Biphasic inhibition of stimulated endogenous dopamine release by 7-OH-DPAT in slices of rat nucleus accumbens

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1 Fast cyclic voltammetry was used to investigate the effect of 7-OH-DPAT (7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin), a putative D₃ receptor agonist, on electrically stimulated endogenous dopamine release in slices of rat nucleus accumbens.

2 7-OH-DPAT inhibited single pulse stimulated dopamine release in a concentration-dependent manner with a maximum inhibition of 95.5%. Analysis of concentration-response curves to 7-OH-DPAT showed that they were biphasic, with the high affinity component contributing 18.0% to the total inhibition and the low affinity component 77.5%. 7-OH-DPAT exhibited a 560 fold selectivity between the high and low affinity components (0.015 nM compared to 8.4 nM).

3 Concentration-response curves to the non-selective D₂/D₃ agonist, apomorphine, were monophasic. The maximum inhibition was 93.1% and the EC₅₀ value 82 nM.

4 The selective D₂ antagonist, haloperidol (30 nM), antagonized the low affinity component of the concentration-response curve to 7-OH-DPAT whilst the high affinity component was essentially unaffected. The pK_B values calculated for the high and low affinity components were 7.89 and 9.45 respectively.

5 In conclusion, these results demonstrate that 7-OH-DPAT inhibits stimulated dopamine release by acting at two different sites. Furthermore, the results are consistent with the hypothesis that the high and low affinity components of the concentration-response curve to 7-OH-DPAT may reflect activation of functional D₃ and D₂ release-regulating autoreceptors respectively. However, the possibility that the biphasic nature of the curve may reflect different subtypes of the D₂ receptor cannot be excluded.

Keywords: Dopamine release; dopamine D₃ receptor; dopamine D₂ receptor; 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT); apomorphine; fast cyclic voltammetry; rat nucleus accumbens

Introduction

Dopamine D₂ receptors have been shown to be located on the axon terminals of A9 and A10 dopaminergic neurones where they are involved in modulating dopamine release and synthesis (for review see Starke *et al.*, 1989). The recent molecular cloning of the gene for a novel D₂-like dopamine receptor, designated the D₃ receptor (Sokoloff *et al.*, 1990), has instigated research into potential functional roles of the D₃ receptor. Messenger RNA (mRNA) distribution studies have shown that the localizations of D₂ and D₃ receptors are anatomically distinct. In contrast to D₂ receptors, D₃ receptors are preferentially distributed in the limbic system with highest densities in the Islands of Calleja, olfactory tubercles and nucleus accumbens (NAc) (Sokoloff *et al.*, 1990). In addition, low concentrations of D₃ receptor mRNA are also detected in the dopamine cell bodies of the rat substantia nigra and ventral tegmental area raising the possibility that the D₃ receptor may act as a dopamine autoreceptor (Bouthenet *et al.*, 1991).

Stable expression of rat and human D₂ and D₃ receptors in Chinese hamster ovary (CHO) recombinant cell lines has established further pharmacological differences between the D₂ and D₃ receptors. For instance, dopamine and other D₂ receptor agonists (e.g. quinpirole), generally display higher affinities at the D₃ receptor. In contrast, many dopamine receptor antagonists tend to exhibit a preference for D₂ receptors. In fact, the only available D₂ antagonists showing limited selectivity (3 to 4 fold) at D₃ receptors are (+)-UH232 (*cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-dipropylamino)tetralin) and (+)-AJ76 (*cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin) (Sokoloff *et al.*, 1990). The lack of selective antagonists for this receptor has therefore limited research into

its functional significance. 7-OH-DPAT (7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin) is a selective dopamine receptor agonist (El Mestikawy *et al.*, 1986), which in CHO cell line binding studies has about an 80 fold greater affinity for the D₃ receptor (0.78 nM) than the D₂ receptor (61 nM) (Lévesque *et al.*, 1992). Its affinity at D₄ and D₁ receptors is relatively lower (650 nM and 5300 nM respectively). Binding studies with [³H]-7-OH-DPAT in rat brain have confirmed D₃ receptor distribution indicated by *in situ* D₃ mRNA hybridisation (Bouthenet *et al.*, 1991) and also shown low concentrations of D₃ receptor binding sites in the caudate putamen (CPU) (Lévesque *et al.*, 1992).

