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In vivo evidence for preferential role of dopamine D_3 receptor in the presynaptic regulation of dopamine release but not synthesis

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Abstract

Brain microdialysis was used to investigate the effects of the putative dopamine D_3 receptor agonist (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) on dopamine release, metabolism and synthesis in the dorsal striatum and nucleus accumbens of awake rats. The drug administered i.p. dose dependently decreased the release, metabolism and synthesis of dopamine in both brain areas. The potency of 7-OH-DPAT to decrease dopamine release was found to be higher in the nucleus accumbens than in the dorsal striatum (ED_{50} for nucleus accumbens 0.0096 mg/kg, i.p.; for dorsal striatum 0.068 mg/kg, i.p.). Dopamine metabolism, assessed by measuring 3,4-dihydroxyphenylacetic acid extracellular levels, and dopamine synthesis, determined as 3,4-dihydroxyphenylalanine output following perfusion with the L-aromatic acid decarboxylase inhibitor 3-hydroxybenzylhydrazine (10^{-5} M), were decreased at higher dose ranges of 7-OH-DPAT (ED_{50} for decrease of 3,4-dihydroxyphenylalanine output in nucleus accumbens 0.124 mg/kg, i.p.; in dorsal striatum 0.101 mg/kg, i.p.). The hypomotility of rats induced by 7-OH-DPAT in doses of 0.002–0.25 mg/kg, i.p., was shown to correlate with the decreased dopamine release in the nucleus accumbens. Pretreatment of animals with 7-OH-DPAT at the putative dopamine D_3 receptor 'selective' dose of 0.05 mg/kg, i.p., was found to prevent the increase of dopamine release but not the increase in metabolism in the dorsal striatum of freely moving rats induced by (+)-AJ76, *cis*-(+)-(1S,2R)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin HCl (7 mg/kg, i.p.) and haloperidol (0.1 mg/kg, i.p.). Local application of 7-OH-DPAT by addition into the perfusing medium also resulted in a preferential decrease of dopamine release in the nucleus accumbens as compared with the dorsal striatum (EC_{50} for nucleus accumbens 1.9 nM; for dorsal striatum 11.3 nM). The present results give further support to the hypothesis that the dopamine D_3 autoreceptor is preferentialy involved in the presynaptic regulation of dopamine release, while the D_2 autoreceptor controls dopamine synthesis.

Keywords: Dopamine release; Dopamine synthesis; Dopamine D_3 receptor; Dopamine D_2 receptor; Microdialysis; 7-OH-DPAT (7-hydroxy-2-(di-*n*-propylamino)tetralin)

1. Introduction

Recently dopamine D_1-like (D_1, D_5) and D_2-like (D_2, D_3 and D_4) receptor genes have been identified by molecular cloning (see for reviews: Gingrich and Caron, 1993; Seeman and Van Tol, 1994). Considerable current interest focuses on the dopamine D_3 receptor, particularly because of its preferential distribution in limbic projections of the midbrain dopamine system involved in emotional behaviour and cognition (Sokoloff et al., 1990). The dopamine D_3 receptor has been implicated in the pathophysiology of some psychiatric disorders, such as schizophrenia, and has been suggested to play a role as a primary modulator of cocaine reinforcement (Sokoloff et al., 1990; Caine and Koob, 1993; Gurevich et al., 1994).

Although the dopamine D_3 receptor was cloned and pharmacologically described in 1990, the real functional role and the second-messenger coupling of this receptor subtype is not yet well characterized (Sokoloff et al., 1990; Svensson et al., 1994a; Tang et al., 1994a; Sautel et al., 1995). It has been shown that the dopamine D_3 receptor, like the D_2 one, is located both pre- and postsynaptically on mesolimbic and nigrostriatal dopamine neurons and therefore might be considered as an dopamine autoreceptor (Sokoloff et al., 1990). Dopamine autoreceptors are known to mediate the presynaptic regulation of impulse flow in dopaminergic neurons as well as dopamine release and synthesis in nerve terminal regions (Carlsson, 1975; Di

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Chiara et al., 1977; Arbilla and Langer, 1981; Roth, 1983). It was reported recently that autoreceptors regulating firing rates of substantia nigra dopamine neurons may belong to the dopamine D_3 receptor subtype (Bergstrom et al., 1994; Devoto et al., 1995; Lejeune and Millan, 1995). There are also some data suggesting differential roles of dopamine D_3 and D_2 receptors in the presynaptic feed-back regulation of dopamine synthesis and release (Damsma et al., 1993a; Waters et al., 1993a; O'Hara et al., 1994; Tang et al., 1994b). The correlation observed between the relative potencies of dopamine antagonists at dopamine D_3 and D_2 receptors and their ability to affect preferentially dopamine release or synthesis/metabolism in the dorsal striatum of freely moving rats led us to hypothesize that the dopamine D_3 autoreceptor is predominantly involved in the modulation of dopamine release while the D_2 type regulates dopamine synthesis in dopaminergic nerve terminal regions (Gainetdinov et al., 1994).

