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Effects of 8-OHDPAT and 5-HT_{1A} antagonists WAY100135 and WAY100635, on guinea-pig behaviour and dorsal raphe 5-HT neurone firing

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1 The effects of 5-HT_{1A} antagonists on guinea-pig behaviour and dorsal raphe neuronal activity were investigated.

2 WAY100135 (10 mg kg^{-1}, s.c.) and WAY100635 (1 mg kg^{-1}, s.c.) significantly reduced the behaviours induced by 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) (1 mg kg^{-1}, s.c.) indicative of post-synaptic 5-HT_{1A} receptor antagonism. WAY100635 (10 mg kg^{-1}, s.c.) alone induced ear twitches, which were antagonized by ketanserin (1 mg kg^{-1}, s.c.), but no other overt behaviours.

3 WAY100635 (0.125 mg kg^{-1}, i.v.) produced a right-ward shift in the dose-related inhibition of neuronal firing by 8-OHDPAT (5–100 $\mu g\ kg^{-1}$, i.v.) but did not affect the maximum inhibition induced by 8-OHDPAT indicating competitive antagonism between 8-OHDPAT and WAY100635 at the 5-HT_{1A} somato-dendritic autoreceptor in the dorsal raphe nucleus of the guinea-pig. WAY100635 also produced a dose-related increase in the basal firing of 5-HT neurones in the dorsal raphe nucleus and restored the firing of dorsal raphe neurones previously inhibited by 8-OHDPAT (10 $\mu g\ kg^{-1}$, i.v.).

4 The results indicate that WAY100635 is a competitive 5-HT_{1A} antagonist in the guinea-pig. Furthermore WAY100635 can increase 5-HT neuronal firing, suggesting that it blocks a 5-HT_{1A} receptor-mediated inhibitory tone acting on guinea-pig 5-HT neurones resulting in increased 5-HT release and 5-HT_2 receptor-mediated behaviours.

Keywords: 5-HT_{1A} antagonists; dorsal raphe nucleus; somatodendritic autoreceptor; behavioural syndrome in guinea-pig; 8-OHDPAT; WAY100135; WAY100635

Introduction

The relative selective 5-HT_{1A} agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OHDPAT) (Arvidsson *et al.*, 1981; Hjorth *et al.*, 1982) elicits several components of the 5-hydroxytryptamine (serotonin) syndrome such as head weaving, reciprocal forepaw treading and flat body posture in rats (Tricklebank *et al.*, 1984) though the role of 5-HT_7 receptors remains to be determined (Lovenberg *et al.*, 1993). Presynaptic 5-HT depletion does not affect the 8-OHDPAT-induced syndrome in either rats or mice (Dourish *et al.*, 1985; Yamada *et al.*, 1988) indicating that the elements of the 5-HT syndrome are mediated via postsynaptic 5-HT_{1A} receptors. An increase in 5-HT synthesis and hence 5-HT levels (Hess & Doepfner, 1961; Grahame-Smith 1971a; Ortmann *et al.*, 1980) also elicits the syndrome, as do the direct agonists 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) (Grahame-Smith 1971b; Green *et al.*, 1981), 5-methoxytryptamine (Green *et al.*, 1976), (+)-lysergic acid diethylamide (LSD) (Trulson & Jacobs, 1976a) and quipazine (Green *et al.*, 1976; 1981). Furthermore 5-HT releasers such as fenfluramine and *p*-chloroamphetamine (Trulson & Jacobs, 1976b; Deakin & Green, 1978) also cause elements of the 5-HT syndrome.

There is behavioural evidence to suggest species differences in the response to 8-OHDPAT. In mice the behavioural syndrome occurs only when 8-OHDPAT is administered at high doses intravenously, whereas in rat it occurs in response to low doses administered by subcutaneous, intraperitoneal and intravenous routes (Yamada *et al.*, 1988). The syndrome can be antagonized by the 5-HT_1 antagonists, spiperone and metergoline, in both species, but to a greater extent in the mouse, again indicating that the behaviours are due to direct agonism at 5-HT_1 receptors. The introduction of the selective 5-HT_{1A} antagonists, WAY100135 (N-tert-butyl-3-(4-(2-methoxyphenyl)piperazine-1-yl)-2phenylpropanamidedihydrochloride) (Fletcher *et al.*, 1993a,b) and the more potent WAY100635 (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(pyridinyl) cyclo hexanecarboxamide trihydro-chloride (Fletcher *et al.*, 1994), make it possible to determine more precisely elements of the 5-HT behavioural syndrome produced by 5-HT_{1A} receptor stimulation. 5-HT_{1A} receptors are also located presynaptically where they act as the somato-dendritic autoreceptor in the dorsal raphe nucleus (DRN) of the guinea-pig (Mundey *et al.*, 1994a).

