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Involvement of 5-HT_6 receptors in nigro-striatal function in rodents

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1 4-Amino-N-(2,4 bis-methylamino-pyrimidin-4-yl) benzene sulphonamide (Ro 04-6790) is a potent, selective and competitive antagonist for the 5-HT_6 receptor which can be detected in the cerebro-spinal fluid (CSF) of rats following intraperitoneal administration. Since 5-HT_6 receptor mRNA and 5-HT_6 receptor-like immunoreactivity have been shown to be present in the striatum, the purpose of the present study was to evaluate the effect of 5-HT_6 receptor antagonism on haloperidol- and SCH 23390-induced catalepsy in mice and on the turning behaviour of rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle.

2 Ro 04-6790 (3, 10 and 30 mg kg^{-1} i.p.) did not induce catalepsy and had no effect on catalepsy induced by either haloperidol or SCH 23390.

3 Ro 04-6790 (3, 10 and 30 mg kg^{-1} i.p.) did not itself induce rotational behaviour in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle nor did it affect the rotational behaviour induced by either L-Dopa or amphetamine.

4 5-HT_6 receptor antagonism inhibited the rotational behaviour of 6-OHDA lesioned rats induced by treatment with the muscarinic antagonists scopolamine and atropine.

5 The data support earlier conclusions from experiments with antisense oligonucleotides that the 5-HT_6 receptor is involved in the control of acetylcholine neurotransmission in the rat brain.

Keywords: 5-HT_6 receptor; Ro 04-6790; acetylcholine; dopamine; turning; 6-OHDA lesions; catalepsy

Introduction

5-Hydroxytryptamine is a modulatory neurotransmitter in the brain and its effects are mediated through at least 14 distinct receptors. These comprise a ligand-gated ion channel (the 5-HT_3 receptor) and 13 G-protein coupled receptors (Boess & Martin, 1994; Hoyer & Martin, 1997).

The 5-HT_6 receptor was first isolated from rat striatal mRNA by reverse transcription and polymerase chain reaction with degenerate oligonucleotide 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 rat histamine H_2 receptor (Ruat *et al*., 1993). Subsequently, the human 5-HT_6 receptor was cloned (Kohen *et al*., 1994). In rats, the highest levels of 5-HT_6 receptor mRNA are 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). In addition to these regions, 5-HT_6-like immunoreactivity was also identified in frontal and entorhinal cortex and the molecular layer of the cerebellum (Gérard *et al*., 1997).

The 5-HT_6 receptor is positively coupled to adenylyl cyclase and can be radiolabelled with [^{125}I]-LSD, [^{3}H]-LSD and [^{3}H]-5-HT (Monsma *et al*., 1993; Boess *et al*., 1997a; reviewed by Sleight *et al*., 1997). The putative binding site of 5-HT for this receptor has been studied using site-directed mutagenesis (Boess *et al*., 1997b). Until recently, however, the only study exploring the functional significance of the receptor *in vivo* used antisense oligonucleotides which should abolish or reduce the expression of the 5-HT_6 receptor protein. Intracerebroventricular treatment of rats with 5-HT_6 specific antisense oligonucleotides produced a behavioural syndrome consisting of yawning, stretching and chewing which could be antagonized by atropine but not by haloperidol (Bourson *et al*., 1995; Sleight *et al*., 1996).

Many non-selective compounds such as tricyclic antidepressants, antipsychotic agents, tryptamine and ergoline derivatives bind to the 5-HT_6 receptor with high affinity (Monsma *et al*., 1993; Roth *et al*., 1994; Boess *et al*., 1997a). In particular, clozapine and related atypical antipsychotics have high affinity for the 5-HT_6 receptor (Roth *et al*., 1994). It has been suggested that the high affinity of clozapine for the 5-HT_6 receptor which is localized in the striatum, could account for its lack of extrapyramidal side-effects (Roth *et al*., 1994).

More recently, we have presented results with two potent and selective 5-HT_6 receptor antagonists 4-amino-N-(2,4 bis-methylamino-pyridin-4-yl)benzene sulphonamide (Ro 63-0563) and 4-amino-N-(2,4 bis-methylamino-pyrimidin-4-yl) benzene sulphonamide (Ro 04-6790). Ro 04-6790 penetrated the brain and induced a behavioural syndrome similar to that seen with 5-HT_6 receptor antisense treatment (Sleight *et al*., 1998).