(±)-7-Hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) (Feenstra et al., 1983; Mulder et al., 1987) has been identified as a high-affinity and selective ligand at dopamine D_3 receptors expressed in Chinese hamster ovary (CHO) cells; the K_i values for dopamine D_2, D_4 and D_1 receptor subtypes are approximately 2, 3 and 4 orders of magnitude higher, respectively (Levesque et al., 1992). Therefore 7-OH-DPAT is commonly used for studying dopamine D_3 receptor-mediated functions (Caine and Koob, 1993; Daly and Waddington, 1993; Ahlenius and Salmi, 1994; Damsma et al., 1993a,b; Svensson et al., 1994a,b; Yamada et al., 1994; Devoto et al., 1995; Gilbert and Cooper, 1995). To investigate further the role of dopamine D_3 autoreceptor in the presynaptic regulation of dopamine synthesis and release in mesolimbic and nigrostriatal targeting regions we used 7-OH-DPAT in microdialysis studies with freely moving rats. The effect of the drug on locomotor activity in rats was also evaluated.

2. Materials and methods

2.1. Subjects and surgery

Male adult Wistar rats weighing 250–300 g were used. They were housed in groups with free access to food and water and kept on a 12-h light/dark cycle. Brain dialysis was performed as previously described (Imperato et al., 1988; Gainetdinov et al., 1994). The rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed in a stereotaxic frame (David Kopf Instruments). The dialysis fibre (0.32 mm outer diameter, 15000 Da cut off, AN 69-HF, Hospal-Dasco, Bologna, Italy), covered with Super-Epoxy glue along the whole of its length except for a region corresponding to the dorsal striatum or nucleus accumbens and held straight by an internal tungsten wire, was implanted transversally. The coordinates used for the implantation of the microdialysis tube were for dorsal striatum: AP +2.0; V −4 and for nucleus accumbens: AP +2.5; V −6 according to the bregma and dura surface (Paxinos and Watson, 1982). Following surgery the animals were returned to their home cages with free access to food and water.

2.2. Apparatus

The high-performance liquid chromatography (HPLC) apparatus, consisting of a BAS LC-4B chromatograpic station (Bioanalytical System, West Lafayette, IN, USA) equipped with a 20 μl injection loop and injector Rhehodyne 7125 (Rhehodyne, Cotati, USA), was used to detect monoamines. A syringe pump (Braun Perfusor VI, Germany) was used to perfuse Ringer solution through the brain. An automated locomotor activity meter box (Rodeo-2, Russia) was used to measure the locomotion of the rats.

2.3. Drugs

In the present study (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin, 7-OH-DPAT (RBI, USA); 3-hydroxybenzylhydrazine dihydrochloride, NSD-1015 (Sigma, USA); haloperidol (Janssen Pharmaceutica, Beerse, Belgium) and *cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-propylamino) tetralin HCl, (+)-AJ76 (Upjohn, Kalamazoo, USA) were used. Haloperidol was dissolved in a few drops of glacial acetic acid and made up to volume with saline (pH adjusted to 5.0 with 0.1 M NaOH). All the other drugs were dissolved in 0.9% physiological saline. To dissolve (+)-AJ76, the solution was heated and sonicated. The drugs and saline were administered intraperitoneally in a volume of 2 ml/kg body weight. For local application 7-OH-DPAT and NSD-1015 were dissolved in Ringer solution and perfused through the microdialysis probe.

2.4. Procedures

24 h after surgery, the dialysis probe was connected to a syringe pump and perfused at 2.7 μl/min with Ringer solution (composition (mM) NaCl 147, $CaCl_2$ 1.5, KCl 4, pH 6.0). After a 1-h settling period, the perfusate was collected every 20 min. At least 4 control samples were taken before the drug was administered either by i.p. injection or by local application through the microdialysis probe. The effect of the drugs was monitored for a period of 2 h after i.p. administration and for 1 h during local application. Perfusate samples were assayed for dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and 3,4-dihydroxyphenylalanine (DOPA) using HPLC with electrochemical detection.

Dopamine and DOPAC were separated on a reverse-phase column (Ultrasphere ODS, 5 μm, 4.6 × 150 mm) with a mobile phase consisting of 0.1 M citrate-phosphate buffer containing 1.1 mM octanesulfonic acid, 0.1 mM EDTA and 9% acetonitrile (pH 3.7) and detected by a

glassy carbon working electrode set at +0.8 V. The method was sufficiently sensitive to detect about 7 fmol of dopamine. For the DOPA determination we used the same column and mobile phase with modification (i.e. 2.2 mM octanesulfonic acid, pH 3.3). To study the biosynthesis of dopamine in vivo, 10^{-5} M of NSD-1015, a decarboxylase L-aromatic acid inhibitor, was added to the perfusate (Westerink et al., 1990). Under this condition, a stable DOPA output was achieved following 2 h of infusion. After that four subsequent samples were taken as a basal control and then the effect of 7-OH-DPAT administration on DOPA output was analysed. The localisation of the dialysis probe was verified at the end of the experiment.

