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Investigation of stretching behaviour induced by the selective 5-HT$_6$ receptor antagonist, Ro 04-6790, in rats

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1 The present study examined the effects of the selective 5-HT$_6$ receptor antagonist 4-amino-N-(2, 6 bis-methylamino-pyrimidin-4-yl)-benzene sulphonamide (Ro 04-6790) on locomotor activity and unconditioned behaviour in male Sprague Dawley rats (230–300 g).

2 In non-quantified behavioural observations, animals treated with Ro 04-6790 (3, 10 or 30 mg kg^{-1}, i.p) showed no overt behavioural signs except a dose-dependent reduction in locomotor activity and a behavioural syndrome of stretching, yawning and chewing. The latter behaviour was most pronounced between 30 and 90 min following the administration of Ro 04-6790.

3 Detailed analysis of the stretching and yawning behaviour showed that Ro 04-6790 (3, 10 or 30 mg kg^{-1}, i.p.) dose-dependently induced stretching. The number of stretches observed following treatment with either Ro 04-6790 (10 mg kg^{-1} i.p.) or Ro-04-6790 (30 mg kg^{-1}, i.p.) was significantly greater than that observed in saline-treated rats. The yawning behaviour, however, was not dose-dependent nor was the number of yawns in any of the drug treated groups significantly greater than in those treated with saline.

4 Pretreatment (30 min) with the non-selective muscarinic antagonists scopolamine (0.1, 0.3 or 1 mg kg^{-1}, i.p.) and atropine (0.3, 1 or 3 mg kg^{-1}, s.c.) but not methylatropine (1, 3 or 10 mg kg^{-1}, s.c) significantly inhibited stretching induced by Ro 04-6790 (30 mg kg^{-1}, i.p.).

5 The dopamine D$_2$-like receptor antagonist, haloperidol (0.03, 0.1 or 0.3 mg kg^{-1}, s.c.) given at the same time as Ro 04-6790 (30 mg kg^{-1}, i.p.) had no effect on the stretching induced by the 5-HT$_6$ antagonist.

6 These data suggest that systemic injection of the 5-HT$_6$ antagonist, Ro 04-6790, produces a stretching behaviour that appears to be mediated by an increase in cholinergic neurotransmission in the CNS and which could be a useful functional correlate for 5-HT$_6$ receptor blockade. There is no evidence for dopamine D$_2$-like receptor involvement in this behaviour.

Keywords: 5-HT$_6$; stretching; Ro 04-6790; 4-amino-N-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene sulphonamide; behaviour; locomotor activity; yawning; chewing

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HT$_6$ receptor, 5-hydroxytryptamine$_6$ receptor; ACTH, adrenocorticotrophic hormone; AO, antisense oligonucleotides; D$_2$ receptor, dopamine$_2$ receptor; [^{3}H]-LSD, [^{3}H]-lysergic acid diethylamide; H$_2$ receptor, histamine$_2$ receptor; i.c.v., intracerebroventricular; MSH, α-melanocyte stimulating hormone; Ro 04-6790, 4-amino-N-(2,6bis-methylamino-pyrimidin-4-yl)-benzene sulphonamide; Ro 63-0563, 4-amino-N-(2,6bis-methylamino-pyridin-4-yl)-benzene sulphonamide; SO, scrambled antisense oligonucleotides

