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European Journal of Pharmacology 303 (1996) 151–162

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Differential serotoninergic and dopaminergic activities of the (*R*)- and the (*S*)-enantiomers of 2-(di-*n*-propylamino)tetralin

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Received 9 November 1995; revised 8 January 1996; accepted 16 January 1996

Abstract

Racemic 2-(di-*n*-propylamino)tetralin ((*R,S*)-DPAT), which lacks phenolic or other aromatic substituents, induces both dopaminergic (sniffing, licking and gnawing) and serotoninergic (forepaw treading and flat body posture) behavioural responses. The present study shows that s.c. administration of (*R*)-DPAT induces typical 5-HT_{1A} receptor agonist behaviours. These effects are blocked by the 5-HT_{1A} receptor antagonist (*S*)-5-fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin ((*S*)-UH-301). Administration of (*S*)-DPAT induces dopaminergic behaviours, which are fully antagonised by raclopride, a dopamine D_2 receptor antagonist. Both enantiomers induce hypothermia, (*R*)-DPAT being antagonised by (*S*)-UH-301, whereas (*S*)-DPAT is antagonised by raclopride. The accumulation of 5-hydroxytryptophan and DOPA (3,4-dihydroxyphenylalanine) after decarboxylase inhibition that reflects presynaptic actions on 5-HT (5-hydroxytryptamine, serotonin) and dopamine neurons, respectively, are inhibited by both enantiomers of DPAT. (*R*)-DPAT is more potent than (*S*)-DPAT as an inhibitor of 5-hydroxytryptophan accumulation whereas (*S*)-DPAT is more potent than (*R*)-DPAT as an inhibitor of DOPA accumulation. Thus, in functional tests of postsynaptic actions (*R*)-DPAT behaves as a 5-HT_{1A} receptor agonist and (*S*)-DPAT as a dopamine D_2 receptor agonist. Presynaptically, (*R*)-DPAT shows selectivity for 5-HT_{1A} receptors and (*S*)-DPAT for dopamine D_2 receptors. Receptor binding studies, utilizing [^{3}H]8-hydroxy-2-(di-*n*-propylamino)tetralin and [^{3}H]quinpirole as radioligands for 5-HT_{1A} and dopamine D_2 receptors, respectively, showed (*R*)-DPAT to have a 3-fold higher affinity than (*S*)-DPAT for 5-HT_{1A} receptors, whereas (*S*)-DPAT had a 6-fold higher affinity than (*R*)-DPAT for dopamine D_2 receptors. Thus, the results from receptor binding studies support the conclusion that (*R*)- and (*S*)-DPAT are agonists showing selectivity for 5-HT_{1A} and dopamine D_2 receptors, respectively. Taken together, these findings may explain previous controversies with regard to the pharmacology of racemic DPAT and re-emphasise the necessity to study pure enantiomers of chiral compounds.

Keywords: 2-(Di-*n*-propylamino)tetralin; Chiral compound; 5-HT_{1A} receptor; Dopamine D_2 receptor; (Racemate); (Rat); (Stereoisomer)

1. Introduction

Tetralin (1,2,3,4-tetrahydronaphthalene) derivatives, especially 2-aminotetralin and numerous ring- and/or N-substituted derivatives thereof, have been investigated for pharmacological activity for at least 70 years (Cloetta and Waser, 1923). A variety of actions, including cardiovascular, thermoregulatory, hormonal and CNS effects, have been reported. McDermed et al. (1975) found that aromatic hydroxylations and aliphatic N-substitutions (N-di-*n*-propylation in particular) provided potent dopamine receptor agonists. Later, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) was discovered to be a potent and selective serotonin 5-HT_{1A} receptor agonist in contrast to the 5-, 6- and 7-monohydroxy-2-(di-*n*-propylamino)tetralins, which are potent dopamine receptor agonists devoid of serotoninergic activity (Arvidsson et al., 1981). Aminotetralins are chiral compounds. Studies of the enantiomers of 8-OH-DPAT have shown that (*R*)-8-OH-DPAT is a potent and full agonist, whereas (*S*)-8-OH-DPAT is less potent and a partial agonist on 5-HT_{1A} receptors (Björk et al., 1989; Cornfield et al., 1991; Yu et al., 1993). The activity of the highly potent dopamine receptor agonist 5-hydroxy-2-(di-*n*-propylamino)tetralin (5-OH-DPAT) resides in the (*S*)-enantiomer (McDermed

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PII S0014-2999(96)00051-9

et al., 1976), the (*R*)-enantiomer being less potent and showing dopamine receptor antagonist properties in some systems (Karlsson et al., 1990).

Racemic 2-(di-*n*-propylamino)tetralin ((*R,S*)-DPAT; coded TL-68 in the earlier literature) has been categorised as a dopamine receptor agonist both postsynaptically (McDermed et al., 1975; Cannon et al., 1977; Costall et al., 1977; Fuller and Snoddy, 1981) and presynaptically (Feenstra et al., 1983; Van Oene et al., 1984). A moderate affinity for dopamine D_2 receptors in receptor binding studies (Cannon et al., 1978; Rusterholz et al., 1979; Seeman et al., 1985; Beart et al., 1987) has indicated that (*R,S*)-DPAT acts via this receptor subtype. It does not seem to have dopamine D_1 receptor agonist properties in a functional test (Sheppard et al., 1978).

There are a number of conflicting findings and unresolved issues with regard to the pharmacology of (*R,S*)-DPAT in the literature. Stereotyped behaviours induced by (*R,S*)-DPAT were not antagonised by the combined treatment with reserpine and α-methyl-*p*-tyrosine (an inhibitor of catecholamine synthesis), which led McDermed et al. (1975) to conclude that the compound is a directly acting dopamine receptor agonist, a finding contradicted by Cannon et al. (1977). A study of (*R,S*)-DPAT on contralateral circling in rats with unilaterally lesioned nigro-striatal pathways mainly supported a direct dopaminergic action of (*R,S*)-DPAT, but a partial inhibition by α-methyl-*p*-tyrosine remained unexplained (Rusterholz et al., 1979). A possible metabolic activation of (*R,S*)-DPAT on systemic administration has been suggested, since direct injection of the compound into the nucleus accumbens or the striatum in rats did not elicit increased locomotor activity or stereotyped behaviours (Costall et al., 1977; Cannon et al., 1978). However, the findings that i.c.v. and intraarterial, but not i.v., administrations of (*R,S*)-DPAT induced hypotension and bradycardia (Sharabi et al., 1978) and that (*R,S*)-DPAT acts on peripheral dopamine receptors in vitro in cats (Ilhan et al., 1984) and in rats (Beaulieu et al., 1984) argue for the drug itself to be the active agent.

The present study of the differential pharmacology of (*R*)- and (*S*)-2-(di-*n*-propylamino)tetralin ((*R*)- and (*S*)-DPAT) was prompted by the unexpected finding that (*R*)-DPAT appeared as a 5-HT_{1A} receptor agonist and (*S*)-DPAT as a dopamine D_2 receptor agonist in the screening of newly synthesised pure enantiomers of a series of 2-aminotetralins (Liu et al., 1993). The objectives were to characterise (*R*)-, (*S*)- and (*R,S*)-DPAT with respect to their serotoninergic and dopaminergic activities in behavioural, biochemical and receptor binding tests. The prototype 5-HT_{1A} receptor agonist, (*R*)-8-OH-DPAT (Arvidsson et al., 1981) and the potent and selective dopamine D_2 receptor agonist (*S*)-5-OH-DPAT (Karlsson et al., 1990) were chosen as positive controls and model compounds in most experiments. It is concluded from the results of the present investigations that racemic DPAT has mixed dopaminergic and serotonergic actions, which might help explain previous contradictory findings regarding its pharmacological characteristics.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (250–280 g; ALAB, Stockholm, Sweden) were used in all experiments. Animals were kept at 23 ± 1°C with lights on between 06:00 and 18:00 h. Four rats were housed in each cage (55 × 35 × 20 cm) and were habituated in the laboratory for at least a week before being used. They were allowed ad libitum access to food and water. All experiments were performed between 09:00 and 15:00 h. Each animal was used only once. Whenever suitable, behavioural symptoms, body temperature and amine turnover were studied sequentially in the same animal. Thus, behavioural scores were recorded at 6, 12 and 30 min after drug administration, body temperature was then measured, and the animal was decapitated as soon as possible thereafter for determination of 5-HT and dopamine turnover. In some cases, however, separate groups of animals were used, e.g. for time-response studies of behavioural effects, changes in body temperature (see sections 2.3 and 2.4), or studies of 5-HT and dopamine synthesis (see section 2.6).

