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Characteristics of stably expressed human dopamine D_{1a} and D_{1b} receptors: Atypical behavior of the dopamine D_{1b} receptor

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Abstract

Human dopamine D_{1a} and D_{1b} receptors were stably expressed in Baby Hamster Kidney (BHK) or Chinese Hamster Ovary (CHO) cells. [^{3}H]SCH23390 saturation experiments indicated the presence of only a single binding site in the D_{1a} expressing cell line with a K_d of 0.5 nM. In D_{1b} expressing cell lines, two binding sites were observed with K_d values of 0.5 and 5 nM in CHO cells and 0.05 and 1.6 nM in BHK cells, respectively. Neither of the receptors affected Ca^{2+} metabolism whereas they both were coupled in a stimulatory fashion to adenylyl cyclase. The pharmacological profile of both the D_{1a} and D_{1b} receptors as assessed from inhibition of specific [^{3}H]SCH 23390 binding was classical D_1-like. Thus, benzazepine derivatives as well as the atypical neuroleptics, clozapine and fluperlapine, exhibited high affinity whereas D_2 selective compounds like sulpiride and spiperone had low affinity for these receptors. Besides SCH 23390, only NNC 112, fluphenazine and bulbocapnine were able to discriminate between the two states of the D_{1b} receptor. In case of the D_{1a} receptor, the K_i values obtained in binding experiments were very similar to K_i values obtained from inhibition of dopamine stimulated adenylyl cyclase. In the D_{1b} expressing cell line, the K_i values obtained from inhibition of the dopamine stimulated adenylyl cyclase indicated a significantly better correlation with the state of the D_{1b} receptor showing high affinity for antagonists. In agreement with observations from binding experiments, dopamine was around 20 fold more potent in stimulating adenylyl cyclase via the D_{1b} receptor as compared to the D_{1a} receptor. Further,adenylyl cyclase in the D_{1a} expressing cell line was stimulated by the benzazepine agonists SKF 75670 and SKF 38393 with potencies and efficacies very similar to these previously observed in rat striatum. On the other hand, in the D_{1b} expressing cell line these benzazepine agonists exhibited potencies and efficacies different from previously obtained data from rat brain. In summary, both in their pharmacology and functional coupling, the D_{1a} and the D_{1b} receptors appear to exhibit a classical D_1-like profile with the markedly high affinity for the atypical neuroleptic clozapine and a stimulatory coupling to adenylyl cyclase. The somewhat better correlation between D_{1a} data than D_{1b} data and rat striatal D_1 receptor data supports findings from in situ hybridization studies on a relatively higher abundance of the D_{1a} receptor in this brain region. Thus, the D_{1a} and the D_{1b} receptors are classical D_1-like. However, the lack of coupling to phospholipase C of these two receptors may point to still another D_1-like receptor to be identified.

Key words: Dopamine D_{1a} receptor; Dopamine D_{1b} receptor; cDNA; Clonal cell line; Baby Hamster Kidney cells; Chinese Hamster Ovary cell; Clozapine; Ca^{2+}

1. Introduction

Classically dopamine receptors have been divided into D_1 and D_2 subtypes (Kebabian and Calne, 1979). However, during the 1980s biochemical, behavioral and electrophysiological experiments suggested the two subtype schemes to be too simple. Consequently, a number of additional subtypes was postulated (Andersen et al., 1990). Shortly thereafter, as a result of the application of molecular biology, most of the suggested subtypes were cloned. At present, five different dopamine receptor genes are known. These genes give rise to five main products and a number of splice

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variants (Sibley and Monsma, 1992). The identified dopamine receptors can still be grouped into a D_1 and D_2 family on basis of their pharmacology. At present, the D_1 family contains the D_1 or D_{1a} receptor (Dearry et al., 1990; Sunahara et al., 1990a; Zhou et al., 1990) and the recently cloned D_{1b}, $D_{1\beta}$ or D_5 receptor (Grandy et al., 1991; Sunahara et al., 1990b; Tiberi et al., 1991; Weinshank et al., 1991). The D_2 receptor family comprises the classical D_2 receptor (Bunzow et al., 1988), the D_3 receptor (Sokoloff et al., 1990), the D_4 receptor (Van Tol et al., 1991) and a number of splice variants of these genes (Sibley and Monsma, 1992).

The D_{1a} and D_{1b} receptors are very homologous at the amino acid level, as the overall identity between the two receptors is 62%. However, the homology increases to 86% if only the putative transmembrane regions are taken into consideration and further, to 97% if conservative substitutions are included. Only limited characterization of the two receptors has been reported to date – the major differences being the higher affinity of dopamine for the D_{1b} receptor as compared to the D_{1a} receptor (Grandy et al., 1991; Sunahara et al., 1990b; Tiberi et al., 1991; Weinshank et al., 1991).

The aim of the present study was to characterize in detail the pharmacological and functional profile of cell lines stably expressing the human D_{1a} or D_{1b} receptors.