Sokoloff *et al.* (1990) found that in contrast to the D₂ receptor, stimulation of the rat D₃ receptor transfected into CHO cells did not inhibit adenylate cyclase activity. Subsequent studies on the human D₃ receptor transfected into various other cell lines have confirmed this finding and that it fails to couple to other second messenger systems including release of arachidonic acid, phosphoinositide hydrolysis, whole cell potassium currents and calcium mobilization (Freedman *et al.*, 1994; MacKenzie *et al.*, 1994; Tang *et al.*, 1994a). However, a few studies have shown coupling of human D₃ receptors to a functional response. Potenza *et al.* (1994) showed that activation of human D₃ receptors transfected into cultured *Xenopus laevis* melanophores causes pigment granule aggregation. This action is thought to be mediated via inhibition of cyclic AMP accumulation. In the NG108-15 cell line, stimulation of transfected human D₃ receptors depresses calcium currents (Seabrook *et al.*, 1994) and stimulates *c-fos* expression and mitogenesis (Pilon *et al.*, 1994). These effects are sensitive to the action of pertussis toxin, suggesting the involvement of G_i and/or G_o G-proteins.

Evidence for a functional role for D₃ receptors, using 7-OH-DPAT or other D₃-selective ligands, comes mainly from be-

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havioural studies. Caine & Koob (1993) have proposed that the D₃ receptor may be involved in the reinforcing effect of cocaine. Other behavioural studies have suggested that activation of D₃ receptors is associated with hypolocomotor activity (Daly & Waddington, 1993; Waters *et al.*, 1993; Svensson *et al.*, 1994), induction of yawning (Damsma *et al.*, 1993) and hypothermia in rats (Millan *et al.*, 1994). However, the assignment of *in vivo* behavioural responses to mediation by any receptor sub-type remains highly speculative (Large & Stubbs, 1994).

Biochemical studies have suggested that striatal dopamine synthesis-inhibiting autoreceptors and release-regulating autoreceptors may be of the D₃ rather than the D₂ type (Meller *et al.*, 1993; Damsma *et al.*, 1993). Furthermore, Tang *et al.* (1994b) have shown that stimulation of rat D₂ and human D₃ receptors transfected into the MN9D mesencephalic cell line causes inhibition of dopamine release whilst activation of transfected rat D₄ receptors is without effect. This cell line has the ability to synthesize and release dopamine and possesses many of the characteristics of CNS dopaminergic neurones. This observation suggests that the D₃ receptor may have the capacity to act as a release-regulating autoreceptor within the CNS.

In this study, the possible role of a functional dopamine D₃ receptor acting as a release-regulating autoreceptor in the rat NAc was investigated. Fast cyclic voltammetry (FCV) was used to examine the inhibition of dopamine release in brain slices by the selective D₃ agonist, 7-OH-DPAT, and the non-selective D₂/D₃ agonist, apomorphine. FCV is an electrochemical technique which has been used to detect endogenous dopamine release (Bull *et al.*, 1990; Palij *et al.*, 1990; Bull & Sheehan, 1991; Trout & Kruk, 1992; Patel *et al.*, 1992a). Release may be measured following single pulse electrical stimulation in the absence of a reuptake inhibitor. This avoids endogenously released transmitter activating the release-inhibiting autoreceptors and interfering with the action of the exogenous agonist (Starke *et al.*, 1989; Limberger *et al.*, 1991). This method can therefore be used for quantitative determination of the potency of agonists and affinity constants for competitive antagonists (Palij *et al.*, 1990; Bull & Sheehan, 1991). In addition, the temporal resolution of FCV can be exploited to measure the kinetics of reuptake of the released transmitter (Trout & Kruk, 1992; Patel *et al.*, 1992b; Hafizi *et al.*, 1992; Davidson & Stamford, 1993).

Methods

Preparation of brain slices

Slices were prepared as previously described (Bull *et al.*, 1990; Trout & Kruk, 1992; Patel *et al.*, 1992a). Briefly, coronal brain slices (350 µm thick) containing the nucleus accumbens were prepared from male Wistar rats (230 to 300 g). Slices were placed in a Perspex brain slice chamber and superfused (1.2 ml min⁻¹) with artificial cerebrospinal fluid (ACSF: composition in mM; NaCl 124.0, KCl 2.0, KH₂PO₄ 1.25, MgSO₄ 2.0, NaHCO₃ 25.0, CaCl₂ 2.0, (+)-glucose 11.0). This solution was saturated with 5% CO₂ in O₂ and maintained at 32°C. Slices were illuminated from below and, with the aid of a binocular dissection microscope, recording and stimulating electrodes were positioned in the slice 80 µm below the surface in the core region of the NAc.