2.2. Drugs

The compounds studied (Fig. 1) or used, (±)-(*R,S*)-, (+)-(*R*)- and (−)-(*S*)-2-(di-*n*-propylamino)tetralin hydrochloride ((*R,S*)-, (*R*)- and (*S*)-DPAT), (+)-(*R*)-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrochloride ((+)-(*R*)-8-OH-DPAT), (−)-(*S*)-5-hydroxy-2-(di-*n*-propylamino)tetralin hydrochloride ((−)-(*S*)-5-OH-DPAT),

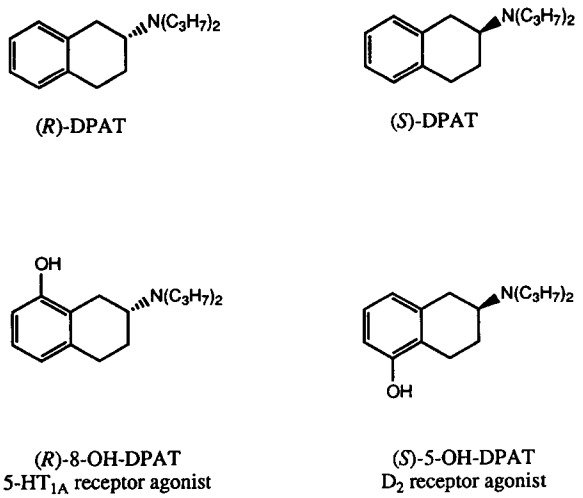

Fig. 1. Chemical structures of the test compounds used.

(−)-(*S*)-5-fluoro-8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide) ((−)-(*S*)-UH-301), were synthesised at the Department of Organic Pharmaceutical Chemistry (Uppsala University, Sweden). Raclopride was synthesised at Astra Arcus (Södertälje, Sweden). Reserpine and 3-hydroxybenzylhydrazine hydrochloride (NSD1015) were purchased from Sigma Chemical and D,L-α-methyl-*p*-tyrosine methyl ester hydrochloride (H44/68) from Labkemi (Stockholm, Sweden). Reserpine was dissolved in a minimal quantity of glacial acetic acid and made up to volume with 0.9% NaCl. All other compounds were dissolved in 0.9% NaCl, occasionally with gentle warming and stirring. All compounds were injected s.c. Injection volumes were 2 ml/kg.

2.3. Behavioural observations

The behavioural observations were performed for 30–60 min after injection of (*R,S*)-, (*R*)-, (*S*)-DPAT, reference compounds or saline, and each animal was observed for 30 s at predetermined intervals, usually 6, 12, 30 and sometimes 60 min. In some behavioural experiments, rats were pretreated with reserpine (5 mg/kg s.c., 18 h previously) in order to minimise a possible indirect mode of action of the drugs, i.e. via release of endogenous 5-HT and dopamine. Rats pretreated with reserpine also constitute a more sensitive assay for directly acting 5-HT_{1A} and dopamine receptor agonists. Saline-treated controls, (*R*)-8-OH-DPAT (1.0 μmol/kg, i.e. 0.28 mg/kg, as a positive control for 5-HT_{1A} receptor actions) and (*S*)-5-OH-DPAT (0.05 μmol/kg, i.e. 0.014 mg/kg, as a positive control for dopamine D_2 receptor actions) were run in parallel in each experiment. The investigators were not blind to drugs given. A 30-item check-list covering a number of drug-induced behaviours of various types (e.g. serotoninergic, dopaminergic and cholinergic) was used. Particular attention was paid to the 5-HT syndrome (flat body posture, forepaw treading and hindlimb abduction) and dopamine D_2 receptor agonist-like behaviours (sniffing, chewing, licking and biting), hereafter called the dopamine D_2 syndrome. Scoring was generally made as follows: absent (0), equivocal (1) and definitely present (2). The maximum score for each symptom was used for calculation of the total scores for the 5-HT syndrome (maximum total score = 6) and the dopamine D_2 syndrome (maximum total score = 8), respectively, for each animal.

2.4. Body temperature

Body temperature was determined by insertion of a thermistoprobe (Ellab Instruments, Copenhagen, Denmark) into the colon, 2.5–3.0 cm from the anal orifice. Baseline values were measured before the injections and recordings were made at predetermined intervals after the injections of the test compounds or vehicle.

2.5. Determination of 5-HT and dopamine turnover

Rats were decapitated within 5–10 min after the last behavioural rating and body temperature measurement at 30 min after drug administration. Brain regions (hippocampus, hypothalamus, corpus striatum and limbic system (rhinal fissure landmark)) were rapidly dissected out and frozen (−20°C) until assayed. Changes in the ratios 5-hydroxyindoleacetic acid over 5-hydroxytryptamine (5-HT) and 3,4-dihydroxyphenylacetic acid over dopamine were taken as indications of changes in 5-HT and dopamine turnover, respectively.

2.6. Serotonin and dopamine synthesis

5-HT and dopamine syntheses were estimated by measuring the accumulation of 5-hydroxytryptophan and 3,4-dihydroxyphenylalanine (DOPA), respectively, after inhibition of aromatic L-amino acid decarboxylase by NSD1015 (Carlsson et al., 1972). The animals were pretreated with reserpine (5 mg/kg s.c.) at 18 h before, and NSD1015 (60 mg/kg, i.e. 287 μmol/kg, s.c.) was injected at 30 min after administration of the test compounds. The rats were decapitated at 30 min after NSD1015. The hippocampus, the hypothalamus and the corpus striatum were rapidly dissected out and stored at −20°C until assayed.

2.7. Biochemical analyses

All samples were stored at −20°C for not more than one week before being assayed. The frozen tissues were weighed and homogenised (Ultra Turrax; Janke and Kunkel, Staufen, Germany) in 1 ml of 0.1 M perchloric acid and α-methyl-5-hydroxytryptophan was added as an internal standard. After centrifugation (12000 rpm, i.e. 18600 × *g*, at 4°C for 10 min) and filtration, 20 μl of the supernatant was injected into a high-performance liquid chromatograph with an electrochemical detector (HPLC-EC) to analyse 5-hydroxyindoleacetic acid, 5-hydroxytryptophan, 5-HT, 3,4-dihydroxyphenylacetic acid, DOPA, dopamine and homovanillic acid. The HPLC system consisted of a PM-48 pump (Bioanalytical Systems, BAS) with a CMA/240 autoinjector (injection volume 20 μl), a precolumn (15 × 3.2 mm, RP-18 Newguard, 7 μ), a column (100 × 4.6 mm, SPHERI-5, RP-18, 5 μ), and an amperometric detector (LC-4B, BAS, equipped with an Ag/AgCl reference electrode and a MF-2000 cell) operating at a potential of +0.85 V. The mobile phase (pH 2.6) was freshly prepared as follows: phosphate/citric acid buffer (7.5 mM K_2HPO_4, 26 mM citric acid), sodium octylsulphate (40 mg/l), 10% EDTA (4 drops/l) and 3–10% methanol (depending on the condition of column). The mobile phase was filtered and degassed before use. The flow rate was 1 ml/min and the temperature of the column was kept constant at 35°C. Standard curves for

each analysis were prepared for each experiment and standards were run intermittently in order to continuously ascertain the accuracy of the analyses.