In the present study we have attempted to determine whether the 5-HT_6 receptor is responsible for the lack of extrapyramidal side-effects of clozapine and related compounds by examining the effect of Ro 04-6790 in two models of extrapyramidal function; catalepsy in mice and rotations in rats with unilateral 6-hydroxydopamine (6-OHDA) lesions of the medial forebrain bundle.

Methods

Animals

Male mice (Ibm:MORO) and male rats (Ibm:RORO) were used in these studies and were obtained from Biological Research Laboratories Ltd (BRL) of Füllinsdorf (Switzer-

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land). A 12 h light-dark cycle was maintained in the rooms with all tests being performed during the light phase. Ambient temperature was approximately 21°C and the relative humidity 55–65%. Access to food ('KLIBA' standardized pellets No. 25-343, Kaisersaugst, Switzerland) and tap water was *ad libitum*.

Unilateral 6-OHDA lesion of the medial forebrain bundle

Rats were pretreated with desmethylimipramine (25 mg kg^{-1} i.p) and 30 min later, the rats were anaesthetized with a combination of 150 μl Ketasol® (100 mg ml^{-1} ketamine) and 150 μl Rompun® (20 mg ml^{-1} Xylazin) and placed in a stereotaxic frame (David Kopf, U.S.A.). 6-OHDA was dissolved in saline containing ascorbic acid (0.2 mg ml^{-1}) to a concentration of 4 μg μl^{-1}. Two microlitres of this solution were slowly injected into the right medial forebrain bundle at the coordinates AP = −2.0 mm, L = +1.5 mm from the bregma according to the atlas of Pellegrino & Cushman (1967). The injection was made over a period of 5 min 30 s, the needle was left in place for a further 30 s to allow diffusion of compound away from the injection site. Rotational behaviour was measured automatically in rotometers (Columbus Instruments, Ohio, U.S.A.) as the number of 360° turns towards (ipsilateral) or away (contralateral) from the lesioned side. Rats were allowed to recover for 2 weeks and were then tested for their rotational response to apomorphine (0.05 mg kg^{-1}, s.c.). Only rats displaying a vigorous contralateral turning response were used in subsequent experiments. This corresponds to a >90% reduction in the dopamine content in the lesioned striatum and an 83% reduction in striatal dopa decarboxylase activity (Robin *et al*., 1985; Palfreyman *et al*., 1985).

The experiments were designed such that each animal received each treatment in a random order with a washout period of at least 4 days.

Effect of Ro 04-6790 in 6-OHDA lesioned rats

In an initial experiment, Ro 04-6790 was tested alone to determine whether a 5-HT_6 antagonist would induce turning behaviour in the 6-OHDA lesioned rat. 6-OHDA lesioned rats were treated with either vehicle or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.) and rotational behaviour was measured for a 4 h period beginning immediately after administration of the compound.

Effect of Ro 04-6790 on amphetamine-induced turning

Separate groups of 6-OHDA lesioned rats were pretreated with either vehicle or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.). and 15 min later the animals were given amphetamine (1 mg kg^{-1} i.p.). The rotational behaviour was measured for a 2 h period beginning immediately after administration of amphetamine.

Muscarinic antagonist-induced turning

Rotational behaviour induced by the muscarinic antagonists atropine and scopolamine was studied by constructing dose-response curves with each compound. 6-OHDA lesioned rats were treated with either vehicle or scopolamine (0.1, 0.3 or 1 mg kg^{-1} i.p.) and rotational behaviour measured for a 3 h period beginning immediately after the administration of scopolamine. In a separate experiment animals were treated with either vehicle or atropine (0.1, 0.3, 1 or 3 mg kg^{-1} s.c.) and again rotational behaviour measured for a 3 h period beginning immediately after the administration of atropine.