Locomotor activity recordings were carried out using a square open-field arena (480 × 480 × 225 mm), equipped with three rows of photocells sensitive to infrared light, placed 40, 115 and 195 mm above the floor. The open-field was enclosed in a ventilated, sound-proof box. Measurements were done in the dark, between 10:00 a.m. and 16:00 p.m. Each non-operated animal was habituated to the open field box for a period of 2 h prior to challenge with vehicle or 7-OH-DPAT. The locomotor activity of rats was determined for two periods of 30 min (before and 5–35 min after the treatment). Interruptions of sensors by horizontal movements generated data that were collected automatically by an analyser and the results were recorded.

2.5. Data analysis

For each group of experiments, the average basal values obtained in at least four samples before drug treatment were considered as 100%. Values obtained during drug treatment were expressed as a percentage of the basal level. Data are presented as the means ± S.E.M. and were analysed statistically by Mann-Whitney U-test (two-tailed). Significance at the $P < 0.05$ level and below is reported. The ED_{50} values (with 95% confidence intervals) were calculated from the median values employing a computerised analysis of non-linear regression (Fig. P, BioSoft, USA).

For graphical representation of the results only the point of maximal response of the dopamine, DOPAC and DOPA output to the drug treatments is depicted.

3. Results

The basal concentrations of dopamine and DOPAC in the rat dialysates were: 191.1 ± 0.7 fmol/20 min for dopamine, 23.5 ± 1.5 pmol/20 min for DOPAC from the dorsal striatum (mean ± S.E.M., $n = 73$) and 110.4 ± 16.9 fmol/20 min for dopamine, 16.4 ± 0.8 pmol/20 min for DOPAC from the nucleus accumbens (mean ± S.E.M., $n = 58$). The administration of saline did not significantly affect basal dopamine and DOPAC extracellular levels in either regions (data not shown).

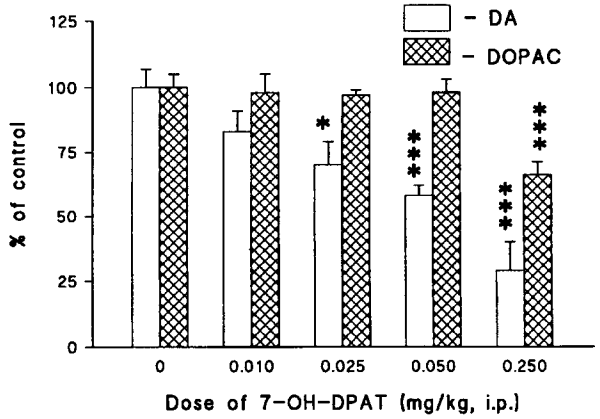

Fig. 1. Effect of 7-OH-DPAT i.p. administration on dopamine and DOPAC extracellular levels in the dorsal striatum of freely moving rats. The maximal responses of the dopamine and DOPAC output to the drug treatment (20–60 min following administration), expressed as a percentage of pre-injection baseline control, are presented. Means ± S.E.M. are shown ($n = 5$–6). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus controls.

3.1. Effect of i.p. administration of 7-OH-DPAT on extracellular dopamine and DOPAC levels in the dorsal striatum and nucleus accumbens of freely moving rats

The drug dose dependently decreased dopamine release in the dorsal striatum and nucleus accumbens of freely moving rats (Figs. 1 and 2). The decrease of dopamine release in the nucleus accumbens was observed over a low dose range of 7-OH-DPAT: ED_{50} values were calculated as 0.068 mg/kg, i.p., for the dorsal striatum and 0.0096 mg/kg, i.p., for the nucleus accumbens (Table 1). The threshold doses for inhibition of dopamine release in vivo were found to be 0.025 mg/kg, i.p., for the dorsal striatum and 0.005 mg/kg, i.p., for the nucleus accumbens. How-

