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Full Paper

Modulation of Adenylyl Cyclase Activity in Rat Striatal Homogenate by Dopaminergic Receptors

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Abstract. We have characterized the modulation of adenylyl cyclase (AC) activity by ligands of dopaminergic receptors in rat striatal homogenate and compared the results with receptor-ligand binding affinities. Despite the fact that rat striatum contains high level of both dopamine D_1 and D_2 receptors, only the D_1-specific AC activation by agonists could be determined. All D_1-receptor agonists (dopamine, dihydrexidine, and A 77636) used were able to increase cAMP accumulation in a concentration-dependent manner, while D_1-receptor antagonists (SCH23390, SKF83566, and butaclamol) blocked the effects induced by the aforementioned agonists. At the same time, the D_2-receptor agonist quinpirole and antagonist sulpiride had no effect on cAMP accumulation in striatal homogenate neither on the basal level nor on the activated level of AC, while inhibited [^{3}H]raclopride binding to these membranes. Comparing the ligands of the D_1 receptor in modulating the activity of AC and displacing D_1-receptor–specific radioligand [^{3}H]SCH23390 binding revealed that the ligands modulate both of these processes with similar affinities. It indicates that under given experimental conditions, only dopamine D_1-receptor–mediated stimulation of AC activity can be measured in membrane homogenate of rat striatum, while dopamine D_2-receptor effects remain fully hidden.

Keywords: adenylyl cyclase, cAMP, dopamine D_1 receptor, subtype selective assay, rat striatum

Introduction

Dopamine (DA) is an important neurotransmitter molecule that is associated with regulation of many important functions in the central nervous system and periphery. DA is acting through a family of G-protein–coupled DA receptors of which five different subtypes have been discovered to date (1). This family of receptors is further divided into two subfamilies based on their pharmacological and functional properties. The D1-family is comprised of D_1 and D_5 receptors and generally they couple to the G_s-family of G-proteins, which stimulate adenylyl cyclase (AC) activity. D_2, D_3, and D_4 receptors belong to the D2-family, and these receptors preferentially couple to the $G_{i/o}$-family of G-proteins, activation of which leads to inhibition of AC activity.

In the striatum, an area that is involved in the control of locomotion, cognition, emotion, and many other functions, high expression of both D_1 and D_2 receptors has been found (2, 3). These receptors are involved in such pathological conditions as schizophrenia, Alzheimer's disease, and Parkinson's disease; and for this reason, they have been targets of intensive research for many decades already. Moreover, the dopaminergic system is also involved in behavioural peculiarities connected with stress, anxiety, and other factors (4). To better understand the role and involvement of different subtypes of dopaminergic receptors in these processes, signal transduction pathways of the receptors in individually different animals should be studied. Using specific radioligands to determine the number of binding sites of different receptor subtypes in certain tissues has been accepted for decades as a describer of a given neurotransmitter system. Unfortunately, such experiments do not give direct information about the state of the signal transduction system. Studies directed to the determination of the activities of G-proteins or modulation of the activities of effector systems involved in the signalling pathway should overcome the foregoing limitations. One of the most direct approaches to

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characterize receptor-dependent effector activation would be the measurement of [^{35}S]GTPγS binding to G-proteins (5) or hydrolysis of guanosine 5'-triphosphate (6). The [^{35}S]GTPγS binding assay has also been successfully used for the characterization of D_2 receptors in striatal membranes (7), and it is applied as a marker of signal transduction for this receptor. However, this assay is selective for $G_{i/o}$-coupled receptors, and receptors coupled with other subtypes of G-proteins can only be picked up by methods based on antibodies (8). The latter approach has also been used to characterize D_1 dopamine receptors in cell cultures as well as in rat brain tissues (9, 10). An alternative approach for the characterization of signal transduction via G-protein–coupled receptors is to measure the modulation of AC activity that would reflect signalling of both, $G_{s/olf}$- and $G_{i/o}$-coupled receptors (11). This, in turn, means that all dopaminergic receptors in striatal tissue may modulate the response of AC, and only careful selection of specific ligands or experimental conditions would enable pick-up of a selective signal. The aim of the present study was to elaborate a methodology for separate measurement of signal transduction of $G_{s/olf}$- and $G_{i/o}$-coupled DA receptors in crude homogenate of rat striatum. Herewith, we present the experimental conditions for cAMP accumulation measurements that enabled us to reveal the modulation of AC activity specific to D_1 receptor, keeping the D_2-specific modulation fully hidden.

