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ORIGINAL INVESTIGATION

Marie L. Woolley · Charles A. Marsden · Andrew J. Sleight · Kevin C. F. Fone

Reversal of a cholinergic-induced deficit in a rodent model of recognition memory by the selective 5-HT$_6$ receptor antagonist, Ro 04-6790

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Abstract *Rationale:* Accumulating evidence suggests a potential role for the 5-HT$_6$ receptor in cognitive function and the potential use of 5-HT$_6$ receptor antagonists in the treatment of learning and memory disorders. *Objectives:* The aim of the current study was to investigate the effect of the selective 5-HT$_6$ receptor antagonist, Ro 04-6790, on both the performance of normal adult rats and restoration of a pharmacological disruption of memory function produced by the non-selective muscarinic receptor antagonist, scopolamine, or the dopamine D$_2$ receptor antagonist, raclopride, in a rodent model of recognition memory. *Methods:* Passive, perceptually based, recognition memory was assessed using a novel object discrimination task. Following habituation to an arena, rats were presented with two identical objects during trial 1 (T$_1$) and a novel and familiar object during trial 2 (T$_2$). The time spent exploring the two objects in each trial was measured and novel object discrimination assessed in T$_2$. *Results:* In the absence of drug all rats spent an equal time exploring the two identical objects in T$_1$ but more time exploring the novel object in T$_2$. Scopolamine (but not *N*-methylscopolamine) and raclopride both produced a dose-dependent reduction in novel object discrimination whilst the 5-HT$_6$ receptor antagonist, Ro 04-6790, had no effect on discrimination when given alone but completely reversed the scopolamine- but not the raclopride-induced deficit. *Conclusion:* This study demonstrates that acute administration of Ro 04-6790 reverses a cholinergic but not a dopaminergic deficit in a rodent model of recognition memory and provides further support for a role of the 5-HT$_6$ receptor in the regulation of cognitive function.

Keywords Memory · Rats · Ro 04-6790 · 5-HT$_6$ receptor antagonists

M. L. Woolley · C. A. Marsden · K. C. F. Fone
Institute of Neuroscience, School of Biomedical Science, Queen's Medical Centre, University of Nottingham, Nottingham, NG7 2UH, UK

A. J. Sleight
PRBD-N, F. Hoffmann La-Roche, 4070 Basel, Switzerland

M. L. Woolley (✉)
Bau 72/129, PRBD-N, F. Hoffmann La-Roche, 4070 Basel, Switzerland
e-mail: Marie.Woolley@roche.com
Tel.: +41-61-6870932
Fax: +41-61-6881895

Introduction

The 5-HT$_6$ receptor is one of the most recent additions to the fifteen mammalian 5-HT receptors identified to date (Hoyer et al. 2002). Following the discovery of the rodent 5-HT$_6$ receptor using molecular biology (Monsma et al. 1993: Ruat et al. 1993), identification of the human analogue quickly followed (Kohen et al. 1996). These two receptor proteins comprise a linear chain of 438 (rat) and 440 (human) amino acids with a typical seven transmembrane spanning G-protein linked structure, are positively coupled to adenylyl cyclase and are 89% homologous. Research into the functional role of the receptor was initially hampered due to a lack of selective ligands and early studies made use of antisense oligonucleotides to reduce 5-HT$_6$ receptor expression (Bourson et al. 1995; Bentley et al. 1997; Yoshioka et al. 1998; Hamon et al. 1999; Otano et al. 1999). However, recently a number of selective 5-HT$_6$ receptor antagonists have been characterized (Sleight et al. 1998; Bromidge et al. 1999; Issac et al. 2000; Lee et al. 2000; Tsai et al. 2000; Slassi et al. 2000; Bös et al. 2001; Russell et al. 2001), providing more selective tools with which to probe the functional role of this receptor. To date, the receptor has been implicated in psychotic disorders (Monsma et al. 1993; Roth et al. 1994; Tsai et al. 1999; Yu et al. 1999; Pouzet et al. 2002), affective disorders (Roth et al. 1994; Yau et al. 1997; Vogt et al. 2000), anxiety (Yoshioka et al. 1998; Hamon et al. 1999; Otano et al. 1999), epilepsy (Routledge et al. 2000) and potentially the regulation of food consumption (Bentley et al. 1997, 1999b; Woolley et al.

2001), but the most compelling evidence suggests a role for the receptor in cognitive function.

Both chronic i.c.v. injection of a 5-HT_6 receptor specific antisense oligonucleotide (A.O.; Bourson et al. 1995) and acute systemic administration of 4-amino-*N*-(2,6) bis-methyl-amino-pyrimidin-4-yl-benzene sulphonamide (Ro 04-6790, Bentley et al. 1999a) produced a specific behavioural syndrome of stretching, which was blocked by atropine but not by haloperidol, suggesting that the 5-HT_6 receptor may regulate central cholinergic, but not dopaminergic, neurotransmission. Consistent with this proposal, Ro 04-6790 blocked scopolamine-induced ipsilateral rotations but had no effect on apopmorphine-induced contralateral rotations in 6-hydroxydopamine (6-OHDA) lesioned rats (Bourson et al. 1998). More recently, microdialysis studies have demonstrated elevated levels of acetylcholine in the hippocampus and frontal cortex of the conscious rat following treatment with either Ro 04-6790 (Shiraz-Southall et al. 2002) or the structural analogue Ro 65-7199 (Sleight et al. 1999). Given the well documented cholinergic link to memory function (Bartus et al. 1982), a 5-HT_6 receptor-cholinergic interaction could account for the modulation of cognition seen with 5-HT_6 receptor antagonists.

