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Facilitation of 8-OHDPAT-induced forepaw treading of rats by the 5-HT_2 agonist DOI

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The potency of the serotonin$_{1A}$ (5-HT_{1A}) agonist, 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), to induce forepaw treading was increased 20-fold after co-treatment with the 5-HT_2 agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). DOI induced head twitches which were inhibited by 8-OHDPAT. The putative 5-HT_{1B} agonist, 1-(3-trifluoromethylphenyl)piperazine (TFMPP), had a weak effect on the responses to DOI or 8-OHDPAT. The forepaw treading induced by 8-OHDPAT plus DOI was inhibited by high doses of (−)-alprenolol, ketanserin or ritanserin, but was not influenced by the β-adrenoceptor antagonist, ICI 118.551, or the 5-HT_3 antagonist, ICS 205-930. A non-effective dose of (−)-alprenolol increased the inhibitory effect of ketanserin and ritanserin. These results indicate a complex and different interaction between 5-HT_{1A} and 5-HT_2 receptors in the expression of two behavioural responses mediated by 5-HT.

5-HT_{1A} receptor agonists; 5-HT_{1B} receptor agonists; 5-HT_2 receptor agonists; Head twitches; Forepaw treading

1. Introduction

It is widely accepted that serotonin (5-HT) receptors can be divided into a number of types of which the 5-HT_{1A} and 5-HT_2 subclasses are most thoroughly characterized (Glennon, 1987). Selective agonists, e.g. 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI; Glennon, 1987), and antagonists have been developed for 5-HT_2 receptors. In contrast, only agonists with 5-HT_{1A} selectivity are available, e.g. 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT; Hjorth et al., 1982).

Various behavioural effects are mediated by 5-HT receptors in vivo, including the so-called '5-HT syndrome' (forepaw treading, head weaving, flat posture, tremor) and head twitches (Green and Grahame-Smith, 1976; Sloviter et al., 1978; Lucki et al., 1984; Tricklebank, 1985; Glennon, 1987). Experimental evidence indicates that head twitches are mediated by 5-HT_2-receptor stimulation (Niemegeers et al., 1983; Tricklebank, 1985). It has been suggested that parts of the 5-HT syndrome are mediated by 5-HT_{1A} receptors (Tricklebank et al., 1985a). The possibility of an interaction between 5-HT_{1A} and 5-HT_2 receptors in the expression of these behaviours was suggested by Goodwin and Green (1985). However, this has not been investigated with selective agonists and was the subject of the present study.

2. Materials and methods

2.1. Animals

Male Wistar (Mol:Wist) SPF rats weighing 170-220 g were used. The rats were housed conventionally in Macrolon type III cages in groups

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of 4-5 in animal rooms at 21 ± 1° C with a relative humidity of 55 ± 5%, air exchange (16 times per hour) and a day/night cycle (light on 6 a.m.-6 p.m.). They had free access to commercial food pellets and tap water.

2.2. *Behavioural observations*

The rats were injected s.c. with test compounds or saline and were placed individually in Perspex observation cages (12 × 25 cm). Forepaw treading behaviour was scored as absent (score 0), weak or periodic (score 1) or continuous (score 2). Each rat was scored 15, 30, 45 and 60 min after injection. The scores were accumulated for each rat. Furthermore, the head twitches were counted between 30 and 40 min after injection. ED_{50} values were calculated by log-probit analysis after the effect of each dose had been expressed as per cent of the maximum possible score above the baseline scores obtained in the control groups. The maximum score for forepaw treading was 8, and 11 episodes were considered as a maximum effect for head twitches. Comparisons between groups were made by means of the Kruskal-Wallis analysis of variance followed by individual comparisons based on the Mann-Whitney U-test.

2.3. *Receptor binding*

[^{3}H]Ketanserin binding to cortical 5-HT_2 receptors was determined as follows: the tissue was homogenized (Ultra Turrax, 10 s) in 5 ml of ice-cold 50 mM tris-buffer pH 7.7 (at 25° C). The centrifuge tubes used in this step were rinsed in ethanol by sonication for 10 min. The homogenate plus a 5 ml rinse of the homogenizer was centrifuged twice at 20000 × g for 10 min at 4° C, with rehomogenization of the pellet in 5 + 5 ml ice cold buffer. The final pellet was homogenized in 450 volumes (w/v) ice-cold buffer.