Materials and Methods

Chemicals

The following reagents were obtained from the indicated commercial sources: [*N*-Methyl-^{3}H]*R*-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride ([^{3}H]SCH23390) and [5',8-^{3}H]adenosine-3',5'-cyclic monophosphate ([^{3}H]cAMP) (GE Amersham Biosciences, Little Chalfont, UK); [^{3}H]raclopride (74 Ci/mmol; Perkin Elmer Life and Analytical Sciences, Boston, MA, USA); (1*R*-*cis*)-1-(aminomethyl)-3,4-dihydro-3-tricyclo[3.3.1.13,7]dec-1-yl-[14]-2-benzopyran-5,6-diol hydrochloride (A 77636), 8-bromo-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol hydrochloride (SKF83566), (*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1*H*-3-benzazepine hydrochloride (SCH23390), 4-[2-[[6-amino-9-(*N*-ethyl-*β*-D-ribofuranuronamidosyl)-9*H*-purin-2-yl]amino]ethyl]benzenepropanoic acid hydrochloride (CGS21680), dihydrexidine (DHX), isobutylmethylxanthine (IBMX), 4-(3-butoxy-4-methoxyphenyl) methyl-2-imidazolidone (Ro20-1724), quinpirole, sulpiride, and forskolin (Tocris Cookson, Ltd., Bristol, UK); butaclamol, bovine serum albumin (BSA), and dopamine (DA) (Sigma-Aldrich Chemie GmbH, Steinheim, Germany); and guanosine-5'-(3-thio)-triphosphate (GTPγS), phosphoenolpyruvate (PEP), and pyruvate kinase (PK) (Roche Diagnostics, Mannheim, Germany). All other reagents were of analytical grade from regular suppliers.

Membrane preparations

Striatum tissues of Wistar rats were separated and collected as described previously (12). For biochemical experiments, the tissues were homogenized in 50 volume (ww/v) of ice cold 2.5 mM Tris-HCl buffer (pH 7.4) containing 2 mM EGTA by a Bandelin Sonoplus sonicator (three passes, 10 s each; Bandelin Electronic GmbH, Berlin, Germany). For AC assays, the suspension was diluted twice with 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM EGTA, divided into aliquots and stored at −80°C until use. For radioligand binding assays, the membranes of the suspension were washed twice by centrifugation at 16,000 rpm for 40 min at 4°C and homogenized in 30 volumes (ww/v) of 50 mM Tris-HCl buffer (pH 7.4) containing 2 mM EGTA. The final suspension (32 mg tissue/ml) was divided into aliquots and stored at −80°C until use.

Adenylyl cyclase assay

The assay was carried out in a reaction medium containing 30 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 1 mM ATP, 10 μM GTP, 0.75 mM EGTA, 7.5 mM KCl, 100 mM NaCl, 0.1 mM IBMX, 0.1 mM Ro20-1724, 100 μg/ml bacitracin, 0.03% BSA, and ATP regenerating system (10 mM PEP and 30 μg/ml PK). The reaction was started by transferring tubes containing membrane homogenate (approx. 12 μg protein/ml) with the ligand of interest from an ice bath to a 30°C water bath, followed by a 15-min incubation. The reaction was terminated by adding a solution containing EDTA (final concentration of 25 mM) and subsequent boiling of samples for 5 min. The content of accumulated cAMP in the samples was measured by competition binding with [^{3}H]cAMP to cAMP binding protein (13). Bound radioactivity was determined by rapid filtration through GF/B glass-fibre filters (Whatman Int., Ltd., Maidstone, UK) using a Brandell cell harvester and three washes of 3 ml of ice-cold washing buffer containing 100 mM NaCl and 20 mM phosphate buffer (pH 7.5) as described previously (14). Nonspecific binding of [^{3}H]cAMP was determined in the absence of the binding protein.

Radioligand binding assays

In saturation binding experiments, the homogenate of rat striatal membranes (1.9 mg tissue/ml) in 50 mM

Tris-HCl buffer (pH 7.4) containing 2 mM EGTA was incubated with increasing concentrations of [^{3}H]SCH23390 (0.06 – 7 nM) for 45 min at 30°C. The reaction was stopped by rapid filtration through a GF/B glass fiber filter (Whatman) and four washings with 3 ml ice-cold washing buffer [20 mM potassium phosphate buffer (pH 7.5), 100 mM NaCl]. Bound radioactivity was measured as described earlier (15). Nonspecific binding of [^{3}H]SCH23390 was determined with 1 μM SKF83566. In displacement binding experiments, different concentrations of the ligand were incubated with a fixed concentration of [^{3}H]SCH23390 (1.7 – 2.1 nM in different experiments) for 45 min at 30°C.