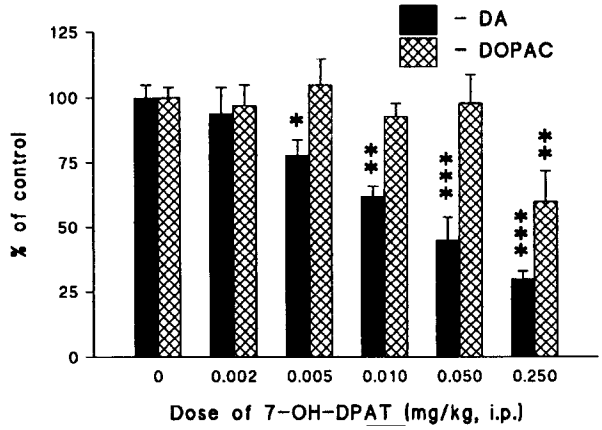

Fig. 2. Effect of 7-OH-DPAT i.p. administration on dopamine and DOPAC extracellular levels in the nucleus accumbens of freely moving rats. The maximal responses of the dopamine and DOPAC output to the drug treatment (20–60 min following administration), expressed as a percentage of pre-injection baseline control, are presented. Means ± S.E.M. are shown ($n = 5$–6). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus controls. (Mann-Whitney U-test).

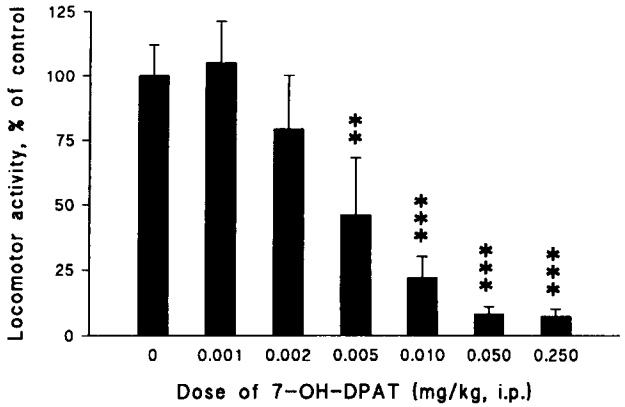

Fig. 3. Effect of 7-OH-DPAT i.p. administration on locomotor activity of rats habituated to their environment, measured 5–35 min following injection. The data expressed as a percentage of saline-injected control (255 ± 31 counts per 30 min). Means ± S.E.M. are shown ($n = 5$–6). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus controls. (Mann-Whitney U-test).

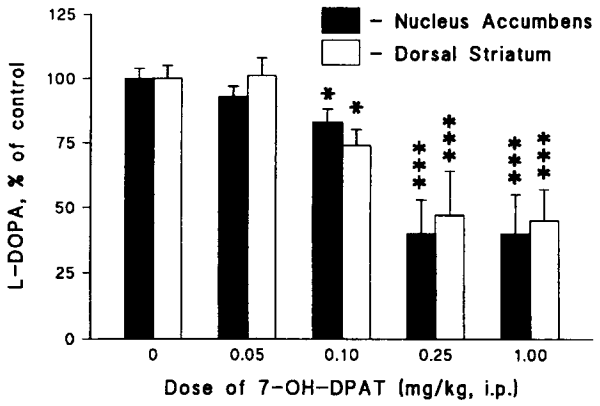

Fig. 4. Effect of 7-OH-DPAT i.p. administration on the extracellular levels of DOPA in the nucleus accumbens and dorsal striatum of freely moving rats during perfusion of NSD-1015 (10^{-5} M) via microdialysis probe. The maximal responses of the DOPA output to the drug treatment (40–80 min following administration), expressed as a percentage of pre-injection baseline control, are presented. Means ± S.E.M. are shown ($n = 5$–6). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ versus controls (Mann-Whitney U-test).

ever, the maximal decrease of dopamine release observed following 0.25 mg/kg of 7-OH-DPAT was approximately equal in both regions, down to 30% of basal level. The drug at this dose also significantly suppressed the DOPAC level in dialysates from the nucleus accumbens and dorsal striatum to a similar extent, down to 60–66% of control (Figs. 1 and 2). 7-OH-DPAT at higher doses (1 and 6 mg/kg, i.p.) did not induce any further decrease of dopamine or DOPAC dialysate levels in either region studied (data not shown).

3.2. *Effect of 7-OH-DPAT on locomotor activity of rats habituated to their environment*

The basal level of locomotor activity of rats habituated to their environment was found to be 266 ± 31 counts per 30 min ($n = 32$). The administration of saline did not significantly modify the locomotor activity of rats. 7-OH-DPAT over the dose range 0.002–0.25 mg/kg markedly decreased the locomotor activity of animals (Fig. 3). At the maximal inhibitory effect, activity was down to 7% of control. The ED_{50} of 7-OH-DPAT for suppression of locomotion was calculated as 0.0044 mg/kg, i.p. (Table 1).