The aim of the following experiments was to investigate the effects of 8-OHDPAT and selective 5-HT_{1A} antagonists on 5-HT_{1A} receptor-mediated behaviour and DRN neuronal firing in the guinea-pig.

Methods

Drug-induced behavioural studies

Animals Male Dunkin-Hartley guinea-pigs (390–450 g) (Tuck, Essex) were housed individually in racks with free access to food and water (food pellets, CRM, SDS, Cambridge) under a 12 h light/dark schedule at 20±1°C. Preliminary studies showed that group-housed guinea-pigs which were suddenly isolated displayed prolonged immobility so they were housed singly to minimize any disturbance caused by being placed singly in observation rings prior to testing. Light in-

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tensity in the testing room was 750 lux (white light). On the morning of the experiments the guinea-pigs were not disturbed, any necessary cleaning was done the day before and all testing took place during the light phase of the light/dark cycle.

Behavioural studies Overt behaviour was monitored in a perspex arena 16 x 12 placed on absorbent paper. Six arenas in a row allowed six individual guinea-pigs to be observed simultaneously with a high degree of accuracy. Six guinea-pigs were monitored for a period of 1 h in each trial. For each experiment 36 guinea-pigs were randomly assigned to 6 coded treatment groups such that the tester was unaware of the drugs being injected. The experiments were carried out so that half of each treatment group was tested in the morning and the other half in the afternoon. This helped to eliminate any errors incurred due to diurnal variations in behaviour or metabolism.

8-OHDPAT behavioural syndrome scoring system After the administration of 8-OHDPAT to guinea-pigs in preliminary studies, various behaviours were noted, categorised and subsequently scored as follows: motor stimulation (0–3), flat body posture (0–2), and head jerks (0–2) where 0 = absent, 1 = present, 2 = moderate, 3 = continuous. Tremor (0–1), forepaw treading (0–1), hindlimb abduction (0–1) were scored as absent (0) or present (1). This method allowed an individual guinea-pig to attain a maximum score of 10 per scoring session.

The identification of behaviours elicited by 8-OHDPAT in the guinea-pig 8-OHDPAT was administered at doses of 0.01, 0.1, 0.3, 1.0 and 3.0 mg kg^{-1}, s.c. and behaviours compared with saline-treated controls. Behaviour was scored as described every 10 min for 60 min.

Antagonism of 8-OHDPAT-induced behavioural effects by the selective 5-HT_{1A} antagonists, WAY100135 and WAY100635 Both the behavioural effects of the antagonists alone and their effects on 8-OHDPAT-induced behaviour were investigated using WAY100135 (10, 30 mg kg^{-1}, s.c.) and WAY100635 (1, 10 mg kg^{-1}, s.c.). The antagonists were administered 30 min prior to 8-OHDPAT (1 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}, s.c.) and behaviour scored every 10 min for 60 min.

Comparison of the effect of 1-(2,5-dimethoxy-4-indophenyl)-2-aminopropane (DOI) and WAY100635 on behaviour in the guinea-pig Guinea-pigs were given either saline, DOI (0.1, 0.3, 1.0, 3.0 and 6.0 mg kg^{-1}, s.c.) or WAY100635 (1, 10 mg kg^{-1}, s.c.). The effect of the predominantly selective 5-HT_2 antagonist, ketanserin (Niemegeers *et al.*, 1983) on the behavioural effects of DOI or WAY100635. The animals were scored for wet dog shakes (whole body shakes) for 30 min.

Effects of WAY100635 on behaviour induced by carbidopa plus 5-hydroxytryptophan (5-HTP) In an attempt to determine whether increased extracellular 5-HT was involved in the behavioural effects produced by WAY100635 (1 mg kg^{-1}, s.c.) guinea-pigs were given carbidopa (25 mg kg^{-1}, i.p.) at time 0 and then 5-HTP (10 or 50 mg kg^{-1}, s.c.) 30 min later, WAY100635 (1 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}, s.c.) was administered 30 min later, after which the guinea-pigs were observed for wet dog shakes until 90 min after the first injection.

Data analysis

Data were analysed by a 2-way analysis of variance (ANOVA) with repeated measures, and *post-hoc* comparison, where appropriate, was carried out using Fisher's Protected LSD. $P<0.05$ was considered significant. Dunnett's t test was also used where appropriate as indicated in the results section.