Introduction

The 5-hydroxytryptamine$_6$ (5-HT$_6$) receptor is one of 14 receptors which mediate the effects of the neurotransmitter, 5-hydroxytryptamine (5-HT, Hoyer & Martin, 1997). The rat receptor was cloned by reverse transcription and polymerase chain reaction with degenerate primers derived from conserved regions of known G-protein coupled receptors (Monsma *et al*., 1993) or by low stringency screening with probes derived from the histamine H$_2$ receptor (Ruat *et al*., 1993). Subsequently, the human receptor was identified (Kohen *et al*., 1994). 5-HT$_6$ mRNA is present in olfactory tubercle, nucleus accumbens, striatum and hippocampus (Monsma *et al*., 1993; Ruat *et al*., 1993; Ward *et al*., 1995; Gérard *et al*., 1996). The localization of the 5-HT$_6$ receptor protein has been studied with polyclonal antibodies raised to a synthetic peptide corresponding to part of the C terminal region (Leu^{398}-Val^{415}) of the 5-HT$_6$ receptor protein. In addition to the regions expressing 5-HT$_6$ mRNA, 5-HT$_6$-like immunoreactivity was found in the frontal and entorhinal cortex and the molecular layer of the cerebellum (Gérard *et al*., 1997). Electron microscopy showed that the immunoreactivity is localized on distal dendrites of pyramidal and granular cells in the hippocampus and on medium spiny neurones in the striatum (Gérard *et al*., 1997).

Although the 5-HT$_6$ receptor has a distinct pharmacological profile, with a high affinity for clozapine-related compounds (Roth *et al*., 1994; Boess *et al*., 1997), *in vivo* investigation of receptor function has been hindered by the lack of selective agonists or antagonists. Chronic intracerebroventricular (i.c.v.) treatment with an antisense oligodeoxynucleotide (A.O.) produced a behavioural syndrome comprising of yawning, stretching and chewing (Bourson *et al*., 1995). This behavioural syndrome was not observed in either saline or scrambled oligodeoxynucleotide (S.O.) treated animals but was accompanied by a 30% reduction in the number of [^{3}H]-

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Lysergic acid diethylamide ([^{3}H]-LSD) binding sites (measured in the presence of 300 nM spiperone). Therefore, it was proposed that this behaviour is a result of a reduction in the expression of the 5-HT$_6$ receptor in the CNS.

Recently, potent and selective 5-HT$_6$ receptor antagonists, 4-amino-N-(2,6 bis-methylamino-pyrimidin-4-yl)-benzene sulphonamide (Ro 04-6790) and 4-amino-N-(2,6 bis-methylamino-pyridin-4-yl)-benzene sulphonamide (Ro 63-0563) have been characterized (Sleight *et al*., 1998). Both of these compounds are competitive antagonists at recombinant 5-HT$_6$ receptors. The latter has been radiolabelled and 5-HT$_6$ receptor binding sites have been identified in the striatum of both rats and pigs (Boess *et al*., 1998). Ro 04-6790 has an affinity (pK_i) of 7.3 for both the rat and human 5-HT$_6$ receptor, has over two log units of selectivity with respect to 23 other receptor binding sites (including eight other 5-HT receptor subtypes and all five muscarinic receptor subtypes) and can be measured in the cerebro-spinal fluid of rats following systemic administration (Sleight *et al*., 1998). Interestingly, Ro 04-6790 produced a similar behavioural syndrome to that produced by 5-HT$_6$ antisense oligonucleotide treatment in rats that had been habituated to the observation cages for 4 days prior to being administered with Ro 04-6790. In these animals, stretching behaviour could be dose-dependently produced by Ro 04-6790 although yawning failed to reach statistical significance when compared to saline treated animals (Sleight *et al*., 1998).

In the present report, we detail experiments undertaken to evaluate the consequences of 5-HT$_6$ receptor antagonism by studying the effect of Ro 04-6790 on locomotor activity and unconditioned behaviour with particular emphasis on behaviour indicative of depressant, stimulant and autonomic properties (Irwin, 1968). In addition we also examined whether the stretching behaviour could be seen in animals that have not been habituated to the observation cages and the mechanisms that mediate this response.

Preliminary results from this paper were presented at the XIIIth International Congress of Pharmacology and at the 4th IUPHAR Satellite Meeting on Serotonin and will be published in the proceedings of the meetings.

Methods

Animals

Male Sprague Dawley rats (Füllinsdorf, Switzerland) weighing 230–300 g were housed in groups of two on a 12 h light-dark cycle (lights on at 07.00 h) and given food and water *ad libitum*. Room temperature (21°C ± 1°C) and humidity (55–65%) were kept constant.