Triplicate incubation tubes kept on ice received 100 μl of drug solution in water (or water for total binding) and 1800 μl of tissue suspension (final tissue content corresponds to 4 mg original tissue). The binding experiment was initiated by the addition of 100 μl of [^{3}H]ketanserin and placing the tubes in a 37° C water bath. After incubation for 30 min the samples were filtered under vacuum (0-50 mBar) through Whatman GF/F filters (25 mm). The tubes were rinsed with 5 ml ice-cold buffer which was then poured on the filters. Thereafter, the filters were washed with 2 × 5 ml of buffer. The filters were placed in counting vials and Picofluor ^{TM}15, 4 ml, was added. After shaking for 1 h and 2-h storage in the dark the radioactivity was determined by liquid scintillation counting. The non-specific binding was determined in the presence of 1 μM of mianserin.

Inhibition of the binding of [^{3}H]8-OHDPAT or [^{3}H]5-HT to rat brain membranes was determined by methods analogous to the [^{3}H]ketanserin assay. Briefly, the rat brains were homogenized in 10 ml of 50 mM Tris buffer pH 8.0 (at 25° C) containing 120 mM NaCl, 4 mM $CaCl_2$ and 4 mM $MgCl_2$. The homogenate was centrifuged (20000 × g, 10 min, 4° C). The pellet was homogenized in 10 ml of the same buffer, incubated for 10 min at 37° C and centrifuged as above. The final pellet was homogenized in 100 volumes (w/v) of the buffer containing 10 μM of pargyline. Aliquots (10 mg tissue) were incubated for 15 min at 37° C with 1 nM of [^{3}H]8-OH-DPAT or 2 nM [^{3}H]5-HT (for 5-HT_{1A} and 5-HT_{1B} binding, respectively) alone or in the presence of test compound in a total volume of 1200 μl. After incubation, the samples were treated like the [^{3}H]ketanserin samples. Non-specific binding was obtained in the presence of 10 μM 5-HT (5-HT_{1A}) or 0.1 mM TFMPP (5-HT_{1B}).

2.4. *Drugs*

The following drugs were used: 8-hydroxy-2-(di-n-propylamine)tetralin hydrobromide (8-OHDPAT, molecular weight (mw) 328; synthesized by Dr. J. Perregaard in the Department of Medicinal Chemistry, H. Lundbeck A/S); 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI, mw 358; Research Biochemicals Inc., USA); 1-(3-trifluoromethylphenyl)piperazine dihydrochloride (TFMPP, mw 303; obtained from Aldrich Biochemicals as free base and converted to the dihydrochloride at H. Lundbeck A/S). (−)-Alprenolol tartrate (mw 399, Hässle, Sweden); ICI 118.551 (erythro-DL-1-(7-methylindan-4-

yloxy)-3-isopropylamino-butan-2-ol hydrochloride, mw 311, ICI, UK); ICS 205-930 (3α-tropanyl)-1H-indole-3-carboxylic acid ester, mw 284, Sandoz, Switzerland); ketanserin, mw 395, and ritanserin, mw 478 (both Janssen, Belgium). Serotonin creatinine sulphate (Sigma, USA); [G-^{3}H]5-hydroxytryptamine creatinine sulphate (spec. act. 18 Ci/mmol, Amersham International plc.); [ethylene-^{3}H]ketanserin hydrochloride (spec. act. 77 Ci/mmol, New England Nuclear, FRG); [2,3(n)-^{3}H]2-(N_1,N_1-dipropylamino)-8-hydroxy-1,2,3,4-tetrahydronaphthalene [^{3}H]8-OHDPAT, spec. act. 200 Ci/mmol, Amersham International plc).

ICS 205-930 was dissolved in a minimum amount of 0.1 N hydrochloric acid; ketanserin and ritanserin were dissolved in a minimum amount of tartaric acid. The solutions were diluted with saline. All other compounds were dissolved in saline. The injection volumes were 5 mg/kg body weight.

3. Results

3.1. Receptor binding

The results are shown in table 1. 8-OHDPAT and (−)-alprenolol had a selective affinity for [^{3}H]OHDPAT binding sites whereas DOI, ketanserin and ritanserin had a selective affinity for [^{3}H]ketanserin binding sites. These compounds had weak affinities for the putative 5-HT_{1B} receptor labelled by [^{3}H]5-HT. Finally, TFMPP had a preferential affinity for [^{3}H]5-HT binding sites but only a marginally weaker affinity for [^{3}H]OHDPAT and [^{3}H]ketanserin binding sites.

