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D_2 dopamine receptors and modulation of spontaneous acetylcholine (ACh) release from rat striatal synaptosomes

[1]M.V. Clos, A. García-Sanz, N.M. Vivas & A. Badia

Department de Farmacologia i Terapéutica, Facultat de Medicina, Universitat Autònoma de Barcelona, 08193-Bellaterra, Barcelona, Spain

1 The effect of two $D_{3/2}$ dopamine receptor agonists, LY-171555 (quinpirole) and 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT) on spontaneous [^{3}H]-acetylcholine ([^{3}H]-ACh) release were investigated in rat striatal synaptosomes.

2 Quinpirole and 7-OH-DPAT inhibited in a concentration-dependent manner the basal efflux of [^{3}H]-ACh with similar E_{max} (maximal inhibitory effect) values ($29.95 \pm 2.91\%$ and $33.19 \pm 1.21\%$, respectively). Significant differences were obtained between the pEC_{50} ($-\log$ of molar concentration) of quinpirole (7.87 ± 0.12) and 7-OH-DPAT (7.21 ± 0.17; $P < 0.01$).

3 Different concentrations (0.3–10 nM) of haloperidol ($D_{2/3}$ dopamine receptor antagonist) shifted to the right the concentration-response curves elicited by quinpirole and 7-OH-DPAT, without modifications in the E_{max}.

4 Slopes of a Schild plot obtained with haloperidol in the presence of quinpirole and 7-OH-DPAT were not signficantly different from unity (0.85 ± 0.05 and 1.17 ± 0.11, respectively) and consequently haloperidol interacted with a homogeneous receptor population. The pK_B values of haloperidol obtained from Schild regression were 9.96 ± 0.15 (in presence of quinpirole) and 9.90 ± 0.09 (in presence of 7-OH-DPAT).

5 Specific binding of [^{3}H]-YM-09151-2 to membranes of striatal synaptosomes and cells expressing D_2 and D_3 dopamine receptors was inhibited by haloperidol. Analysis of competition curves revealed the existence of a single population of receptors. There were no differences between the estimated pK_i ($-\log$ of molar concentration) values for synaptosomes (8.96 ± 0.02) and cells expressing D_2 receptors (8.81 ± 0.05), but the pK_i value from cells expressing D_3 dopamine receptors differed significantly (8.48 ± 0.06; $P < 0.01$).

6 In conclusion, the data obtained in the present study indicate that quinpirole and 7-OH-DPAT, two $D_{3/2}$ dopamine receptor agonists, inhibit the spontaneous [^{3}H]-ACh efflux and this effect is competitively antagonized by haloperidol and probably mediated through dopamine D_2 receptors.

Keywords: Presynaptic dopamine receptors; D_2 dopamine receptors; D_3 dopamine receptors; spontaneous ACh release; quinpirole; 7-OH-DPAT; haloperidol; synaptosomes

Introduction

It has been established that acetylcholine (ACh) as well as muscarinic agonists cause feedback regulation of neurotransmitter release from cholinergic nerve terminals through presynaptic autoreceptors (Nordstrom & Bartfai, 1980; Marchi *et al*., 1981). This autoregulation appears to work under stimulating conditions. In addition, in basal forebrain slices the spontaneous ACh release is modulated by two muscarinic agents, atropine and pirenzepine, suggesting that a M_1 muscarinic receptor could mediate this effect (Suzuki *et al*., 1988). Similar results have been observed in striatal slices where atropine and pirenzepine regulated the ACh release at rest conditions, but not oxotremorine (Dolezal & Wecker, 1990). This modulation observed in both striatal and forebrain slices on ACh release in non-stimulating conditions has been ascribed to the presence of intrinsic impulse activity (Suzuki *et al*., 1988; Dolezal & Wecker, 1990). Although the functional role of neurotransmitter release during non-stimulating conditions is not well established, it has been proposed that in the absence of axonal firing, a continuous release of neurotransmitter could be important in maintaining the sensitivity and proper trophic function of the postsynaptic region (Adam-Vizi, 1992).