The binding of [^{3}H]raclopride was performed as described before (16). In brief, the suspension of membranes (5.4 mg tissue/ml) was incubated with different concentrations of [^{3}H]raclopride (0.1 – 6 nM) for 60 min at 25°C, and the incubation was terminated by rapid filtration as described above. Nonspecific binding of [^{3}H]raclopride was determined with 10 μM (+)-butaclamol. In displacement binding experiments, different concentrations of the ligand were incubated with 2.8 nM [^{3}H]raclopride for 60 min at 25°C.

Data analysis

All data were analyzed by means of nonlinear least squares method using the program GraphPad PRISM™ (GraphPad Software, Inc., La Jolla, CA, USA). Data are each presented as the mean ± S.E.M. of at least two independent determinations.

Results

DA, an endogenous agonist of dopaminergic receptors, caused a concentration-dependent increase in the production of cAMP in crude homogenate of rat striatum. At 1-μM concentration, DA did not produce significant changes in basal cAMP level, but 100 μM DA caused already substantial cAMP accumulation of 12 (fmol/μg tissue)/min (Fig. 1). Both, D_1 and D_2 subtypes of dopamine receptors are highly expressed in the striatal region of rat brain (2, 3) and both of them modulate the activity of AC. However, at the experimental conditions studied, we could not detect any D_2-specific inhibition of cAMP formation despite the presence of these receptors. The presence of receptors was confirmed by specific binding of the D_2-specific radioligand [^{3}H]raclopride, whose affinity ($K_D = 2.3 \pm 0.2$ nM) and number of binding sites ($B_{max} = 21 \pm 1$ fmol/mg tissue (ww)) were in good agreement with our previous data obtained for characterization of dopamine D_2 receptors (16, 17). Quinpirole, a D2-family–selective agonist, had no significant influence at concentrations up to 10 μM

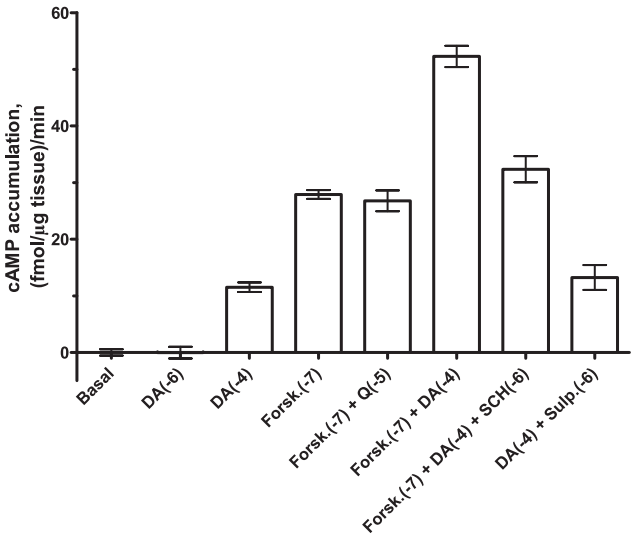

Fig. 1. Modulation of cAMP accumulation in rat striatal homogenate by dopamine and forskolin. Homogenates of rat striatum (11.3 μg/ml) were incubated with different combinations of modulators, 1 μM dopamine [DA(-6)], 100 μM dopamine [DA(-4)], 10 μM quinpirole [Q(-5)], 0.1 μM forskolin [Forsk.(-7)], 1 μM SCH23390 [SCH(-6)], and 1 μM sulpiride [Sulp.(-6)] for 15 min at 30°C, and the accumulated amount of cAMP was measured as described in the Materials and Methods section. Data are each presented as accumulation of cAMP (total – basal) with S.E.M. as error bars of three independent experiments carried out in triplicate.

neither on the cAMP accumulation generated by direct activation of AC with 0.1 μM forskolin (Fig. 1) nor on the adenosine A_{2A} receptor–mediated activation of AC (with 1 μM CGS21680, data not shown). We could not detect any specific effect of D2-family–selective antagonist on the basal or DA-activated cAMP accumulation (Fig. 1). However, DA significantly increased cAMP accumulation even in the presence of forskolin, in which case the increase was even higher (Fig. 1). Moreover, all these DA-induced effects were fully inhibited by the D1-family–selective antagonist SCH23390 (1 μM), while sulpiride (1 μM), an antagonist selective to D_2 receptors, had no effect (Fig. 1). The functionality of D_2 receptors was confirmed by the displacement of [^{3}H]raclopride binding by quinpirole, which was described by a 2-site model [$pK_H = 7.93 \pm 0.15$ (12 nM), $pK_L = 6.51 \pm 0.15$ (306 nM), $\alpha_H = 0.51 \pm 0.08$], high affinity of sulpiride ($pK_i = 8.67 \pm 0.06$), and low affinity of SCH23390 ($pK_i = 6.24 \pm 0.05$). These data are in agreement with data obtained earlier for these ligands in characterization of dopamine D_2 receptors in rat striatum by displacement of [^{3}H]raclopride binding (18) as well as with functional assays in activation of [^{35}S]GTPγS binding (7).