3.2. Effects of agonists

The results are shown in fig. 1. 8-OHDPAT induced forepaw treading with an ED_{50} of 8.0 μmol/kg = 2.5 mg/kg s.c. Following simultaneous treatment with DOI (7 μmol/kg = 2.5 mg/kg s.c.) the potency of 8-OHDPAT was increased approximately 20-fold. The ED_{50} was 0.41 μmol/kg = 0.13 mg/kg.

DOI did not induce typical forepaw treading when given alone but it did induce head twitches. The latter were significantly inhibited by 8-OHDPAT in doses from 0.24 μmol/kg, with an ED_{50} value of 0.47 μmol/kg = 0.15 mg/kg s.c.). Complete blockade was not obtained. Dose-response experiments with DOI showed that head twitches were induced with an ED_{50} value of 0.51 μmol/kg = 0.17 mg/kg s.c., while forepaw treading was absent. Forepaw treading was facilitated after combination of DOI with a threshold dose of 8-OHDPAT (1.9 μmol/kg = 0.63 mg/kg s.c.) and an ED_{50} of 0.27 μmol/kg = 0.097 mg/kg was obtained with DOI. Head twitches were significantly inhibited by 8-OHDPAT at all dosages of DOI.

TFMPP was tested at two dose levels (1.0 and 4.2 μmol/kg = 0.31 and 1.3 mg/kg, s.c.; data not shown). Neither head twitches nor forepaw treading were induced by TFMPP given alone. A slight increase in forepaw treading score followed the highest dose of TFMPP after combination with 8-OHDPAT (1.9 μmol/kg). The scores after 8-OHDPAT and after 8-OHDPAT + TFMPP were 1.8 ± 0.63 and 3.3 ± 0.37, respectively (mean ± S.E.M., n = 8, P = 0.05). The high dose of TFMPP inhibited the head twitch response to DOI (7.0 μmol/kg, s.c.). These were 11 ± 1.2 and 6.3 ± 1.3 (mean ± S.E.M., n = 4, P = 0.03) head twitches after DOI and DOI + TFMPP, respectively.

3.3. Effects of antagonists

The effects of various antagonists on the response to combined treatment with 8-OHDPAT (1.9 μmol/kg) and DOI (7.0 μmol/kg) are shown

TABLE 1

Affinity of 5-HT agonists and antagonists for 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptors. See methods for details.

	IC_{50} (nM)		
	[^{3}H]OHDPAT	[^{3}H]5-HT	[^{3}H]Ketanserin
Agonists			
8-OHDPAT	3.5	410	9200
DOI	11000	3000	110
TFMPP	460	97	360
Antagonists			
(−)Alprenolol	87	14000	6900
Ketanserin	5700	1500	2.0
Ritanserin	3800	910	0.40