3.3. *Effect of i.p. administration of 7-OH-DPAT on the DOPA output in the dorsal striatum and nucleus accumbens of freely moving rat*

DOPA was not detectable in the dialysates from untreated rats. Local application of the L-aromatic acid decarboxylase inhibitor NSD-1015 (10^{-5} M) resulted in a gradual increase of DOPA in the dialysates from both brain regions and a stable state plateau was reached approximately 2 h after the perfusion was started (data not shown). The basal levels of DOPA under these conditions were 3.2 ± 0.648 pmol/20 min in the dorsal striatum (mean ± S.E.M., $n = 28$) and 2.087 ± 0.351 pmol/20 min in the nucleus accumbens (mean ± S.E.M., $n = 28$). The DOPA output in both regions was not modified significantly by i.p. injection of saline (data not shown). 7-OH-DPAT

Table 1
Summary of the effects of 7-OH-DPAT i.p. administration on locomotor activity and in vivo neurochemical parameters in rats

Effect of i.p. administration of 7-OH-DPAT	ED_{50} (mg/kg)
Inhibition of locomotor activity	0.0044 (0.0033–0.0058)
Inhibition of dopamine release in the nucleus accumbens	0.0096 (0.004–0.023)
Inhibition of dopamine release in the dorsal striatum	0.068 (0.029–0.163)
Inhibition of DOPA output in the nucleus accumbens	0.124 (0.067–0.234)
Inhibition of DOPA output in the dorsal striatum	0.101 (0.080–0.128)
Effect of 7-OH-DPAT local application	**EC_{50} (nM in perfusate)**
Inhibition of dopamine release in the nucleus accumbens	1.9 (1.3–2.7)
Inhibition of dopamine release in the dorsal striatum	11.3 (4.7–26.9)

ED_{50} values of 7-OH-DPAT i.p. administration were calculated from the data presented in Figs. 1–4 and are given as mg/kg, i.p., followed by 95% confidence interval. EC_{50} values of 7-OH-DPAT for decreasing dopamine release in the rat brain areas were calculated from the data presented in Fig.6 and are given as nM in perfusing solution (followed by 95% confidence interval).

administered i.p. decreased dialysate DOPA levels in a similar manner in both brain regions studied, with the maximum decrease being to about 40% of the basal levels (Fig. 4). The ED_{50} values for the drug to decrease DOPA levels were found to be 0.101 mg/kg, i.p., for the dorsal striatum and 0.124 mg/kg, i.p., for the nucleus accumbens (Table 1). The threshold dose for decreasing dopamine synthesis in vivo in both regions appeared to be the same, 0.1 mg/kg, i.p.

3.4. Effect of 7-OH-DPAT pretreatment on dopamine receptor antagonist-induced increase of dopamine release and metabolism in the dorsal striatum of freely moving rats

7-OH-DPAT (0.05 mg/kg, i.p.) elicited a marked decrease in dopamine release (down to 58% to control) but failed to affect the extracellular level of DOPAC in the dorsal striatum of freely moving rats. (+)-AJ76 (7 mg/kg i.p.) increased dopamine and DOPAC levels up to 206% and 131% of control, respectively, while haloperidol (0.1 mg/kg, i.p.) elevated the dopamine level up to 142% and DOPAC up to 194% of control. Pretreatment with 7-OH-DPAT 20 min prior to haloperidol or (+)-AJ76 was found to prevent the increases of dopamine but not those of DOPAC produced by both dopamine receptor antagonists (Fig. 5).

3.5. Effect of local application of 7-OH-DPAT on dopamine release in the dorsal striatum and nucleus accumbens of freely moving rats

7-OH-DPAT added to the perfusing medium resulted in a decrease in dopamine release in both brain regions studied (Fig. 6). The effect of the drug at concentrations of 2–50 nM was more pronounced in the nucleus accumbens than in the dorsal striatum ($P < 0.05$). The EC_{50} values of the drug in this regard were calculated as 1.9 nM for the nucleus accumbens and 11.3 nM for the dorsal striatum (Table 1). The threshold concentration of 7-OH-DPAT which reduced dopamine levels in the dialysate of the dorsal striatum was 5 nM, while even at 2 nM the drug significantly decreased dopamine release in the nucleus accumbens (down to 62% of control). This difference was not observed at high concentrations of 7-OH-DPAT, i.e. 100 and 1000 nM decreased dopamine release similarly in both regions to about 30% to control.