Drugs

All drugs (apart from 5-HTP, Sigma) used in behavioural studies were synthesized in the Department of Medicinal Chemistry, Wyeth Research (U.K.), Taplow and were administered s.c. in a volume of 1 ml kg^{-1}. Antagonists were given 30 min before the agonist with the two drugs given on opposite sides of neck. 8-OHDPAT, DOI and 5-HTP was dissolved by gentle warming in 0.9% saline. WAY100135 (racemate) was dissolved in HPMC (hydroxypropylmethyl cellulose-5% in water) with 10% PEG (polyethyleneglycol) and WAY100635 in HPMC alone. Once dissolved all drugs were adjusted to pH 7.4. All doses are stated as the base equivalent. In studies involving WAY100135 and WAY100635, the controls received an equivalent volume of HPMC, and in 8-OHDPAT studies, saline.

Electrophysiology

Male Dunkin-Hartley guinea-pigs (300–400 g) were anaesthetized with urethane (1.3 g kg^{-1}, i.p.) and the jugular vein was cannulated for intravenous drug administration. Single barrelled glass microelecrodes with an *in vitro* impedance of 4–6 MΩ and filled with 2 M NaCl containing 2% pontamine sky blue dye, were stereotaxically implanted into the dorsal raphe nucleus (DRN). The coordinates, taken from lambda, were: anterior +0.7–1.00 mm, lateral 0.0 mm, ventral 6.5–7.5 mm (Mundey *et al.*, 1994a). Unit activity was amplified by a high impedance amplifier with filters (Neurolog, Digitimer) and separated from background noise with a variable threshold spike trigger. Integrated firing rate was completed in 10 s sampling periods from an electrical rate meter triggered by the individual action potentials. At the end of the recording a negative 20 μA cathodal current was passed through the electrode to eject the dye from the tip permitting histological verification of the recording site. The brains were removed, fixed in 10% formalin solution and sectioned (40 μm) to locate the blue dye spots. 8-OHDPAT and WAY100635 were dissolved in 0.9% saline solution and were administered intravenously in a 1.0 ml kg^{-1} injection volume.

Results

Behavioural studies

General behaviour of saline-treated guinea-pigs in the observation arena Most of the guinea-pigs, when initially placed into the observation arena, displayed a natural investigative behaviour for at least a minute, consisting mainly of circling and rearing, while the remainder instantly became immobile, a common response for guinea-pigs placed in a novel environment. After these initial responses all the guinea-pigs were relatively inactive with occasional periods of grooming and chewing.

The behavioural effect of 8-OHDPAT. 8-OHDPAT (0.01, 0.1, 0.3, 1.0 and 3.0 mg kg^{-1}, s.c.) induced dose-related behavioural effects which included the following:- tremor, continuous episodic jerky movement, head lifting, shuffling, sniffing, biting, selective flattening of the hind quarters, head rotation, head jerks, hindlimb abduction and forepaw treading. This behaviour was quantified by use of the scoring method described in the Methods section (Figure 1).

The first signs of the 8-OHDPAT-induced 5-HT syndrome occurred within 50–70 s of administration. Selective flattening of the hind quarters and tremor were the first obvious features, with increased rearing and sniffing of the air. Flat body posture was most pronounced at the highest dose (3.0 mg kg^{-1}) (Figure 1) and within 2 min these animals had clear impairment of hind limb function accompanied by whole body jumps and head jerks. This extensive almost myoclonic behaviour began to decline after 15 min. The guinea-pigs had increased defae-

cation and urination at the top dose. At 1 h the animals still showed increased levels of rearing and exploration with tremor.

At the lowest dose (0.01 mg kg^{-1}, s.c.) tremor was evident within 3 min. Hindlimb lifting and a degree of shuffling were displayed for up to 20 min after which the animals sat completely still; a dose of 0.1 mg kg^{-1}, s.c. was high enough to induce selective flattening of the hind quarters, an increase in rearing and head weaving 5 min after administration. Most of the behaviours had subsided by 40 min leaving only tremor by 60 min.

With an intermediate dose of 8-OHDPAT (0.3 mg kg^{-1}, s.c.) by 2 min the guinea-pigs displayed tremor, selective flattening of hind quarters, increased rearing, head jerks, head weaving, forepaw treading, shuffling and splaying of the legs. All the behaviours described above were apparent with the 1.0 mg kg^{-1} dose, but to a greater extent and accompanied by slight ataxia. On the basis of these results, 8-OHDPAT (1.0 mg kg^{-1}, s.c.) was selected as the submaximal dose for subsequent antagonist studies.