Measurement of locomotor activity

The computerized Digiscan 16 Animal Activity Monitoring System (Omnitech, Columbus, Ohio, U.S.A.) was used to measure locomotor activity. Data were obtained simultaneously from eight Digiscan chambers. Each activity monitor consisted of a Plexiglas box (40 × 40 × 30.5 cm) surrounded by horizontal and vertical infrared sensor beams. The cages were connected to a Digiscan analyser working in conjunction with a personal computer to interpret the photobeam interruptions. With this system 19 different parameters could be measured, such as horizontal and vertical activity (e.g. total number of interruptions of the horizontal and vertical sensors, respectively, during a given period).

Animals were treated with either saline or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.) and immediately placed in the Digiscan chambers. Locomotor activity was measured between 08.00 and 12.00 h for a total of 3 h.

Behavioural observations

Behavioural observations were made between 08.00 and 12.00 h. Rats were placed into transparent boxes (55 × 34 × 18 cm) in groups of three and treated with either saline ($n = 6$) or Ro 04-6790 (3, 10 or 30 mg kg^{-1}, i.p.; $n = 6$ per dose). Behavioural signs were observed for 3 h with emphasis on behaviour indicative of depressant, stimulant and autonomic properties (Irwin, 1968). All experiments were performed on a blind basis.

Measurement of stretching and yawning behaviour induced by Ro 04-6790

Groups of eight rats were treated with either saline (1 ml kg^{-1}, i.p.) or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.) and were placed into observation cages in groups of four (one animal from each treatment group). Thirty min later the number of stretches and yawns were counted for 1 h.

Effect of scopolamine on stretching induced by Ro 04-6790

Groups of eight rats were treated with either scopolamine (0.1, 0.3 or 1 mg kg^{-1}, i.p.) or saline (1 ml kg^{-1}, i.p.). Thirty min later all animals were given Ro 04-6790 (30 mg kg^{-1}, i.p.) and immediately placed in observation cages in groups of four. After a further 30 min, the number of stretches and yawns were recorded for 1 h. In addition a group of eight animals was treated with scopolamine (1 mg kg^{-1}, i.p.) followed 30 min later by saline to determine whether scopolamine itself induced stretching and yawning.

Effect of atropine and methylatropine on stretching induced by Ro 04-6790

Groups of eight rats were treated with either atropine (0.3, 1 or 3 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}, s.c.). Thirty min later all animals were given Ro 04-6790 (30 mg kg^{-1}, i.p.) and immediately placed in observation cages in groups of four. After a further 30 min, the number of stretches and yawns were recorded for 1 h. In addition a group of eight animals was treated with atropine (3 mg kg^{-1}, i.p.) followed 30 min later by saline to determine whether atropine itself induced stretching and yawning.

In a separate set of animals, groups of eight rats were treated with either methylatropine (1, 3 or 10 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1}). Thirty min later all animals were given Ro 04-6790 (30 mg kg^{-1}, i.p.) and immediately placed in observation cages in groups of four. After a further 30 min, the number of stretches and yawns were recorded for 1 h. In addition a group of eight animals were treated with methylatropine (10 mg kg^{-1}, i.p.) followed 30 min later by saline to determine whether methylatropine itself induced stretching and yawning.

Effect of haloperidol on stretching induced by Ro 04-6790

Groups of eight rats were treated with either haloperidol (0.03, 0.1 or 0.3 mg kg^{-1}, s.c.) or saline (1 ml kg^{-1} s.c.). and at the

same time all animals were treated Ro 04-6790 (30 mg kg^{-1}, i.p.) and immediately placed in observation cages in groups of four. Thirty min later, the number of stretches and yawns were recorded for 1 h. In addition a group of eight animals were treated with haloperidol (0.3 mg kg^{-1}, i.p.) and saline to determine whether haloperidol itself induced stretching and yawning.