Fast cyclic voltammetry

Fast cyclic voltammetry was used to monitor electrically evoked dopamine efflux by measuring the current generated at a carbon fibre microelectrode (exposed tip 8 µm in diameter, 20 to 50 µm in length) when dopamine is oxidised. Using a Millar voltammeter (PD Systems Ltd., UK), 1½ cycles of a 100 Hz triangular waveform (−1.0 to +1.4 V peak to peak, 15 ms duration) were applied to the carbon fibre

microelectrode twice a second. Changes at the peak oxidation potential for dopamine (+595 mV relative to a Ag/AgCl reference electrode) were monitored by a 'sample and hold' circuit: this signal was output to a chart recorder and to a Cambridge Electronic Design (CED) 1401 computer interface. The 'sample and hold' circuit recorded changes at the oxidation current with time. Data were captured and digitally stored as files using CED software (Signal Averager and Chart). In addition, the applied voltage waveform and the waveform showing the current flowing in the recording electrode were monitored on a digital storage oscilloscope (Nicolet 310).

Electrical stimulation

Bipolar tungsten wire stimulating electrodes (tip separation 200 µm) were placed about 200 to 300 µm away from the carbon fibre recording electrode. The stimulating electrodes were connected to a Digitimer DS2 constant voltage stimulator controlled by a Neurolog (Digitimer) modular timing system. Dopamine efflux was evoked by single electrical pulses (0.1 ms duration, 20 V amplitude) applied every 2 min.

Protocol

Responses to single pulse stimulation were allowed to stabilize under control conditions for at least 60 min after which a dopamine autoreceptor agonist (7-OH-DPAT or apomorphine) was added to the superfusion fluid at increasing concentrations. Each drug concentration was superfused until its effect on responses achieved a plateau or for a minimum of 20 min if no effect was observed. Concentration-response curves to 7-OH-DPAT were constructed either in the absence or presence of a dopamine autoreceptor antagonist (haloperidol or (+)-AJ76) added at least 60 min prior to the first concentration of agonist. Dopamine release was studied by agreement with calibration curves obtained when the carbon fibre microelectrode was exposed to known concentrations of dopamine post-experimentally.

Analysis of data

Concentration-response data (% inhibition of peak dopamine efflux) were fitted to the following equation:

$$E = \sum_{i=1}^n \frac{E_{\max,i} 10^{\log_{10}[D]}}{10^{\log_{10}EC_{50,i}} + 10^{\log_{10}[D]}}$$

where E = response (% inhibition of stimulated dopamine efflux), E_{max} = maximum response, EC₅₀ = concentration required to produce half-maximum response, [D] = agonist concentration, n = 1 (monophasic model) or 2 (biphasic model) and i refers to the appropriate component of the curve. Curves were fitted by a least-squares iterative method using the Solver function in Microsoft Excel. Each set of data was fitted to both a one component (n = 1 in the above equation) and a two component model (n = 2). Slopes were constrained to unity. The goodness of fit to a biphasic over a monophasic model was evaluated by the *F* statistic from the residual sums of squares (Kenakin, 1993). Pharmacological parameters such as the EC₅₀ and E_{max} values were calculated from individual fitted curves. The pK_B values were calculated from concentration-ratios at the EC₅₀ level in the absence and presence of antagonist using the following equation:

$$pK_B = \log_{10} (\text{concentration-ratio} - 1) - \log_{10} (\text{antagonist concentration})$$

Data are expressed as the arithmetic mean ± s.e. mean, except EC₅₀ values, which are expressed as the geometric mean with

95% confidence limits (CL). Student's *t* test (two-tailed, unpaired) was used to compare $\log_{10}$ EC_{50} and E_{max} values obtained for 7-OH-DPAT in the absence and presence of antagonists. The number of experiments conducted is referred to by *n*, each of which was performed in a brain slice prepared from a different animal.