2. Materials and methods

2.1. Materials

The cDNAs encoding the human dopamine D_{1a} and D_{1b} receptors was obtained from Drs. M.G. Caron, Duke University, NC, USA and B.F. O'Dowd, University of Toronto, Ontario, Canada; respectively. Chinese Hamster Ovary (CHO, CRL 1793) and Baby Hamster Kidney (BHK, CRL 8544) cells were obtained from the American Tissue Culture Company. All other chemicals used were obtained from commercial suppliers.

2.2. Drugs

The following drugs were dissolved in water whenever possible, otherwise in methanol: Bulbocapnine (+)- and (−)-Butaclamol (RBI, MA, USA); Chlorpromazine hydrochloride (Sigma Chemicals, St. Louis, MO, USA); Clozapine (Sandoz Pharma, Basel, Switzerland); Dopamine (Sigma); *cis*- and *trans*-Flupentixol (H. Lundbeck, Copenhagen, Denmark), Fluphenazine (RBI); Fluperlapine (Sandoz); Haloperidol, Spiperone and Ketanserin (Jansen Pharma, Copenhagen, Denmark); NNC 112, NNC 687 and NNC 756 (Novo Nordisk, Bagsvaerd, Denmark); SCH 23390 (Schering-Plough, Bloomfield, NJ, USA); SKF 38393 and SKF 75670 (RBI) and (±)-sulpiride (Sigma).

2.3. Generation of cell lines

Cells were grown in Dubelco's modified Eagles medium (DMEM) supplemented with 10% heat inactivated fetal calf serum, 1% non-essential amino acids, 1% glutamax and 1% penicillin/streptomycin (pen/strep) (hereafter termed: standard medium) in 150 cm^2 dishes. Confluent cells were split to achieve a 20–30% confluency on the day of transfection. Before transfection, cells were washed twice in phosphate-buffered salt solution (PBS). Twenty ml/150 cm^2 dish of DMEM supplemented with 1% pen/strep were added. A 25 : 1 (mol/mol) mixture of pCMV5 containing the D_{1a} cDNA (Dearry et al., 1990) or pCd containing the D_{1b} cDNA (Sunahara et al., 1990b) and a plasmid bearing the resistance marker for either neomycin or methotrexate was then transfected into cells using lipofectin® (BRL) according to the manufacture's protocol. Twenty hours after transfection, the medium was changed to the standard medium supplemented further with either G 418 (0.1–5 mg/ml) or methotrexate (0.2–20 μM). Resistent colonies were subcloned using conventional cloning cylinders. Subclones showing specific [^{3}H]SCH 23390 binding were subjected to several rounds of subcloning and increasing levels of G 418 or methotrexate. The resulting monoclonal cell lines used in the detailed pharmacological and functional characterization in this paper were termed D_{1a} BHK-subclone 3B and D_{1b} CHO-subclone 3D, respectively.

2.4. Membrane preparation

Confluent cells were scraped off and centrifuged at $10.000 \times g$ for 10 min at 4°C. Cell membranes used for binding or adenylyl cyclase assays were prepared as described previously for rat striatum (Andersen et al., 1985). In brief, pelleted cells were homogenized using a glass-teflon homogenizer in 1 ml/150 cm^2 dish of 10 mM imidazole-HCl (pH 7.4) containing 2 mM EDTA and centrifuged at $25.000 \times g$ for 20 min at 4°C. The pellet was resuspended in the same volume of the above buffer and the step was repeated for a total of three times. Final pellet was resuspended in 2 mM imidazole-HCl (pH 7.4) containing 2 mM EGTA.

2.5. [^{3}H]SCH 23390 binding assay

Binding assays were performed as described in Andersen et al. (1985). In brief, 100 μl of the above membrane preparation were mixed with 600 μl of 16.67 mM imidazole-HCl (pH 7.4) containing 16.67 mM theophylline, 1 mM EGTA and 10 mM $MgSO_4$;

100 μl [^{3}H]SCH 23390; 200 μl of water/test compound/1 μM *cis*-flupentixol (total binding/compound testing/non-specific binding) and incubated at 30°C for 60 min. The reaction was terminated by rapid filtration through Whatman GF/B filters under vacuum. The filters were washed immediately with 2 × 10 ml 0.9% saline and the radioactivity trapped in the filters was determined in a scintillation counter. For details concerning testing of antagonists and agonists see Andersen and Braestrup (1986) and Andersen and Jansen (1990).

2.6. Adenylyl cyclase assay

Dopamine sensitive adenylyl cyclase activity was assayed in membranes essentially as described previously (Andersen et al., 1985). A mixture of 50 μl membrane preparation (see section 2.3), 300 μl of 16.67 mM imidazole-HCl (pH 7.4) containing 16.67 mM theophylline, 1 mM EGTA and 10 mM $MgSO_4$ was combined with 50 μl GTP (final concentration 15 μM) and solutions of agonists and antagonists as indicated and preincubated on ice for 15 min. ATP (final concentration 1.5 mM) was added and the samples were incubated for 15 min at 30°C. The reaction was terminated by boiling for 3 min followed by a 20 min centrifugation at 2.800 × *g*. CyclicAMP was measured in the clear supernatant according to the method of Geisler et al. (1977). For further details on the testing of agonists or antagonists, see Andersen and Braestrup (1986) and Andersen and Jansen (1990).

