-induced rotation by methiothepin and metergoline in guinea-pigs. Antagonists were given s.c. 30 min before unilateral intra-nigral injection of 5-CT (10 µg). Contralateral (hatched columns) and ipsilateral (solid columns) rotation were recorded for 3 h post 5-CT injection. Significant differences (* $P < 0.05$) compared to 5-CT/vehicle (Veh) were calculated by the unpaired t test. Figures in parentheses above each column refer to the number of animals used in each treatment group; vertical bars show s.e.

Figure 5 Effect of ritanserin, ondansetron and haloperidol on 5-carboxamidotryptamine (5-CT)-induced rotation following intra-nigral injection in guinea-pig. Ritanserin (1 mg kg^{-1}), ondansetron (0.5 mg kg^{-1}), haloperidol (0.3 mg kg^{-1}) or vehicle were given s.c. 30 min before unilateral intra-nigral injection of 5-CT ($10 \mu\text{g}$). Contralateral (hatched columns) and ipsilateral (solid columns) rotations were recorded for 3 h. Significant differences ($*P < 0.05$) compared to 5-CT/vehicle were calculated by unpaired *t* test. Figures in parentheses above each column refer to the number of animals used in each treatment group; vertical bars show s.e.

rotational response to 5-CT was exclusively contralateral. However, at doses of methiothepin or metergoline that significantly blocked the contralateral response, some ipsilateral rotation was observed (Figure 5).

Peak rotational responses to intra-nigral 5-CT occurred at 90–120 min post administration. The effect of the antagonists, given alone, on locomotor activity was monitored for 120 min. At doses that significantly antagonized 5-CT-induced contralateral rotation, methiothepin (1 mg kg^{-1} , s.c.), metergoline (10 mg kg^{-1} , s.c.) and haloperidol (0.3 mg kg^{-1} , s.c.) did not significantly reduce spontaneous activity in the guinea-pig (Table 2). Furthermore, no overt signs of sedation were seen in any of these groups except in the metergoline- and methiothepin-pretreated groups where 2/7 of the animals displayed hind limb abduction with a lowered body position.

Discussion

The present studies demonstrate that the unilateral micro-infusion of the non-selective 5-HT₁ receptor agonist 5-CT, into the SN of the guinea-pig elicits a marked contralateral rotation. Various lines of evidence support an involvement of 5-HT_{1D} receptors in this response. Firstly, the rank order of potency for a range of agonists to induce this behaviour following intra-nigral injection i.e. 5-CT > sumatriptan > RU24969 > 8OH-DPAT, is in good agreement with their relative potencies at inhibiting adenylate cyclase activity in calf SN, a functional 5-HT_{1D} model (Schoeffter *et al.*, 1988; Schoeffter & Hoyer, 1989). This rank order is also similar to

their relative affinities for 5-HT_{1D} binding sites (Waeber *et al.*, 1988; Schoeffter & Hoyer, 1989). Furthermore, the selective 5-HT_{1C}/5-HT₂ receptor agonist ($\pm$)-DOI (Glennon *et al.*, 1984; Hoyer, 1988) and the 5-HT₃ receptor agonist 2-Me 5-HT (Richardson *et al.*, 1985) were ineffective at a dose of $25 \mu\text{g}$, suggesting that these 5-HT receptor subtypes are not involved.

Secondly, the rotations induced by 5-CT were blocked by the non-selective 5-HT₁/5-HT₂ receptor antagonists methiothepin and metergoline at doses which failed to produce any marked behavioural effects. Pretreatment with the 5-HT_{1C}/5-HT₂ receptor antagonist, ritanserin, at 1 mg kg^{-1} failed to block the response to 5-CT. Previous studies have shown that this dose of ritanserin antagonizes ($\pm$)-DOI-induced wet dog shakes and hyperactivity in the guinea-pig, a putative central 5-HT₂ model in this species (Cole *et al.*, 1990). The 5-HT₃ receptor antagonist, ondansetron, was similarly ineffective against 5-CT-induced contralateral rotation at a pharmacologically relevant dose in the guinea-pig (Costall *et al.*, 1987). Thus, the antagonist studies are consistent with an involvement of a 5-HT₁ receptor subtype in the rotational response to 5-CT.

Finally, all drug microinfusions were made into the SN of the guinea-pig, a tissue containing 5-HT receptors belonging primarily, if not exclusively, to the 5-HT_{1D} subtype (Waeber *et al.*, 1989b). Microinfusions of 5-CT just outside this structure failed to induce significant rotation. This strongly suggests an involvement of the SN in the behavioural effects observed.