Effect of Ro 04-6790 on turning induced by either scopolamine or atropine

The effect of Ro 04-6790 on atropine- and scopolamine-induced rotational behaviour was studied. Animals were pretreated with either vehicle or Ro 04-6790 (3, 10, 30 mg kg^{-1} i.p.). Fifteen minutes later the rats were treated with scopolamine (1 mg kg^{-1} i.p.) Rotational behaviour was measured for a 3 h period beginning immediately after the administration of scopolamine. In a separate experiment animals were treated with either vehicle or Ro 04-6790 (3, 10, 30 mg kg^{-1} i.p.) and 15 min later with atropine (3 mg kg^{-1} s.c.). Rotational behaviour was measured for a 2 h period beginning immediately after the administration of atropine.

Haloperidol- and SCH 23390-induced catalepsy in mice

Male (Ibm:MORO) mice were used in these experiments. At the time of testing body weight was approximately 25 g. Catalepsy was measured in two tests. The first, by placing the forepaws of the mouse over a horizontal metal bar 3 cm above the floor of the cage; and the second, by placing the mouse on a stainless steel grid held at an angle of 60° to the horizontal.

The time to step down from the bar or the time taken to move any paw from the grid was recorded with a stop watch in two trials. The maximal time over both trials was 4 min. Groups of eight mice were treated with either vehicle or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.). Other groups of eight mice were treated with either vehicle or Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.) 30 min after treatment with haloperidol (0.5 mg kg^{-1}). Catalepsy experiments were then conducted 30 min after the treatment with Ro 04-6790. In separate experiments Ro 04-6790 was administered 15 min before treatment with SCH 23390 and catalepsy experiments performed after a further 15 min. In this way all catalepsy measurements were started 30 min after treatment with the 5-HT_6 antagonist. All experiments were performed on a blind basis.

Drugs and solutions

Compounds were obtained from the following sources: Ro 04-6790, atropine, SCH 23390 (F. Hoffmann-La Roche, Switzerland), scopolamine HBr, desmethylimipramine, 6-hydroxydopamine, amphetamine (Sigma, U.S.A.), Ketasol® (Gräub AG, Bern, Switzerland), haloperidol (Haldol® Janssen, Switzerland) and Rompun® (Bayer AG, Germany).

Ro 04-6790, atropine, scopolamine, SCH 23390, desmethylimipramine were dissolved in sterile saline (0.9% w/v). Solutions of haloperidol were prepared to the required concentrations from 5 mg kg^{-1} Haldol® ampoules using saline. All drug solutions were freshly prepared.

Statistics

Rotational data were analysed with two-way analysis of variance with repeated measures. If statistical significance was reached between groups, differences were studied using the Scheffe test.

Data from catalepsy experiments were first analysed with the Kruskal-Wallis analysis of variance followed by Mann-Whitney *U*-test.

Results

Effect of Ro 04-6790 on rotational behaviour

Ro 04-6790 (3, 10 and 30 mg kg^{-1} i.p.) was studied in rats with unilateral 6-OHDA lesions of the medial forebrain bundle. Ro 04-6790 produced neither ipsilateral nor contralateral turning behaviour (results not shown). In addition, Ro 04-6790 (3, 10 and 30 mg kg^{-1} i.p) had no effect on amphetamine (1 mg kg^{-1} i.p.)-induced ipsilateral rotations (Figure 1).

Ipsilateral rotations could be induced by muscarinic antagonists. Atropine (0.1, 0.3, 1, 3 mg kg^{-1} s.c.) and scopolamine (0.1, 0.3, 1 mg kg^{-1} i.p) induced ipsilateral rotations (Figure 2). This effect seems to be an all or nothing effect which may be due to the fact that muscarinic receptors have a modulatory effect on dopamine neurotransmission and hence on the rotational behaviour. Ro 04-6790 (3, 10, 30 mg kg^{-1} i.p.) administered 15 min before either scopolamine (1 mg kg^{-1} i.p.; Figure 3) or atropine (3 mg kg^{-1} s.c.; Figure 4) reduced the number of ipsilateral rotations induced by the muscarinic antagonists. Irrespective of the muscarinic antagonist used, this effect of 5-HT_6 receptor blockade became significant ($P<0.05$) following pretreatment with Ro 04-6790 (30 mg kg^{-1} i.p.).