Antagonism of 8-OHDPAT-induced behavioural effects by the selective 5-HT_{1A} antagonists, WAY100135 and WAY100635

WAY100135 (10 mg kg^{-1}, s.c.) or WAY100635 (1 mg kg^{-1}, s.c.) given 30 min before 8-OHDPAT (1 mg kg^{-1}, s.c.) completely prevented any syndrome behaviour (Figure 2). WAY100135 (10 mg kg^{-1}, s.c.) or WAY100635 (1 mg kg^{-1}, s.c.) given alone produced no specific behaviours; however, at a higher dose of WAY100635 (10 mg kg^{-1}, s.c.), the guinea-pigs displayed decreased activity within 5 min of administration and, whilst the other treatment groups would react to an auditory stimulus, these showed no reaction. This implied that the guinea-pigs were mildly sedated, but if touched they reacted instantly and darted around the arena indicating that normal coordinated movement was unaffected. An unexpected observation was that the high dose of WAY100635 induced ear twitching which was seen 20–45 min after antagonist administration and occured either in bursts of a few (3–4) in each ear or as a single twitch in a one ear.

Comparison of the effect of DOI and WAY100635 on behaviour in the guinea-pig and the effect of ketanserin DOI (0.1–3.0 mg kg^{-1}, s.c.) induced a dose-related increase in head and wet dog shakes (Figure 3); these were observed within 2 min of administration (Figure 4). Another behaviour observed was a form of back muscle contraction associated with a shiver down

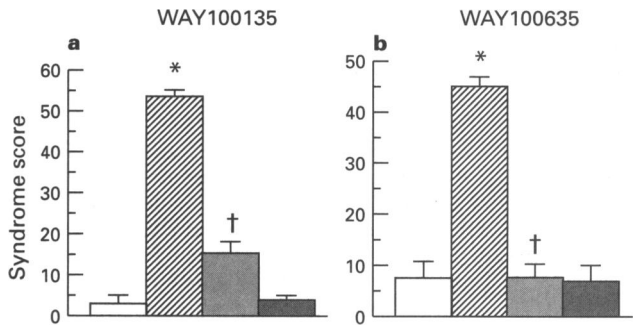

Figure 2 Antagonism of 8-OHDPAT (1.0 mg kg^{-1}, s.c. hatched column)-induced behaviour by WAY100135 (10 mg kg^{-1}, s.c., stippled column) given 30 min before 8-OHDPAT (a) and by WAY100635 (1.0 mg kg^{-1}, s.c., solid column) (b). Each column represents the mean syndrome score (±s.e.mean, $n=6$). The antagonists alone had no effect on behaviour (solid columns). *Significantly different from saline control (open column); †significant antagonism by WAY100135 or WAY100635 to 8-OHDPAT alone ($P<0.05$, Dunnett's t test).

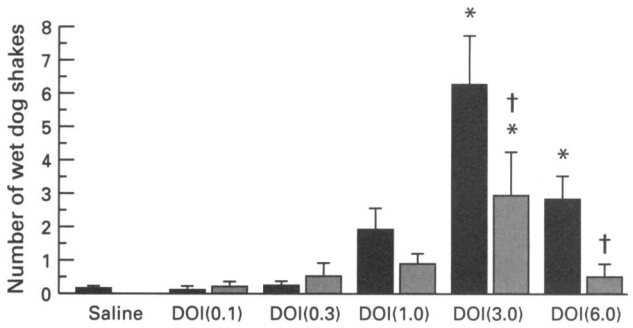

Figure 3 Production of wet dog shakes by DOI (0.1–6.0 mg kg^{-1}, s.c.) alone (solid columns), and their antagonism by the pretreatment with ketanserin (1 mg kg^{-1}, s.c.) (stippled columns). Each column represents the mean number of wet dog shakes (±s.e.mean, $n=6$). There was a dose-related increase in wet dog shakes with DOI (0.1–3.0 mg kg^{-1}, s.c.) with a reduced response at the top dose (6.0 mg kg^{-1}, s.c.). Ketanserin significantly reduced this behaviour. *Significantly different from control; †significant antagonism by ketanserin ($P<0.05$, Dunnett's t test).

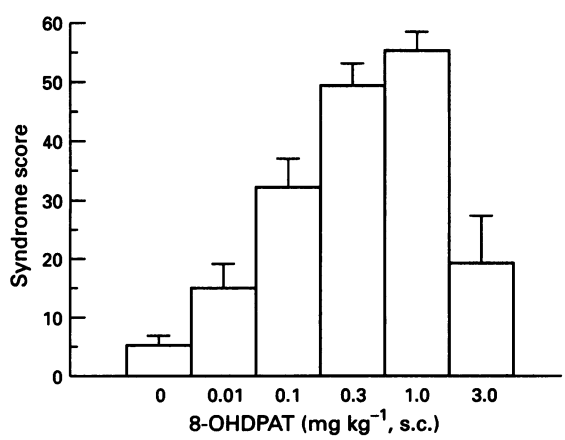

Figure 1 Dose-related 8-OHDPAT behavioural syndrome in guinea-pigs. Each column represents the mean syndrome score (±s.e.mean, $n=6$). Each animal was scored as described in the methods every 5 min for the first 15 min, and then every 10 min for 30 min. These data were sampled at the 15 min time point. (Maximum score = 60).