Chemicals

Methylatropine, scopolamine and atropine were synthesized at Hoffmann-La Roche, Switzerland. Ro 04-6790 was synthesized by Dr Michael Bös at Hoffmann-La Roche. Haloperidol was purchased from Janssen Beerse (Belgium). All compounds were dissolved in sterile saline (0.154 M).

Statistical Analysis

All behavioural data was analysed by Kruskal-Wallis analysis of Variance followed by Mann-Whitney *U*-test. Level of significance was set at $P<0.05$ and all data are presented as mean $\pm$ s.e.mean.

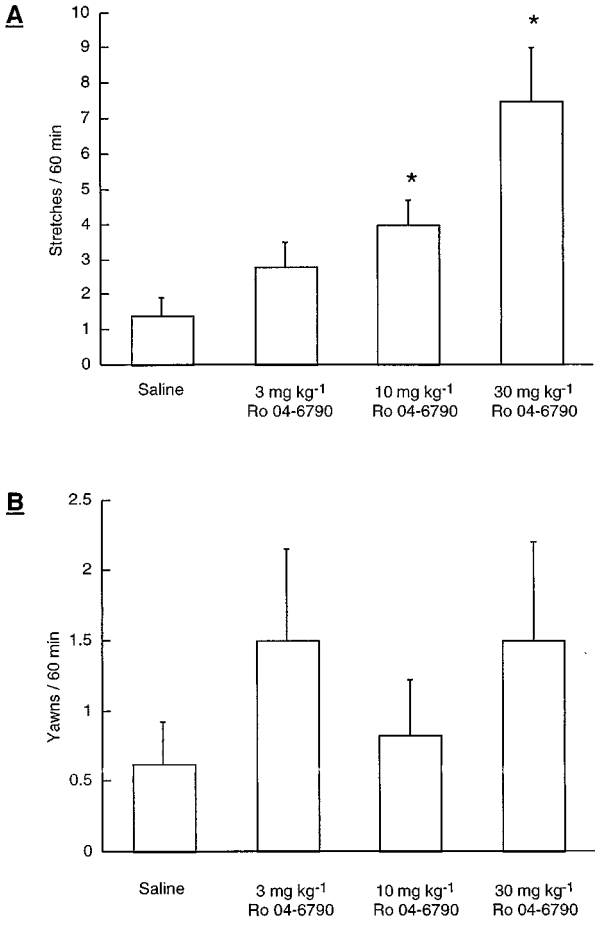

Figure 1 Number of stretches (A) and yawns (B) observed after treatment with either Ro 04-6790 (3, 10 or 30 mg kg^{-1}, i.p.) or saline. Rats were treated with either Ro 04-6790 or saline and placed in observation cages in groups of four. Thirty min later, the animals were observed for 1 h and the number of stretches (A) and yawns (B) recorded. (A) Results are expressed as the total number of stretches (mean $\pm$ s.e.mean, $n=8$) observed in 1 h. *$P<0.05$ compared to saline treated controls (Mann Whitney *U*-test following a Kruskal Wallis ANOVA, $H_{(5,6)}=20.1$, $P=0.0002$). (B) Results are expressed as the total number of yawns (mean $\pm$ s.e.mean, $n=8$) observed in 1 h. No significant differences were found between any of the groups ($P>0.05$).

Results

Effect of Ro 04-6790 on locomotor activity

Ro 04-6790 produced a dose-dependent reduction in both horizontal and vertical activity. This effect was statistically significant following treatment with Ro 04-6790 (30 mg kg^{-1}, i.p.; $P<0.05$; results not shown) compared to saline treated rats.

Behavioural observations

Treatment with Ro 04-6790 did not induce any overt behaviour other than a decrease in locomotor activity and the appearance of a behavioural syndrome of yawning, stretching and chewing. This syndrome was most pronounced between 30 and 90 min following the administration of Ro 04-6790. Consequently, in all subsequent experiments the number of stretches and yawns were counted over a 60 min period beginning 30 min after the administration of Ro 04-6790. Although chewing was also observed in rats following treatment with Ro 04-6790, it was not quantified in subsequent experiments.