We previously demonstrated enhanced retention (but not acquisition) of a learnt platform position in normal adult rats following chronic treatment with a specific 5-HT_6 receptor-directed A.O. and the selective 5-HT_6 receptor antagonist, Ro 04-6790, after acquisition training in the Morris water maze (Bentley et al. 1997; Woolley et al. 2001). This was also seen with other 5-HT_6 receptor antagonists (5-chloro-*N*-(4-methoxy-3-piperazin-1-yl-phenyl)-3-methyl-2-benzothio-phene sulphonamide (SB-271046) and N-(2,5-dibromo-3-fluorophenyl)-4-methoxy-3-piperazin-1-yl benzene sulphonamide (SB-357134; Rogers et al. 1999; Rogers and Hagan 2001), suggesting that the 5-HT_6 receptor may regulate long-term memory in normal adult rats. More recently, Stean et al. (2002) demonstrated enhanced acquisition as measured by path length, in addition to enhanced retention of a learnt platform position in the Morris water maze following chronic administration of SB-357134 (10 mg/kg PO, twice daily, 7 days prior to training), implying a role for the 5-HT_6 receptor in both the learning and mnemonic processes involved in this spatial learning task. However, only few preliminary investigations have examined the effect of 5-HT_6 receptor antagonists on rodent models of impaired memory function, in order to determine their potential utility as therapeutic agents for the treatment of Alzheimer's disease (Rogers et al. 1999, 2000; Menses 2001).

Assessing passive perceptually based recognition memory, the novel object discrimination task takes advantage of the spontaneous preference of rodents for novelty and does not require reinforcement of behaviour by food reward. The latter aspect is of importance, since both i.c.v. injection of a 5-HT_6-receptor directed A.O. (Bentley et al. 1997) and systemic injection of a selective 5-HT_6 receptor antagonist Ro 04-6790 (Bentley et al. 1999b; Woolley et al. 2000) cause hypophagia that would confound interpretation of food motivated operant tasks (Meneses et al. 2001). Thus, using an ITI of 1 min, the novel object discrimination task used in the current study assesses short-term recognition memory. Importantly, the pharmacological validity of the rodent novel object discrimination task to predict novel compounds with potential clinical advantage has also been demonstrated since Aricept (donepezil, E2020), currently used for the symptomatic relief in Alzheimer's disease, was found to reverse an age-related deficit in this task (Ni et al. 2001).

However, to date the neural substrates of novel object discrimination have not been conclusively defined. Several groups have suggested participation of the perirhinal and entorhinal cortices (Wiig and Bilkey 1995; Ennaceur et al. 1996, 1997; Aggleton et al. 1997; Ennaceur 1998), cortical association areas (Steckler et al. 1998) and the globus pallidus (Ennaceur 1998). In contrast, the role of the hippocampus is more controversial (Steckler et al. 1998). Thus, although electrolytic lesions of the septal-hippocampal pathway have no effect on novel object discrimination (Ennaceur 1998) conflicting results have been obtained following lesions of the fimbria-fornix pathway (Ennaceur and Aggleton 1994; Ennaceur et al. 1996, 1997; Mostafa and Ennaceur 2001) and radiofrequency lesions of the hippocampus (Clark et al. 2000; Aingie et al. 2002; Mumby et al. 2002). Yet, profound hippocampal lesion by ischaemia does induce impairment (Woods and Philips 1991), which becomes apparent with intertrial intervals between 1 and 10 min (Clark et al. 2000; Mumby et al. 2002), consistent with it being involved in recognition memory but not the appreciation of novelty per se. One possible explanation for this apparent disparity is that novel object discrimination impairment is only induced following severe hippocampal lesions and intertrial intervals of greater than 1 min, as used herein.

The current study examines the effect of Ro 04-6790 on novel object discrimination, both when given alone and following the impairments induced by a muscarinic or a dopamine D_2 receptor antagonist.

Materials and methods

Animals and drug treatment

Adult male Lister hooded rats (Biomedical Services Unit, University of Nottingham) weighing 200–400 g were housed in groups of four on a 12 h light/dark cycle (lights on 7.00 a.m.) and given food and water ad libitum. Room temperature (21±1°C) and humidity (55–65%) were kept constant. Rats were randomly assigned to one of 14 treatment groups (n=10–12 per group). Pretreatment comprised scopolamine hydrobromide (0.1, 0.5 or 1 mg/kg IP), *N*-methylscopolamine (0.25 or 0.5 mg/kg IP), raclopride L-tartrate (0.1, 0.3 or 0.5 mg/kg IP), or physiological saline as the vehicle in all cases (0.154 M, 1 ml/kg IP). Twenty minutes later, rats were treated with either saline (IP), or the selective 5-HT_6 receptor antagonist Ro 04-6790 (10 or 30 mg/kg IP).

Each individual group of 10–12 rats was tested twice 7 days apart with a different pair of pretreatment/treatment conditions. On the second test, each rat was given the opposite pretreatment so that all rats received both treatments, such as saline and a chosen drug dose and thus served as their own control. Each rat was only re-tested once in order to avoid habituation to the two objects used for all studies. Further repeated testing with alternative objects was not performed, to avoid potential habituation to the task. All experiments were performed in accordance with the UK Animals (Scientific Procedures) Act 1986, by an observer who was unaware of the treatment given.