In a wide range of animal models, dopamine has been implicated in playing a primary role in the mediation of circling behaviour (reviewed in Pycock, 1980). For instance, unilateral electrical stimulation of the SN elicits contralateral rotation in rats (which may be blocked by dopamine antagonists e.g. haloperidol), which is associated with increased striatal dopamine metabolism on the stimulated side only (Arbuthnott & Crow, 1971; Arbuthnott & Ungerstedt, 1975). The present studies also suggest that 5-CT-induced contralateral rotation in the guinea-pig is similarly dependent upon activation of ascending nigrostriatal dopamine fibres. Thus, haloperidol antagonized this rotational response at a dose that failed to affect behaviour in naive animals. This would imply that the antagonism was not simply due to sedation. One interpretation of these data is that an agonist action at nigral 5-HT_{1D} receptors results in stimulation of ascending dopaminergic fibres to elicit rotation. This is the first demonstration of a functional interaction between 5-HT_{1D} receptors and dopaminergic systems. At present the nature of this interaction is unclear, although a recent study (Waeber *et al.*, 1989a) may suggest that nigral 5-HT_{1D} receptors are not localized on dopamine cell bodies. The histological verification of injection sites in this study (Figure 3) is consistent with this hypothesis. Thus, the greatest circling response occurred following injection into the nigra reticulata, a region containing little dopamine (dopamine cell bodies are in the nigra compacta). Based on the effects of lesions in striatum, Waeber *et al.* (1990) have suggested that 5-HT_{1D} receptors appear to be presynaptically located on terminals of the pathway descending from the striatum to nigra reticulata.

Because of the time-consuming nature of the surgical operations necessary for this type of experiment, we studied the rotational responses to repeated injections of 5-CT in order to determine whether the animals could be re-used. There was a clear desensitization to a second challenge dose of 5-CT given 7 days after the first. Increasing the interval between dosing to 21 days failed to restore the second response to the initial level. Whether this is a pharmacological or behavioural desensitization, or simply due to tissue necrosis following the first injection, remains to be established but in view of this observation all the studies reported were carried out in experimentally naive animals.

An interesting finding from these studies was the ipsilateral rotation observed at doses of 5-CT and sumatriptan lower than those required to induce contralateral rotation. The

Table 2 Effects of metergoline, methiothepin and haloperidol on locomotor activity in the guinea-pig

Treatment	(mg kg^{-1} , s.c.)	Locomotor activity counts in 2 h	P value
Vehicle	—	235 ± 66	—
Metergoline	10.0	210 ± 47	0.76
Methiothepin	1.0	341 ± 26	0.14
Haloperidol	0.3	202 ± 51	0.70

*Unpaired Students *t* test.

Six to eight animals were used in each treatment group.

small magnitude of this response made pharmacological characterization using antagonists virtually impossible. However, following pretreatment with metergoline and methiothepin, a significant ipsilateral rotation was unmasked in animals treated with a dose of 5-CT that in control animals produced exclusively contralateral rotation. This may indicate that the ipsilateral rotation produced by 5-CT is not mediated via 5-HT₁/5-HT₂ receptors. Since both methiothepin and metergoline have affinity for the dopamine D₂-binding site (Leysen *et al.*, 1981) it could be argued that they prevent 5-CT-induced rotation indirectly by blocking D₂-binding sites (cf. haloperidol). However, unlike methiothepin and metergoline, haloperidol attenuates 5-CT-induced contralateral rotation but does not reveal the ipsilateral rotation response. This would suggest that haloperidol is acting at a different site(s) from metergoline and methiothepin. It would be interesting to examine the effects of central 5,7-dihydroxytryptamine (5,7-DHT) lesions on both the ipsi- and contralateral rotation response to 5-CT. Such studies may provide further insight into the mechanisms underlying these rotational responses. Following intra-nigral infusion in the rat, 5-HT-induced

contralateral rotation is potentiated by 5,7-DHT lesions (Oberlander *et al.*, 1981) suggesting the involvement of post-synaptic 5-HT receptors within the substantia nigra. Based on both the functional and anatomical similarity between 5-HT_{1D} and 5-HT_{1B} receptors (see Introduction) this equivalent response in the rat may represent an *in vivo* 5-HT_{1B} receptor model.

In conclusion, 5-CT-induced contralateral rotation in the guinea-pig may represent the first animal model for the study of 5-HT_{1D} receptors *in vivo*. Although the results for the agonists strongly implicate an action at the 5-HT_{1D} site, definitive classification is not possible without selective 5-HT_{1D} antagonists. A functional involvement of 5-HT_{1D} receptors with dopaminergic systems seems evident, a finding supported by the anatomical distribution of this receptor subtype (Waeber *et al.*, 1989a,b). This may suggest a role for selective 5-HT_{1D} receptor compounds in the treatment of movement disorders (see Waeber & Palacios, 1989).

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