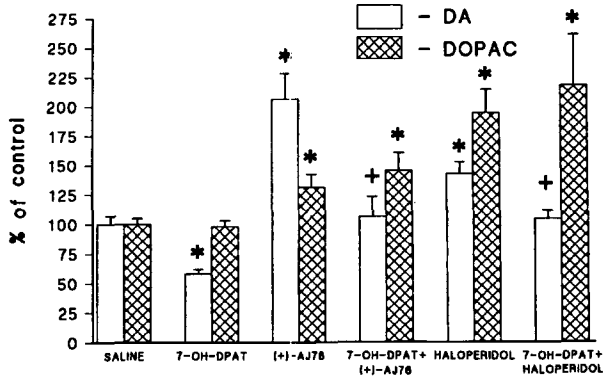

Fig. 5. Effect of 7-OH-DPAT (0.05 mg/kg, i.p.) pretreatment on the (+)-AJ76 (7 mg/kg, i.p.)- and haloperidol (0.1 mg/kg, i.p.)-induced increase of dopamine and DOPAC extracellular levels in the dorsal striatum of freely moving rats. 7-OH-DPAT was injected 20 min prior to (+)-AJ76 or haloperidol. The maximal responses of the dopamine and DOPAC output to the drug treatments (40–80 min following administration; for haloperidol-induced elevation of DOPAC level – 100–120 min following administration), expressed as a percentage of pre-injection baseline control, are presented. Means ± S.E.M. are shown ($n = 5$–6). * $P < 0.05$ versus controls; + $P < 0.05$ versus the effects of (+)-AJ76 or haloperidol (Mann-Whitney U-test).

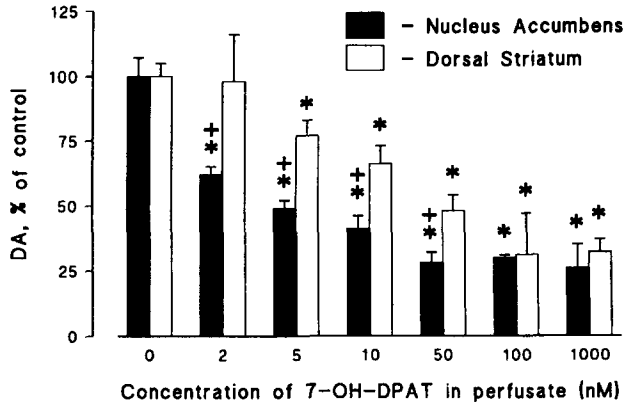

Fig. 6. Effect of local application of 7-OH-DPAT via addition to perfusing medium on dopamine extracellular levels in the nucleus accumbens and dorsal striatum of freely moving rats. The maximal responses of the dopamine output to the drug treatment, expressed as a percentage of baseline control, are presented. Means ± S.E.M. are shown ($n = 4$–5). * $P < 0.05$; versus controls; + $P < 0.05$ between the effects of the drug in the nucleus accumbens and the dorsal striatum (Mann-Whitney U-test).

4. Discussion

7-OH-DPAT dose dependently decreased the release, synthesis and metabolism of dopamine in the nucleus accumbens and dorsal striatum of freely moving rats. These neurochemical effects, characteristic of those observed after stimulation of dopamine D_2-like receptors, were shown previously in a number of studies (Feenstra et al., 1983; Zetterström and Ungerstedt, 1984; El Mestikawy et al., 1986; Imperato et al., 1988; Stamford et al., 1991; Svensson et al., 1994a) and are commonly explained by a compensatory negative feed-back mechanism which involves dopamine autoreceptors situated on nerve terminals and cell bodies as well as dopamine receptors located on postsynaptic structures (Carlsson, 1975; Roth, 1983). Although all of these dopamine receptors at different sites seem to be involved in the observed effects of dopamine receptor agonists, nerve terminal dopamine autoreceptors of the D_2 family are suggested to play the most important

role (Roth, 1983; Imperato et al., 1988; Timmerman et al., 1990; Santiago and Westerink, 1991).

A decrease of dopamine release in the striatum in vivo following systemic 7-OH-DPAT was reported earlier (Mulder et al., 1987; Damsma et al., 1993a,b; Rivet et al., 1994; Devoto et al., 1995). A new finding of the present study is that 7-OH-DPAT administered i.p. inhibited dopamine release in the nucleus accumbens over a lower dose range than in the dorsal striatum of freely moving rats (Figs. 1 and 2, Table 1). This observation is of importance due to the known preferential limbic distribution of dopamine D_3 receptors (Sokoloff et al., 1990; Levesque et al., 1992; Herroelen et al., 1994). The drug is also more potent in the nucleus accumbens than in the dorsal striatum in inhibiting dopamine release during local application (Fig. 6, Table 1). The preferential decrease of dopamine release in the nucleus accumbens versus dorsal striatum following either systemic or local administration of 7-OH-DPAT clearly indicates the functional limbic selectivity of the drug in vivo, which might serve as evidence of involvement of dopamine D_3 receptors in this event. This observation is in line with in vitro data reported on the effects of 7-OH-DPAT on electrically evoked dopamine release from brain slices, which also suggest that the contribution of D_3 receptors in the nucleus accumbens to the presynaptic regulation of dopamine release is much greater than in the striatum (Yamada et al., 1994). It is important to note that some other dopamine agonists (Stamford et al., 1991), as well as dopamine D_3 receptor-preferring antagonists (+)-AJ76 and (+)-UH232 (Waters et al., 1993a), also demonstrated higher potency in affecting dopamine release in vivo in the nucleus accumbens than in the striatum of rats.