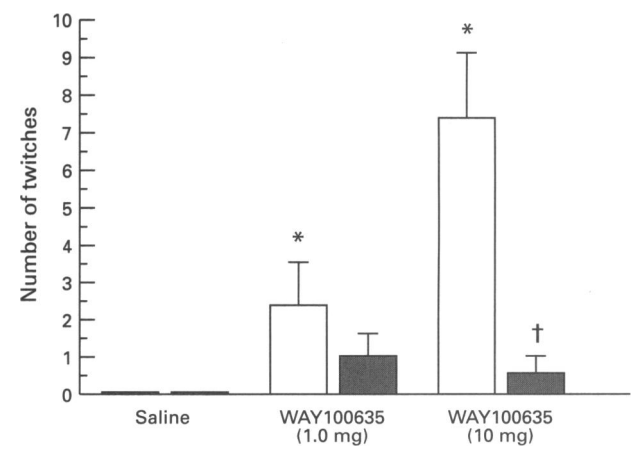

Figure 4 Occurrence of ear twitches following administration of WAY100635 (1 and 10 mg kg^{-1}, s.c.) alone (open columns) and their antagonism by ketanserin (1 mg kg^{-1}, s.c.) (solid columns). Each column represents the mean number of twitches (±s.e.mean, $n=8$). *Significantly different from saline controls; †significant antagonism of ear twitches by ketanserin ($P<0.05$, Dunnett's t test).

the back. The guinea-pigs also displayed dose-related forepaw licking, rearing, sniffing, digging, some flat body posture and stretching of the body across the width of the arena whilst yawning. The majority of the behaviours were not apparent by 20 min except for the back muscle contractions which declined within 30 min. DOI did not induce ear twitches similar to those seen after the high dose of WAY100635. Pretreatment with ketanserin (1.0 mg kg^{-1}, s.c., 30 min before DOI), significantly attenuated the wet dog shakes induced by DOI (0.1–3.0 mg kg^{-1}, s.c.) (Figure 3). WAY100635 (10 mg kg^{-1}, s.c.) induced ear twitches as previously described and these were also antagonized by ketanserin (1.0 mg kg^{-1}, s.c.) (Figure 4).

Effects of WAY100635 on behaviour induced by carbidopa plus 5-HTP Carbidopa (25 mg kg^{-1}, i.p.) plus 5-HTP (150 mg kg^{-1}, s.c.) induced significant wet dog shake behaviour in guinea-pigs from 60 min after 5-HTP administration. 5-HTP (100, 150 and 200 mg kg^{-1}, s.c.) also caused severe involuntary body jerks rather than classical wet dog shakes from 30–40 min post drug administration. The number of body jerks varied widely throughout the treatment group, but as many as 40–60 per min were observed. The first overt behavioural response to 5-HTP administration was a simple head nodding which was superseded by wet dog shakes. WAY100635 (1 mg kg^{-1}, s.c.) alone induced no more wet dog shakes than saline controls (1.8±0.8); however when administered 30 min after 5-HTP (50 mg kg^{-1}, s.c.), the number of wet dog shakes counted for 15 min from 15 min after the last injection increased from 9.0±2.1 in the animals given carbidopa and 5-HTP to 14.2±1.7 in those also given WAY100635.

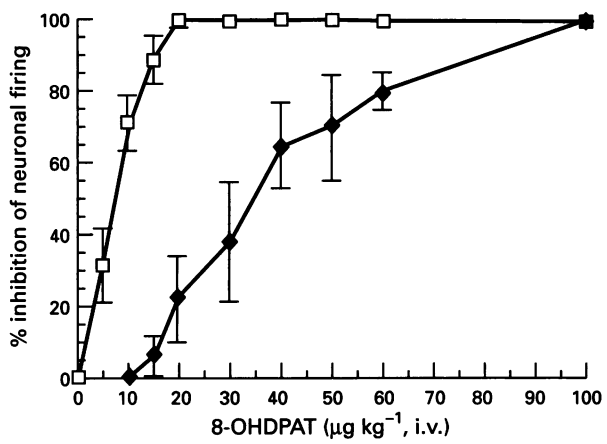

Figure 5 Dose-related inhibition (ED_{50} = 6.6±1.5 μg kg^{-1}, i.v.) of slow firing neurones (0.2–4.0 spikes s^{-1}) in the guniea-pig DRN by 8-OHDPAT (5–100 μg kg^{-1} i.v.) (□) and the competitive antagonism of this by the 5-HT_{1A} antagonist, WAY100635 (0.125 mg kg^{-1}, i.v.) (◆). The ED_{50} for 8-OHDPAT after prior treatment with WAY100635 (0.125 mg kg^{-1}, i.v.) increased to 34.8±5.6, μg kg^{-1}, i.v. from 6.6±1.5 μg kg^{-1}. Points are mean values±s.e.mean (n = 6).