2.7. Whole cell cAMP assay

Cells were grown in 24 well plates until 60–90% confluency was obtained. The cells were washed twice with 1 ml/well of PBS. One ml DMEM supplemented with 100 μM isobutylmethylxanthine was added to each well. Following 15 min equilibration period at 37°C, agonists were added and the cells were incubated at 37°C for 10 min. The reaction was terminated by addition of 25 μl of 2 M NaOH. Following a 15 min incubation at room temperature, the mixture was neutralized by addition of 25 μl of 2 M HCl. The whole tray was centrifuged for 20 min at 2800 × *g*. CyclicAMP was measured in the supernatant using a commercial cAMP RIA kit (Amersham).

2.8. Imaging of cytosolic free calcium concentration

Cells were plated on glass coverslips and grown to 20–40% confluency. The cells were loaded with the acetoxymethyl ester of Fura-2 (1 μM, 15 min, 25°C). After loading, the cells were washed twice with physiological salt solution (PSS: 140 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM Hepes; pH 7.4) and transferred to the experimental chamber mounted on an inverted multimode fluorescence microscope (Biological Detection Systems). The cells were continuously perfused at room temperature with PSS containing test compound and viewed with a 40 × oil immersion objective (NA 1.3, Zeiss Acrostigmat). The cells were sequentially excited with 340 nm and 380 nm UV light and the emitted Fura-2 fluorescence (505 nm) was collected with an intensified CCD camera (Hamamatsu). Video signals were digitized at 512 × 474 pixels by a PixelStore frame grabber (Perceptics) in a Macintosh Quadra computer. Calculation of cytosolic free calcium concentration was performed as described previously (Grynkiewicz et al., 1985) taking into account the ration between the 340 and 380 nm images.

2.9. Data analysis

Determination of EC_{50} values from agonist dose-response curves for stimulation of adenylyl cyclase was analyzed using FigP (Elsevier Biosoft). Binding data were analyzed using EBDA-scarfit (Elsevier Biosoft).

3. Results

3.1. Saturation experiments

Saturation experiments with increasing concentrations of [^{3}H]SCH 23390 performed on membranes from the D_{1a} BHK-subclone 3B clearly indicated saturable binding with the presence of only a single binding site (Fig. 1a). The K_d was calculated to be 0.5 ± 0.1 nM and the B_{max} to be 4.8 ± 2.6 pmol/mg protein (means ± S.D.; $n = 8$). The wild type BHK cell line showed no specific [^{3}H]SCH 23390 binding (data not shown). Similar experiments performed on the D_{1b} expressing CHO-subclone 3D surprisingly revealed the presence of more than a single binding site for the radioligand as indicated by the curvilinear Scatchard plot (Fig. 1b and Table 1). To clarify whether this was dependent upon the level of receptor expression, the methotrexate concentration in the media was increased from 2 μM to 20 μM. This treatment resulted after 2–3 passages in a 3–4 fold increase in the D_{1b} receptor expression level. Nevertheless, both the ratio between and the affinities for the high (K_1) and low (K_2) affinity site was unchanged (Table 1). Further, addition of GTP (300 μM) had no effect on the affinity of [^{3}H]SCH 23390 for the two sites or the ratio between them (not shown). The presence of endogenous D_1-like receptors in this cell line was excluded since no specific [^{3}H]SCH 23390 binding was detected in wild type CHO cells (not shown). Thus, most likely, the two sites identified by [^{3}H]SCH 23390 reflect different states of the D_{1b} receptor. To further investigate the putative two state

Table 1
Scatchard analysis of cell lines stably expressing the dopamine D_{1b} receptor. Saturation experiments were done using 8–15 different concentration of [^{3}H]SCH 23390. Data are shown as means ± S.D. The number of independent experiments is shown in brackets

Cell line	K_1 (nM)	K_2 (nM)	B_{max_1} (fmol/mg protein)	B_{max_2} (fmol/mg protein)
CHO [b] ($n=2$)	0.51 ±0.12	5.2±1.4	110±28	500± 35
CHO [c] ($n=3$)	0.54 ±0.05	4.9±1.0	405±31	1670± 76
BHK-4 ($n=2$)	0.075±0.031 [a]	1.7±0.3	320±55	6740±391
BHK-7 ($n=2$)	0.030±0.012 [a]	1.6±0.3	70±18	812± 97

[a] High affinity component analyzed using [^{125}I]SCH 23982. The CHO cell line is the subclone-3D in [b] 1 μM methotrexate or [c] 20 μM methotrexate.

phenomenon, BHK cells was transfected with the D_{1b} cDNA. A number of D_{1b} BHK-subclones were selected and tested in details. However, also in the D_{1b} BHK-subclones, Scatchard analysis of saturation experiments exhibited a curvilinear nature. The high affinity component showed an even higher affinity for [^{3}H]SCH 23390 in these BHK cell lines (see Table 1).