The other finding of this study is that the hypomotility induced by 7-OH-DPAT in doses of 0.002–0.25 mg/kg, i.p., appears to correspond to decreases in dopamine release in the nucleus accumbens but not in the dorsal striatum (Figs. 1–3, Table 1). This is in contrast to the data of Svensson et al. (1994a,b), which showed a lack of correlation between the ability of the drug to reduce dopamine release in the nucleus accumbens and inhibition of locomotion. They reported that locomotion in rats was inhibited by the active enantiomer *R*-(+)-7-OH-DPAT over a dose range markedly lower than that used in our study (ED_{50} was reported to be 0.4 nmol/kg, i.e. approximately 0.13 μg/kg, s.c. (Svensson et al., 1994a,b)). No effect on dopamine release in either the nucleus accumbens or striatum was found with this extremely low dose. This observation, as well as the inability of putative dopamine D_3 receptor antagonist U 99194A to affect dopamine release or synthesis at doses which induce behavioural stimulation in rats (Waters et al., 1993b), led the authors to hypothesize that the functional dopamine D_3 receptor is a postsynaptic receptor mediating inhibition of locomotion in rats (Svensson et al., 1994a,b; see for review Stähle, 1992). However, it should be noted that the other dopamine receptor agonists studied, i.e. apomorphine, (+)-3-hydroxy-*N*-*n*-propyl-phenylpiperidine ((+)-3-PPP), quinpirole and pramipexole, have been found to decrease significantly dopamine release in striatum and nucleus accumbens at ED_{50} doses causing inhibition of locomotion in rats (Svensson et al., 1994a). In the present study we observed a decrease of locomotion following substantially higher doses of racemic 7-OH-DPAT (ED_{50} = 0.0044 mg/kg,i.p.). Approximately the same potency of 7-OH-DPAT for inhibiting locomotion in rats has been reported also by other researchers (Damsma et al., 1993a; Ahlenius and Salmi, 1994). It is possible that the model used by Svensson et al. (1994a,b) is more sensitive than the presently used one.

There are a number of observations suggesting that dopamine release and synthesis are under the control of different types of dopamine terminal autoreceptors (Arbilla and Langer, 1981; Mulder et al., 1987; Gainetdinov et al., 1994). The fact that dopamine synthesis studied in vivo was affected by 7-OH-DPAT administration at doses higher than those that decreased dopamine release gives further support to this notion. The decrease of dopamine metabolism, assessed by extracellular DOPAC level, was detected only at doses which also suppressed dopamine synthesis. Therefore, the present data serve as additional evidence that extracellular DOPAC concentrations may be considered as an index of dopamine synthesis (Zetterström et al., 1988). It is important that no difference was found in the suppression of dopamine synthesis/metabolism in vivo by 7-OH-DPAT in the dorsal striatum versus the nucleus accumbens. This observation confirms the earlier data showing also that the potency of 7-OH-DPAT, as well as some other dopamine receptor agonists, in decreasing dopamine biosynthesis, as measured by DOPA accumulation, is approximately the same in the rat striatum and nucleus accumbens (Ahlenius and Salmi, 1994; Booth et al., 1994; Svensson et al., 1994a; Aretha et al., 1995). This is in agreement with the rather homogenous distribution of dopamine D_2 receptors over striatal and mesolimbic regions (Joyce et al., 1991; Boundy et al., 1993; Gingrich and Caron, 1993).

Recent ex vivo studies have provided conflicting results on the question whether dopamine D_2 or D_3 receptors are coupled to dopamine synthesis (Meller et al., 1993; Ahlenius and Salmi, 1994; Booth et al., 1994; Aretha et al., 1995). The present in vivo data, showing the lower potency of 7-OH-DPAT in suppressing dopamine synthesis as compared to dopamine release, as well as some published earlier (Gainetdinov et al., 1994; Svensson et al., 1994a; see Sautel et al., 1995), give more evidence for the preferential involvement of dopamine D_2 but not D_3 autoreceptors in the presynaptic modulation of dopamine synthesis. Another line of argument in favour of our hypothesis was presented recently in a study with transfected D_2 and D_3 receptors in the dopamine-producing mouse mesencephalic cell line MN9D. It was shown that

dopamine D_3 receptor stimulation resulted in a more pronounced inhibition of dopamine release than D_2 receptor stimulation did, while only D_2 but not D_3 receptors were involved in the regulation of tyrosine hydroxylase activity in this cell line (O'Hara et al., 1994; Tang et al., 1994b). Indeed, most studies have found a weak or lack of coupling of dopamine D_3 receptors to the cAMP transduction pathway (Sokoloff et al., 1990; Freedman et al., 1993; Chio et al., 1994; Tang et al., 1994a; Lajiness et al., 1995) suggested to be involved in autoreceptor-mediated tyrosine hydroxylase phosphorylation (El Mestikawy et al., 1986; Santiago and Westerink, 1990).