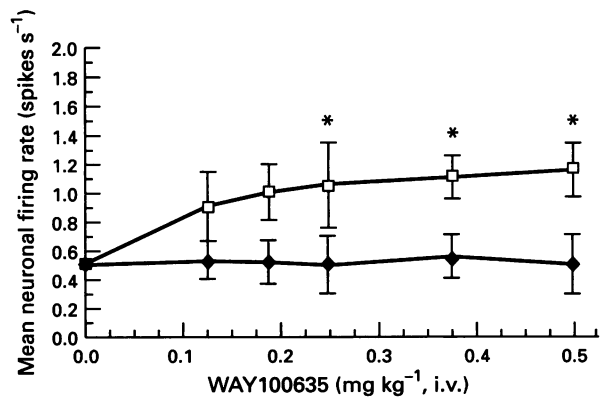

Figure 6 WAY100635 (0.125–0.5 mg kg^{-1}, i.v.) (□) increases basal rate of firing of 5-HT neurones in the guinea-pig DRN. The neurones used for both the saline (◆) and WAY100635 were first shown to be sensitive to 8-OHDPAT (10 μg kg^{-1}, i.v.). Each point is the mean firing rate±s.e.mean (n = 8). *Significantly different from saline controls, $P<0.05$, Dunnett's t test.

Electrophysiology

Effect of 8-OHDPAT and WAY100635 on DRN 5-HT neuronal firing In the guinea-pig 5-HT neurones were initially identified using criteria similar to those for the rat, namely that extracellular spikes of certain raphe units displayed a slow (0.2–4.0 spikes s^{-1}) regular firing rate and these neurones were inhibited by 8-OHDPAT (10 μg kg^{-1}, i.v.) for between 20–30 min. The action potential had a long duration (1.5–2.5 ms) with amplitudes ranging from 0.2–0.3 mV. Units with a different type of firing pattern were also found in the DRN with rates up to 2–3 times faster. These faster firing cells were not inhibited by 8-OHDPAT (0.5–100 μg kg^{-1}, i.v.) and only neurones which were inhibited by 8-OHDPAT (10 μg kg^{-1}, i.v.) were used for further experiments. Histological analysis revealed that all units recorded which were inhibited by 8-OHDPAT were located within the midline of the guinea-pig DRN. 8-OHDPAT produced a dose-related inhibition of the slow firing neurones in the guinea-pig DRN (Figure 5). WAY100635 (0.125 mg kg^{-1}, i.v.) antagonized the 8-OHDPAT (0.5–100 μg kg^{-1}, i.v.) induced inhibition of neuronal firing (Figure 5) and shifted the 8-OHDPAT dose-response curve to the right. The dose-related inhibition of unit activity by 8-OHDPAT (5–100 μg kg^{-1}, i.v.) produced an ED_{50} of 6.6±1.5 μg kg^{-1}, i.v. and in the presence of WAY100635 (0.125 mg kg^{-1}, i.v.), this increased to 34.8±5.6 μg kg^{-1}, i.v.

Under basal conditions WAY100635 (0.125–0.5 mg kg^{-1}, i.v.) alone increased the firing rate in a shallow dose-related manner in neurones previously shown to be sensitive to 8-OHDPAT (10 μg kg^{-1}, i.v.) (Figure 6). This increase generally occurred 1–3 min after administration and for a dose of 0.125 mg kg^{-1}, i.v., the firing remained at an elevated level for between 10–15 min and up to 30 min with 0.5 mg kg^{-1}, i.v. WAY100635 also restored the neuronal firing in a cell which was previously inhibited by 8-OHDPAT (10 μg kg^{-1}, i.v.) and when administered together with 8-OHDPAT, the inhibition of firing by 8-OHDPAT (10 μg kg^{-1}, i.v.) was completely blocked with no detectable change in firing.

Discussion

Guinea-pigs displayed a dose-related increase in the behavioural syndrome produced by the 5-HT_{1A} agonist, 8-OHDPAT (0.01–1.0 mg kg^{-1}, s.c.) and in subsequent studies the 5-HT_{1A} antagonist, WAY100135 (10 mg kg^{-1}, s.c.) and WAY100635 (1 mg kg^{-1}, s.c.) produced full antagonism of the syndrome induced by 8-OHDPAT. This indicated that the majority of the 8-OHDPAT-induced behaviours in the guinea-pig result from postsynaptic 5-HT_{1A} receptor stimulation similar to results reported in rodents (Tricklebank *et al.*, 1984; Dourish *et al.*, 1985; Yamada *et al.*, 1988). The role of other 5-HT receptor subtypes with which 8-OHDPAT has affinity (e.g. 5-HT_7, Lovenberg *et al.*, 1993) needs clarification. The behaviours produced by 8-OHDPAT in the guinea-pig were similar to those described in rats; the only difference being that in the guinea-pig, low doses induced tremor, which occurs only at higher doses in rats (Tricklebank *et al.*, 1984), and guinea-pigs displayed more head movements and hyperactivity rather than the hindlimb abduction and forepaw treading seen in rats.