3.2. Dopamine inhibition of [^{3}H]SCH 23390 binding

Inhibition of specific [^{3}H]SCH 23390 binding by dopamine occurred in the D_{1a} BHK-subclone 3B with Hill slopes below unity. However, by adding 15 μM GTP, the Hill slope changed to unity and the obtained K_i was similar to the K_L observed in the absence of GTP (Fig. 2a and Table 2). The dopamine inhibition of specific [^{3}H]SCH 23390 binding from the D_{1b} CHO-subclone 3D was investigated using both high and low concentrations of radioligand. Thus, at a concentration of 0.2 nM [^{3}H]SCH 23390, only the high affinity component was evaluated whereas both the high and the low affinity components were evaluated at a radioligand concentration of 10 nM. In both cases, inhibition of specific binding by dopamine occurred with Hill slopes below unity in the absence of GTP. Upon addition of GTP, these shallow inhibition curves was shifted towards Hill slopes at unity (Fig. 2b and c). The affinity of dopamine for the two states of the D_{1b} receptor was not significantly different (Table 2).

3.3. D_{1a} and D_{1b} receptor associated second messenger systems

Dopamine dose-dependently increased the cAMP level in both whole cells and in membrane preparations. In agreement with observations from binding experiments, the potency of dopamine in stimulating adenylyl cyclase was approximately 20 fold higher for the D_{1b} than the D_{1a} receptor (18 ± 3 nM ($n=7$) versus 320 ± 21 nM ($n=9$), respectively; means ± S.D.). Further, no difference was found between the potency of dopamine in membrane or whole cell assays (see Fig. 3a, b and c).

The dopamine receptor agonists with a benzazepine structure, e.g., SKF 38393 and SKF 75670 are known as partial agonists in rat striatal homogenates (Andersen et al., 1987; Andersen and Jansen, 1990). Both drugs were also partial agonists on the D_{1a} expressing cell line with potencies and efficacies compatible with previous observations in rat striatal tissue (100 ± 8 nM; 60% ($n=2$) and 3 ± 0.1 nM, 30% ($n=3$); respectively). On the other hand, in the D_{1b} expressing cell line, SKF 38393 was a full agonist with lower potency than dopamine (EC_{50} = 75 ± 15 nM, $n=2$) whereas SKF 75670 still appeared to be a partial agonist (EC_{50} = 23 ± 4 nM; 50%, $n=2$).

Dopamine (10 μM or 1 μM) had no effect on the cytosolic calcium concentration neither in the D_{1a}-BHK 3B cell line, the D_{1b}-CHO 3D cell line nor in nontransfected cell lines. In contrast, cells transfected with the M1 muscarinic receptor cDNA, showed a clear dose-

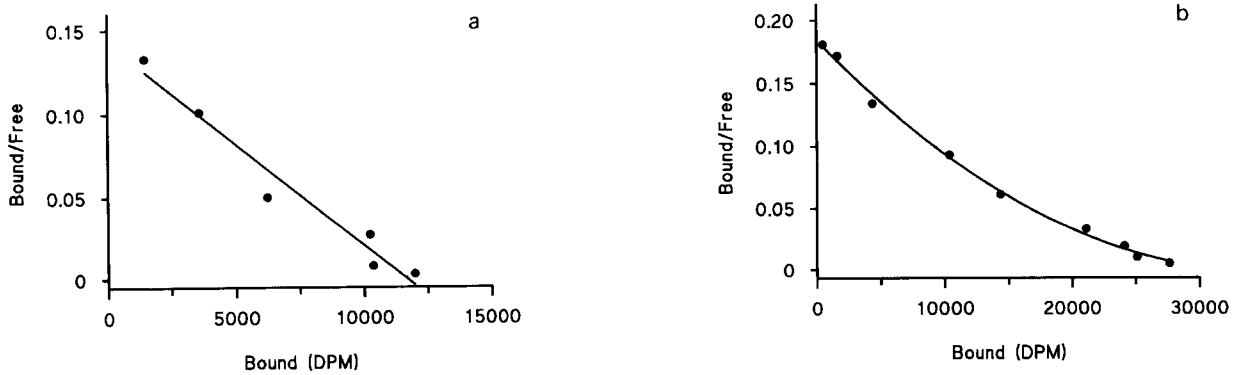

Fig. 1. Scatchard analysis of [^{3}H]SCH 23390 saturation experiments (0.01–50 nM) performed on membranes from the D_1a expressing BHK cell line (Fig. 1a) and the D_1b expressing CHO cell line (Fig. 1b). The data shown is from a representative experiment; This yielded a K_d of 0.5 nM and Bmax of 4.5 pmol/mg protein for the D_1a receptor and a K_1 = 0.3 nM and K_2 = 2.4 nM and corresponding B_{max} values of 0.39 and 1.6 pmol/mg protein, respectively, for the D_1b receptor. See text and Table 1 for average data on the kinetic parameters and Materials and methods for further experimental details.