In recent electrophysiological studies in which neuronal firing rate and patch-recordings of dopamine-modulated K^+-channels were used, no differences were found in the potency of 7-OH-DPAT between mesolimbic and nigrostriatal systems at either auto- or postsynaptic receptors (Liu et al., 1994). On the basis of this observation, it was suggested that the selectivity of 7-OH-DPAT at dopamine D_3 receptors is lower than previously reported. Other evidence of less selectivity of 7-OH-DPAT at D_3 versus D_2 dopamine receptors has been found in some functional tests on cloned receptors (Chio et al., 1994; Svensson et al., 1994a; Sautel et al., 1995). Recently published binding data indicate that the dopamine D_3 versus D_2 receptor selectivity of the drug decreases under conditions favouring high-affinity receptor binding for the D_2 receptor (Large and Stubbs, 1994; Seeman and Van Tol, 1994; Burris et al., 1995). It has been shown that 7-OH-DPAT affinity for the dopamine D_2 receptor varies from 10 nM for the high-affinity state to 61 nM for the low-affinity state in different experimental paradigms, while the affinity for the D_3 receptor is as low as 0.78–1.6 nM (Levesque et al., 1992; Freedman et al., 1993; Seeman and Van Tol, 1994). Due to the relatively limited selectivity of 7-OH-DPAT at dopamine D_3 in comparison to D_2 receptors, it is important to establish the doses of the drug which might be considered as D_3 receptor 'selective'. Using the quantitative microdialysis 'point of no net flux' method, we have estimated the interstitial free concentration of 7-OH-DPAT in the dorsal striatum of freely moving rats after i.p. administration of the drug (Gainetdinov et al., 1995). We found that the interstitial free concentrations of 7-OH-DPAT following administration of doses lower than 0.05 mg/kg will not reach the affinity values for dopamine D_2 receptors. It is important that 7-OH-DPAT at these putative dopamine D_3 receptor 'selective' doses produces decreases of dopamine release but not synthesis/metabolism in dorsal striatum and nucleus accumbens in vivo. We used 7-OH-DPAT at the dose of 0.05 mg/kg, i.p., to test whether the increases in dopamine release induced by the dopamine D_2- and D_3-receptor-preferring antagonists, haloperidol and (+)-AJ76, respectively, were mediated by D_3 receptors (Fig. 5). The fact that 7-OH-DPAT pretreatment prevented the increase in dopamine release but not that of its metabolite produced by both antagonists gives further support for a preferential role of dopamine D_3 receptors in the presynaptic modulation of dopamine release.

To verify the nerve terminal localisation of dopamine autoreceptors involved in the inhibition of dopamine release by 7-OH-DPAT, we used the local application of the drug in the dorsal striatum and nucleus accumbens via perfusion through the microdialysis probe. This approach has been suggested to allow a study of the effect of the drug solely at autoreceptors located on nerve terminals, excluding an involvement of autoreceptors situated on the cell body as well as postsynaptic receptors (Timmerman et al., 1990). The potent decrease of dopamine release in both brain regions induced by infusion of 7-OH-DPAT (Fig. 6) confirms the leading role of nerve terminal dopamine release-regulating autoreceptors in the effect observed (Roth, 1983; Imperato et al., 1988; Westerink and De Vries, 1989 ; Santiago and Westerink, 1991). It should be noted that the in vivo recovery of the drug via the microdialysis membrane was established using the 'point of no net flux' method (Gainetdinov et al., 1995) as about 43%. It means that the 'true' extracellular concentration of the drug that produced profound suppression of dopamine release in both nigrostriatal and mesolimbic targeting regions is well below the affinity for the dopamine D_2 receptor. This fact, as well as the marked limbic preference in the effect of infused 7-OH-DPAT, might serve as additional evidence for a preferential role of D_3 terminal autoreceptors in the presynaptic regulation of dopamine release.

In summary, the present results give support to the hypothesis that terminal autoreceptors controlling dopamine release preferentially belong to the D_3 type, while those modulating dopamine synthesis are dopamine D_2 receptors in both the mesolimbic and nigrostriatal regions. We can also conclude that presynaptic autoreceptor regulation of dopamine release but not synthesis in the rat nucleus accumbens appears to be more sensitive to 7-OH-DPAT stimulation than that in the dorsal striatum. Our data give further support to the original autoreceptor theory (Carlsson, 1975) whereby the behavioural inhibition produced by dopamine agonists is a result of stimulation of presynaptic dopamine release-regulating autoreceptors, causing a decrease of dopamine release in the nucleus accumbens.

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