Effect of Ro 04-6790 on haloperidol- and SCH 23390-induced catalepsy

Ro 04-6790 (3, 10 or 30 mg kg^{-1} i.p.) did not induce catalepsy in mice in either the horizontal bar or in the grid test (results not shown). As shown in Figures 5 and 6, pretreatment with Ro 04-6790 neither potentiated nor inhibited the catalepsy induced by either haloperidol (0.5 mg kg^{-1} i.p.) or SCH 23390 (0.5 mg kg^{-1} s.c.).

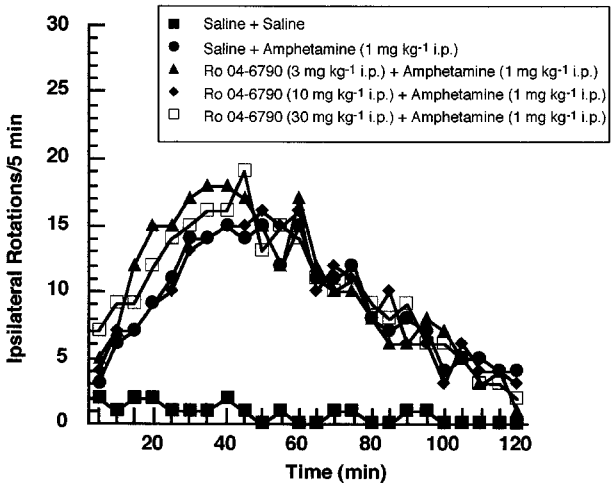

Figure 1 Effect of Ro 04-6790 on amphetamine-induced ipsilateral rotations in rats with unilateral 6-OHDA lesion of the medial forebrain bundle. Vehicle or Ro 04-6790 was administered i.p. 15 min before amphetamine (1 mg kg^{-1} i.p.). Treatment groups were: saline + saline, saline + amphetamine, Ro 04-6790 3 mg kg^{-1} + amphetamine, Ro 04-6790 10 mg kg^{-1} + amphetamine, Ro 04-6790 30 mg kg^{-1} + amphetamine. Rats were placed in the rotometers immediately after the amphetamine administration. Eight rats were used per treatment group.

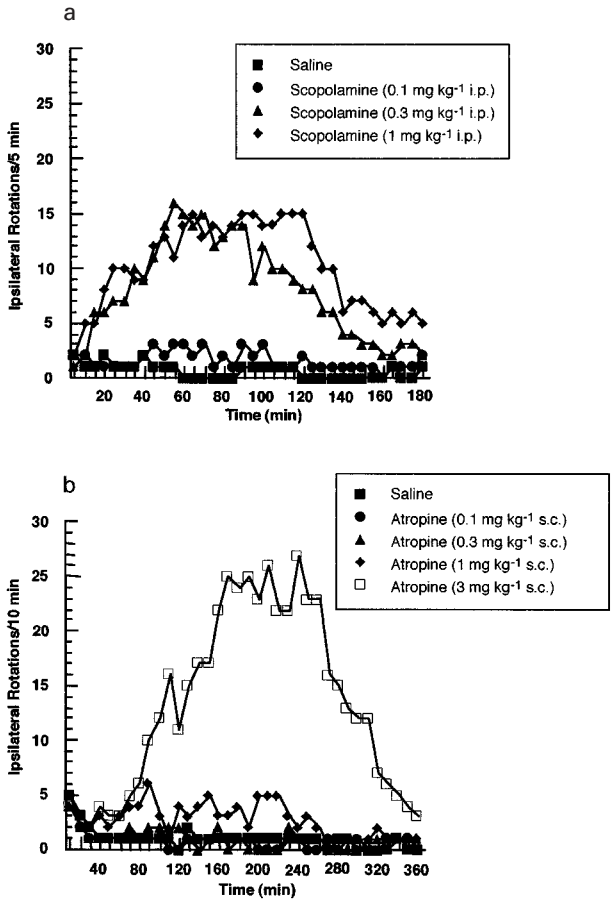

Figure 2 Effect of the muscarinic antagonists, scopolamine (a) and atropine (b) in rats with unilateral 6-OHDA lesion of the medial forebrain bundle. (a) Saline or scopolamine at the doses of 0.1 mg kg^{-1}, 0.3 mg kg^{-1}, 1 mg kg^{-1} was administered i.p. before the rats were placed in the rotometers for 3 h. (b) Saline or atropine at the doses of 0.1 mg kg^{-1}, 0.3 mg kg^{-1}, 1 mg kg^{-1}, 3 mg kg^{-1} were administered subcutaneously immediately before the rats were placed in the rotometers for 6 h. In both experiments, eight rats were used per treatment group.