It is known that nigrostriatal dopaminergic neurones exert a direct inhibitory action on cholinergic interneurones in the striatum, probably through D_2 dopamine receptors located on cholinergic nerve terminals (Stoof & Kebabian, 1982; Stoof *et al*., 1992). Thus, different studies have demonstrated that dopamine and D_2 dopamine receptor agonists are effective in inhibiting ACh release from striatal slices under stimulating conditions (Stoof & Kebabian, 1982; Drukarch *et al*., 1990; Dolezal *et al*., 1992). However, by use of a microdialysis method, the role of D_3 dopamine receptors in the modulation of ACh release in the striatum has recently been proposed (Sato *et al*., 1994; Ueda *et al*., 1995). While the regulation of basal ACh release from striatal slices through autoreceptors is still being investigated, to our knowledge so far, there is little data available about the role of heteroreceptors on the modulation of ACh release under resting conditions.

The aim of the present study was to determine the presynaptic regulation by two $D_{3/2}$ dopamine receptor agonists, 7-OH-DPAT and quinpirole, on spontaneous [^{3}H]-ACh efflux by use of a synaptosomal superfusion method. This methodology is the best approach in the study of presynaptic modulation of neurotransmitter release avoiding the interaction with other neurotransmitters present in the synaptosomal preparation (Raiteri *et al*., 1974). Binding assays were also carried out to analyse the dopamine receptor subtype involved in this effect.

Methods

Preparation of synaptosomes

Male Sprague-Dawley rats (300–350 g) were housed 3 per cage and maintained on a 12 h light/dark cycle with food and

[1] Author for correspondence.

water freely available. The animals were killed by decapitation and the brains were quickly removed and kept on ice during dissection. Crude striatal synaptosomes were prepared essentially as previously described (Clos *et al*., 1994). The synaptosomal pellet was resuspended in a physiological medium with the following composition (in mM): NaCl 125, KCl 3, $MgSO_4$ 1.2, $CaCl_2$ 1.2, $NaHCO_3$ 22, NaH_2PO_4 1 and glucose 10; pH 7.2 to 7.4; and continuously aerated with 95% O_2 and 5% CO_2 at 37°C.

Neurotransmitter release

The release of [^{3}H]-ACh from synaptosomes was measured in a continuous superfusion system as described by Raiteri *et al.* (1974). Briefly, after incubation of synaptosomes (15 min, 37°C) in presence of [^{3}H]-choline (0.03 μM, 69 Ci mmol^{-1}), aliquots of the synaptosomal suspension (0.24 mg protein/chamber) were distributed on 0.65 μm Millipore filters and placed at the bottom of a set of parallel superfusion chambers maintained at 37°C. Superfusion was started with standard medium aerated with 95% O_2 and 5% CO_2 at a rate of 0.62 ml min^{-1} ($t=0$). Synaptosomes were superfused for 20 min to obtain a constant outflow of tritium. When the effects of quinpirole and 7-OH-DPAT were evaluated, four two-min fractions were collected starting at 37.5 min, after the beginning of superfusion. Quinpirole or 7-OH-DPAT were added at the end of the first fraction collected. In experiments evaluating the effects of haloperidol on inhibition by quinpirole and 7-OH-DPAT of tritium release, the antagonist was added 10 min before agonists ($t=29.5$ min).

The amount of radioactivity released in each fraction was expressed as a percentage of total tritium present in the synaptosomes at the onset of the fraction collected. Tritium release on each fraction was expressed as a ratio over the first fraction collected (F_x/F_1). Preliminary studies indicated that the maximal effect of drugs on tritium release was reached in the fourth fraction (data not shown). Thus, the effect of drugs on tritium release was evaluated by calculating the ratio of the percentage of tritium release in fraction 4 (F_4) and the tritium released in the first fraction collected (F_1, in which no drugs were present). This F_4/F_1 ratio was compared with the corresponding F_4/F_1 ratio obtained in control conditions. Results are expressed as a percentage of the control group. Estimates of pEC_{50} ($-$log of molar concentration of the drug producing 50% of its maximal effect) and E_{max} (maximal effect) were calculated for quinpirole and 7-OH-DPAT from concentration-response experiments by non-linear regression analysis by use of the SigmaPlot computer programme (Jandel, San Rafael, CA). The antagonism of haloperidol was evaluated by means of Schild regression.