2.8. Receptor binding assays

5-HT_{1A} receptor affinity was studied using rat hippocampal tissue, which was incubated with [^{3}H]8-OH-DPAT (New England Nuclear, Boston, MA) as radioligand at 37°C for 45 min (Jackson et al., 1995). The dopamine receptors were studied with [^{3}H]quinpirole and [^{3}H]-raclopride (both from New England Nuclear) as radioligands using cloned human dopamine D_{2A} and D_3 receptors expressed in mouse fibroblast (Ltk^-) and Chinese hamster ovary (CHO) cells, respectively. The assay conditions were 60 min at 22°C for [^{3}H]raclopride and 60 min at 30°C for [^{3}H]quinpirole. For details of methods, see Malmberg et al. (1993) and Malmberg and Mohell (1995). α_1-, α_2-, β-Adrenoceptors, muscarinic and 5-HT_2 receptors were studied using [^{3}H]prazosin, [^{3}H]RX821002 (2-(2-methoxy-1,4-benzodioxan-2-yl)-2-imidazoline, [^{3}H]dihydroalprenolol, [^{3}H](−)-3-quinuclidinylbenzilate and [^{3}H]ketanserin as radioligands, respectively, and rat cortex tissue (Jackson et al., 1995). Dopamine D_1 receptors (rat striatum) were labeled with [^{3}H]SCH23390 (7-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7ol; Jackson et al., 1995). The incubations were terminated by rapid filtration and subsequent washing using a Brandell cell harvester. Scintillation cocktail was added and radioactivity determined in a Packard 2500TR liquid scintillation counter. The displacement curves were based on 10–12 concentrations of the test compounds and were analysed by nonlinear regression using the LIGAND program (Munson and Rodbard, 1980).

2.9. Statistical methods

Behavioural scores were assumed to be on an ordinal scale and have been presented as medians of groups of rats. Nonparametric methods were used in tests for statistical significance between groups or trends (Mann-Whitney U test, Kruskal-Wallis test, Spearman's rank correlation test; Abacus Concepts, Statview, Abacus Concepts, Berkeley, CA, 1994). Parametric statistics (ANOVA followed by Tukey's studentized range (HSD) test; Base SAS software, SAS Institute, Cary, NC) were used for all other measurements.

3. Results

3.1. The 5-HT_{1A} behavioural syndrome

Administration of (*R*)-8-OH-DPAT elicited within 3–5 min a typical 5-HT motor syndrome consisting of flat body posture, forepaw treading and hindlimb abduction (Fig. 2A). A dose-dependent (1–32 μmol/kg s.c.) 5-HT syndrome was displayed by (*R*)-DPAT (Spearman's $\rho = 0.83$; $P < 0.001$) and by (*R,S*)-DPAT ($\rho = 0.93$; $P < 0.001$) (Fig. 2A). The behavioural symptoms appeared within 6 min after injection. A 30-fold higher dose of (*R*)-DPAT had to be given in order to produce a response similar to (*R*)-8-OH-DPAT. (*S*)-DPAT caused only flat body posture (score 1) at a dose of 32 μmol/kg s.c. (Fig. 2A). (*S*)-5-OH-DPAT did not elicit the 5-HT syndrome at the dose used (Fig. 2A).

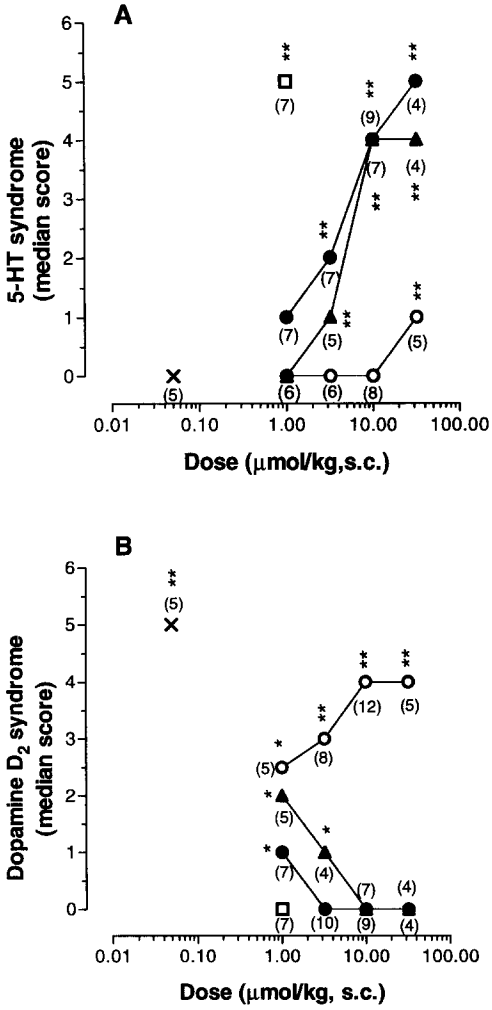

Fig. 2. The 5-HT syndrome (A) and dopamine D_2 syndrome (B) in rats treated with (*R*)-8-OH-DPAT (□), (*R*)- (●), (*R,S*)- (▲) and (*S*)-DPAT (○) and (*S*)-5-OH-DPAT (×). The data are expressed as the median scores of the behavioral syndromes which are described in section 2. The numbers within brackets indicate the number of rats tested. Statistics: Kruskal-Wallis test followed by Mann-Whitney U test. * $P < 0.05$ and * * $P < 0.01$ vs. groups treated with saline.

The 5-HT_{1A} receptor antagonist (*S*)-UH-301 completely blocked the 5-HT syndrome induced by (*R*)-8-OH-DPAT or (*R*)-DPAT (Fig. 3A). In contrast, the dopamine D_2 receptor antagonist raclopride did not attenuate the 5-HT syndrome induced by the two compounds.

In rats pretreated with reserpine, the 5-HT syndrome scores after (*R*)-8-OH-DPAT and (*R*)-DPAT were increased in comparison with saline controls ($P < 0.01$; Fig.

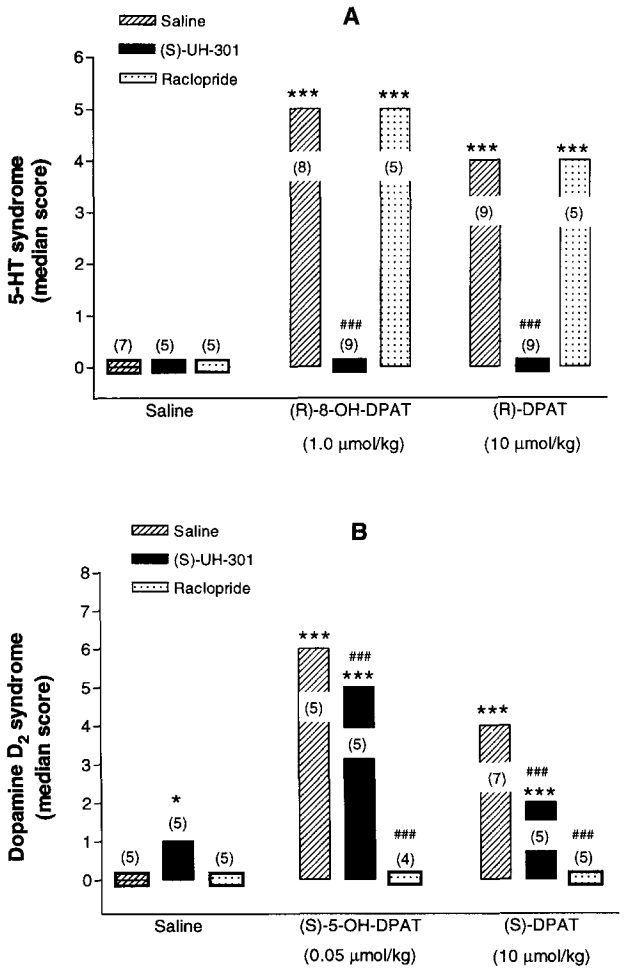

Fig. 3. Effects of (*S*)-UH-301 or raclopride on the 5-HT syndrome after (*R*)-8-OH-DPAT and (*R*)-DPAT (A) and on the dopamine D_2 syndrome after (*S*)-5-OH-DPAT and (*S*)-DPAT (B). (*S*)-UH-301 (32 μmol/kg s.c., black columns), saline (hatched columns) or raclopride (3.2 μmol/kg s.c., stippled columns) was injected 10 min or 60 min, respectively, before saline, (*R*)-8-OH-DPAT (1.0 μmol/kg s.c.), (*S*)-5-OH-DPAT (0.05 μmol/kg s.c.), (*R*)-DPAT or (*S*)-DPAT (each in a dose of 10 μmol/kg s.c.). The data are expressed as the median scores. The numbers within brackets indicate the number of rats tested. Statistics: Kruskal-Wallis test followed by Mann-Whitney *U* test. $^{*}P < 0.05$ and $^{***}P < 0.001$ vs. the groups treated with saline + saline; $^{###}P < 0.001$ vs. groups treated with saline + the respective agonist.