Drugs

Salts for the ACSF (see above for composition) were of Analar grade and obtained from BDH Ltd. Drugs were obtained from the following sources: dopamine HCl, apomorphine and haloperidol were obtained from Sigma Chemical Company Ltd., ($\pm$)-7-OH-DPAT (7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin) was obtained from Research Biochemicals Incorporated (RBI, distributed under the NIMH special synthesis programme) and (+)-AJ76 (*cis*-(+)-(1*S*,2*R*)-5-methoxy-1-methyl-2-(*n*-propylamino)tetralin) was donated by Dr K. Svensson, University of Göteborg, Sweden. All drugs were dissolved initially in deionised water and then diluted further in ACSF, except haloperidol, which was initially dissolved in tartaric acid (0.01 M).

Results

Using FCV it was possible to detect endogenous dopamine efflux in response to a single electrical pulse from rat brain slices containing the NAc. The rapid transient responses following stimulation reached a maximum within 1 s and returned to pre-stimulation levels within 5 s. The amount of dopamine evoked by a single pulse in the NAc was 118 ± 8 nM ($n=21$), determined by post-experimental calibration of the recording electrode, and was stable for up to 10 h (data not shown).

7-OH-DPAT (0.01 nM to 3 μ M) inhibited single pulse stimulated dopamine efflux in a concentration-dependent manner, the maximum inhibition being $95.5 \pm 0.6\%$. Figure 1 shows 'sample and hold' traces of evoked dopamine efflux obtained in the absence and presence of increasing concentrations of 7-OH-DPAT. Analysis of the concentration-response data by curve fitting revealed that the inhibition of dopamine efflux by 7-OH-DPAT could be fitted significantly better to a biphasic concentration-response curve than a monophasic curve ($F=142.9$, d.f.=2.8; $P<0.005$) (Figure 2). The relative EC_{50} and E_{max} values calculated by curve fitting for the high and low affinity components of the curve are shown in Table 1.

The non-selective D_2/D_3 receptor agonist, apomorphine (0.1 nM to 10 μ M) also inhibited evoked dopamine efflux in a concentration-dependent manner. However, in contrast to 7-OH-DPAT, analysis of the data by curve fitting showed that a biphasic curve was not a significant improvement over a monophasic one ($F=0.62$, d.f.=2.7; $P>0.05$) (see Figure 3 and Table 1 for calculated EC_{50} and E_{max} values).

In the presence of 30 nM haloperidol, a selective D_2 receptor antagonist (Sokoloff *et al.*, 1990), the two components of the concentration-response curve to 7-OH-DPAT were differentially affected without any significant reduction in the total inhibition achieved ($95.5 \pm 0.6\%$ in the absence of haloperidol ($n=7$) compared to $93.7 \pm 1.3\%$ in the presence of haloperidol

Figure 1 Sample and hold traces showing the concentration-dependent effect of the selective D_3 agonist 7-OH-DPAT on stimulated dopamine efflux in the rat nucleus accumbens. Dopamine (DA) efflux was evoked by single electrical pulses (0.1 ms duration, 20 V amplitude). Data were captured from a single experiment using a Cambridge Electronic Design (CED) 1401 analogue to digital interface with CED Chart and Signal Averager software.

Figure 2 Concentration-response curve for the inhibition of stimulated dopamine (DA) efflux in rat brain slices containing the nucleus accumbens by 7-OH-DPAT. Values are expressed as percentage inhibition of peak dopamine efflux (mean $\pm$ s.e. mean), against log concentration of agonist ($n=7$). The mean data were best fitted to a two component model (solid line).

($n=5$; $P>0.05$), (Figure 4). The EC_{50} value for the high affinity component of the curve was not significantly altered (0.015 nM (CL 0.007–0.032) in the absence of haloperidol ($n=7$) compared to 0.050 nM (CL 0.012–0.204) in the presence of haloperidol ($n=5$, $P>0.05$)) although there was a significant increase in the E_{max} value of this phase ($18.0 \pm 2.1\%$ to $30.2 \pm 3.2\%$; $P<0.05$, $n=7$ and 5 respectively). In contrast, there was a significant right-ward displacement of the low affinity component (EC_{50} : 724 nM (CL 493–1062) in the presence of haloperidol ($n=5$) compared to 8.4 nM (CL 6.3–11.1) in the absence of haloperidol ($n=7$, $P<0.001$)), accompanied by a significant reduction in the E_{max} value of this component ($77.5 \pm 2.7\%$ ($n=7$) to $63.4 \pm 3.0\%$ ($n=5$); $P<0.01$). The pK_B values calculated for haloperidol at the high and low affinity sites were 7.89 and 9.45 ($n=5$) respectively.