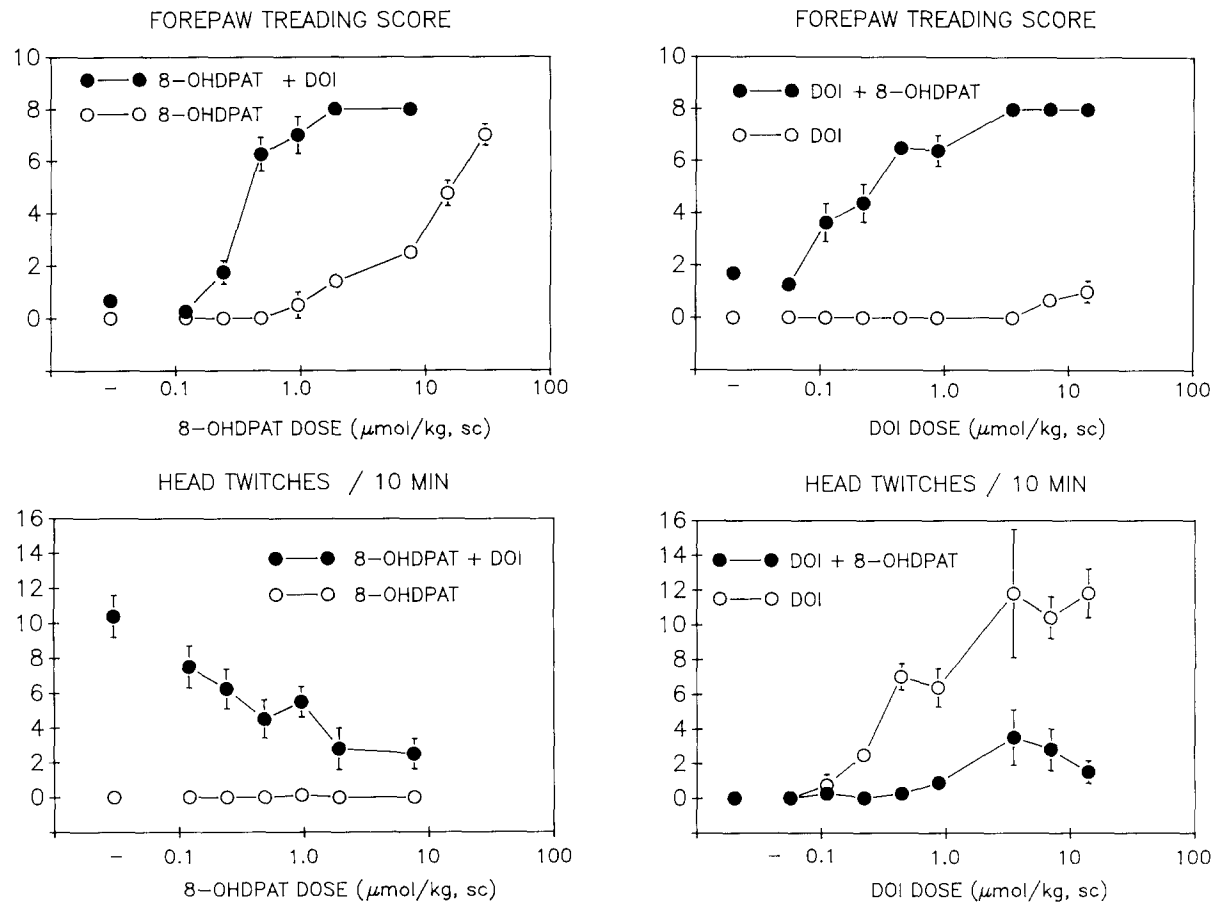

Fig. 1. Effects of 8-OHDPAT and DOI, alone and in combination, on forepaw treading and head twitches in rats. Left: 8-OHDPAT injected s.c. alone (○) or simultaneously with DOI (7 μmol/kg s.c.; ●). Forepaw treading (upper panel) was scored 15, 30, 45 and 60 min after injection as 0, 1 or 2 and the scores were added. The maximum score was thus 8. The effect of saline (○) and DOI (●) given alone is shown as single points to the left. Head twitches (lower panel) were counted in the same groups of rats from 30-40 min after injection. Right: DOI was injected alone (○) or simultaneously with 8-OHDPAT (1.9 μmol/kg s.c.; ●) and behaviour was evaluated as described above. Each point represents the mean ± S.E.M. of data from 4-8 rats. The control values are pooled results from a total of 12-16 rats.

in table 2. The 5-HT_2 antagonists, ketanserin and ritanserin, induced a dose-dependent inhibition of the forepaw treading response. The head twitch response to DOI + 8-OHDPAT was already low (see section 3.2) and was further decreased by ketanserin and ritanserin. Forepaw treading was also inhibited by (−)-alprenolol whereas the head twitches were slightly facilitated. The β-adrenoceptor antagonist, ICI 118.551, and the 5-HT_3 antagonist, ICS 205-930, had no effect on either forepaw treading or head twitches in comparison with the controls.

Finally, the effect of combined treatment with a non-effective dosage of (−)-alprenolol and either ketanserin or ritanserin was studied. Figure 2 shows that (−)-alprenolol increased the inhibitory effect of both compounds on forepaw treading, leading to almost complete blockade of the re-

TABLE 2

Effect of antagonists on forepaw treading and on head twitches induced by combined treatment with 8-OHDPAT and DOI. The antagonists were injected s.c. 30 min or 2 h (ritanserin) before combined treatment with 8-OHDPAT (1.9 μmol/kg s.c.) and DOI (7.0 μmol/kg). Parallel control groups treated with DOI alone had a forepaw treading score of 3.1% and 11.4±0.89 head twitches (n = 24). 8-OHDPAT given alone induced a forepaw treading score of 32±6.1%. No head twitches were observed.