Stretching and yawning induced by Ro 04-6790

Intraperitoneal injection of Ro 04-6790 produced a dose-dependent increase in the number of stretches (Figure 1A) from 1.4 $\pm$ 0.6 in saline-treated rats to 4.0 $\pm$ 0.7 (10 mg kg^{-1}, $P<0.05$) and 7.5 $\pm$ 1.5 in rats treated with either Ro 04-6790 (10 mg kg^{-1}, $P<0.01$) or Ro 04-6790 (30 mg kg^{-1}, $P<0.05$), respectively. In contrast, there was no dose-related increase in the number of yawns and furthermore, the difference between the number of yawns counted in saline and Ro 04-6790 treated groups was not statistically significant (Figure 1B).

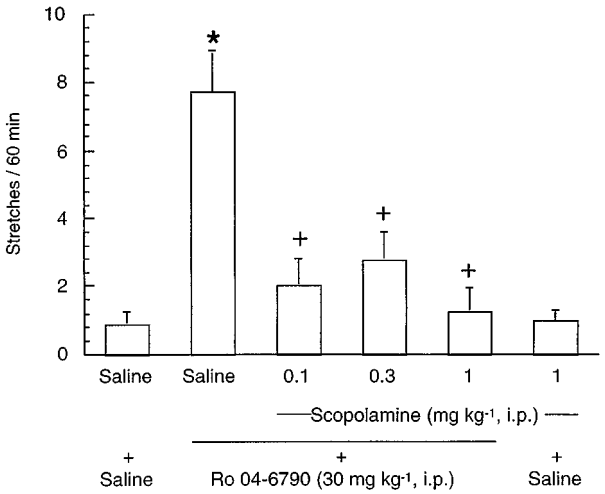

Figure 2 Effect of scopolamine (0.1, 0.3 or 1 mg kg^{-1}, i.p.) pretreatment on stretching induced by Ro 04-6790. Rats were pretreated with scopolamine and 30 min later with either Ro 04-6790 (30 mg kg^{-1}, i.p.) or saline and placed in observation cages in groups of four. After a further 30 min the animals were observed for 1 h and the number of stretches recorded. Results are expressed as the total number of stretches (mean $\pm$ s.e.mean, $n=8$) observed in 1 h. *$P<0.05$ compared to saline/saline treated rats and +$P<0.05$ compared to saline/Ro 04-6790 (30 mg kg^{-1}, i.p.) treated rats (Mann-Whitney *U*-test following a Kruskal Wallis ANOVA, $H_{(5,6)}=20.5$, $P=0.001$).