The putative D_3 receptor antagonist, (+)-AJ76 (1 μ M), also

Table 1 EC_{50} (nM) and E_{max} values obtained for 7-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) and apomorphine on dopamine efflux evoked by single pulse stimulation in rat nucleus accumbens (NAc)

Drug	EC_{50} (nM) (geometric mean)	E_{max} (%) (mean $\pm$ s.e. mean)	n
7-OH-DPAT (High)	0.015 (0.007–0.032)	18.0 ± 2.1	7
7-OH-DPAT (Low)	8.4 (6.3–11.1)	77.5 ± 2.7	7
Apomorphine	82 (50–136)	93.1 ± 1.3	5

Individual values were calculated by curve fitting; EC_{50} values are expressed as geometric means with 95% confidence intervals whereas E_{max} values are expressed as arithmetic means $\pm$ s.e. mean.

Figure 3 Concentration-response curve for the inhibition of stimulated dopamine (DA) efflux in rat brain slices containing the nucleus accumbens by apomorphine. Values are expressed as percentage inhibition of peak dopamine efflux (mean $\pm$ s.e.mean), against log concentration of agonist ($n=5$). The mean data were best fitted to a one component model (solid line).

Figure 4 Concentration-response curves for the inhibition of stimulated dopamine (DA) efflux in rat brain slices containing the nucleus accumbens by 7-OH-DPAT in the absence ($\bullet$, $n=7$) and presence ($\circ$, $n=5$) of haloperidol (30 nM). Values are expressed as percentage inhibition of peak dopamine efflux (mean $\pm$ s.e.mean), against log concentration of agonist. Each curve was individually fitted to mean data using a two component model (solid lines).

displaced the low affinity component of the 7-OH-DPAT concentration-response curve (EC_{50} : 8.4 nM (CL 6.3–11.1) in the absence of (+)-AJ76 ($n=7$) compared to 105 nM (CL 23–49) in the presence of (+)-AJ76 ($n=4$), $P<0.05$) and had a small non-significant effect at the high affinity site (EC_{50} : 0.015 nM (CL 0.007–0.032) in the absence of (+)-AJ76 ($n=7$) compared to 0.017 nM (CL 0.008–0.038) in the presence of (+)-AJ76 ($n=4$), $P>0.05$). In addition, there was no significant change in the E_{max} values at the high ($18.0 \pm 2.6\%$) and low ($77.8 \pm 3.5\%$) affinity components, or in the total inhibition ($95.8 \pm 1.4\%$), $P>0.05$ and $n=4$ in all cases (Figure 5). The pK_B values calculated at the high and low affinity sites were 5.05 ($n=3$) and 7.06 ($n=4$) respectively.

In order to establish the effect of 7-OH-DPAT on dopamine reuptake, the half decay times, i.e. the time taken for the observed peak dopamine efflux to decline to half its value were measured. In the presence of 10 nM 7-OH-DPAT (a concentration which inhibits dopamine efflux by more than 50%), the mean half decay time did not significantly increase (1167 ± 167 ms in the presence of 7-OH-DPAT compared to 1250 ± 281 ms under control conditions, $P>0.05$; $n=6$).

Discussion

The possibility of a functional dopamine D_3 receptor acting as an axon terminal release-regulating autoreceptor was investigated using the selective D_3 receptor agonist 7-OH-DPAT (Lévesque *et al.*, 1992). Although it is difficult to establish positively a role for the D_3 receptor in the absence of selective antagonists for this receptor, 7-OH-DPAT can be used to provide evidence consistent with this possibility. Effects were examined on stimulated dopamine release evoked by single electrical pulses in the absence of a reuptake inhibitor. In this way, the possibility of released dopamine activating autoreceptors and thus interfering with the action of the exogenous agonist was avoided. Agonist concentration-response curves were therefore constructed in the absence of competition from the endogenous ligand.