Pretreatment	Dose (μmol/kg s.c.) [a]	Forepaw treading % max. score (mean ± S.E.M.)	Head twitches counts/10 min (mean ± S.E.M.)
Saline	– (24)	100	2.9 ±0.48
Ketanserin	0.40 (8)	88± 7.0	1.3 ±0.37
	1.6 (12)	66± 4.6 [c]	0.58±0.33 [c]
	6.3 (8)	17± 6.7 [c]	0 [c]
Ritanserin	0.33 (8)	61± 6.0 [c]	0.25±0.25 [c]
	1.3 (4)	31± 3.6 [c]	0 [b]
(−)-Alprenolol	6.3 (8)	100	4.6 ±1.3
	25 (12)	93± 6.2	5.7 ±0.99
	50 (8)	38±12 [c]	7.5 ±1.2 [d]
ICI 118.551	64 (4)	100	0.25±0.25
ICS 205-930	4.4 (4)	98± 2.0	1.8 ±1.4

[a] Number of animals shown in parentheses. [b] $P < 0.05$. [c] $P < 0.01$ significant inhibition compared with parallel control groups. [d] $P < 0.05$ significant increase compared with parallel control groups.

sponse. The head twitches were inhibited in all groups receiving ketanserin or ritanserin (data not shown).

4. Discussion

The results indicate that forepaw treading behaviour is facilitated by an interaction between 5-HT_{1A} and 5-HT_2 receptors.

Forepaw treading is part of the 5-HT syndrome and is the symptom which we find most reliable to score. Previous evidence suggests that 5-HT_{1A} receptors mediate this response (Tricklebank, 1985; Tricklebank et al., 1985a,b). This suggestion is consistent with our results showing that the maximum response is induced by the specific 5-HT_{1A} agonist 8-OHDPAT, whereas the preferential 5-HT_{1B} agonist, TFMPP, and the 5-HT_2 agonist, DOI, are ineffective. However, co-treatment with 8-OHDPAT and DOI increased the potency of 8-OHDPAT markedly. This suggests that stimulation of 5-HT_2 receptors has a permissive role for the expression of the forepaw treading syndrome.

This may also explain why 5-HT_2 antagonists (e.g. ketanserin and ritanserin; Leysen and Gommeren, 1986) have some inhibitory effect on the forepaw treading syndrome induced by 8-OHDPAT or 5-methoxy-N,N-dimethyltryptamine, a non-selective 5-HT_1/5-HT_2 agonist (Tricklebank et al., 1985a,b; see also Goodwin and Green, 1985). Ketanserin and ritanserin also inhibited the response induced by DOI plus 8-OHDPAT (table 2). A similar inhibitory effect was found with the 5-HT_1 (and β-adrenoceptor) antagonist (−)-alprenolol (Middlemiss, 1986; table 1). Supplementary evidence for a 5-HT_{1A}/5-HT_2 interaction is provided by the marked synergistic effect of (−)-alprenolol and either ketanserin or ritanserin as inhibitors of forepaw treading (fig. 2). Involvement of β-adrenoceptors is unlikely since the β-adrenoceptor antagonist, ICI 118.551, was ineffective as in other models of 5-HT_1 receptor function

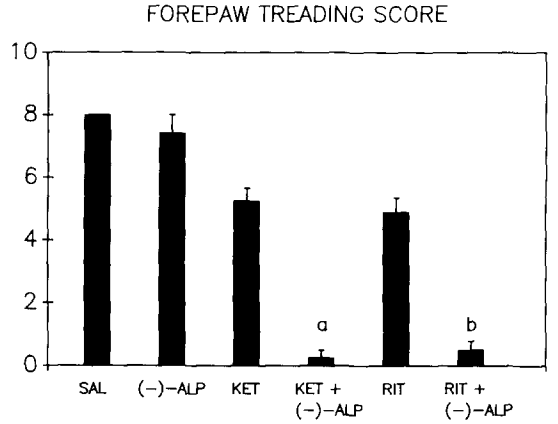

Fig. 2. Effects of antagonists alone or in combination on the forepaw treading induced by 8-OHDPAT plus DOI. Ketanserin (1.6 μmol/kg s.c.) and (−)-alprenolol (25 μmol/kg s.c.) were injected 30 min before, and ritanserin (0.33 μmol/kg s.c.) was injected 2 h before 8-OHDPAT (1.9 μmol/kg s.c.) plus DOI (7.0 μmol/kg s.c.). Scoring was according to the legend to fig. 1. The results are the means ± S.E.M. from 4 (combination treatments) or 8 rats (other groups). [a] $P < 0.01$ significant inhibition compared with ketanserin and [b] $P < 0.01$ significant inhibition compared with ritanserin.