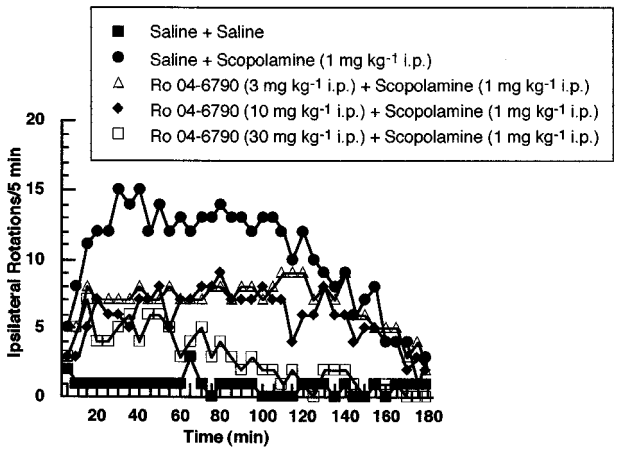

Figure 3 Effect of Ro 04-6790 on scopolamine-induced ipsilateral rotations in rats with unilateral 6-OHDA lesion of the medial forebrain bundle. Saline or Ro 04-6790 was administered i.p. 15 min before scopolamine (1 mg kg^{-1}, i.p.). Treatment groups were: saline + saline, saline + scopolamine, Ro 04-6790 3 mg kg^{-1} + scopolamine, Ro 04-6790 10 mg kg^{-1} + scopolamine, Ro 04-6790 30 mg kg^{-1} + scopolamine. Eight rats per treatment group were used.

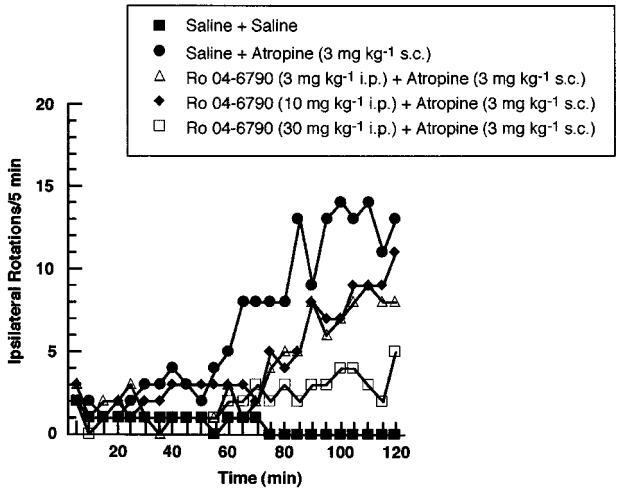

Figure 4 Effect of Ro 04-6790 on atropine-induced ipsilateral rotations in rats with unilateral 6-OHDA lesion of the medial forebrain bundle. Saline or Ro 04-6790 was administered i.p. 15 min before atropine (3 mg kg^{-1}, s.c.). Treatment groups were: saline + saline, saline + atropine, Ro 04-6790 3 mg kg^{-1} + atropine, Ro 04-6790 10 mg kg^{-1} + atropine, Ro 04-6790 30 mg kg^{-1} + atropine. Eight rats were used per treatment group.

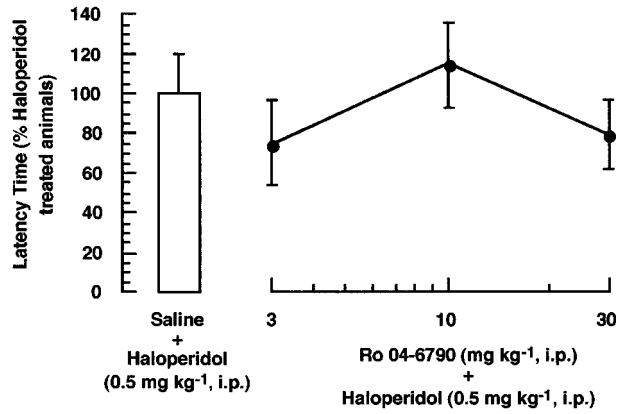

Figure 5 Effect of Ro 04-6790 on haloperidol-induced catalepsy in mice. Ro 04-6790 was administered 30 min after haloperidol and catalepsy was evaluated 60 min after administration of haloperidol. Eight mice per treatment group were used.