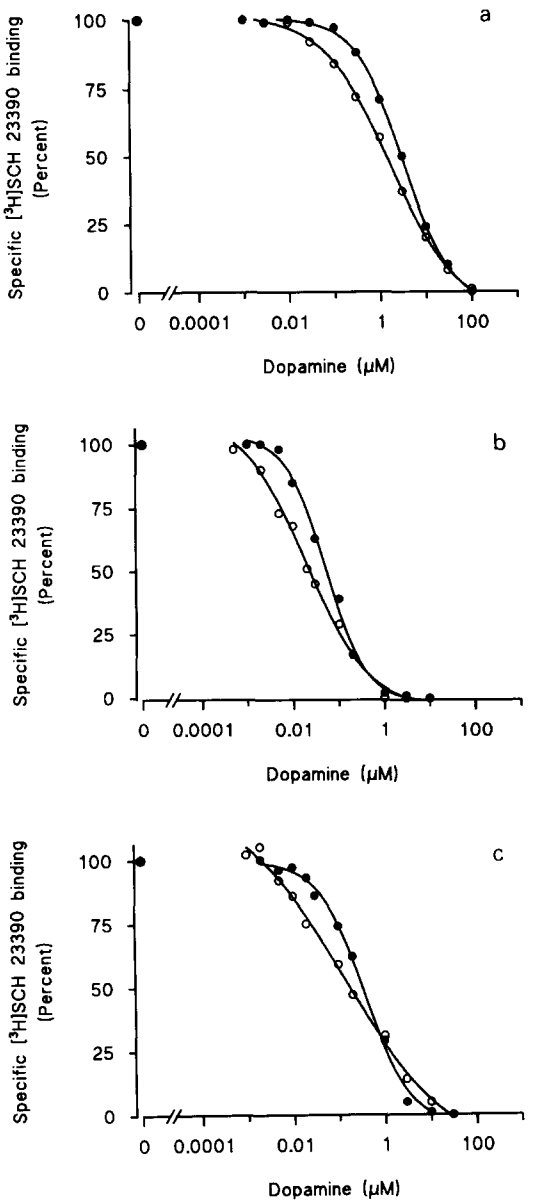

Fig. 2. The effect of GTP (15 uM) on dopamine inhibition of specific [^{3}H]SCH 23390 binding to membranes from the D_{1a} expressing cell line (Fig. 2a) or the D_1b expressing cell line using low (0.2 nM, Fig. 2b) or high (10 nM, Fig. 2c) radioligand concentrations. For experimental details see Materials and methods and Andersen and Jansen (1990). Data shown are from single experiments and the constants were; 2a: $K_H = 79$ nM, $K_L = 1{,}800$ nM, $R_H = 25\%$, $R_L = 75\%$; $K_i = 1900$ nM; 2b: $K_H = 2.1$ nM, $K_L = 72$ nM, $R_H = 25\%$, $R_L = 75\%$, $K_i = 68$ nM; 2c: $K_H = 2.4$ nM, $K_L = 181$ nM, $R_H = 30\%$, $R_L = 70\%$, $K_i = 83$ nM. For average data see Table 2. The symbols are: no GTP added; 15 μM GTP added.

dependent increase in the intracellular calcium concentration following application of carbacholine (Fig. 4).

3.4. Pharmacological profile of the D_{1a} and D_{1b} receptors

The affinities of various pharmacologically active drugs for inhibiting [^{3}H]SCH 23390 binding and

Table 2
Effect of GTP on the inhibition of specific [^{3}H]SCH 23390 binding by dopamine. Results are shown as means ± S.D. The number of separate experiments is shown in brackets. For further details concerning experimental design see legend Fig. 1, Materials and methods and Andersen and Jansen (1990)

Receptor	[^{3}H]SCH 23390 binding				
	K_H (nM)	K_L (nM)	R_H (%)	R_L (%)	K_i (nM)
D_{1a} (3)	8.4 ± 17	1400 ± 200	25	75	1700 ± 200
D_{1b} (2) [a]	2.7 ± 0.8	87 ± 12	25	75	77 ± 15
D_{1b} (3) [b]	2.8 ± 0.7	123 ± 51	33	67	93 ± 13

[a] Low or [b] high [^{3}H]SCH 23390 concentrations used in the assay.