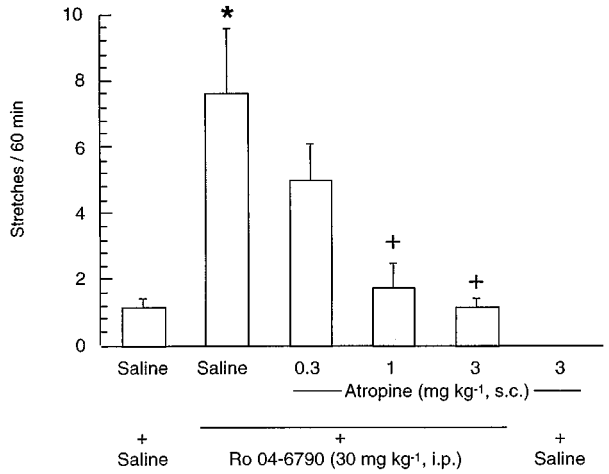

Figure 3 Effect of atropine (0.3, 1 or 3 mg kg^{-1}, s.c.) pretreatment on stretching induced by Ro 04-6790. Rats were pretreated with atropine and 30 min later with either Ro 04-6790 (30 mg kg^{-1}, i.p.) or saline and placed in observation cages in groups of four. After a further 30 min the animals were observed for 1 h and the number of stretches recorded. Results are expressed as the total number of stretches (mean ± s.e.mean, $n=8$) observed in 1 h. *$P<0.05$ compared to saline/saline treated rats and +$P<0.05$ compared to saline/Ro 04-6790 (30 mg kg^{-1}, i.p.) treated rats (Mann-Whitney U-test following a Kruskal Wallis ANOVA, $H_{(5,6)}=30.3$, $P=0.0001$).

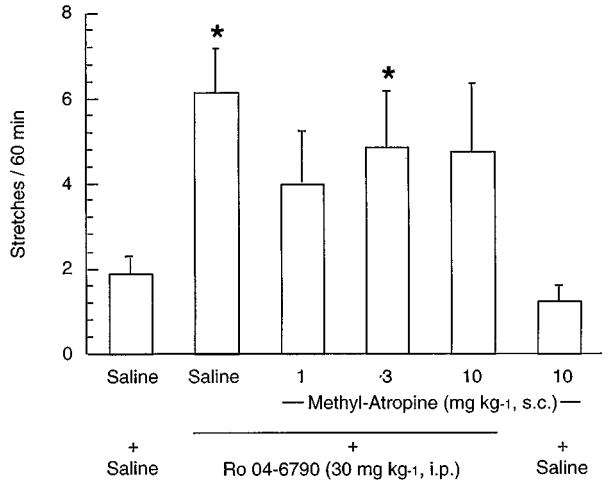

Figure 4 Effect of methylatropine (1, 3 or 10 mg kg^{-1}, s.c.) pretreatment on stretching induced by Ro 04-6790. Rats were pretreated with methylatropine and 30 min later with either Ro 04-6790 (30 mg kg^{-1}, i.p.) or saline and placed in observation cages in groups of four. After a further 30 min the animals were observed for 1 h and the number of stretches recorded. Results are expressed as the total number of stretches (mean ± s.e.mean, $n=8$) observed in 1 h. *$P<0.05$ compared to saline/saline treated rats (Mann-Whitney U-test following a Kruskal Wallis ANOVA, $H_{(5,6)}=19.2$, $P=0.0018$). There was no significant difference in the number of stretches observed in animals treated with Ro 04-6790 and animals treated with Ro 04-6790 and the various doses of methylatropine.

Effect of scopolamine on Ro 04-6790-induced stretching

Pretreatment with the non-selective muscarinic antagonist, scopolamine, did not by itself induce stretching. It did, however, significantly reduce stretching induced by Ro 04-6790 (30 mg kg^{-1}, i.p.; $P<0.05$; Figure 2). All three doses of scopolamine tested completely inhibited the stretching induced by Ro 04-6790 and therefore lower doses would have to be tested to calculate an ED_{50} value and to determine whether the effects of this muscarinic antagonist showed dose-dependency.