Mg^{2+} has been found to play a crucial role in the GPCR-dependent signal transduction system and its

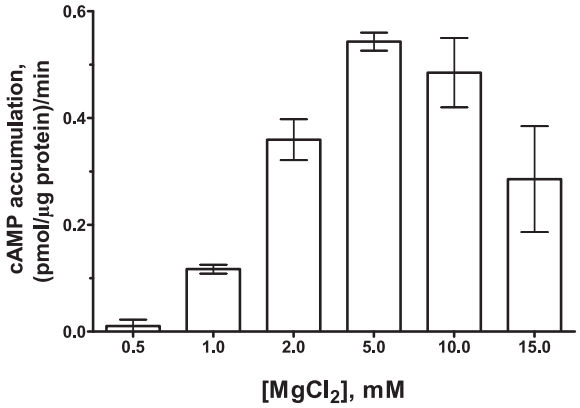

Fig. 2. Influence of Mg^{2+} concentration on the DA-dependent cAMP accumulation in the rat striatal homogenate. Homogenate of rat striatum (11.3 μg/ml) in the presence of indicated concentrations of $MgCl_2$ was incubated with or without 1 mM DA for 15 min at 30°C and total, and basal levels of cAMP accumulation were measured. Data are each presented as the mean of DA-specific accumulation of cAMP (total – basal) with S.E.M. as error bars of two independent experiments carried out in triplicate.

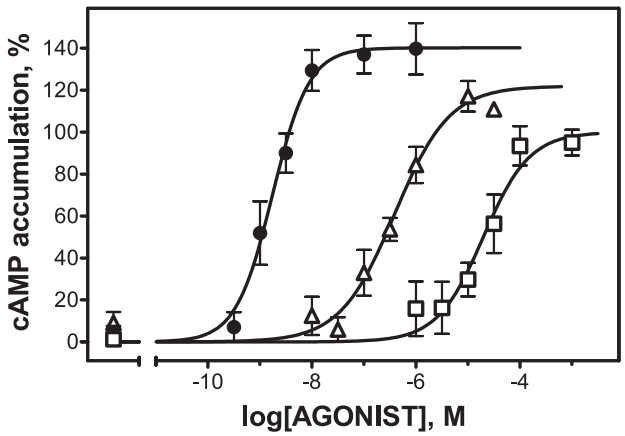

Fig. 3. Concentration-dependent activation of cAMP accumulation in rat striatal homogenate by D_1-receptor agonists. Homogenate of rat striatum (11.3 μg/ml) was incubated with the indicated concentrations of A 77636 (circle), DHX (triangle), and DA (sqaure) for 15 min at 30°C, and accumulated cAMP concentrations were measured as described in Materials and Methods. Data are each presented as a percentage of the maximal DA effect (± S.E.M. as error bars) from two independent experiments carried out in triplicate.

presence is required at least in micromolar concentration range to achieve a response (19). With adenosine A_{2A} receptors, we have previously shown that careful selection of optimal Mg^{2+} concentration allows achievement of considerably higher receptor-dependent effects in the cAMP accumulation assays (20). In the present system, we could not determine any DA-dependent cAMP accumulation at Mg^{2+} concentrations of 0.5 mM and below. Increasing the concentration of Mg^{2+} caused a proportional increase in both basal and DA-activated levels of cAMP accumulation. The maximum of DA-dependent cAMP accumulation was already achieved at 5 mM Mg^{2+} (Fig. 2), while the basal cAMP accumulation continued to increase at least up to 15 mM Mg^{2+} (data not shown). High basal level of cAMP accumulation at higher Mg^{2+} concentrations resulted in relatively higher scattering of experimental points (Fig. 2); therefore, all the following experiments were carried out in the presence of 5 mM Mg^{2+}, if not stated otherwise. The presence of Mg^{2+} was also required for forskolin-dependent accumulation of cAMP, which gave the best effect/basal ratio at 1 – 3 mM Mg^{2+}. However, no significant effects of D_2-specific–modulators, quinpirole and sulpiride, could be detected on the forskolin-activated cAMP level at any Mg^{2+} concentrations used (0 – 15 mM).