Table 3
Pharmacological characteristics of D_{1a} and D_{1b} dopamine receptors stably in BHK and CHO cells, respectively. In binding experiments 5 to 15 different drug concentrations were used. Hill slopes were not significantly different from unity if only a single K_i value is given in the stable. K_i values for inhibition of dopamine stimulated adenylyl cyclase were obtained by Schild analysis using three different inhibitor concentrations and five to seven different concentrations of dopamine. All values are means (S.D. values < 10% of mean) from at least two independet experiments. For further details see Andersen and Braestrup, 1986 and Materials and methods

Compound	D_{1a}		D_{1b}	
	[^{3}H]SCH 23390 K_i (nM)	Adenylyl cyclase K_i (nM)	[^{3}]SCH 23390 K_i (nM)	Adenylyl cyclase K_i (nM)
Benzazepines				
NNC 112	0.24	0.65	K_1 0.07 K_4	0.2
NNC 687	5.8	4.5	5.8	6.3
NNC 756	0.34	0.5	0.6	0.3
SCH 23390	0.5	0.9	K_1 0.5 K_2 4.9	0.95
SKF 38393 [a]	107	[b]	58	[b]
SKF 75670 [a]	9.4	[b]	7.2	[b]
Thioxanthenes				
cis-Flupentixol	0.7	0.9	0.65	0.65
trans-Flupentixol	670	nt	540	nt
Phenothiazines				
Fluphenazine	1.5	2	K_1 2 K_2 241	2.1
Chlorpromazine	16	23	33	46
Butyrophenones				
Haloperidol	10	15	27	30
Spiperone	179	170	504	608
Dibenzepines				
Fluperlapine	39	47	137	150
Clozapine	18	25	35	35
Miscellaneous				
(+)-Butaclamol	0.2	0.3	0.3	0.4
(−)-Butaclamol	> 5000	nt	> 5000	nt
Bulbocapnine	85	145	K_1 251 K_2 1175	333
(+)-Sulpiride	22000	nt	3800	nt
Dopamine [a]	1700	[b]	85	[b]
Ketanserin	23	nt	85	nt

[a] agonists; K_i values obtained in the presence of 15 uM GTP. [b] See section 3.3 for details on EC_{50} and efficacy values; nt, not tested.

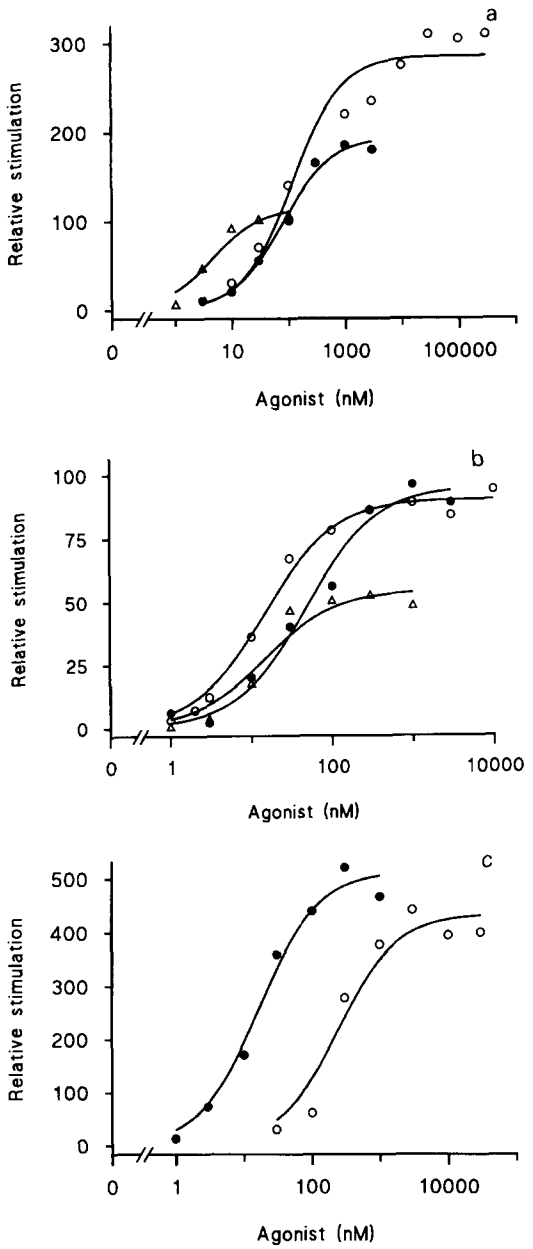

Fig. 3. Dose-response curves for agonist stimulation of adenylyl cyclase by the D_1a or D_1b receptor in membranes (Fig. 3a and Fig. 3b, respectively) or in whole cells (Fig. 3c). Data shown are from representative experiments, for average data see text. Specific activity were 0.8 pmol/min mg protein (D_{1a} membranes), 5.5 pmol/min mg protein (D_{1b} membranes), 0.46 pmol/min well (D_{1a} cells) and 0.74 pmol/min well (D_{1b} cells). Symbols are: a and b: ○, dopamine; △, SKF 75670; ●, SKF 38393; and c: -●, D_{1b} cells and ○, D_{1a} cells.

dopamine stimulated adenylyl cyclase in the D_{1a} and D_{1b} expressing cell lines are presented in Table 3. Due to the presence of two affinity states for the radioligand in the D_{1b} expressing cell lines, testing of compounds was done on these cells using both 0.2 nM and 10 nM [^{3}H]SCH 23390 as already described in section 3.2.