Preparation of purified synaptosomes

Purified synaptosomes from striatum were obtained by the method described by Pastor *et al.* (1995). Briefly, the striatum was rapidly dissected on ice and homogenized with a Teflon-glass Potter homogenizer in 40 volumes of 0.32 M sucrose buffered at pH 7.4 with 0.01 M sodium phosphate (PB). Subsequent steps were carried out at 4°C. Homogenates were centrifuged for 10 min at 1000 *g*. The volume of the supernatant layer (S_1) was measured, layered onto PB 1.2 M sucrose and centrifuged for 15 min at 105,000 *g* in a Sorvall ultracentrifuge. The interface material was removed, diluted to the S_1 volume with PB 0.32 M sucrose, layered onto PB 0.8 M sucrose and centrifuged under the same conditions. The pellet obtained was strongly resuspended in 30 ml cold buffer (50 mM Tris-HCl, 1 mM EDTA, pH 7.4) to obtain a preparation enriched in synaptosomal membranes. This suspension was centrifuged at 50,000 *g* for 10 min. Pellets were washed twice by resuspension and centrifugation under the aforementioned conditions. Final pellets were resuspended in the same buffer at 1–2 mg protein ml^{-1} and frozen at -70°C until use. Protein content was determined, before freezing, with the Bio-Rad dye reagent and bovine γ-globulin as standard (Bradford, 1976).

Tissue culture and cell membrane preparation

Chinese hamster ovary (CHO) cells expressing the human D_2 and D_3 receptors were grown and maintained in DMEM with 10% of foetal bovine serum, G418 (500 μg ml^{-1}) in 37°C incubators with an atmosphere of 5% CO_2 until membrane preparation. The medium was removed and cells were washed once with 10 ml of PBS (in mM: NaCl 145, KCl 5, $CaCl_2$ 0.7, $MgCl_2$ 0.5 and phosphate 10, pH 7.4; 4°C), which were then also removed. Five ml of a solution containing 10 mM Tris and 5 mM EDTA (4°C) were added to each culture dish, to detach the cells. Afterwards, the culture dishes were scraped with a spatula. The solution containing the cells was centrifuged at 48,000 *g* for 20 min at 4°C. The pellet was homogenized with a polytron (setting 1, 5 s) in 10 ml of 10 mM Tris-HCl (pH 7.4). Aliquots of 1 ml were stored at -70°C, for subsequent binding assays.

[^{3}H]-YM-09151-2 binding assays

Membrane suspensions were thawed quickly and kept on ice before use. The membranes were resuspended in incubation buffer to obtain approximately the following concentrations: 12.5 μg protein ml^{-1} for synaptosomal membranes, 13 μg protein ml^{-1} for membranes containing D_2 receptors and 7 μg protein ml^{-1} for those containing D_3 receptors. The composition of incubation buffer was the following (in mM): Tris 50, EDTA 5, $MgCl_2$ 5, KCl 5, $CaCl_2$ 1.5 and NaCl 120; pH 7.4.

For saturation experiments, aliquots of membrane suspensions were incubated in triplicate with increasing concentrations (range 50 pM–2 nM) of [^{3}H]-YM-09151-2 in 2 ml (final volume) of incubation buffer and maintained at 25 ± 0.5°C for 60 min. The potency of haloperidol in competing with [^{3}H]-YM-09151-2 for the specific binding was determined by incubation of a single concentration of [^{3}H]-YM-09151-2 (0.05 nM for synaptosomal and D_2 receptors and 0.1 nM for D_3 receptors) in the absence or presence of 15–17 concentrations of the competing drug (range: 0.01 nM–0.1 μM). Incubations were stopped by rapid vacuum filtration (Brandel M24R Cell Harvester) over glass fibre filters (Schleicher & Schuell, No. 30) presoaked in 0.1% polyethylenimine for at least 30 min before filtration. The filters were rapidly rinsed three times with 5 ml of ice-cold Tris-HCl buffer. After the filters had been dried at 70°C for 2 h, the radioactivity retained was measured by liquid scintillation spectrometry in a LKB (1209 Rackbeta) counter with a counting efficiency of 60%. Non-specific binding was determined in the presence of 0.1 μM haloperidol and usually amounted to 5–10% of the total [^{3}H]-YM-09151-2 binding at a concentration close to its K_D value.