Behavioural testing

The novel object discrimination test used in the present study was a modification of that described by Ennaceur and Delacour (1988). The apparatus comprised a clear Perspex box as the arena, measuring 39×23.5 cm with 30 cm high walls. The objects to be discriminated were plastic bottles (8 cm high×5 cm outer diameter) covered in white masking tape (familiar objects) or black and white striped masking tape (novel object). Each bottle was inverted and secured with Blue Tac through holes in the floor located 10 and 5 cm from either side and 5 cm from the end wall in opposite corners of the arena. The weight of each bottle was such that the rats could not displace it.

Twenty-four hours prior to testing, each rat was habituated to the arena for 60 min in the absence of any object. A total of 12 arenas were used and each rat was tested in the arena that it was habituated to. On test days, each group of rats received drug or saline pretreatment 20 min prior to the second drug or saline treatment and 20 min later testing began. Each rat was placed in the arena for 3 min in the absence of the objects for a second brief habituation period and then for two consecutive 3-min trial periods (T_1 and T_2, respectively). All three encounters with the arena were separated by an inter-trial interval (ITI) of 1 min, during which the rat was returned to the home cage. In the first trial (T_1), rats were exposed to two objects of identical size, shape and pattern (objects a_1 and a_2). In the second trial (T_2) one of the bottles "a_2" was replaced with a bottle of identical size and shape but with alternating horizontal black and wipe stripes ("b", the novel object), whilst a_1 was replaced with an object identical to those used in T_1 (the familiar object, "a"). During T_1 and T_2 the exploration of either object was defined as the time spent (s) sniffing, licking, chewing or touching it with the nose or within 1 cm of it with moving vibrissae and was recorded separately for each object by stopwatch. Sitting on the object was not regarded as exploratory activity (but rarely occurred). Between each session, the bottles were wiped with 20% (v/v) ethanol to remove any olfactory cues. Experiments were performed in constant light at 200 lux at floor level in the arena between 09.00 and 13.00 hours.

Statistical analysis

Within-group comparisons of time (s) spent exploring each of the two identical objects (a_1 and a_2) in trial 1 (T_1) and the novel (b) versus the familiar (a) objects in trial 2 (T_2) were analysed using the Student's paired *t*-test. The effect of treatment on overall exploratory time in T_1 and T_2 and the time spent at the novel and familiar object was compared with their appropriate controls and also analysed using the Student's paired *t*-test.

Materials

Scopolamine hydrobromide and *N*-methylscopolamine, were purchased from Sigma Chemicals (Poole, Dorset, UK), raclopride L-tartrate from RBI chemicals (Poole, Dorset, UK), and Ro 04-6790 was a gift from F. Hoffmann La-Roche (Basel, Switzerland).

Results

Pretreatment with scopolamine hydrobromide

Following treatment with saline all three groups of rats spent an equal time exploring the two identical objects (a_1 and a_2) in the first trial (T_1, Table 1), but a significantly

Table 1 Effect of increasing concentrations of the non-selective muscarinic antagonist, scopolamine hydrobromide, the quaternary amonium derivative, *N*-methylscopolamine, the dopamine D_2 receptor antagonist, raclopride and the selective 5-HT_6 receptor antagonist, Ro 04-6790, both given alone and as a combined treatment with scopolamine or raclopride, on the time spent (s, mean±SEM) exploring the two identical objects (a_1 and a_2) in trial 1. For each separate drug study, the saline data were pooled, as there was no significant difference between the groups

Treatment group	Treatment (mg/kg IP)	Number of rats	Total exploration of a_1 (s)	Total exploration of a_2 (s)
Scopolamine	Saline	35	25±2	27±2
	0.1 scopolamine	12	26±3	29±2
	0.5 scopolamine	12	18±3	19±2
	1.0 scopolamine	11	19±3	20±8
N-Methylscopolamine	Saline	24	21±2	22±1
	0.25 scopolamine	12	14±3	19±3
	0.5 scopolamine	12	16±2	16±3
Raclopride	Saline	36	24±1	21±3
	0.1 raclopride	12	24±8	23±3
	0.3 raclopride	12	16±2	17±1
	0.5 raclopride	12	18±3	16±3
Ro 04-6790	Saline	21	31±2	30±1
	10 Ro 04-6790	12	29±2	29±2
	30 Ro 04-6790	9	21±5	21±5
0.5 scopolamine+ 10 Ro 04-6790	Saline	12	17±2	21±3
	0.5 scopolamine	24	20±2	21±1
	0.5 scopolamine+10 Ro 04-6790	12	23±2	24±2
1.0 scopolamine+ 10 Ro 04-6790	Saline	12	25±3	27±4
	1.0 scopolamine	24	26±2	27±2
	1.0 scopolamine+10 Ro 04-6790	12	27±2	27±2
0.1 raclopride+ 10 Ro 04-6790	Saline	12	22±3	19±2
	0.1 raclopride	24	22±2	20±2
	0.1 raclopride+10 Ro 04-6790	12	14±2	13±1
0.3 raclopride+ 10 Ro 04-6790	Saline	12	21±1	20±1
	0.3 raclopride	24	17±1	18±1
	0.3 raclopride+10 Ro 0-46790	12	20±1	19±1