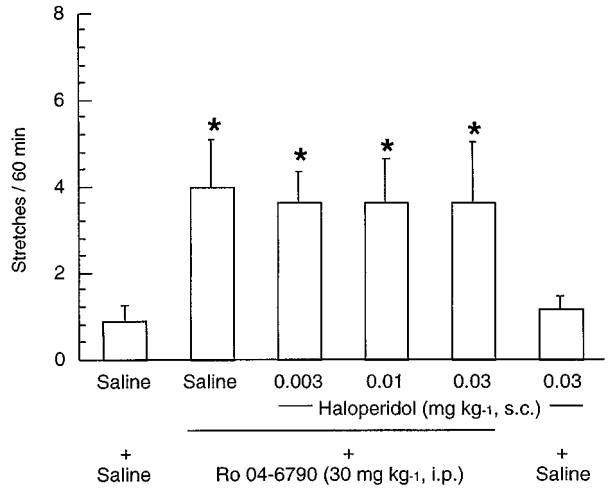

Figure 5 Effect of haloperidol (0.003, 0.01 or 0.03 mg kg^{-1}, s.c.) pretreatment on stretching induced by Ro 04-6790. Rats were treated with haloperidol and either Ro 04-6790 (30 mg kg^{-1}, i.p.) or saline and placed in observation cages in groups of four. After 30 min the animals were observed for 1 h and the number of stretches recorded. Results are expressed as the total number of stretches (mean ± s.e.mean, $n=8$) observed in 1 h. *$P<0.05$ compared to saline/saline treated (Mann-Whitney U-test following a Kruskal Wallis ANOVA, $H_{(5,6)}=13.2$, $P=0.022$).

Effect of atropine and methylatropine on Ro 04-6790-induced stretching

Atropine did not itself induce stretching, however atropine pretreatment dose-dependently decreased stretching induced by Ro 04-6790 (30 mg kg^{-1}, i.p.; Figure 3). The attenuation of Ro 04-6790-induced stretching, was statistically significant following the administration of either atropine (1 mg kg^{-1}, s.c.; $P<0.05$) or atropine (3 mg kg^{-1}, s.c.; $P<0.05$). In contrast, methylatropine pretreatment had no significant effect on Ro 04-6790 (30 mg kg^{-1}, i.p)-induced stretching (Figure 4).

Effect of haloperidol on Ro 04-6790-induced stretching

As shown in Figure 5, pretreatment with haloperidol, had no significant effect on stretching induced by Ro 04-6790 (30 mg kg^{-1}, i.p.).

Discussion

The aim of the present study was to evaluate the effect of the selective 5-HT$_6$ receptor antagonist, Ro 04-6790 on spontaneous rat behaviour in a novel environment and to determine whether any observed effect was centrally or peripherally mediated. These data suggest that systemic administration of Ro 04-6790 induces a stretching behaviour, which is centrally mediated. These data would also suggest that *in vivo*, the receptor is either constitutively active or is under tonic activation of the endogenous neurotransmitter, 5-HT, since the administration of 5-HT$_6$ antagonists alone induces a behavioural syndrome. Although yawning was also observed following treatment with Ro 04-6790, it was not significantly greater in any of the drug treated groups than in the control groups. In addition, chewing was observed in animals treated with Ro 04-6790 but this behaviour was not quantified. In a

recent publication we showed that Ro 04-6790 induces stretching behaviour in rats habituated to the observation cages (Sleight *et al*., 1998), however, the present data suggest that prolonged habituation is not necessary.

Pretreatment with the muscarinic antagonists, atropine and scopolamine which cross the blood-brain barrier, prevented the stretching induced by the 5-HT$_6$ antagonist. Therefore, it is proposed that blockade of the 5-HT$_6$ receptor facilitates cholinergic neurotransmission which, in turn gives rise to the stretching behaviour. Methylatropine which does not penetrate into the brain (Herz *et al*., 1965) had no effect on the Ro 04-6790-induced stretching, suggesting that the behaviour is centrally mediated. This is in agreement with previous reports showing that the 5-HT$_6$ receptor is predominantly expressed in the CNS and not in the periphery (Monsma *et al*., 1993). In addition, although dopamine D$_2$-like receptors have been implicated in this type of behaviour (Argiolas & Melis, 1998), haloperidol failed to attenuate the stretching induced by Ro 04-6790. This suggests that dopamine D$_2$-like receptors are not involved in mediating the stretching response to 5-HT$_6$ receptor blockade.