Ligand binding data were analysed with the RADLIG programme package (Biosoft, Cambridge, U.K.). The dissociation constant (K_D) and the total number of binding sites (B_{max}) were estimated from saturation experiments. The K_D values of [^{3}H]-YM-09151-2 measured for dopamine receptors were subsequently used for analysis of the competition data. The affinity constant of haloperidol (K_i) was estimated from the results of displacement experiments. Data from competition curves were analysed with both one-site and two-site models. The appropriate model was achieved by an approximated F test as previously described (Munson & Rodbard, 1980). K_i values (M) were expressed as negative logarithms (pK_i).

Statistical analysis

Results represent mean $\pm$ s.e.mean. Statistical differences between groups were analysed with Student's t test (unpaired); $P<0.05$ was considered to be signficant.

Drugs

The following drugs were used: [^{3}H]-choline (69 Ci mmol^{-1}) [^{3}H]-YM-09151-2 ((±)-cis-N-(1-benzyl-2-methylpyrrolidin-3-yl)-5-chloro-2-methoxy-4-methylamino benzamide, 76 Ci mmol^{-1}), obtained from Amersham International (Buckinghamshire, U.K.), LY-171555 (quinpirole), 7-hydroxy-N,N-di-n-propyl-2-aminotetraline (7-OH-DPAT), obtained from RBI (Natick, MA, U.S.A.) and dissolved in standard medium. Haloperidol was dissolved in a standard medium containing methanol ($\leqslant$0.01%) and was obtained from Janssen Pharmaceutica N.V., (Beerse, Belgium). Dulbecco's modified Eagle's medium (DMEM) and foetal calf serum were from Biological Industries (Kibbutz Beit Haemek, Israel) and G418 was obtained from Gibco (Grand Island, NY). CHO cells expressing human D_2 dopamine receptors were provided by Dr W.J. Donelan from Duke University Medical Center (Durham, NC, U.S.A.). CHO cells expressing human D_3 dopamine receptors were provided by Dr J.C. Schwartz from INSERM (Paris, France).

Results

The release of tritium is recognized to be largely newly synthesized [^{3}H]-ACh following striatal incubation of [^{3}H]-choline and has been proposed as a valid representation of [^{3}H]-ACh release and cholinergic cellular activity (Molenaar *et al*., 1993). The percentage of tritium released from rat striatal synaptosomes was 2.84±0.08% (per 2 min) of the total remaining radioactivity in the first fraction collected (F_1). In the absence of added drugs, the ratio of % tritium of the fourth over the first fraction (F_4/F_1) was 0.95±0.01.

Effect of dopamine receptor agonists on tritium efflux

The effects of $D_{3/2}$ dopamine receptor agonists, quinpirole (1 nM–3 μM) and 7-OH-DPAT (1 nM–10 μM) on spontaneous tritium efflux are shown in Figures 1 and 2. A significant concentration-dependent decrease in tritium efflux was observed. The E_{max} values calculated by non-linear regression of concentration-response curves were similar for both agonists, 29.95±2.91% and 33.19±1.21%, respectively. The estimated pEC_{50}s 7.87±0.12 (quinpirole) and 7.21±0.12 (7-OH-DPAT) were significantly different ($P<0.05$).

The inhibitory effect of agonists on tritium efflux was studied in the presence of different concentrations (0.3–10 nM) of haloperidol, a $D_{2/3}$ dopamine receptor antagonist. Previous experiments showed that haloperidol alone did not modify the tritium efflux at the concentrations tested (results not shown). The addition of an antagonist in the superfusion medium shifted the concentration-response curves elicited by either quinpirole or 7-OH-DPAT to the right, without modifications in the E_{max} (Figures 1 and 2). This displacement indicates that haloperidol may act as a competitive antagonist of the inhibitory effect of dopamine receptor agonists on tritium efflux. Schild regression was used to analyse the antagonism of haloperidol against both agonists. The slope value of the Schild plot was not significantly different from unity (0.85±0.05 and 1.17±0.11 in the presence of quinpirole and 7-OH-DPAT, respectively). The pK_B values were calculated from Schild regression assuming a slope of unity. In the presence of quinpirole and 7-OH-DPAT, the pK_B values of haloperidol were 9.96±0.15 and 9.90±0.09, respectively.