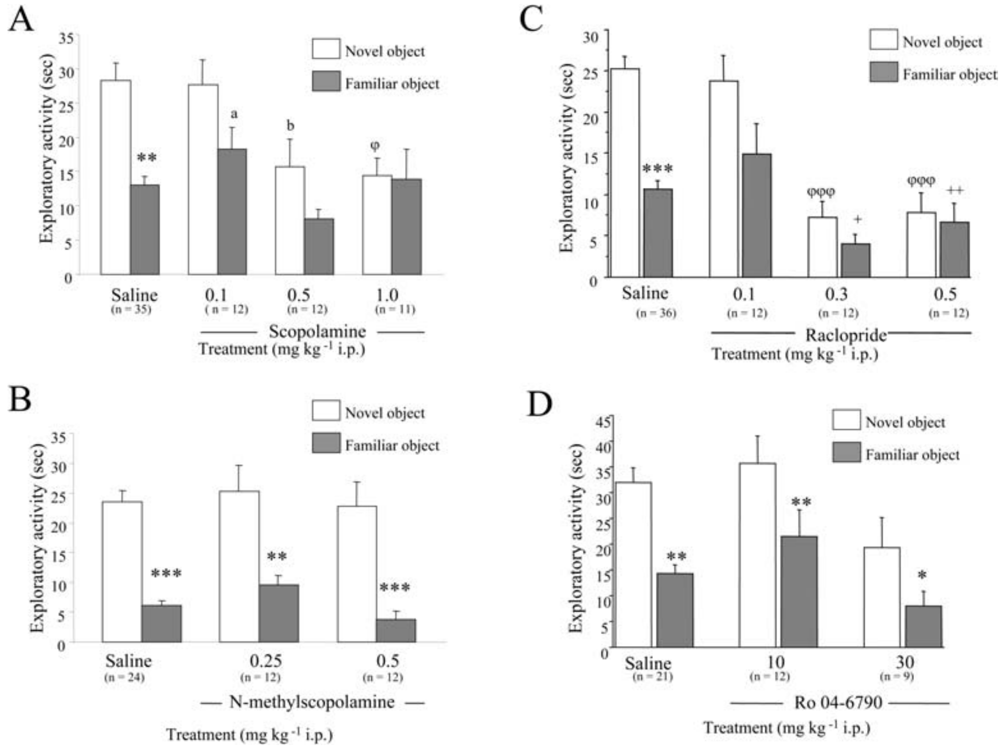

Fig. 1 Effect of increasing concentrations (as indicated, mg/kg, IP) of the non-selective muscarinic antagonist, scopolamine hydrobromide (**A**), the quaternary ammonium derivative, *N*-methylscopolamine (**B**), the dopamine D_2 receptor antagonist, raclopride (**C**) and the selective 5-HT_6 receptor antagonist, Ro 04-6790 (**D**), on the time spent (s, mean±SEM) exploring the novel versus the familiar object during trial 2. Within each separate drug study, each group of rats received a single drug dose and saline separated by an interval of 1 week and the saline data were pooled for clarity of presentation as there was no significant difference between the three groups. *$P<0.05$, **$P<0.01$, ***$P<0.001$, $^{a}P=0.07$ compared with time spent at the novel object in the same treatment group. $^{\phi}P<0.05$, $^{\phi\phi\phi}P<0.001$, $^{b}P=0.06$ compared with time spent exploring the novel object following saline treatment in the same group of rats. $^{+}P<0.05$, $^{++}P<0.01$ compared with time spent at the familiar object following saline treatment in the same group of rats

greater time exploring the novel (b) versus the familiar object (a) in the second trial (T_2, $P<0.01$, in each case, Fig. 1A), showing that they were able to discriminate the novel object during the choice trial. Following treatment with scopolamine (0.1–1 mg/kg IP), all rats also spent an equal time exploring the two identical objects (a_1 and a_2) in T_1 (Table 1). However, scopolamine (0.1, 0.5, and 1 mg/kg) caused a dose-dependent impairment of novel object discrimination in T_2, such that rats spent equivalent times exploring the novel and the familiar objects (Fig. 1A). This effect was most pronounced with the two higher doses of scopolamine, since the difference in time spent exploring the novel versus the familiar object following pretreatment with the lower dose of (0.1 mg/kg) scopolamine just missed significance ($P=0.07$). Notably, scopolamine (0.5 and 1.0 mg mg/kg) tended to reduce the overall exploratory activity in T_2, although when compared with saline treatment in the same rats, this did not reach significance. However, further analysis shows that scopolamine (0.5 and 1.0 mg/kg) selectively reduced the time spent at the novel ($P=0.06$ for 0.5 mg/kg and $P<0.05$ for 1.0 mg/kg scopolamine) and not the familiar object when compared with saline pretreatment in the same rats (Fig. 1A). Thus 0.5 and 1.0 mg/kg were the doses of scopolamine chosen to examine the effect of Ro 04-6790 on a scopolamine-induced deficit in novel object discrimination.

Pretreatment with *N*-methylscopolamine

Following treatment with saline rats spent an equal time exploring the two identical objects during T_1 and significantly longer exploring the novel versus the familiar object during the choice trial ($P<0.001$, Fig. 1B). Similarly, following treatment with N-methylscopolamine (both 0.25 and 0.5 mg/kg), all rats spent an equal time exploring the two identical objects during T_1 (Table 1). In contrast to scopolamine (above), treatment with *N*-methylscopolamine (0.25 or 0.5 mg/kg), which does not penetrate into the CNS, had no effect on the ability of the rats to discriminate the novel object in T_2. Thus all rats given *N*-methylscopolamine, irrespective of dose, spent a significantly longer time exploring the novel versus the familiar object ($P<0.01$ for 0.25 and $P<0.001$ for 0.5 mg/kg *N*-methylscopolamine, Fig. 1B) Notably, *N*-methylscopolamine also had no effect on total object exploration time in either trial (Table 2).