The most striking behaviour in the guinea-pig produced by a high dose of 8-OHDPAT (3.0 mg kg^{-1}, s.c.) was whole body jumps with associated vocalisations. This behaviour is similar

to myoclonic jerks produced by 5-HTP and carbidopa in the guinea-pig (Klawans *et al.*, 1973) indicating that there may be a role of the 5-HT_{1A} receptor in the eliciting of these characteristic actions as previously suggested by Luscombe *et al.* (1981). The 5-HT_{1B} agonist, RU24969, evokes myoclonus (Luscombe *et al.*, 1982a, b) but this drug also has affinity for the 5-HT_{1A} receptors (Hamon *et al.*, 1986). In a previous study the non-selective 5-HT_1 antagonist, metergoline, blocked myoclonic behaviour whereas other antagonists such as methysergide and mianserin only had weak effects (Chadwick *et al.*, 1978; Luscombe *et al.*, 1981). In summary, there is evidence indicating involvement of postsynaptic 5-HT_{1A} receptor activation in the 5-HT behavioural syndrome in rats, mice (Tricklebank *et al.*, 1984; Dourish *et al.*, 1985; Yamada *et al.*, 1988), and guniea-pig while the present study clearly demonstrates that both WAY100135 and WAY100635, selective antagonists at the 5-HT_{1A} receptor (Fletcher *et al.*, 1993a, b), blocked not only the classical components of the 5-HT behavioural syndrome but also the myoclonus elicited by 8-OHDPAT. The results also indicate that WAY100635 is at least 10 fold more potent than WAY100135 as a postsynaptic 5-HT_{1A} antagonist. Having established that WAY100135 and WAY100635 antagonized postsynaptic 5-HT_{1A} receptors, the ability of these compounds to antagonize presynaptic 5-HT_{1A} receptor function was investigated.

A previous electrophysiological study (Mundey *et al.*, 1994a) demonstrated that the presynaptic somato-dendritic autoreceptors in the guinea-pig DRN were of the 5-HT_{1A} subtype and that the 5-HT_{1A} antagonist, WAY100135, blocked the agonist effects of 8-OHDPAT on the somato-dendritic autoreceptor in the guinea-pig dorsal raphe nucleus but had no significant effect on basal neuronal firing when administered alone. In the present study WAY100635 (0.125 mg kg^{-1}, i.v.) shifted the dose-response for the effects of 8-OHDPAT on neuronal firing to the right without effect on the maximum inhibition elicited by 8-OHDPAT; evidence of competitive antagonism of the 8-OHDPAT response by WAY100635 at the 5-HT_{1A} somato-dendritic autoreceptors in the dorsal raphe nucleus of the guinea-pig. The 8-OHDPAT-induced inhibition was also reversed by WAY100635 so that if the neuronal activity was already inhibited with 8-OHDPAT, administration of WAY100635 counteracted this and neuronal firing rapidly resumed. Therefore WAY100635 not only antagonized the inhibitory effect of 8-OHDPAT on dorsal raphe neuronal firing, but also restored firing of an inhibited cell.