Binding assays

Saturation experiments with the dopamine receptor antagonist, [^{3}H]-YM-09151-2, showed that the specific binding of [^{3}H]-YM-09151-2 was a saturable process with high affinity. Moreover, non-linear regression analysis of the saturation isotherms was consistent with the presence of an homogeneous population of receptors, in all preparations. A Hill coefficient near unity was obtained indicating a lack of cooperativity and confirming the fact that [^{3}H]-YM-09151-2 binds to a single population of receptors. The K_D and B_{max} values obtained are shown in Table 1.

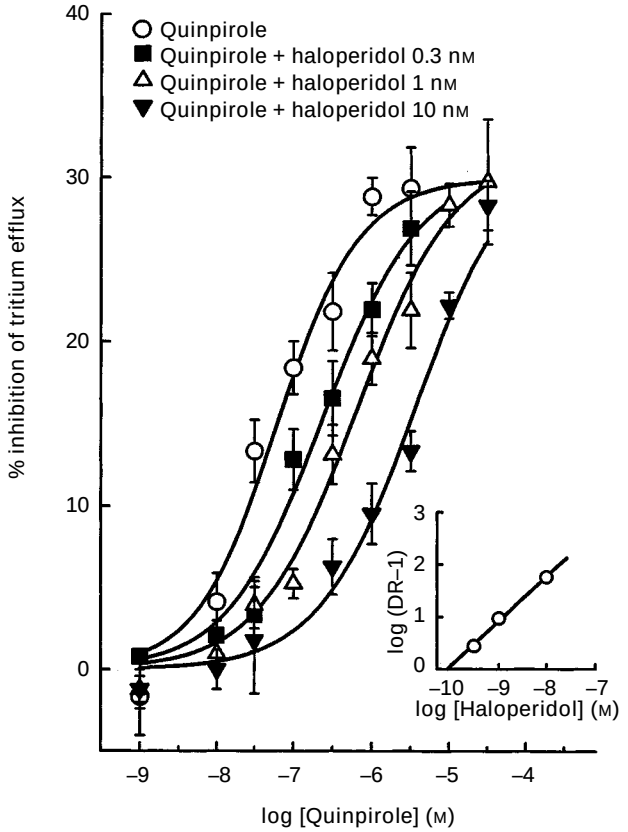

Figure 1 Concentration-response curves for the inhibitory effect of quinpirole on the spontaneous release of [^{3}H]-ACh from rat striatal synaptosomes and its antagonism by haloperidol. Inset: Schild plot for haloperidol (DR indicates the dose-ratio). Data represent the mean of at least 4 experiments run in triplicate; vertical lines show s.e.mean.

Specific binding of [^{3}H]-YM-09151-2 to membranes of striatal synaptosomes and cells expressing D_2 and D_3 dopamine receptors was inhibited by haloperidol. Displacement curves were monophasic with pseudo-Hill coefficients close to unity (Table 2). Iterative non-linear curve-fitting analysis (Munson & Rodbard, 1980) of competition curves revealed the existence of a single population of receptors. There were no differences between the estimated pK_i values (Table 2) for synaptosomal membranes and cells expressing D_2 receptors. However, the respective pK_i differed from the affinity constant obtained in membranes from cells expressing D_3 receptors ($P<0.01$).

Discussion

The results obtained in the present study showed that, in rat striatal synaptosomes, quinpirole and 7-OH-DPAT ($D_{3/2}$ dopamine receptor agonists) inhibited spontaneous [^{3}H]-ACh release in a concentration-dependent manner. This modulatory effect seems to be mediated by D_2 dopamine receptors as inferred from the Schild regression analysis and binding assay results. These data correlate with those obtained in rabbit striatal slices (Dolezal *et al*., 1992), where 1 μM quinpirole decreased [^{3}H]-ACh release under resting conditions. When the concentration-response curves of agonists were analysed, signficant differences between pEC_{50} of quinpirole and 7-OH-DPAT were observed. These results could be ascribed to differences in their affinities for $D_{3/2}$ dopamine receptors (Sokoloff *et al*., 1990; 1992; Levesque *et al*., 1992).