Table 2 Effect of scopolamine, *N*-methylscopolamine, raclopride and the 5-HT_6 antagonist, Ro 04-6790 at the doses indicated (mg/kg IP) on total object exploration time (s, mean±SEM) in trial 1 (T_1) and trial 2 (T_2) (i.e. the total time spent at the two familiar objects during T_1 and the novel and familiar object during T_2). For each separate drug study the saline data were pooled, as there was no significant difference between the groups

Dose-response curve	Treatment (mg/kg IP)	Number of rats	Total object exploration (s) in T_1	Total object exploration (s) in T_2
Scopolamine	Saline	35	52±3	41±3
	0.1 scopolamine	12	54±4	46±4
	0.5 scopolamine	12	36±4	24±5
	1.0 scopolamine	11	40±5	28±7
N-Methylscopolamine	Saline	24	43±3	29±2
	0.25 scopolamine	12	33±5	37±6
	0.5 scopolamine	12	33±5	23±4
Raclopride	Saline	36	46 ±2	36±2
	0.1 raclopride	12	46±5	39±5
	0.3 raclopride	12	33±3*	11±3**
	0.5 raclopride	12	34±6*	15±5***
Ro 04-6790	Saline	21	62±3	54±4
	10 Ro 04-6790	12	58±4	49±9
	30 Ro 04-6790	9	50±8	39±6

*P<0.05, **P<0.01 and ***P<0.001 compared with saline pretreatment in the same rats

Pretreatment with raclopride

As seen previously, following treatment with saline, rats spent an equal time exploring the two identical objects in the first trial (T_1, Table 1) but a significantly greater time (P<0.001) exploring the novel versus the familiar object in the second trial (Fig. 1C). In contrast, treatment with raclopride, irrespective of dose, impaired novel object discrimination in T_2 (Fig. 1C) without altering the pattern of exploration in T_1 (Table 1). Notably, pretreatment with raclopride (0.3 mg/kg and 0.5 mg/kg) also reduced the total exploratory activity in T_1 when compared with that of saline pretreatment (P<0.05 for both groups, Table 2). This effect was also seen in T_2 (P<0.01 for 0.3 mg/kg and P<0.001 for 0.5 mg/kg, Table 2), and further analysis showed that raclopride reduced both the time spent exploring the novel (P<0.001 in both cases) and the familiar object (P<0.05 for 0.3 mg/kg and P<0.01 for 0.5 mg/kg, Fig. 1C) in T_2, suggesting that these doses caused a non-selective reduction in exploration rather than a selective attenuation of working memory. Thus the lower doses (0.1 and 0.3 mg/kg) of raclopride were chosen to examine the effect of Ro 04-6790 on a raclopride-induced deficit in novel object discrimination.

Treatment with Ro 04-6790

Following treatment with saline, both groups of rats spent an equivalent time exploring the two familiar objects during T_1 (Table 1) but significantly longer exploring the novel versus the familiar object during T_2 (P<0.01, Fig. 1D). Treatment with Ro 04-6790 had no effect on novel object discrimination, such that even following the highest dose (30 mg/kg) of this 5-HT_6 antagonist, rats spent an equal time exploring the two identical objects in T_1 (Table 1) and significantly longer exploring the novel versus the familiar object in T_2 (P<0.01 for 10 mg/kg Ro 04-6790 and P<0.05 for 30 mg/kg Ro 04-6790, Fig. 1D). Thus, Ro 04-6790 had no effect on the ability of the rats to discriminate the novel object in T_2. In contrast to the effect of the lowest dose of Ro 04-6790, treatment with 30 mg/kg tended to reduce total object exploration time during T_2 (Table 2), but this did not reach significance.

Ro 04-6790 reverses a scopolamine- but not a raclopride-induced deficit in novel object discrimination

As expected from the dose-response study, pretreatment with both 0.5 and 1 mg/kg scopolamine impaired the ability of the rats to discriminate the novel from the familiar object in T_2 (Fig. 2A, B), without altering the pattern of exploration in T_1 (Table 1), such that in both cases rats spent an equal time exploring the novel and familiar objects in the choice trial. However, in both cases, treatment with Ro 04-6790 (10 mg/kg IP) completely reversed the scopolamine-induced deficit in novel object discrimination (Fig. 2A, B) such that rats receiving combined treatment of scopolamine (0.5 or 1.0 mg/kg) and Ro 04-6790 spent a significantly longer time exploring the novel versus the familiar object in T_2 (P<0.01 for 0.5 mg/kg scopolamine + Ro 04-6790 and P<0.001 for 1.0 mg/kg scopolamine + Ro 04-6790).

Notably, when given in combination with scopolamine (0.5 mg/kg) in T_2, Ro 04-6790 (10 mg/kg) increased the total exploratory activity compared with that in the same rats receiving scopolamine alone (P<0.05, Table 3). Although it failed to reach significance, Ro 04-6790 produced a similar increase in the response in rats receiving the higher dose of scopolamine (1.0 mg/kg, Table 3). Further analysis showed that in both cases, this effect was accounted for by a significant increase in time spent exploring the novel object during T_2 (P<0.05 in both cases, Fig. 2A and 2B) following combined treatment with scopolamine and Ro 04-6790, consistent with the rats selectively redirecting their exploration towards the novel object in the choice trial.