4A). The inhibitor of catecholamine synthesis, α-methyl-*p*-tyrosine, was accompanied by an increased serotonin syndrome score after (*R*)-DPAT in comparison with saline-treated rats but not in reserpine-treated rats (Fig. 4A).

3.2. The dopamine D_2 agonist-like behavioural syndrome

(*S*)-5-OH-DPAT (0.05 μmol/kg s.c.), a dopamine D_2 receptor agonist, elicited clear-cut chewing, biting and sniffing but no licking in rats (Fig. 2B). (*S*)-DPAT induced a dose-dependent (1–32 μmol/kg s.c., Spearman's $\rho = 0.59$; $P < 0.001$) dopamine D_2 syndrome (Fig. 2B) with all symptoms present within 5–10 min after the administration. (*R,S*)-DPAT produced occasional biting and chewing in the dose range of 1.0–3.2 μmol/kg s.c. but not at higher doses (Fig. 2B).

Raclopride, a dopamine D_2 receptor antagonist, completely blocked the dopamine D_2 syndrome induced by (*S*)-DPAT and (*S*)-5-OH-DPAT (Fig. 3B). The 5-HT_{1A} receptor antagonist (*S*)-UH-301 reduced, but did not block, the (*S*)-5-OH-DPAT or (*S*)-DPAT-induced dopamine D_2 syndromes (Fig. 3B).

The effect of (*S*)-5-OH-DPAT after reserpine pretreatment was similar to controls (Fig. 4B). However, jerks and yawns appeared after (*S*)-5-OH-DPAT in reserpine-treated rats. The response to (*S*)-DPAT was increased in reserpine-treated rats (Fig. 4B) and jerk and yawns appeared as for (*S*)-5-OH-DPAT. Inhibition of catecholamine synthesis with α-methyl-*p*-tyrosine did not antagonise the (*S*)-DPAT-induced dopamine D_2 syndrome in rats (Fig. 4B). After reserpine, α-methyl-*p*-tyrosine abolished jerks and yawns induced by (*S*)-DPAT (data not shown), but not

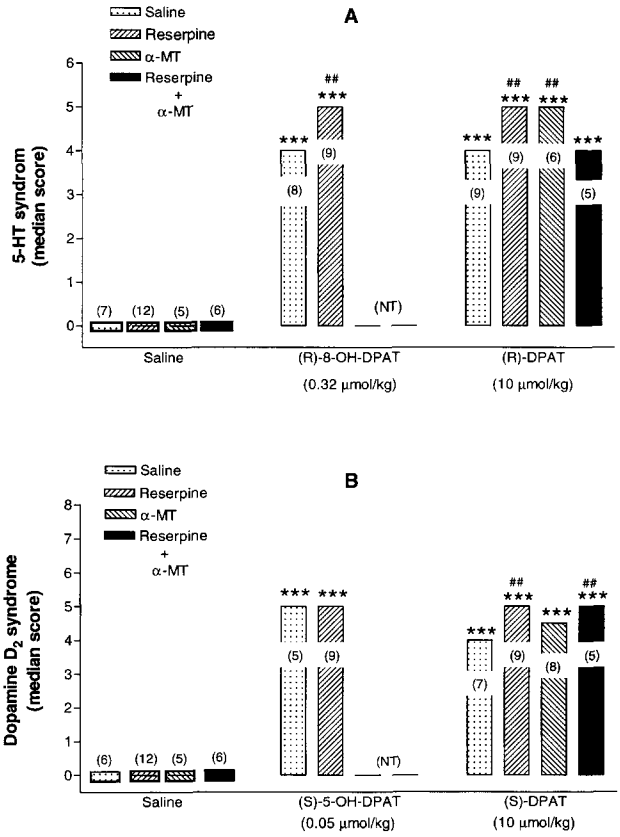

Fig. 4. The 5-HT syndrome (A) or dopamine D_2 syndrome (B) after (*R*)-8-OH-DPAT, (*R*)-DPAT, (*S*)-DPAT and (*S*)-5-OH-DPAT in rats pretreated with saline, reserpine, α-MT (α-methyl-*p*-tyrosine) or reserpine + α-MT, respectively. Rats were given either reserpine (5 mg/kg s.c., left oblique hatched columns) at 18 h or α-MT (250 mg/kg i.p., right oblique hatched columns) at 2 h or both (reserpine at 18 h and α-MT at 1 h, black columns) before administration of the test compounds. The data are expressed as the median scores of the behavioural syndromes. The numbers within brackets indicate the number of rats tested. NT, not tested. Statistics: Kruskal-Wallis test followed by Mann-Whitney *U* test. $^{***}P < 0.001$ vs. group treated with saline + saline; $^{##}P < 0.01$ vs. groups treated with saline + the respective agonist.

other dopamine D_2 receptor agonist-like behaviours (Fig. 4B).

3.3. Effects of (R,S)-, (R)- and (S)-DPAT on body temperature

The 5-HT_{1A} receptor agonist (*R*)-8-OH-DPAT and the dopamine D_2 receptor agonist (*S*)-5-OH-DPAT both induced a decrease in body temperature (Fig. 6). (*R,S*)-, (*R*)- and (*S*)-DPAT all induced hypothermia at doses between 1 and 32 μmol/kg (Fig. 5B). The maximal decrease in body temperature for (*R*)- and (*S*)-DPAT was seen at a dose 3.2 and 10 μmol/kg, respectively. At higher doses, however, the hypothermia tended to be attenuated. (*R*)-DPAT was significantly more effective in decreasing the body temperature than both (*R,S*)- and (*S*)-DPAT at doses of 1.0 ($P < 0.01$) and 3.2 μmol/kg ($P < 0.05$), respectively. The drop in body temperature appeared to have a more rapid onset after (*R*)-DPAT in comparison with (*S*)-DPAT (Fig. 5A). The maximal hypothermic response was seen at 40 min after both enantiomers of DPAT at a dose of 3.2 μmol/kg. The body temperature was back to normal at 2 h after injection.

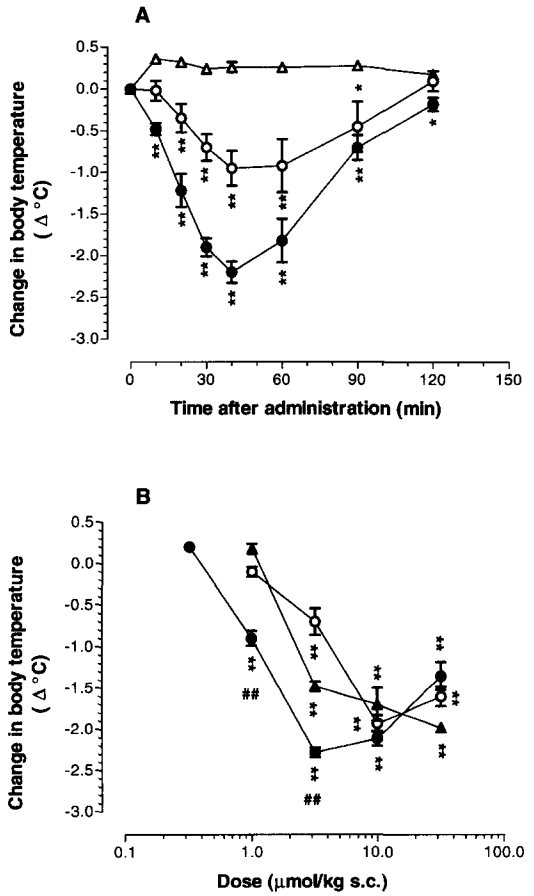

Fig. 5. A: Time-response curves for (*R*)- (●), (*S*)-DPAT (○) (each in dose of 3.2 μmol/kg s.c.) and saline (△) induced changes in body temperature (mean ± S.E.M., $n = 6$). B: Dose-response relationships of (*R*)- (●), (*R,S*)- (▲) and (*S*)-DPAT (○) for the changes in body temperature from the preinjection values at 30 min after administration (mean ± S.E.M., $n = 5$/group). The change in body temperature of the saline-treated rats was 0.28 ± 0.05°C ($n = 10$). Statistics: ANOVA followed by Tukey's studentized range test. * $P < 0.05$, ** $P < 0.01$ compared with saline; $^{\#\#}$ $P < 0.01$ comparison between the two enantiomers at the same dose.