(Tricklebank et al., 1985b; Middlemiss, 1986). The 5-HT_3 receptor antagonist ICS 205-930 (Richardson et al., 1985; Kilpatrick et al., 1987) is also ineffective.

The interpretation of our results was complicated by recent observations showing that DOI has similar affinities for 5-HT_2 and 5-HT_{1C} sites (Hoyer and Karpf, 1988). Titeler et al. (1988), however, found a 40-fold higher affinity for 5-HT_2 than for 5-HT_{1C}. Moreover, ritanserin, which is considered to be a selective 5-HT_2 antagonist (Leysen and Gommeren, 1986), has a high 5-HT_{1C} affinity (Hoyer and Karpf, 1988). However, ketanserin shows at least 50-fold selectivity for 5-HT_2 versus 5-HT_{1C} receptors (Hoyer et al., 1985; Hoyer and Karpf, 1988). The potent inhibitory effect of ketanserin against the forepaw treading induced by DOI plus 8-OHDPAT thus favors 5-HT_2 rather than 5-HT_{1C} sites as the important mediator of the effect of DOI. This conclusion is further supported by the lack of effect of TFMPP which has a high 5-HT_{1C} affinity (Hoyer, 1988) and is active in an in vivo model suggested for 5-HT_{1C} activity (Kennett and Curzon, 1988).

The facilitatory effect of 5-HT_2 stimulation raises the question of whether the effect of 8-OHDPAT given alone depends on endogenous 5-HT activation of 5-HT_2 receptors. This would be analogous to similar findings from study of dopamine (DA) D-1 and D-2 receptors. It is known that endogenous activity at D-1 receptors or treatment with a D-1 agonist is necessary for the expression of the behavioural stimulation induced by a D-2 agonist. Following subchronic DA depletion D-1 and D-2 receptors become supersensitive and function independently of each other (for review, see Clark and White, 1987). The observation that the forepaw treading induced by 8-OHDPAT was preserved in rats treated for 3 days with an inhibitor of 5-HT synthesis (Tricklebank et al., 1985a) argues against the above hypothesis. However, adaptational increases in the sensitivity of 5-HT_1 receptors have been observed as early as 1 day after lesions of 5-HT neurones (Ortmann et al., 1981). This indicates that adaptational changes may also take place after the subchronic inhibition of 5-HT synthesis and thus influence the outcome of the behavioural experiment. Clearly further work is needed before acceptance or rejection of these hypotheses.

The receptor interaction for the head twitch syndrome is different, since stimulation of 5-HT_{1A} receptors leads to inhibition of the response to a 5-HT_2 agonist. It cannot be excluded that the inhibition is caused by behavioural competition with forepaw treading, since the dose-response curves for the two responses mirror each other (fig. 1). A similar observation is that the inhibition of DOI-induced head twitches by 8-OHDPAT was partially reversed, in parallel to the inhibition of forepaw treading after treatment with (−)-alprenolol. However, preliminary results indicate that partial 5-HT_{1A} agonists such as buspirone and ipsapirone inhibit head twitches without inducing forepaw treading (Arnt, unpublished observations). An opposite 5-HT_{1A}/5-HT_2 interaction is also observed in the regulation of body temperature in rats (Gudelsky et al., 1986) and thus a similar balance model is possible for the head twitch syndrome.

The involvement of 5-HT_{1B} receptors in the expression of these behaviours is unlikely to be of importance. The preferential 5-HT_{1B} agonist TFMPP had a maximum effect at 5-HT_{1B} receptors at the doses used in the present study (Arnt, 1989) but had weak effects which showed similarities to those of 8-OHDPAT and DOI. However, since the selectivity of TFMPP for 5-HT_{1B} versus 5-HT_{1A} and 5-HT_2 receptors is limited (Glennon, 1987; present results, table 1), the weak effect of TFMPP may also depend on non-specific stimulation of 5-HT receptors.

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