Statistical analysis of data from concentration-response curves obtained in the NAc showed that 7-OH-DPAT inhibited evoked dopamine release in a biphasic manner and that the two components resolved contributed different amounts to the total inhibition. This initial finding indicates that there may be a mixed population of dopamine autoreceptors in the NAc: the high affinity component of the curve may be due to activation of D_3 receptors whereas the low affinity component may be due to D_2 receptors. In contrast, monophasic concentration-response curves were obtained for the non-selective D_2/D_3 agonist, apomorphine (Sokoloff *et al.*, 1990). This observation

Figure 5 Concentration-response curves for the inhibition of stimulated dopamine (DA) efflux in rat brain slices containing the nucleus accumbens by 7-OH-DPAT in the absence ($\bullet$, $n=7$) and presence ($\circ$, $n=4$) of (+)-AJ76 (1 μ M). Values are expressed as percentage inhibition of peak dopamine efflux (mean $\pm$ s.e.mean), against log concentration of agonist. Each curve was individually fitted to mean data using a two component model (solid lines).

is also consistent with a mixed D_2/D_3 receptor population and furthermore, eliminates the possibility that the biphasic curve to 7-OH-DPAT might be an artifact of the experimental technique used. Interestingly, visual examination of the experimental data from a recent study demonstrating the inhibitory effect of 7-OH-DPAT on stimulated dopamine release in the NAc suggests that the concentration-response curve to 7-OH-DPAT may be biphasic (Yamada *et al.*, 1994). This would be in agreement with our own data. However, it is unclear why an analysis to show this was not made.

In the presence of haloperidol, a D_2 antagonist displaying about 20 fold selectivity for D_2 over D_3 receptors (Sokoloff *et al.*, 1990), the biphasic nature of the concentration-response curve to 7-OH-DPAT became more pronounced. In this functional assay, haloperidol antagonized the high and low affinity components of the concentration-response curve to 7-OH-DPAT with similar affinities (K_B 12.9 nM and 0.35 nM respectively) to those reported at rat D_3 and D_2 receptors (K_i 9.8 nM and 0.45 nM respectively) in CHO cell lines (Sokoloff *et al.*, 1990).

In the present study it was found that the E_{max} values for the two components were changed in the presence of haloperidol without any change in the total inhibition achieved. The E_{max} for the high affinity component was increased and that for the low affinity component reduced. This effect was surprising as

haloperidol is considered to be a competitive antagonist and as such would be expected to affect the EC_{50} value without altering the E_{max} . However, it is unclear whether the changes in the E_{max} values for the high and low affinity components are both direct effects of the antagonist. It may be that the antagonist causes a decrease in the E_{max} for the low affinity component and the E_{max} for the high affinity component increases as a consequence of the unchanged total inhibition, or *vice versa*.

(+)-AJ76, a putative D_3 antagonist with 3 fold selectivity over D_2 receptors (Sokoloff *et al.*, 1990) was also unable to shift significantly the high affinity component of the concentration-response curve to 7-OH-DPAT. This appears to be inconsistent with the suggestion that the high affinity component is due to activation of the D_3 receptor. However, it should be noted that this compound is not a highly selective D_3 antagonist, its weak selectivity being demonstrated only in binding and not in functional studies. A recent study by Rivet *et al.* (1994) showed that basal dopamine flux into dialysate was markedly reduced in both the striatum and the NAc by systemic administration of 7-OH-DPAT. This effect was partially blocked by pretreatment with S 14297 (+)-[7-(*N,N*-dipropylamino)-5,6,7,8-tetrahydro-naphtho(2,3b)dihydro,2,3-furane] a D_3 antagonist displaying about 23 fold selectivity for human D_3 receptors over D_2 in transfected CHO cells (Gobert *et al.*, 1994; Millan *et al.*, 1994). This compound could not be made available to us for the present study.

The R-(+)-isomer has been shown to be the active isomer of 7-OH-DPAT (Baldessarini *et al.*, 1993; Damsma *et al.*, 1993). In this study the EC_{50} values obtained for racemic 7-OH-DPAT at the two sites were much lower (0.015 nM and 8.4 nM) than K_i values obtained for D_3 (0.78 nM) and D_2 (61 nM) receptors in transfected CHO cell lines (Lévesque *et al.*, 1992). In addition, there was a greater selectivity of the drug between the two sites (560 fold) than those reported (about 80 fold) between D_3 and D_2 receptors. The reasons why such discrepancies exist may be because the affinities determined in recombinant cell lines measure receptor occupancy whereas in the present study a functional response was measured. This response depends not only on receptor occupancy but also on other factors such as efficacy and receptor reserve. It has been shown that there is a 50–60% receptor reserve for terminal release-regulating autoreceptors in the NAc and CPu (Yokoo *et al.*, 1988), which may account for the lower EC_{50} values observed in this study. A greater receptor reserve for the D_3 receptor would also contribute to the greater selectivity of 7-OH-DPAT between the two components.