The hypothermia induced by (*R*)-8-OH-DPAT and (*R*)-DPAT was significantly attenuated by the 5-HT_{1A} receptor antagonist (*S*)-UH-301 (Fig. 6A). The dopamine D_2 receptor antagonist raclopride did not antagonise the decrease in body temperature after (*R*)-DPAT, but attenuated the hypothermic effect of (*R*)-8-OH-DPAT (Fig. 6A). Raclopride markedly reduced the hypothermic response induced by (*S*)-DPAT (Fig. 6B). The drop in body temperature induced by (*S*)-5-OH-DPAT, however, disappeared completely after pretreatment with raclopride. The 5-HT_{1A} receptor antagonist (*S*)-UH-301 attenuated, but did not block, the hypothermia induced by (*S*)-5-OH-DPAT and (*S*)-DPAT (Fig. 6B).

3.4. Effects of (R,S)-, (R)- and (S)-DPAT on 5-HT and dopamine turnover

As shown in Table 1, (*R*)-8-OH-DPAT inhibited 5-HT turnover in the hippocampus at a dose of 1.0 μmol/kg s.c. (*R*)-DPAT decreased hippocampal 5-HT turnover in a dose-dependent (1–32 μmol/kg s.c.) manner. (*R,S*)-DPAT at a dose of 32 μmol/kg also reduced 5-HT turnover, whereas there was no change after (*S*)-DPAT. (*S*)-5-OH-DPAT did not affect 5-HT turnover.

Similarly to (*S*)-5-OH-DPAT, (*S*)-DPAT and (*R,S*)-DPAT caused a dose-dependent (1–32 μmol/kg s.c.) decrease in dopamine turnover in the corpus striatum as well as a decrease in the levels of homovanillic acid (Table 1). (*R*)-DPAT inhibited dopamine turnover at 10 μmol/kg but not at 32 μmol/kg, i.e. the effect was neither consistent nor dose-dependent. (*R*)-8-OH-DPAT did not have any effect on homovanillic acid levels or on dopamine turnover.

The decrease in 5-HT turnover in the hippocampus, as well as in the hypothalamus, after (*R*)-8-OH-DPAT and (*R*)-DPAT was blocked by (*S*)-UH-301 (Table 2). However, (*S*)-UH-301 did not antagonise the reduction of dopamine turnover in the striatum, nor in the limbic system, in rats treated with (*S*)-DPAT.

3.5. Effects of (R)- and (S)-DPAT on 5-hydroxytryptophan and DOPA accumulations in reserpine-treated rats (Table 3)

(*R*)-8-OH-DPAT (0.32 μmol/kg s.c.) significantly reduced the accumulation of 5-hydroxytryptophan in the hippocampus, the hypothalamus and the corpus striatum in reserpine-pretreated rats without changing the accumulation of striatal DOPA. (*R*)-DPAT dose-dependently (3.2–10 μmol/kg s.c.) reduced the accumulation of 5-hydroxytryptophan in the brain regions studied. The accumulation of DOPA in the striatum also decreased by 25–30% after

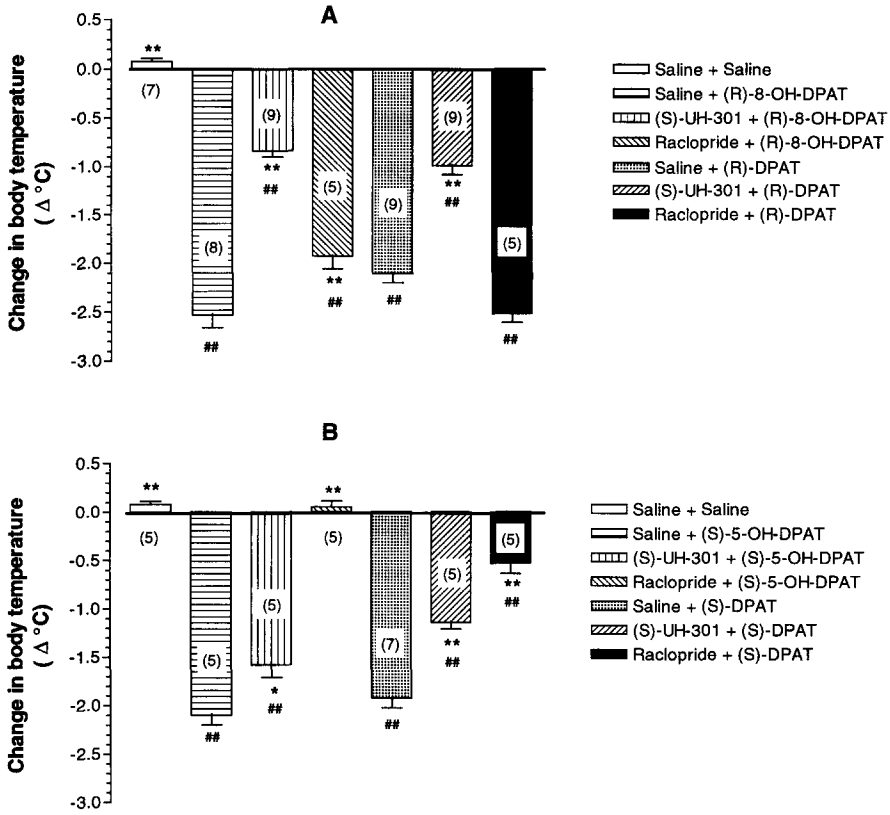

Fig. 6. Effects of (*S*)-UH-301 or raclopride on (*R*)-8-OH-DPAT and (*R*)-DPAT (A) and on (*S*)-DPAT and (*S*)-5-OH-DPAT (B) induced decreases in body temperature. (*S*)-UH-301 (32 μmol/kg s.c.) or raclopride (3.2 μmol/kg s.c.) was injected 10 min or 60 min, respectively, before the test compounds. The changes in body temperature were recorded at 30 min after administration of the tetralins (mean ± S.E.M., $n = 5$). Statistics: ANOVA followed by Tukey's studentized range test. $^{\#\#} P < 0.01$ vs. saline. $^{*} P < 0.05$, $^{**} P < 0.01$ vs. (*R*)-8-OH-DPAT, (*R*)-DPAT, (*S*)-DPAT or (*S*)-5-OH-DPAT.

Table 1
Effects of (*R*)-8-OH-DPAT, (*R*)-, (*R,S*)-, (*S*)-DPAT and (*S*)-5-OH-DPAT on 5-HT turnover in the hippocampus and dopamine turnover in the striatum

Compound	Dose (μmol/kg s.c.)	*n*	Hippocampus (% of control)			Striatum (% of control)			
			5-HIAA	5-HT	5-HIAA/5-HT	DOPAC	HVA	DA	DOPAC/DA
(*R*)-8-OH-DPAT	1.0	6	76 ± 2	122 ± 8	68 ± 2 [b]	107 ± 10	100 ± 5	100 ± 7	105 ± 3
(*R*)-DPAT	1.0	4	111 ± 4	105 ± 3	100 ± 3	92 ± 12	92 ± 4	105 ± 14	90 ± 6
	3.2	4	90 ± 10	110 ± 9	76 ± 3	84 ± 2	81 ± 3	91 ± 6	89 ± 5
	10	3	71 ± 6	112 ± 9	65 ± 0 [b]	70 ± 0 [b]	84 ± 3	100 ± 4	67 ± 3 [a]
	32	4	77 ± 10	122 ± 9	64 ± 4 [b]	84 ± 5	89 ± 7	95 ± 7	85 ± 5
(*R,S*)-DPAT	1.0	4	107 ± 5	90 ± 9	119 ± 16	80 ± 5	77 ± 6 [b]	89 ± 4	90 ± 5
	3.2	4	94 ± 2	108 ± 6	88 ± 6	90 ± 5	71 ± 5 [a]	122 ± 10	74 ± 5 [a]
	10	4	99 ± 2	113 ± 1	88 ± 3	61 ± 4 [a]	65 ± 3 [a]	114 ± 8	57 ± 7 [a]
	32	4	73 ± 6	106 ± 10	68 ± 2 [b]	61 ± 3 [a]	64 ± 6 [a]	115 ± 2	53 ± 2 [a]
(*S*)-DPAT	1.0	6	102 ± 4	101 ± 5	98 ± 5	80 ± 9	65 ± 10 [a]	89 ± 10	87 ± 2
	3.2	5	83 ± 8	97 ± 11	84 ± 8	97 ± 8	60 ± 6 [a]	133 ± 13	72 ± 4 [a]
	10	4	69 ± 9	87 ± 4	78 ± 13	70 ± 4	33 ± 4 [a]	107 ± 12	63 ± 4 [a]
(*S*)-5-OH-DPAT	0.05	4	110 ± 9	115 ± 9	96 ± 5	60 ± 3 [a]	51 ± 3 [a]	114 ± 3	53 ± 3 [a]