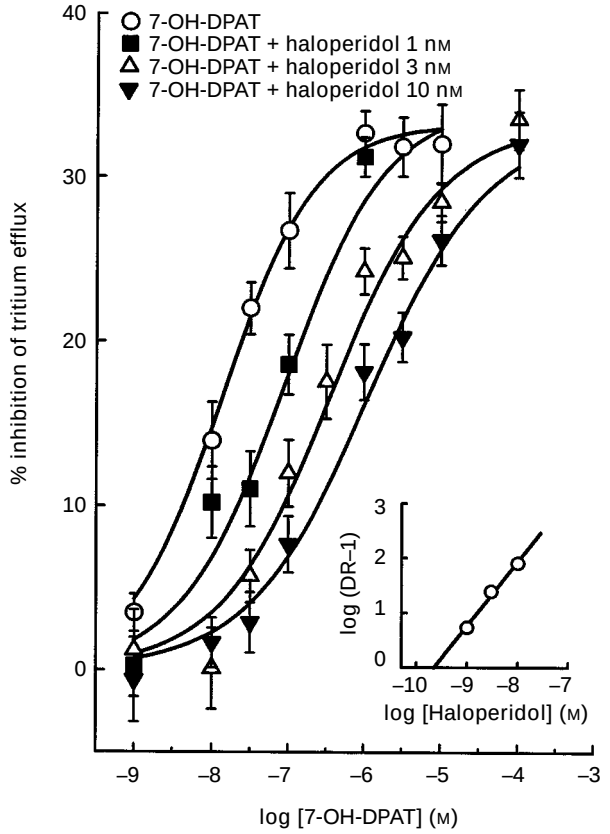

Figure 2 Concentration-response curves for the inhibitory effect of 7-OH-DPAT on the spontaneous release of [^{3}H]-ACh from rat striatal synaptosomes and its antagonism by haloperidol. Inset: Schild plot for haloperidol (DR indicates the dose-ratio). Data represent the mean of at least 4 experiments run in triplicate; vertical lines show s.e.mean.

Table 1 Characterization of [^{3}H]-YM-09151-2 binding to membranes obtained from rat purified striatal synaptosomes and CHO cells transfected with the D_2 or D_3 human receptors

	K_D (pM)	B_{max} (pmol mg^{-1} protein)	n
Synaptosomes	39.15±9.04	0.41±0.03	4
CHO-D_2	65.66±7.37	0.38±0.03	4
CHO-D_3	147.0±2.06*	0.45±0.71	6

The values shown are the mean±s.e.mean of n experiments run in triplicate. *$P<0.05$ vs synaptosomes (Student's t test).

Table 2 Inhibition of [^{3}H]-YM-09151-2 binding to dopamine receptors by haloperidol, in mebranes prepared from purified rat striatal synaptosomes and CHO cells transfected with D_2 or D_3 human receptors

	pK_i	n_H	n
Synaptosomes	8.83±0.02**	0.81±0.09	6
CHO-D_2	8.97±0.05***	0.96±0.05	4
CHO-D_3	8.49±0.06	0.88±0.01	4

Data are expressed as mean±s.e.mean of n experiments performed in triplicate. **$P<0.01$, ***$P<0.0001$ vs CHO-D_3 (Student's t test).