The possibility that the biphasic concentration-response curve for 7-OH-DPAT may be due to factors other than a mixed D_2/D_3 autoreceptor population cannot be ruled out. One possibility is that the effect of 7-OH-DPAT may not be specific for the dopaminergic system. The affinity of 7-OH-DPAT at receptor subtypes other than dopamine receptors is generally relatively lower (K_i in nM: α_1 10 000; α_2 1 610; 5-HT $_{2A}$ > 10 000; 5-HT $_{2C}$ > 10 000; M_1 5 400; and M_2 880) (personal communication from Mark J. Millan, Institut de Recherches Servier, France). In addition, it has been shown, using FCV, that phenylephrine, clonidine, isoprenaline, 5-HT, acetylcholine, muscimol, CCK-8 and neurotensin do not significantly alter evoked dopamine release in slices of rat NAc (Bull & Sheehan, 1991). However, 7-OH-DPAT does have

high affinity for 5-HT $_{1A}$ receptors expressed in CHO cells (K_i 81 nM personal communication from Mark J. Millan) and 72.7 nM in rat hippocampal membrane homogenates (Zhuang *et al.*, 1993). The 5-HT $_{1A}$ receptor is a 5-HT somatodendritic autoreceptor in the dorsal raphe nucleus (O'Connor & Kruk, 1992). The selective 5-HT $_{1A}$ agonist 8-OH-DPAT (8-hydroxy-*N,N*-di-*n*-propyl-2-aminotetralin) does not affect stimulated endogenous dopamine release in the striatum (at concentrations up to 1 μ M), when examined using FCV (O'Connor & Kruk, unpublished observations). It is therefore unlikely that the biphasic nature of the 7-OH-DPAT curve is due to actions of 7-OH-DPAT at other non dopaminergic receptors.

At present we have no knowledge of the affinity of 7-OH-DPAT for the dopamine transporter. However, when using FCV, if a drug inhibits dopamine reuptake the observed peak dopamine detected is potentiated (by as much as 4 to 5 fold) and the rate of its decline prolonged (2 to 3 fold) (Trout & Kruk, 1992; Patel *et al.*, 1992b; Hafizi *et al.*, 1992; Davidson & Stamford, 1993). In these experiments 7-OH-DPAT did not increase the peak dopamine detected, or have any significant effect on the time for half decay.

The D_2 receptor agonist, *N,N*-dipropylamino-5,6-dihydroxytetralin (dpADTN), inhibits stimulated dopamine release in both the CPu and NAc: this inhibition is resistant to the atypical antipsychotic clozapine and is poorly antagonized by haloperidol, sulpiride and metoclopramide (Sheehan *et al.*, 1993). In addition, the inhibitory effect of dpADTN is resistant to pertussis toxin (Bull *et al.*, 1993). It was suggested by Sheehan *et al.* (1993), that dpADTN may inhibit stimulated dopamine release by acting at a novel D_2 -like receptor other than D_3 or D_4 receptors. The possibility therefore exists that the high affinity component of the 7-OH-DPAT concentration-response curve may be due to activation of this novel D_2 -like receptor.

Another possibility is that the two components reflect the two affinity states of the same receptor. In the nucleus accumbens the D_2 receptor exists primarily in the high affinity state (66%) under normal conditions (Richfield *et al.*, 1989). The possibility therefore exists that the high affinity component of the concentration-response curve to 7-OH-DPAT is due to an action mediated by the high affinity state of the receptor and the low affinity component is due to an action mediated via the low affinity state. However, the relative contributions of the high (18.0%) and low (77.5%) affinity components do not correspond to the distribution of D_2 receptors between high and low affinity states. In addition, the action of haloperidol observed in the present study is inconsistent with this possibility, as antagonists are not thought to be able to distinguish between different receptor affinity states (Richfield *et al.*, 1989).

In conclusion, the results of this study are consistent with the existence of at least two receptors, acting as autoreceptors, regulating evoked dopamine efflux in the NAc. These experiments with selective D_2/D_3 ligands suggest that the high affinity component of the concentration-response curve to 7-OH-DPAT, in the NAc, may reflect a functional D_3 autoreceptor, whereas the low affinity component reflects D_2 receptor function. Highly selective D_3 receptor antagonists are required to confirm this. The possibility that there may be an as yet unidentified dopamine autoreceptor cannot be excluded.

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