As expected from the dose-response curve (Fig. 1C), pretreatment with raclopride (0.3 mg/kg IP) abolished novel object discrimination in T_2 (Fig. 3B) without altering the pattern of object exploration in T_1 (Table 1).

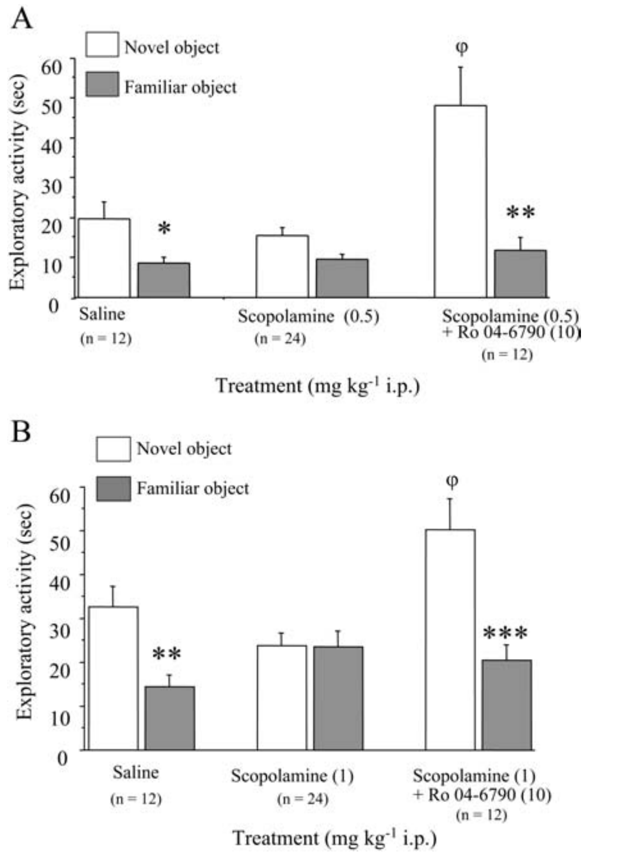

Fig. 2 Effect of combined treatment with Ro 04-6790 (10 mg/kg IP) and 0.5 mg/kg IP scopolamine (**A**) or 1 mg/kg IP scopolamine (**B**) on the time spent (s, mean±SEM) exploring the novel versus the familiar object during trial 2. Within each of the studies the two groups of rats received either scopolamine and saline or scopolamine and Ro 04-6790 separated an interval of 1 week. For clarity of presentation the data from the two groups were pooled for each drug. *P<0.05, **P<0.01, ***P<0.001 compared with time spent at the novel object in the same treatment group. $^{\phi}P$<0.05 compared with time spent exploring the novel object following treatment with scopolamine alone in the same rats

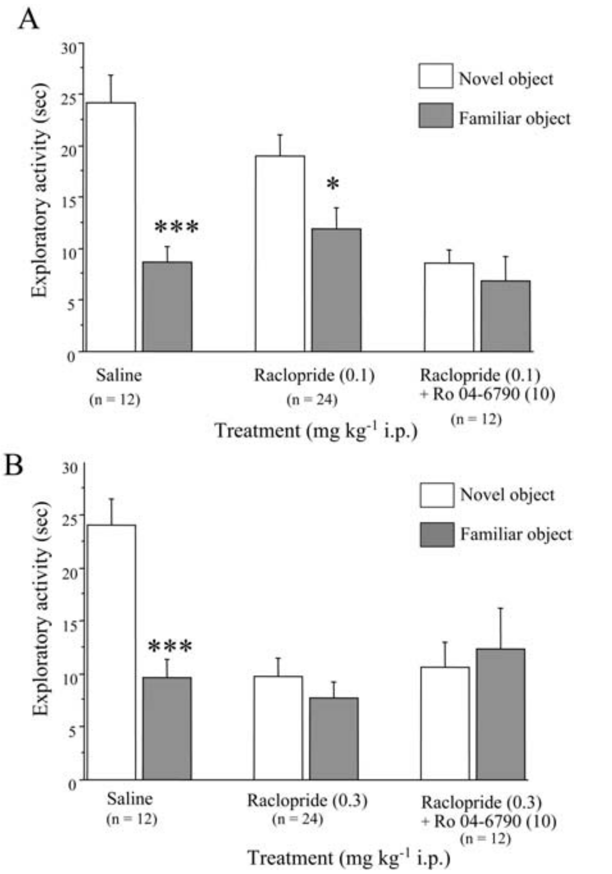

Fig. 3 Effect of combined treatment with Ro 04-6790 (10 mg/kg IP) and 0.1 mg/kg IP raclopride (**A**) or 0.3 mg/kg IP raclopride (**B**) on the time spent (s, mean±SEM) exploring the novel versus the familiar object during trial 2. Within each of the studies each group of rats received either raclopride and or raclopride and Ro 04-6790 separated by an interval of 1 week. For clarity of presentation, the data from the two groups were pooled for each drug. *P<0.05, ***P<0.001 compared with time spent at the novel object in the same treatment group

Table 3 Effect of scopolamine (0.5 and 1.0 mg/kg IP) or raclopride (0.1 or 0.3 mg/kg IP) either given alone or as a combined treatment with Ro 04-6790 (10 mg/kg IP) on the total object exploration time [total time spent at both objects (s, mean±SEM)] in trial 1 (T_1) and trial 2 (T_2)