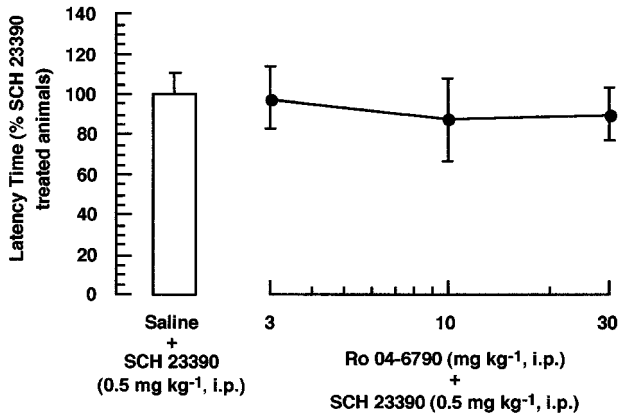

Figure 6 Effect of Ro 04-6790 on SCH 23390-induced catalepsy in mice. Ro 04-6790 was administered 15 min before SCH 23390 catalepsy was evaluated 15 min after administration of SCH 23390. Eight mice per treatment group were used.

Discussion

Until recently the only studies determining the role of the 5-HT_6 receptor *in vivo* have used antisense oligonucleotides which should reduce the expression of the 5-HT_6 receptor (Sleight *et al*., 1996; Bourson *et al*., 1995). In rats treated with 5-HT_6 receptor antisense but not those treated with a scrambled oligonucleotide sequence a behavioural syndrome of yawning, stretching and chewing was observed. Now, however, potent and selective antagonists have become available and allow a more detailed analysis of 5-HT_6 receptor function. 4-Amino-N-(2,4 bis-methylamino-pyridin-4-yl)benzene sulphonamide (Ro 63-0563) and 4-amino-N-(2,4 bis-methylamino-pyrimidin-4-yl)benzene sulphonamide (Ro 04-6790) are highly selective for the 5-HT_6 receptor with affinities of 10 and 30 nM for the 5-HT_6 receptor, respectively (Sleight *et al*., 1998). Additionally, they are competitive antagonists at recombinant human 5-HT_6 receptors with pA_2 values of 7.1 and 6.8, respectively although only Ro 04-6790 can be detected in the cerebro-spinal fluid of rats following i.p. administration. Interestingly, when Ro 04-6790 was administered to rats that had been habituated to observation cages as was the case for animals treated with 5-HT_6 receptor antisense, a behavioural syndrome of yawning and stretching could be measured (Sleight *et al*., 1998).

A large number of antipsychotic agents display high affinity for the 5-HT_6 receptor (Monsma *et al*., 1993; Roth *et al*., 1994) and it has been speculated that the high affinity of clozapine-like compounds for the 5-HT_6 receptor may be responsible in part for the atypical profile of these compounds (Roth *et al*., 1994). Clozapine has been shown to act as an antagonist at the 5-HT_6 receptor (Boess *et al*., 1997a). Therefore the present studies investigated the effect of 5-HT_6 receptor antagonism on catalepsy and in rats with unilateral 6-OHDA lesions of the medial forebrain bundle.

Ro 04-6790 did not induce catalepsy nor did it have any effect on either haloperidol- or SCH 23390-induced catalepsy. In addition, Ro 04-6790 alone did not induce turning behaviour in the unilaterally lesioned rat nor did it potentiate or inhibit ipsilateral rotations induced by amphetamine. Furthermore, Ro 04-6790 had no effect on the contralateral rotations induced by L-Dopa (Bourson, unpublished observations). Therefore, the present data would suggest that 5-HT_6 receptor affinity does not account for the lack of extrapyramidal side-effects characteristic of clozapine-like compounds.