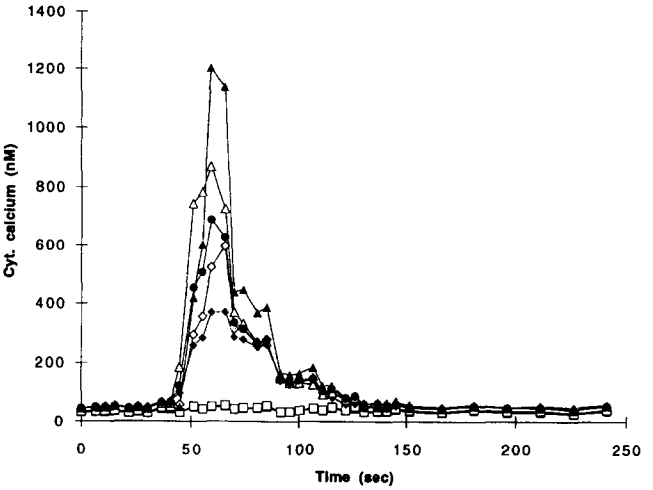

Fig. 4. Intracellular calcium concentrations in BHK or CHO cells. The cells were either transfected with D_1a, D_1b or M1 muscarinic receptor cDNAs. Cells were subsequently challenged with 10 μM (D_{1a} cells) or 1 μM (D_{1b} cells) dopamine or 0.1 μM (♦), 0.3 μM (◇), 1 μM (●), 3 μM (△) and 10 μM (▲) carbachol (M1 transfected cells). Data shown are from a typical experiment.

The pharmacological profile of both the D_{1a} and the D_{1b} receptors exhibit characteristics typical of the D_1-family. Thus, the classical D_1 selective benzazepine compounds like the NNC-compounds and SCH 23390 as well as the non-selective compounds flupentixol and butaclamol were very potent inhibitors of specific [^{3}H]SCH 23390 binding. On the other hand, classical D_2 selective drugs like spiperone and sulpiride exhibited low potency in inhibiting the binding. At low concentrations (0.2 nM) of [^{3}H]SCH 23390 when labeling only the high affinity D_{1b} receptor state, all compounds tested inhibited binding with Hill slopes at unity. At high (10 nM) [^{3}H]SCH 23390 concentrations, when labeling both states of the D_{1b} receptor, a few compounds were able to discriminate between the two

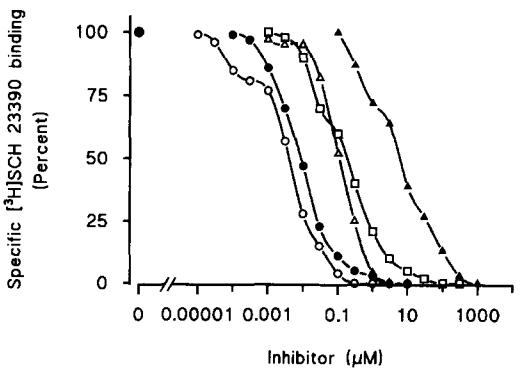

Fig. 5. Inhibition of specific [^{3}H]SCH 23390 binding (10 nM) by NNC 112 (○), NNC 756 (●), fluphenazine (□), chlorpromazine (△) and bulbocapnine (△). Hill slopes for these curves were: NNC 112 (< 0.5), NNC 756 (0.97 ± 0.04), fluphenazine (0.69 ± 0.05), chlorpromazine (1.02 ± 0.02) and bulbocapnine (0.55 ± 0.03). Data shown are from single experiments, for average data see Table 3.

states of the D_{1b} receptor (see Fig. 5). The affinity of the drugs for the two states of the D_{1b} receptor was not affected by addition of GTP (not shown). Further, the high affinity component (K_1) obtained at high radioligand concentration, was identical to the K_i obtained using low radioligand concentration. Interestingly, the atypical neuroleptics clozapine and fluperlapine exhibited fairly high affinity for these receptors with some preference for the D_{1a} receptor (Table 3).

The K_i values for inhibition of dopamine stimulated adenylyl cyclase were obtained from Schild analysis as described previously (Andersen and Braestrup, 1986). None of the antagonists tested affected the unstimulated cAMP levels in membranes from any of the cell lines. The K_i values obtained in the D_{1a} expressing cell line matched the K_i values obtained from the binding experiments. In case of the D_{1b} receptor, the K_i values obtained in the adenylyl cyclase assay correlated significantly better with the K_1 affinity state than with the K_2 affinity state of the receptor ($r = 0.98$ vs. $r = 0.71$, not shown).

4. Discussion

This paper describes for the first time an extensive and comparative pharmacological and functional characterization of the human D_{1a} and D_{1b} receptors stably expressed in mammalian cell lines.

The D_{1a} receptor only displayed a single binding site when stably expressed in BHK cells. On the other hand, the D_{1b} receptor existed in two states with high (K_1) and low (K_2) affinity for [^{3}H]SCH 23390 in both CHO and BHK cells. The pharmacological profile of the [^{3}H]SCH 23390 binding to membranes from these cell lines showed that both the D_{1a} as well as the D_{1b} receptors exhibited D_1 receptor antagonist pharmacology in agreement with previous reports using transient expression systems (Zhou et al., 1990; Dearry et al., 1990; Sunahara et al., 1990a, 1990b; Tiberi et al., 1991; Sunahara et al., 1991; Grandy et al., 1991). However, the potency of agonists in these stable cell lines were higher than observed previously in transient systems (ibid). This probably indicates a more correct G protein coupling in this stably expressing system.