Compounds or saline were injected at 40 min before the animals were killed. Values are shown as percentages (mean ± S.E.M.) of controls. Control levels of 5-HIAA (5-hydroxyindoleacetic acid), 5-HT (ng/g tissue) and the 5-HIAA/5-HT ratio in hippocampus were 439 ± 30, 315 ± 13 and 1.39 ± 0.07 for (*R,S*)-DPAT; and 357 ± 17, 297 ± 16 and 1.28 ± 0.04 for all other compounds. Control levels of DOPAC (3,4-dihydroxyphenylacetic acid), HVA (homovanillic acid) and DA (dopamine) (ng/g tissue) and the DOPAC/DA ratio in stratium were 1181 ± 82, 1187 ± 76, 10144 ± 688 and 0.12 ± 0.02 for (*R,S*)-DPAT; and 1013 ± 50, 1081 ± 30, 8773 ± 343 and 0.12 ± 0.02 for all other compounds. Statistics: One-way ANOVA followed by Tukey's studentized range (HSD) test, $^{a} P < 0.01$, $^{b} P < 0.05$, vs. saline ($n = 10$).

Table 2
Antagonism by (*S*)-UH-301 of (*R*)-8-OH-DPAT, (*R*)- and (*S*)-DPAT induced effects on 5-HT and dopamine turnover

Compound	Dose (μmol/kg s.c.)	*n*	5-HIAA/5-HT (% of control)		DOPAC/DA (% of control)	
			Hippocampus	Hypothalamus	Striatum	Limbic system
Saline + (*R*)-8-OH-DPAT	– 1.0	6	66 ± 4 [a]	66 ± 5 [a]	115 ± 7	114 ± 2
(*S*)-UH-301 + (*R*)-8-OH-DPAT	32 1.0	7	99 ± 3	90 ± 3	112 ± 4	90 ± 4
Saline + (*R*)-DPAT	– 10	5	68 ± 2 [a]	73 ± 5 [b]	104 ± 4	120 ± 8
(*S*)-UH-301 + (*R*)-DPAT	32 10	8	97 ± 4	95 ± 2	104 ± 3	100 ± 5
Saline + (*S*)-DPAT	– 10	5	97 ± 3	91 ± 5	69 ± 5 [a]	58 ± 3 [a]
(*S*)-UH-301 + (*S*)-DPAT	32 10	5	92 ± 2	93 ± 3	67 ± 3 [a]	67 ± 6 [a]
(*S*)-UH-301 + Saline	32 –	4	102 ± 6	91 ± 3	110 ± 6	90 ± 2

(*S*)-UH-301 or saline was injected 50 min and (*R*)-8-OH-DPAT or (*R*)-, (*S*)-DPAT was injected 40 before the animals were killed. Values are shown as percentages of controls, mean ± S.E.M. Control levels of the 5-HIAA (5-hydroxyindoleacetic acid)/5-HT ratio in the hippocampus varied between 1.0 ± 0.09 and 1.48 ± 0.11 and in the hypothalamus between 0.72 ± 0.03 and 0.97 ± 0.05. The DOPAC/DA (3,4-dihydroxyphenylacetic acid/dopamine) ratio in the striatum varied between 0.16 ± 0 and 0.23 ± 0.02 and in the limbic system between 0.19 ± 0.01 and 0.34 ± 0.02. Statistics: One-way ANOVA followed by Tukey's studentized range (HSD) test, [a] $P < 0.01$, [b] $P < 0.05$ vs. saline ($n = 14$).

Table 3
Effects of (*R*)-8-OH-DPAT, (*R*)- and (*S*)-DPAT and (*S*)-5-OH-DPAT on 5-hydroxytryptophan and DOPA accumulation after NSD1015 in rats pretreated with reserpine

Compound	Dose (μmol/ kg s.c.)	*n*	5-Hydroxytryptophan (% of control)			DOPA (% of control)
			Hippocampus	Hypothalamus	Striatum	Striatum
(*R*)-8-OH-DPAT	0.32	6 [a]	35 ± 3 [c]	43 ± 4 [c]	55 ± 4 [c]	110 ± 7
(*R*)-DPAT	1.0	5 [a]	99 ± 9	96 ± 7	87 ± 8	68 ± 4 [c]
	3.2	7 [b]	53 ± 3 [c]	55 ± 4 [c]	52 ± 3 [c]	75 ± 8 [d]
	10	6 [b]	47 ± 5 [c]	49 ± 5 [c]	56 ± 4 [c]	70 ± 9 [c]
(*S*)-DPAT	1.0	6 [a]	109 ± 3	106 ± 5	118 ± 9	33 ± 3 [c]
	3.2	8 [b]	79 ± 11	77 ± 4	74 ± 6	32 ± 2 [c]
	10	6 [b]	65 ± 4 [c]	73 ± 9 [c]	65 ± 12 [c]	70 ± 3 [c]
(*S*)-5-OH-DPAT	0.05	7 [a]	94 ± 6	95 ± 4	99 ± 5	31 ± 4 [c]

Rats were given reserpine (5 mg/kg s.c.) 18 h before administration of the test compounds. The test compounds were injected 60 min and NSD1015 (60 mg/kg s.c.) 30 min before the animals were killed. Values are shown as percentages of controls, mean ± S.E.M. [a] Control levels (ng/g tissue) of 5-hydroxytryptophan were 198 ± 8, 558 ± 26 and 173 ± 14 (hippocampus, hypothalamus and striatum, respectively) and of DOPA in the striatum 3921 ± 136 ($n = 12$). [b] Control levels (ng/g tissue) of 5-hydroxytryptophan were 121 ± 7.7, 443 ± 27 and 156 ± 8.9 (hippocampus, hypothalamus and striatum, respectively) and of DOPA in the striatum 5681 ± 155 ($n = 5$). Statistics: One-way ANOVA following by Tukey's studentized range (HSD) test, [c] $P < 0.01$, [d] $P < 0.05$ vs. control.

Table 4
Affinities of (*R*)-8-OH-DPAT, (*R*)-DPAT, (*S*)-DPAT and (*S*)-5-OH-DPAT for 5-HT_{1A}, D_{2A} and D_3 receptors

Compounds	K_i (nM)			
	[^{3}H]8-OH-DPAT (5-HT_{1A})	[^{3}H]Quinpirole (D_{2A})	[^{3}H]Raclopride (D_{2A})	[^{3}H]Raclopride (D_3)
(*R*)-8-OH-DPAT	0.70 ± 0.06	–	589 ± 48	87.5 ± 8.2
(*R*)-DPAT	11.5 ± 0.1	31.7 ± 4.7	540 ± 90	32.7 ± 4.4
(*S*)-DPAT	38.4 ± 6.6	5.50 ± 0.45	468 ± 81	34.7 ± 2.3
(*S*)-5-OH-DPAT	215 ± 20	0.14 ± 0.01	27.0 ± 3.7	0.89 ± 0.05

5-HT_{1A} receptors were prepared from rat hippocampus. Cloned human dopamine D_{2A} and D_3 receptors were prepared from Ltk^- and CHO cell lines, respectively. The results are mean ± S.E.M. values of 2–4 experiments made in duplicate.