Treatment of unilaterally lesioned rats with muscarinic antagonists such as atropine and scopolamine induces ipsilateral rotations (see Pycock, 1980 for review). Although the 5-HT_6 receptor antagonist had no effect on turning induced by dopamine stimulation, it inhibited the turning induced by both scopolamine and atropine. These data would suggest that there is an interaction between the 5-HT_6 receptor and cholinergic transmission in the striatum that is distal to dopamine transmission although the precise mechanism is not known. A possible mechanism could be an increase in ACh release induced by 5-HT_6 receptor blockade. Alternatively, Gérard *et al*., (1997) suggest that 5-HT_6 receptors may be expressed on GABA spiny neurones in the striatum and therefore interactions between 5-HT_6 and muscarinic receptors may involve GABA neurotransmission.

The present data demonstrate that 5-HT_6 receptor antagonism can produce effects in the 6-OHDA unilaterally lesioned rat, suggesting a role for the 5-HT_6 receptor in striatal function. This is in agreement with the expression of both 5-HT_6 receptor mRNA and 5-HT_6 receptor-like immunoreactivity (Monsma *et al*., 1993; Ruat *et al*., 1993; Ward *et al*., 1995;

Gérard *et al*., 1996; 1997) and suggests the existence of functional 5-HT_6 receptors in this brain region.

It is interesting to note that the present data support earlier suggestions that the 5-HT_6 receptor may have a role in the control of acetylcholine neurotransmission. A reduction in the number of 5-HT_6 receptors by treatment with 5-HT_6 receptor antisense oligonucleotides gave rise to a behavioural syndrome similar to that of a cholinergic agonist that was antagonized by atropine but not by haloperidol (Sleight *et al*., 1996; Bourson *et al*., 1995). Previously published data show that the 5-HT_6 receptor antagonist Ro 04-6790 produces a similar behavioural syndrome (Sleight *et al*., 1998) although it remains to be seen whether the behavioural syndrome induced by Ro 04-6790 is also antagonized by atropine. Finally, the present data also show that 5-HT_6 receptor blockade specifically reverses the effects of muscarinic antagonists in the 6-OHDA unilaterally lesioned rat.

In summary, administration of Ro 04-6790 reversed rotational behaviour induced by the muscarinic antagonists scopolamine and atropine in the 6-OHDA unilaterally lesioned rat but had no effect on rotational behaviour induced by dopaminergic stimulation. These data suggest that the 5-HT_6 receptor is functionally expressed in the rat brain and support the findings obtained with antisense oligonucleotides that the 5-HT_6 receptor is involved in the control of cholinergic neurotransmission.

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References

BOESS, F.G. & MARTIN, I.L. (1994). Molecular biology of 5-HT receptors. *Neuropharmacol.,* **133,** 275 – 317.

BOESS, F.G., MONSMA, F.J., CAROLO, C., MEYER, V., RUDLER, A., ZWINGELSTEIN, C. & SLEIGHT, A.J. (1997a). Functional and radioligand binding characterization of rat 5-HT_6 receptors stably expressed in HEK293 cells. *Neuropharmacol.,* **36,** 713 – 720.

BOESS, F.G., MONSMA, F.J., MEYER, V., ZWINGELSTEIN, C. & SLEIGHT, A.J. (1997b). Interaction of tryptamine and ergoline compounds with threonine 196 in the ligand binding site of the 5-hydroxytryptamine$_6$ receptor. *Molecular Pharmacol.,* **52,** 515 – 523.

BOURSON, A., BORRONI, E., AUSTIN, R.H., MONSMA, F.J. & SLEIGHT, A.J. (1995). Determination of the role of the 5-HT_6 receptor in the rat brain: A study using antisense oligonucleotides. *J. Pharmacol. Exp. Ther.,* **274,** 173 – 180.

GÉRARD, C., EL MESTIKAWY, S., LEBRAND, C., ADRIEN, J., RUAT, M., TRAIFFORT, E., HAMON, M. & MARTRES, M-P. (1996). Quantitative RT-PCR distribution of serotonin 5-HT_6 receptor mRNA in the central nervous system of control or 5,7-dihydroxytryptamine-treated rats. *Synapse,* **23,** 164 – 173.