Treatment group	Treatment (mg/kg IP)	Number of rats	Total object exploration (s) in T_1	Total object exploration (s) in T_2
0.5 scopolamine+10 Ro 04-6790	Saline	12	38±4	28±4
	0.5 scopolamine	24	41±3	25±3
	0.5 scopolamine+10 Ro 04-6790	12	47±3	60±9*
1.0 scopolamine+10 Ro 04-6790	Saline	12	51±7	47±7
	1.0 scopolamine	24	50±3	47±6
	1.0 scopolamine+10 Ro 04-6790	12	54±4	71±9
0.1 raclopride+10 Ro 04-6790	Saline	12	47±5	33±3
	0.1 raclopride	24	42±3	31±3
	0.1 raclopride+10 Ro 04-6790	12	26±2	15±3
0.3 raclopride+10 Ro 04-6790	Saline	12	41±2	34±3
	0.3 raclopride	24	36±2	18±3
	0.3 raclopride+10 Ro 04-6790	12	39±2	23±6

*P<0.05, compared with scopolamine treatment alone in the same rats

Similarly, rats receiving combined treatment with raclopride (0.3 mg/kg) and Ro 04-6790 (10 mg/kg) were also unable to discriminate the novel from the familiar object and spent an equal time at the two objects in T_2 (Fig. 3B). Conversely pretreatment with the lower dose (0.1 mg/kg) of raclopride did not impair novel object discrimination but combined treatment of 0.1 raclopride and Ro 04-6790 further attenuated, and led to a significant impairment of, novel object discrimination (Fig. 3A). Thus, in contrast to the results seen with scopolamine, Ro 04-6790 did not reverse the raclopride-induced deficit in novel object discrimination.

Discussion

The aim of the present study was to investigate the effect of the selective 5-HT_6 receptor antagonist, Ro 04-6790, both when given alone and following a pharmacologically induced deficit in a rodent model of short term recognition memory, the novel object discrimination task. Acute systemic injection of Ro 04-6790 had no effect on novel object discrimination when given alone but it totally reversed the deficit induced following blockade of muscarinic but not dopamine D_2 receptors.

The centrally active, non-selective muscarinic receptor antagonist, scopolamine, has been widely used to demonstrate the cholinergic involvement in memory and cognition in rodents. Peripheral administration of scopolamine produces deficits in a variety of spatial tests such as the delayed matching to position (Dunnett 1985) and the Morris water maze (Harder et al. 1996). In addition, deficits in non-spatial working memory tests such as spontaneous alternation (Pontecorvo et al. 1991), novel object discrimination (Ennaceur and Meliani 1992) and continuous non-matching tasks (Wan et al. 1997) occur following muscarinic antagonist administration. Consistent with these findings, pretreatment with scopolamine at doses equivalent to those used in the aforementioned studies (Dunnett 1985; Ennaceur and Meliani 1992) also produced impaired novel object discrimination in the current study. In contrast, acute, systemic injection of the quaternary ammonium derivative *N*-methylscopolamine, that does not penetrate the blood-brain barrier and therefore has no CNS activity, had no effect on novel object discrimination, consistent with previous reports (Dunnett 1985; Ennaceur and Meliani 1992). Taken together, this suggests that the deficit in novel object discrimination seen with scopolamine is mediated via central muscarinic receptor blockade and not due to a parasympatholytic effect on visual acuity.

In accordance with previous studies demonstrating attenuation of working memory in both spatial (Wilkerson and Levin 1999; Umegaki et al. 2001) and non-spatial tasks (Didriksen 1995), the selective dopamine D_2 receptor antagonist raclopride also caused a dose-dependent impairment of novel object discrimination. This is consistent with the proposed role of dopamine D_2 receptors in the regulation of memory (Noyce et al. 1993). However, the deficit in novel object discrimination seen following treatment with the highest dose (0.3 mg/kg) of raclopride was also accompanied by a reduction in total object exploration, in both trials 1 and 2. Thus, in the current study it seems that the deficit in novel object discrimination seen with raclopride was a consequence of the characteristic hypolocomotor effect of dopamine D_2 receptor antagonists (Jackson and Westlind-Danielsson 1994; Feldman et al. 1997), rather than a selective attenuation of recognition memory.

The selective 5-HT_6 receptor antagonist Ro 04-6790 has been shown to have over 100-fold selectivity for the 5-HT_6 receptor compared with 24 other G-protein coupled receptors, including all muscarinic, dopamine and eight other 5-HT receptors (Sleight et al. 1998). When given alone, Ro 04-6790, had no effect on the ability of the rats to perform discrimination of a novel object. However, it completely reversed the deficit in novel object discrimination produced by scopolamine, suggesting that blockade of 5-HT_6 receptors increases cholinergic function sufficiently to overcome central muscarinic receptor blockade, probably by increasing acetylcholine release (Sleight et al. 1999; Shirizai-Southall et al. 2002). Conversely, Ro 04-6790 did not reverse the dopamine D_2 receptor antagonist-induced deficit in the same task, consistent with previous data indicating that the 5-HT_6 receptor does not modulate central dopaminergic neurotransmission (Bourson et al. 1995, 1998; Bentley et al. 1999a; Dawson et al. 2000, 2001). Since D_2 dopamine receptor blockade following treatment with raclopride in the current study produces a non-selective reduction in exploratory behaviour, the fact that Ro 04-6790 did not reverse this non-specific behavioural disturbance, but did reverse a selective reduction in novel object discrimination following scopolamine, further supports a role for the 5-HT_6 receptor in memory processes.