The present study is consistent with previous work by Bourson *et al*. (1995) using a 5-HT$_6$ receptor-directed antisense oligonucleotides (AO). Chronic (4 days) i.c.v. treatment with AO produced a behavioural syndrome of stretching, yawning and chewing which was attenuated by atropine (0.3, 1.0, 3.0 mg kg^{-1}, s.c.) but not by haloperidol (0.03 mg kg^{-1}, s.c.) pretreatment. The studies with AO thus correctly predicted both that decreased 5-HT$_6$ receptor function induces stretching and that this behaviour can be blocked by cholinergic but not by dopaminergic antagonists. These results show that AO can be a valuable tool for the evaluation of the function of novel receptors for which selective antagonists are not yet available, provided that the correct procedures and controls are used. This is one of the first examples of the successful use of AO to predict function that has subsequently been confirmed with selective antagonists. Most AO studies have examined receptors for which the effects of selective antagonists were already known or the observations with AO have not yet been confirmed pharmacologically (Wahlestedt *et al*., 1993a,b; Zhou *et al*., 1994). While the stretching behaviour observed after AO treatment was confirmed with the 5-HT$_6$ receptor antagonist Ro 04-6790, the yawning seen after Ro 04-6790 treatment was neither dose-dependent nor statistically significant compared with saline treated animals, while it was clearly present after AO treatment. There are a number of possible explanations for this difference between AO treatment and that of a 5-HT$_6$ antagonist. For example, the distribution of the antisense given i.c.v. will be different from that of Ro 04-6790, and therefore the two treatments may be affecting different pools of receptor. Another explanation could be that the treatment of animals with both the AO and SO exhibited non-specific toxic symptoms (Bourson *et al*., 1995) and this may affect the expression of the yawning behaviour. Alternatively, it is possible that either the AO or Ro 04-6790 treatment is not completely specific for the 5-HT$_6$ receptor and may interfere with other receptors and proteins. Clearly although stretching seems to be a result of decreased 5-HT$_6$ receptor function, it is important to study whether other selective 5-HT$_6$ antagonists will produce yawning.

Stretching and yawning have been reported following central administration of adrenocorticotrophic hormone (ACTH) and α-melanocyte stimulating hormone (MSH) and related peptides (Gessa *et al*., 1967). Further investigation into the mechanism surrounding this particular behavioural syndrome has indicated the involvement of a variety of neurotransmitters (acetylcholine; Ferrari *et al*., 1963, dopamine; Ferrari *et al*., 1993), neuropeptides (morphine; Bertolili & Gessa 1981), and inorganic ions such as calcium (Argiolas *et al*., 1990) and nitric oxide (Poggioli *et al*., 1995). Few other studies, however, have examined stretching and yawning separately, but have scored both behaviours together making comparison with the current data difficult. Moreover, investigators observing solely a yawning note that occasionally there is a 'sudden stretching of the forelimbs' proceeding the yawning behaviour (Urba-Holmgren *et al*., 1977; Yamada & Furukawa, 1980). In the current study stretching and yawning were clearly dissociated and only stretching was dose-dependent. Numerous factors may influence expression of such behaviours in particular the observation protocol, size of the observation box, extent of habituation to the environment and the age of the rats used. In addition, further investigation may identify other neurotransmitters that are involved in mediating 5-HT$_6$ receptor antagonist-induced stretching.

In conclusion, we have demonstrated that a 5-HT$_6$ antagonist, Ro 04-6790, induces a stretching behaviour which is centrally mediated and similar to that previously reported following AO treatment. The behavioural syndrome was reversed by muscarinic antagonists suggesting that 5-HT$_6$ receptors modulate cholinergic neurotransmission. In agreement with this finding we have recently reported that 5-HT$_6$ receptor-directed AO treatment enhances acquisition in the Morris Water Maze (Bentley *et al*., 1997), suggesting that this potentiation of acetylcholine neurotransmission may give rise to enhanced cognitive responses. It is not known, however, whether these effects directly involve an increase in the release of acetylcholine from cholinergic neurones in the rat CNS. Nevertheless, the interaction between the 5-HT$_6$ receptor and cholinergic neurotransmission is particularly interesting with respect to disease states such as, Alzheimer's disease, where there is clear evidence of a cholinergic deficit (Bartus *et al*., 1982).

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