GÉRARD, C., MARTRES, M-P., LEFEVRE, K., MIQUEL, M-C., VERGE, D., LANFUMEY, L., DOUCET, E., HAMON, M. & EL MESTIKAWY, S. (1997). Immuno-localization of serotonin 5-HT_6 receptor-like material in the rat central nervous system. *Brain Res.,* **746,** 207 – 219.

HOYER, D. & MARTIN, G. (1997). 5-HT receptor classification and nomenclature: towards a harmonization with the human genome. *Neuropharmacol.,* **36,** 419 – 428.

KOHEN, R., METCALF, M.A., DRUCK, T., HUEBNER, K., SIBLEY, D.R. & HAMBLIN, M.W. (1994). Cloning and chromosomal localization of a human 5-HT_6 serotonin receptor. *Soc. Neurosci. Abstracts,* **20,** 476.8.

MONSMA, F.J., SHEN, Y., WARD, R.P., HAMBLIN, M.W. & SIBLEY, D.R. (1993). Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.,* **43,** 320 – 327.

PALFREYMAN, M.G., MCDONALD, I.A., FOZARD, J.R., MELY, Y., SLEIGHT, A.J., ZREIKA, M., WAGNER, J., BEY, P. & LEWIS, P.J. (1985). Inhibition of monoamine oxidase selectively in brain monoamine nerves using the bioprecursor (E)-β-fluoromethylene-*m*-tyrosine (MDL 72394), a substrate for aromatic L-amino acid decarboxylase. *J. Neurochem.,* **45,** 1850 – 1860.

PELLEGRINO, L.J. & CUSHMAN, A.J. (1967). *A Stereotaxic atlas of the rat brain*. New York, Appleton-Century Crafts.

PYCOCK, C.J. (1980). Turning behaviour in animals. *Neuroscience,* **5,** 461 – 514.

ROBIN, M., FORLER, C. & PALFREYMAN, M.G. (1985). Effect of chronic apomorphine on the development of denervation supersensitivity. *Pharmacol. Biochem. Behav.,* **22,** 547 – 551.

ROTH, B.L., CRAIGO, S.C., CHOUDHARY, M.S., ULUER, A., MONSMA, F.J., SHEN, Y., MELTZER, H.Y. & SIBLEY, D.R. (1994). Binding of typical and atypical antipsychotic agents to 5-hydroxytryptamine-6 and 5-hydroxytryptamine-7 receptor. *J. Pharmacol. Exp. Ther.,* **268,** 1403 – 1410.

RUAT, M., TRAIFFORT, E., ARRANG, J-M., TARDIVEL-LACOMBE, J., DIAZ, J., LEURS, R. & SCHWARTZ, J-C. (1993). A novel serotonin (5-HT_6) receptor: Molecular cloning, localisation and stimulation of cyclic AMP accumulation. *Biochem. Biophys. Res. Com.,* **193,** 269 – 276.

SLEIGHT, A.J., BOESS, F.G., BÖS, M., LEVET-TRAFIT, B., RIEMER, C. & BOURSON, A. (1998). Characterization of Ro 04-6790 and Ro 63-0563: potent and selective antagonists at human and rat 5-HT_6 receptors. *Br. J. Pharmacol.,* **124,** 556 – 562.

SLEIGHT, A.J., BOESS, F.G., BOURSON, A., SIBLEY, D.R. & MONSMA, F.J. (1997). 5-HT_6 and 5-HT_7 receptors: molecular biology, functional correlates and possible therapeutic indications. *Drug News and Perspectives,* **10,** 214 – 224.

SLEIGHT, A.J., MONSMA, F.J., BORRONI, E., AUSTIN, R.H. & BOURSON, A. (1996). Effects of altered 5-HT_6 expression in the rat: Functional studies using antisense oligonucleotides. *Beh. Brain Res.,* **73,** 245 – 248.

WARD, R.P., HAMBLIN, M.W., LACHOWICZ, J.E., HOFFMAN, B.J., SIBLEY, D.R. & DORSA, D.M. (1995). Localization of serotonin subtype 6 receptor messenger RNA in the rat brain by in situ hybridization histochemistry. *Neuroscience,* **64,** 1105 – 1111.

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