WAY100635 produced some further interesting electrophysiological data as it caused a dose-related increase in dorsal raphe neuronal firing; an effect not seen with the less potent analogue, WAY100135 (Mundey *et al.*, 1994b) indicating the existence of a 5-hydroxytryptaminergic tone in the guinea-pig dorsal raphe nucleus possibly modulated by an inhibitory 5-HT_{1A} autoreceptor. The 5-HT_{1A} antagonist, by blocking this site, lifts this inhibitory influence on neuronal firing in the raphe, hence elevating the unit activity and consequently 5-HT synthesis and release. Administration of other 5-HT antagonists such as spiperone (Fornal *et al.*, 1989) and (S)-UH-301 (Arborelius & Svensson, 1992) and (−)-tertatolol (Jolas *et al.*, 1993) can also increase DR neuronal firing in the cat and rat. However when 5-HT turnover was monitored in terminal regions in rats after the administration of (−)-tertatolol (Jolas *et al.*, 1993) and (S)-UH-301 (Bjork *et al.*, 1991), there was no increase as one would expect if a tonic inhibition over the firing of dorsal raphe neurones was prevented. These antagonists however produced only an increase in about a third of the cells tested and so it is possible that any enhancement of 5-HT turnover was undetected as the majority of the cells were under tonic inhibition. Microdialysis studies in the rat have measured the effect of chronic (14 day treatment) and acute effects of WAY100635 (1 mg kg^{-1}, s.c.) on 5-HT release in the hippocampus and demonstrated no significant effects on basal 5-HT levels (Gurling *et al.*, 1994a), however, in another study looking at the diurnal variation in terminal release of 5-HT, WAY100635 increased release on 5-HT during the night but had no effect during the day indicating a diurnal variation in 5-hydroxytryptaminergic tone in the rat governed by the state of arousal (Gurling *et al.*, 1994b). WAY100635 also increased the dorsal raphe 5-HT firing in the freely moving cat when neuronal activity is high but had no effect on firing during sleep when neuronal activity is low (Fornal *et al.*, 1994). The present electrophysiological studies in the guinea-pigs where WAY100635 increased 5-HT neuronal firing in the DRN, were carried out during the day in anaesthetized animals. Thus there appear to be species differences in the 5-hydroxytryptaminergic tone of the DRN and functional responsiveness of the somato-dendritic autoreceptor under different physiological conditions. The guinea-pig data warrant further investigation using measurement of 5-HT release in terminal regions in the freely moving guinea-pig, in order to verify whether there is increased terminal 5-HT release after an acute dose of WAY100635.

The increase in firing produced by WAY100635 raised the question as to whether this was associated with any behavioural respon particular the ear twitching induced by the higher dose c AY100635. Skingle *et al.* (1991) reported a 'fanning' of guinea-pig's ears associated with vocalisation after the administration of 5-HTP which was subsequently described as a type of wet dog shake response. The ear twitch behaviour induced by WAY100635 could be a mild form of the head shakes and wet dog shakes described by Skingle *et al.* (1991) elicited by increased release of 5-HT acting on 5-$HT_{2A/2C}$ receptors. However, the 5-$HT_{2A/2C}$ receptor agonist, DOI failed to produce similar ear twitches whilst causing a range of other behaviours e.g. wet dog shakes which were antagonized by the 5-$HT_{2A/2C}$ antagonists, ketanserin indicating the involvement of 5-HT_2 receptors (Heaton *et al.*, 1988; Fone *et al.*, 1991). While DOI administration did not induce ear twitches, ketanserin fully antagonized the ear twitches induced by the higher doses of the 5-HT_{1A} antagonist, supporting the view that the twitches involve 5-HT_2 receptor activation. It remains to be determined which 5-HT_2 receptor subtype is involved; a question that may be answered with the development of relatively selective antagonists (Baxter *et al.*, 1995).

Assuming the WAY100635-induced increase in dorsal raphe 5-HT neuronal firing resulted in increased terminal 5-HT release, we looked next at the behavioural effect of pharmacologically increasing 5-HT release by giving 5-HTP in combination with the peripheral decarboxylase inhibitor, carbidopa. 5-HTP administered alone or in conjunction with carbidopa induces wet dog shake behaviour in rats (Bedard & Pycock, 1977) and head shakes and tremor in mice (Martin & Sanders-Busk, 1982). 5-HTP (25–200 mg kg^{-1}, s.c.) induced dose-related wet dog shake behaviour in the guinea-pig along with inconsistent body jerks, head nods and fanning of the ears which has been likened to the wet dog shake in the rat (Skingle *et al.*, 1991). Higher doses (150 and 200 mg kg^{-1}, s.c.) produced myoclonus, identified by a rhythmic, and substantial jerking of the entire body as previously described by Volkman *et al.* (1987). WAY100635 given before 5-HTP (50 mg kg^{-1}, s.c.) tended to increase the wet dog shake response supporting the view that WAY100635 enhanced the increase in 5-HT release produced by 5-HTP administration resulting in potentiation of the wet dog shake response.

In conclusion, WAY100135 and WAY100635 antagonized postsynaptic 5-HT_{1A} receptor-mediated behaviour and myoclonus in the guinea-pig. WAY100635 showed competitive antagonism of the 8-OHDPAT-induced inhibition of 5-HT neuronal firing in the DRN and caused a dose-related increase in basal dorsal raphe neuronal firing suggesting the presence of a 5-HT_{1A}-mediated 5-hydroxytryptaminergic tone. High doses of WAY100635 produced ear twitches in guinea-pigs. This behaviour was not produced by administration of DOI or 5-HTP plus carbidopa but was antagonized by ketanserin indicating possible 5-HT_2 receptor mediation; however, the precise mechanism by which the behaviour occurs remains unknown.

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