(*R*)-DPAT. (*S*)-DPAT significantly inhibited the 5-hydroxytryptophan accumulation only at 10 μmol/kg but to a similar degree in the three brain regions studied. However, the DOPA accumulation was significantly reduced in the striatum already at 1 μmol/kg. Thus, the action (*S*)-DPAT on dopamine synthesis was more pronounced than its enantiomer and it displayed the same efficacy as 0.05 μmol/kg of (*S*)-5-OH-DPAT in this respect.

3.6. Receptor binding profiles of (R)- and (S)-DPAT in vitro (Table 4)

(*R*)-DPAT displayed a 3-fold higher affinity than (*S*)-DPAT for the 5-HT_{1A} binding site labeled with [^{3}H]8-OH-DPAT (K_i = 11.5 nM and K_i = 38.4 nM, respectively). In contrast, (*S*)-DPAT displayed a 6-fold higher affinity than (*R*)-DPAT for the dopamine D_2 receptor binding site labeled with [^{3}H]quinpirole as radioligand (K_i = 5.5 nM and K_i = 31.7 nM, respectively). Both enantiomers showed low affinities (K_i = 468 nM and K_i = 540 nM, respectively) for the dopamine D_2 sites labeled with [^{3}H]raclopride as radioligand. In contrast, both (*R*)- and (*S*)-DPAT had high and similar affinities for dopamine D_3 sites labeled with [^{3}H]raclopride. As expected, (*R*)-8-OH-DPAT displayed high affinity for the 5-HT_{1A} site, and (*S*)-5-OH-DPAT showed high affinity and selectivity for the dopamine D_2 vs. the 5-HT_{1A} receptor binding site. (*R*)-8-OH-DPAT showed moderate affinity for dopamine D_3 receptor binding sites.

Both (*R*)- and (*S*)-DPAT had low affinities (K_i = 769 nM and K_i = 493 nM, respectively) for α_2-adrenoceptors and even lower affinities (K_i values of > 1000 nM) for α_1- and β-adrenoceptors, muscarinic, 5-HT_2 and dopamine D_1 receptors.

4. Discussion

4.1. (R)-DPAT

The present study shows that (*R*)-DPAT, similarly to (*R*)-8-OH-DPAT, elicited typical 5-HT_{1A} receptor agonist-like behaviours (forepaw treading, flat body posture, hindlimb abduction; Jacobs, 1976; Ortmann, 1985; Tricklebank et al., 1985) in a dose-dependent fashion. The 5-HT_{1A} syndrome after (*R*)-DPAT was elicited to a similar degree in reserpine-treated, i.e. amine-depleted, rats. The 5-HT_{1A} receptor antagonist (*S*)-UH-301 antagonised the actions of (*R*)-DPAT and of (*R*)-8-OH-DPAT, whereas the dopamine D_2 receptor antagonist raclopride did not. Thus, (*R*)-DPAT appears to be a directly acting 5-HT_{1A} receptor agonist at postsynaptic 5-HT_{1A} receptors. The body temperature was dose- and time-dependently decreased by (*R*)-DPAT, and (*S*)-UH-301 antagonised the hypothermic response of (*R*)-DPAT and (*R*)-8-OH-DPAT to the same extent, indicating that the hypothermic response to (*R*)-DPAT is mediated via 5-HT_{1A} receptors. Raclopride did neither antagonise nor potentiate (*R*)-DPAT in this test. Thus, dopamine D_2 receptors appear not to be involved in the hypothermic response to (*R*)-DPAT. The reason for the small but statistically significant reduction of the hypothermic response to (*R*)-8-OH-DPAT caused by raclopride is not evident. (*R*)-DPAT decreased 5-HT turnover and 5-HT synthesis to the same extent as (*R*)-8-OH-DPAT in all brain regions studied. (*S*)-UH-301 completely antagonised the (*R*)-DPAT as well as the (*R*)-8-OH-DPAT-induced effects on 5-HT turnover. These biochemical findings strongly suggest that (*R*)-DPAT is also an agonist at somatodendritic 5-HT_{1A} autoreceptors.

(*R*)-DPAT induced weak dopamine receptor agonist-like behaviours, but only at the lower dose levels (1 and 3.2 μmol/kg). There were no such symptoms after reserpine treatment, indicating that (*R*)-DPAT is not a direct postsynaptic dopamine D_2 receptor agonist. Dopamine turnover was not decreased by (*R*)-DPAT except after one single dose (10 μmol/kg) of (*R*)-DPAT in one out of two experiments. Striatal dopamine synthesis, however, was reduced by 30% in reserpine-treated rats at all dose levels tested. An efficacious presynaptic dopamine receptor agonist, such as (*S*)-5-OH-DPAT, decreased DOPA accumulation by 70%. Therefore, (*R*)-DPAT behaves as a partial agonist at dopamine autoreceptors. (*R*)-8-OH-DPAT did not appear to affect dopamine autoreceptors at the doses tested (0.32 and 1.0 μmol/kg s.c.).

Taken together, the present findings support the conclusion that (*R*)-DPAT is a 5-HT_{1A} receptor agonist both pre- and postsynaptically. In addition, (*R*)-DPAT displays at least partial agonist activity on dopamine autoreceptors regulating dopamine synthesis, but has apparently no direct dopaminergic actions postsynaptically. These conclusions from functional tests are supported by the receptor binding data, where (*R*)-DPAT shows higher affinity for 5-HT_{1A} receptor than for dopamine D_2 receptor agonist binding sites. Thus, (*R*)-DPAT might be categorised as a 5-HT_{1A} agonist and a partial dopamine D_2 receptor agonist.

4.2. (S)-DPAT

In the present study (*S*)-DPAT behaved as a dopamine D_2 receptor agonist in several functional tests. Thus, it displayed the dopamine D_2 syndrome in normal rats, as well as in rats pretreated with reserpine or α-methyl-*p*-tyrosine or the combined treatment with reserpine and α-methyl-*p*-tyrosine. These findings strongly suggest a direct agonist action by (*S*)-DPAT on postsynaptic dopamine D_2 receptors in agreement with previous reports by McDermed et al. (1975) and Rusterholz et al. (1979), but not by Cannon et al. (1977), using (*R,S*)-DPAT (see section 1). Similar results were obtained with the dopamine D_2 receptor agonist (*S*)-5-OH-DPAT. In addition, (*S*)-DPAT, similarly to (*S*)-5-OH-DPAT, elicited jerks and yawns after reserpine, which is typical for dopamine D_2

receptor agonists (Grabowska-Andén and Andén, 1983). The dopamine D_2 syndrome induced by (*S*)-DPAT and (*S*)-5-OH-DPAT was antagonised by raclopride, a dopamine D_2 receptor antagonist, but not by (*S*)-UH-301, a 5-HT_{1A} receptor antagonist, which results support that the effects are mediated by dopamine D_2 receptors. Biochemically, (*S*)-DPAT reduced brain dopamine turnover and dopamine synthesis to the same extent as (*S*)-5-OH-DPAT, indicating an agonist action on dopamine autoreceptors. (*S*)-UH-301 did not antagonise the effect of (*S*)-DPAT on brain dopamine turnover. Raclopride was not tested, since it increases DOPA accumulation due to its antagonistic action on dopamine D_2 receptors (Ögren et al., 1986). (*S*)-DPAT induced hypothermia similarly to (*R*)-DPAT. However, hypothermia is a response common to both 5-HT_{1A} and dopamine D_2 receptor agonists. The hypothermic response to (*S*)-DPAT, in contrast to (*R*)-DPAT, was fully antagonised by raclopride. (*S*)-UH-301 weakly antagonised the (*S*)-DPAT-induced hypothermia as well as that after the dopamine D_2 receptor agonist (*S*)-5-OH-DPAT. This finding might be due to an agonistic action of (*S*)-DPAT on the 5-HT_{1A} receptors mediating hypothermia, or, more likely, to a weak antidopaminergic action of (*S*)-UH-301. (*S*)-UH-301 has presynaptic agonist activity on central dopamine neurons (Björk et al., 1991), and may behave as a partial dopamine receptor agonist, i.e. showing postsynaptic dopamine D_2 receptor antagonism (see Hjorth et al., 1983). The absence of obvious signs of the 5-HT_{1A} receptor agonist like behaviours in normal and reserpine-treated rats given (*S*)-DPAT appears to argue against 5-HT_{1A} receptor stimulation. However, flat body posture, which is a sign of 5-HT_{1A} receptor agonism, occurred after administration of 10 μmol/kg of (*S*)-DPAT both in normal and reserpine-treated rats. Furthermore, although (*S*)-DPAT did not affect 5-HT turnover, it weakly inhibited 5-HT synthesis indicating a partial 5-HT_{1A} receptor agonistic activity of (*S*)-DPAT on somatodendritic 5-HT_{1A} autoreceptors.