Consistent with the data in the current study, several lines of evidence suggest that 5-HT_6 receptors regulate central cholinergic neurotransmission. For instance, both 5-HT_6-directed A.O. (Bourson et al. 1995) and Ro 04-6790 (Sleight et al. 1998) produced yawning, stretching and chewing that was antagonised by the muscarinic receptor antagonists atropine and scopolamine (Bourson et al. 1995; Bentley et al. 1999a). Routledge et al. (1999) also demonstrated that another 5-HT_6 antagonist, SB-271046 (Bromidge et al. 1999), enhanced physostigmine-induced yawning. Since the behavioural responses were seen after treatment with a 5-HT_6 receptor-directed A.O. or a selective antagonist, it has been proposed that this receptor receives tonic serotonergic input or possesses constitutive activity (Bourson et al. 1995). Furthermore, after unilateral 6-OHDA lesions of the medial forebrain bundle, both atropine- and scopolamine-induced ipsilateral rotations, but not dopamine-induced contralateral rotations, were inhibited by Ro 04-6790 (Bourson et al. 1998). In agreement with a 5-HT_6 receptor-cholinergic interaction in the regulation of cognition, Meneses (2001) demonstrated that Ro 04-6790 (5 mg/kg IP) reversed a scopolamine-induced deficit in learning consolidation in an autoshaping paradigm. Furthermore, two preliminary intracerebral microdialysis reports suggest that Ro 04-6790 and the structural analogue Ro 65-7199, increase cortical and hippocampal extracellular acetylcholine levels (Sleight et al. 1999; Shirazi-Southall et al. 2002, respectively) and reverse scopolamine-induced deficits in the Morris water maze and passive avoidance tests (Sleight et al. 1999; Bös et al. 2001).

Whilst the present study demonstrates a 5-HT_6 receptor-cholinergic interaction, the way in which 5-HT_6 receptor blockade increases acetylcholine release is currently unknown. By using an N-terminal directed specific antibody, we previously characterised the distribution of 5-HT_6-like immunoreactivity (5-HT_6-LI) in the rat brain (Woolley et al. 2000). The overall pattern matched that reported using a C-terminal directed antibody (Gérard et al. 1997) and the selective radioligand [^{125}I] 4-iodo-N-[4-methoxy-3-(4-methylpiperazin-1-yl)phenyl]benzenesulfonamide (SB-258585, Roberts et al. 2002), demonstrating abundant 5-HT_6 receptor protein in several of the neural areas implicated in the novel object discrimination task, including the cortex and hippocampus (Steckler et al. 1998; Clark et al. 2000; Mumby et al. 2002). Furthermore, by using dual labelled immunohistochemistry, very low levels of co-existence were found between the 5-HT_6 receptor and choline acetyltransferase (ChAT)-LI (Woolley et al. 2000) limited to few discrete brain regions, including the medial septal nuclei (17% of 5-HT_6 positive neurones) caudate nucleus (19%), nucleus accumbens (16%) and some areas of the cortex (5–8% in the cingulate, frontal and parietal corticies). Taken together, these data suggest that whilst a direct 5-HT_6 receptor activation of cholinergic neurones may occur in these areas, in some regions the predominant form of interaction may not be direct. Conversely, abundant and extensive co-existence was seen between 5-HT_6-LI and GAD_{67}-LI, a marker of GABAergic neurones in 29 out of the 42 brain regions examined (Woolley et al. 2000). Such co-existence is in accordance with previous evidence of 5-HT_6 receptor-GABA co-existence in the striatum (Ward and Dorsa 1996; Gérard et al. 1997), and suggests that this may be an important method of interaction. Therefore, the 5-HT_6 receptor-regulation of cholinergic neurotransmission may occur via inhibition of GABA and thence disinhibition of acetylcholine release, as previously described for the 5-HT_3 receptor (Ramirez et al. 1996; Diez-Ariza et al. 1998).

Interestingly, Dawson et al. (2001) recently demonstrated that the 5-HT_6 receptor antagonist, SB-271046, caused a tetrodotoxin-sensitive increase in extracellular glutamate release by microdialysis in the frontal cortex and dorsal hippocampus (but not in the striatum or nucleus accumbens) of conscious adult rats. Furthermore, Meneses (2001) demonstrated that Ro 04-6790 partially reversed an impairment in learning consolidation produced by the NMDA receptor antagonist, dizocilpine, consistent with the idea that 5-HT_6 receptor-modulation of glutamate may also contribute to the effect of 5-HT_6 receptor antagonists on memory processing. Interestingly, serotonergic median raphé afferents preferentially innervate inhibitory GABAergic interneurones in the hippocampus and dentate gyrus (Freund et al. 1990; Freund 1992) and thus is consistent with the proposal that 5-HT_6 receptor regulation of glutamate may occur indirectly via inhibition of GABA in this area. Indeed, Dawson et al. (2001) showed that cholinergic-regulation of glutamate release in this area is unlikely, since atropine had no effect on SB-271046-induced extracellular glutamate release. However, the possibility that cholinergic neurotransmission increases as a result of elevated glutamatergic neurotransmission has not been investigated in this region and cannot be ruled out, since cholinergic interneurones are present, albeit at low levels (Vizi and Kiss 1998), and such an interaction has been demonstrated in the rat striatum (Consolo et al. 1996).

In summary, the current study demonstrates that acute systemic administration of Ro 04-6790 selectively reverses a cholinergic-induced deficit in a rodent model of short term recognition memory. Whilst the underlying mechanism of action remains to be elucidated the current findings add further support for a role of the 5-HT_6 receptor in the regulation of memory processes.

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