Dopamine receptors were originally classified into two main groups, the D_1-like (D_1, D_5) and the D_2-like (D_2, D_3, D_4) dopamine receptors (Sibley & Monsma, 1992). It has been suggested that the inhibitory effect of dopaminergic neurones on evoked [^{3}H]-ACh release is mediated by D_2 dopamine receptors (Stoof & Kebabian, 1982; Drukarch *et al*., 1990; Login *et al*., 1995). Quinpirole and 7-OH-DPAT are $D_{3/2}$ dopamine receptor agonists showing approximately 100 times higher affinity for D_3 dopamine receptors than for D_2 dopamine receptors (Sokoloff *et al*., 1990; 1992; Levesque *et al*., 1992). *In vivo* experiments have demonstrated that 7-OH-DPAT and quinpirole decreased cocaine self-administration (Caine & Koob, 1993) and induced hypothermia in rats (Millan *et al*., 1994). The fact that these effects were observed at concentrations near the affinities of both agonists on D_3 dopamine receptors, could indicate that quinpirole and 7-OH-DPAT exert their effects through these receptors (Caine & Koob, 1993; Millan *et al*., 1994). On the other hand, systemic administration of quinpirole was able to inhibit the release of dopamine and ACh in the striatum, suggesting that the agonist did not discriminate between dopamine receptors regulating dopamine and ACh release in this cerebral region (Robertson *et al*., 1993). Moreover, in a microdialysis study carried out in conscious rats sham-operated and lesioned with 6-hydroxydopamine, 7-OH-DPAT and quinpirole modulated the basal ACh release indicating the possible involvement of D_3 dopamine receptors (Sato *et al*., 1994; Ueda *et al*., 1995). In our experimental conditions the effect of both agonists on spontaneous [^{3}H]-ACh release was antagonized by low concentrations of haloperidol, a $D_{2/3}$ dopamine receptor antagonist. In the present study, the slopes of Schild plots obtained with quinpirole and 7-OH-DPAT in the presence of haloperidol were not significantly different from unity which infers that haloperidol interacted with an homogeneous receptor population. Different binding studies have demonstrated that haloperidol shows a high affinity for D_2 dopamine receptors with a K_i 0.45 nM (Sokoloff *et al*., 1990; Levesque *et al*., 1992). The fact that in our experimental conditions the dissociation constant of haloperidol (calculated as pK_B) was lower than 1 nM could indicate that the effect of the antagonist on tritium release inhibition induced by quinpirole and 7-OH-DPAT was probably mediated by D_2 dopamine receptors. These data are in accordance with the lack of D_3 dopamine receptors in the dorsal striatum, where dopamine modulates motor functions (Caine & Koob, 1993). Furthermore, behavioural and biochemical studies regarding the effect of 7-OH-DPAT on D_3 dopamine receptors have concluded that, at pre- and postsynaptic levels, the profile of this agonist corresponds to a D_2 dopamine receptor agonist (Ahlenius & Salmi, 1994).

Radioligand binding data obtained from CHO cells expressing D_2 and D_3 receptors have demonstrated that haloperidol is a dopamine $D_{2/3}$ receptor antagonist with a $K_i \geqslant 1$ nM on D_2 dopamine receptors, and this affinity is 3–10 times higher than that obtained at D_3 dopamine receptors (Sokoloff *et al*., 1990; Levesque *et al*., 1992; Malmberg *et al*., 1993). When membranes from CHO-D_2 and CHO-D_3 cells were used, the haloperidol pK_i value obtained in CHO cells expressing D_2 receptors was significantly higher (8.81±0.05) than in CHO cells expressing D_3 receptors (8.48±0.06; $P<0.001$). These data are similar to those obtained by other authors in the same membrane preparations (Sokoloff *et al*., 1990; Levesque *et al*., 1992; Malmberg *et al*., 1993). Likewise, it has been observed that haloperidol shows a K_i value corresponding to the dopamine D_2 receptor in rat striatal membranes (Assié *et al*., 1993). Purified synaptosomal membranes from striatum, a preparation which is enriched in presynaptic membranes, were used in binding assays to study the interaction of haloperidol with dopamine receptors. In these conditions, iterative non-linear curve-fitting analysis of saturation curves was consistent with the presence of a single population of receptors. Analysis of competition experiments with haloperidol yielded monophasic curves with a pseudo-Hill coefficient (n_H) close to unit and a K_i value similar to

that obtained in CHO-D_2 membranes but which was significantly different from the CHO-D_3 K_i value.

In conclusion, the results obtained in striatal synaptosomes showed that the dopamine $D_{3/2}$ receptors agonists, quinpirole and 7-OH-DPAT, inhibit spontaneous [^{3}H]-ACh release in a concentration-dependent manner. Haloperidol, a $D_{2/3}$ antagonist, competitively blocked the inhibitory effect of agonists and thus both functional and binding approaches seem to indicate that haloperidol exerts its effects through dopamine D_2-receptors. Consequently, the modulatory effects of quinpirole and 7-OH-DPAT on spontaneous [^{3}H]-ACh release from rat striatal synaptosomes are probably mediated by these dopamine receptor subtypes. Moreover, data obtained in our laboratory seem to indicate that spontaneous ACh release from rat striatal synaptosomes is partially Ca^{2+}-dependent (unpublished results). Further experiments are now required to characterize the spontaneous ACh release better, to study which component of this release is modulated by D_2 dopamine receptors and the influence of this modulation on evoked ACh release in rat striatal synaptosomes.

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