In conclusion, (*S*)-DPAT might be categorised as a dopamine D_2 receptor agonist both pre- and postsynaptically, and as a partial presynaptic 5-HT_{1A} receptor agonist. The receptor binding data are consistent with an action on dopamine D_2 receptors as well as on 5-HT_{1A} receptors. More specifically, (*S*)-DPAT has a higher affinity than (*R*)-DPAT for dopamine D_2 receptors (see below), whereas it has a lower affinity for 5-HT_{1A} receptors, which support the functional data.

4.3. Receptor binding data

Binding constants for [^{3}H]raclopride and [^{3}H]quinpirole are similar for human cloned dopamine D_2 receptors expressed in cell lines and dopamine D_2 receptors in rat striatal tissue (Malmberg and Mohell, 1995). Therefore, the present binding data are considered relevant as support for the functional data obtained in rats. Under the experimental conditions used, [^{3}H]quinpirole labeled only the high-affinity state of the dopamine D_{2A} receptors, whereas [^{3}H]raclopride labels both the high- and the low-affinity state (Malmberg and Mohell, 1995). In the present experiments with the dopamine D_2 receptor agonist [^{3}H]-quinpirole, the affinity of (*S*)-DPAT was 6-fold higher than that of (*R*)-DPAT, whereas the affinities measured with the dopamine D_2 receptor antagonist [^{3}H]raclopride were similar. This indicates that the dopamine D_2 receptor efficacy of (*S*)-DPAT is higher than that of (*R*)-DPAT, since there seem to be a positive correlation between the intrinsic activity and the ratio between the affinity values of the low- and high-affinity binding sites for dopamine D_2 agonists (Lahti et al., 1992; see also Malmberg and Mohell, 1995). Thus, the receptor binding data are consistent with the functional data discussed above.

According to the receptor model of the human D_{2A} receptor constructed by Malmberg et al. (1994), both (*R*)- and (*S*)-DPAT should have the potential to interact with the binding site of the dopamine D_{2A} receptor by forming a reinforced hydrogen bond with Asp-114 and an edge to face aromatic interaction with Phe-390 of the receptor protein. However, neither enantiomer has a phenolic hydroxyl group available to donate a hydrogen bond to Ser-193. This observation is intriguing, since this later interaction has often been considered to be crucial for agonist activity. Thus, the hydroxyl group in (*S*)-5-OH-DPAT increases the dopamine D_2 receptor affinity by 40-fold compared to (*S*)-DPAT. It may also be noted that (*R*)-DPAT may interact with the dopamine D_2 receptor in the same binding mode as the dopamine receptor antagonist (1*S*, 2*R*)-UH-232 (Malmberg et al., 1994).

Both (*R*)- and (*S*)-DPAT displayed relatively high and similar affinity for dopamine D_3 receptors with [^{3}H]-raclopride as the radioligand. An agonist action on dopamine D_3 receptors might contribute to the effects of the compounds on dopamine synthesis and turnover via presynaptic dopamine neuron autoreceptors (Meller et al., 1993). In contrast to dopamine D_2 receptors, the dopamine D_3 receptors are not present in the striatum and dopamine D_3 agonists are not known to induce stereotyped behaviours but to inhibit locomotor activity probably via D_3 receptors located in limbic brain structures (Sokoloff et al., 1990; Waters et al., 1993, see also Sokoloff and Schwartz, 1995). Therefore, the stereotyped behaviours induced by (*S*)-DPAT are probably not mediated via dopamine D_3 receptors. However, a possible postsynaptic dopamine D_3 receptor-mediated action by (*S*)-DPAT cannot be excluded, since locomotor activity was not assessed in this study. Raclopride has high affinity for both dopamine D_2 and D_3 receptors (Malmberg et al., 1993; Malmberg and Mohell, 1995). Based on current knowledge (see review by Sokoloff and Schwartz, 1995), the antagonism by raclopride of the (*S*)-DPAT-induced effects is most probably mediated via the antagonist activity at dopamine D_2, rather than D_3 receptors. (*R*)- and (*S*)-DPAT have low affinities

for α_1-, α_2- and β-adrenoceptors, muscarinic, 5-HT_2 and dopamine D_1 receptors indicating that these receptors are not important for the pharmacological effects of the two enantiomers.

4.4. (R,S)-DPAT

(*R,S*)-DPAT induced both 5-HT_{1A} and dopamine D_2 receptor agonist-like behaviours and decreased body temperature in normal rats. In ex vivo biochemical tests, (*R,S*)-DPAT decreased dopamine but not 5-HT turnover. Thus, although there seem to be a mixture of both 5-HT and dopamine receptor-mediated actions after (*R,S*)-DPAT, they are not as prominent as those after the two enantiomers given separately. It may be possible that the dopaminergic and the serotoninergic effects partly counteract each other, which would blur the picture. In retrospect, and with the present knowledge of the pharmacology of the pure enantiomers of DPAT, the difficulties for previous investigators (see section 1) to correctly interpret their findings is understandable. In 1975–80, the 5-HT_{1A} agonist syndrome displayed after 8-OH-DPAT had not been described. Forepaw treading mixed with oral stereotyped behaviour might easily have been interpreted as dopamine receptor-mediated hyperactivity and forelimb stereotyped behaviours. Intracerebral injections of each of the enantiomers in separate experiments and in combination might improve our understanding of the possible interactions between dopaminergic and serotoninergic pathways in the brain and may resolve the contradictory findings reported earlier (McDermed et al., 1975; Costall et al., 1977; Cannon et al., 1977).

The present study does not address the question of a possible metabolic activation of DPAT (see section 1). However, the 5-HT_{1A} and D_2 receptor-mediated symptoms appeared within minutes after s.c. administration, which argue against metabolic activation as a prerequisite for pharmacological activity. Also, previous in vitro findings support an action of nonmetabolised (*R,S*)-DPAT itself as the pharmacological active agent (see section 1). If metabolic activation occurs, the present results would indicate a stereoselective metabolism of (*R*)-DPAT into serotoninergic (5-HT_{1A}) and (*S*)-DPAT into dopaminergic (D_2) compounds. Although such an explanation of the present findings appears remote, it can only be excluded after performing the proper studies.

5. Conclusions

Based on the present results, (*R*)-DPAT may be categorised as a 5-HT_{1A} receptor agonist and (*S*)-DPAT as a dopamine D_2 receptor agonist, both pre- and postsynaptically. In addition, the (*R*)-enantiomer seems to have dopamine autoreceptor partial agonist activity and the (*S*)-antipode displays partial agonist activity on somatodendritic 5-HT_{1A} autoreceptors. Therefore, racemic DPAT could be viewed as a mixed 5-HT_{1A} and dopamine D_2 agonist both pre- and postsynaptically. Previous and present findings suggest that the actions of (*R*)-, (*S*)- and (*R,S*)-DPAT are direct rather than indirect, although a contributory indirect action cannot be excluded. The in vivo effects of racemic DPAT and the pure enantiomers do not appear to be due to metabolic activation.

Acknowledgements

The authors thank Ms. Anne-Maj Gustafsson for excellent help with the biochemical assays, and the receptor binding group at Astra Arcus AB for performing some of the receptor binding assays. The study was supported by the Swedish Board for Technical and Industrial Development, The Swedish Society for Medical Research, the University of Uppsala (Seda och Signe Hermansson Stipendiefond) and by Astra Arcus AB.

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