In accordance with previous reports using rat brain tissue (Andersen and Braestrup, 1986) the stably expressed recombinant receptors also exhibited fairly high affinity for the atypical neuroleptics clozapine and fluperlapine. Of other dopamine receptor family members, only the D_4 receptor show affinity in the same range for clozapine (Seeman, 1992; Van Tol et al., 1991). This supports that blocking of the D_1-family of dopamine receptors may be involved in the antipsychotic action of clozapine-like neuroleptics (see also Andersen and Nielsen, 1990). Certain antagonists were capable of discriminating between the two D_{1b} receptor states identified by the radioligand. On the other hand, dopamine showed similar affinity for the two states (Tables 2 and 3).

Using the previously described procedure (Andersen et al., 1985; Andersen and Braestrup, 1986), we generated K_i values from inhibition of dopamine stimulated adenylyl cyclase in the D_{1a} and D_{1b} expressing cell lines. Contrary to previous observations on rat striatal tissue (Andersen et al., 1985; Andersen and Braestrup, 1986), the K_i values from the adenylyl cyclase experiments matched the K_i values obtained from binding experiments. In case of the D_{1b} receptor, a substantially better correlation was obtained between K_1 values than K_2 values and the K_i values obtained from adenylyl cyclase inhibition studies (Table 3). This clearly indicates that the K_1 affinity state is the state associated with adenylyl cyclase in the D_{1b} expressing CHO cell line.

The D_{1b} receptor was expressed in two different affinity states in both BHK and CHO cells. No indication of more than a single state was observed with the D_{1a} receptor. It is a widely accepted dogma that antagonist ligands label both free and G protein coupled receptor with equal affinity. In agreement with this, the presence of the two D_{1b} receptor affinity states was shown not to be dependent on the level of receptor expression as evidenced by the gene duplication experiments (Table 1) This was further supported by receptor depletion experiments using EEDQ; irrespective of receptor number the affinities for and the ratio between the two states were maintained (not shown). The primary sequence of D_{1a} and D_{1b} receptors are very divergent in the intracellular loops. Interestingly, these loops are known to be responsible for association of receptors with intracellular messenger systems via G proteins (Ostrowski et al., 1992). However, neither the D_{1a} nor the D_{1b} receptors appear in the presently investigated cell lines to be associated directly with intracellular calcium metabolism contrary to a previous report utilizing Ltk-cells (Bouvier et al., 1993). Nevertheless, these divergent loops and the two state characteristics of the D_{1b} receptor may imply coupling of this receptor to an as yet unidentified second messenger system.

Of major interest is the question, which of the presently identified dopamine receptor genes corresponds to the D_1 receptor detected by [^{3}H]SCH 23390 binding in rat striatum. A correlation between the K_i values from rat striatum (data from Andersen et al., 1985; Andersen and Braestrup, 1986; Andersen and Nielsen, 1986; Andersen et al., 1992) and the data from Table 3 indicated a close correlation with the D_{1a} K_i values but a somewhat lower correlation to D_{1b} K_1 values ($r = 0.94$ and $r = 0.81$; not shown) whereas a significantly lower correlation was found between the

rat data and D_{1b} K_2 values ($r = 0.62$; not shown). This indicates, that the site labeled with low [^{3}H]SCH 23390 concentrations in rat striatal tissue primarily has been the D_{1a} receptor. This conclusion agrees well with findings from in situ hybridization studies, indicating that the D_{1a} receptor is the most abundant species in striatum (Dearry et al., 1990; Tiberi et al., 1991; Weiner et al., 1991).

The previously observed discrepancy between K_i value obtained in binding and adenylyl cyclase assays (Andersen and Braestrup, 1986) was not observed in the D_{1a} or D_{1b} expressing cell lines. This, together with reports on D_1-like receptors linked to PI turnover in rat striatal slices (Undie and Friedman, 1990) or expressed in Xenopus Oocytes from rat striatal mRNA (Mahan et al., 1990) may point to yet another member of the D_1 receptor family which remains to be identified.

In summary, human D_{1a} and D_{1b} receptors stably expressed in mammalian cell lines exhibited a pharmacology closely resembling the previously identified D_1 receptor in rat striatum with high affinity for benzazepine derivatives and clozapine-like compounds. The D_{1b} receptor was expressed in two different affinity states. These states have similar affinity for dopamine but different affinity for SCH 23390, NNC 112, fluphenazine and bulbocapnine. The mechanism underlying these states and their physiological significance is presently unknown. Together with data on D_1 receptor pharmacology and function generated on rat striatal tissue, these data may point to yet another D_1 receptor to be identified.

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6. References

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