DA-induced increase in cAMP accumulation was dependent on the ligand concentration, reaching half of the maximal effect 0.54 (pmol/μg protein)/min at 20 μM DA ($pEC_{50} = 4.7 \pm 0.1$, Fig. 3). In addition to DA, D_1-selective agonists likewise were able to stimulate cAMP production. DHX and A 77636 triggered concentration-dependent stimulation of cAMP production with half-maximal effect at a concentration of 400 nM ($pEC_{50} = 6.4 \pm 0.1$) and 2 nM ($pEC_{50} = 8.7 \pm 0.1$), respectively (Fig. 3). For all these cases, a simple one-step sigmoidal curve was preferred over more complicated models in describing log[agonist] *vs* cAMP response data, whereas the Hill slope was not significantly different from unity. However, D_1-selective agonists stimulated cAMP accumulation with higher efficacies. For DHX and A 77636, the levels of cAMP accumulation in relation to the maximal effect of DA were 122 ± 12% and 140 ± 9%, respectively (Fig. 3). All these activations by agonists could be reversed by D_1-receptor antagonists. SCH23390, SKF83566, and butaclamol inhibited the 1 mM DA-stimulated cAMP accumulation in a concentration-dependent manner, while the D_2-selective antagonist sulpiride remained without a significant effect at concentrations up to 10 μM (Fig. 4). Sigmoidal inhibition curves were symmetric with Hill coefficients close to unity, having pIC_{50} values of 7.5 ± 0.1, 7.4 ± 0.1, and 6.6 ± 0.1 for SCH23390, SKF83566, and butaclamol, respectively. Assuming that the inhibition is fully competitive, the apparent affinities of these antagonists in the cAMP assay can be estimated to be 0.7 nM (0.5 – 1.0 nM), 0.8 nM (0.5 – 1.2 nM), and 4.5 nM (2.3 – 7.5 nM) for SCH23390, SKF83566, and butaclamol, respectively.

In addition, we compared the results of cAMP accumulation studies with the receptor-ligand binding properties to assess the relationship between the affinity

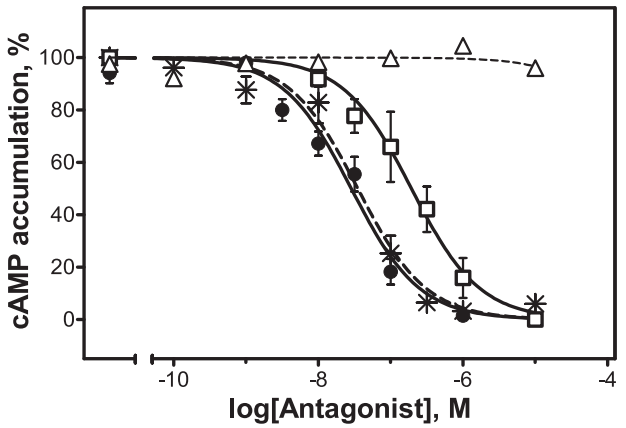

Fig. 4. Concentration-dependent inhibition of DA-stimulated cAMP accumulation in rat striatal homogenate by D_1-receptor antagonists. Homogenate of rat striatum (11.3 μg/ml) was incubated with 1 mM DA and the indicated concentrations of SCH23390 (circle), SKF83566 (asterisk), butaclamol (square), and sulpiride (triangle) for 15 min at 30°C, and accumulated concentrations of cAMP were measured as described in Materials and Methods. Data are each presented as a percentage of the DA effect (± S.E.M. as error bars) from two independent experiments carried out in triplicate.

and potency of the ligands. Therefore, we conducted [^{3}H]SCH23390 binding assays to characterize D_1 receptors in the homogenate of rat striatal membranes. Binding of [^{3}H]SCH23390 was specific and saturable, whereas nonspecific binding remained below 20% at all radioligand concentrations studied. The binding curve of [^{3}H]SCH23390 corresponded to the simplest model of single binding sites with an affinity constant $K_d = 0.75 \pm 0.08$ nM and density of binding sites $B_{max} = 62 \pm 2$ fmol/mg tissue (ww). These values are comparable to the data published earlier for cultured cell lines expressing D_1 receptors (15) as well as for brain membranes (21, 22), respectively. All the dopaminergic ligands that were able to modulate AC activity also caused a concentration-dependent inhibition of [^{3}H]SCH23390 binding. The inhibition of [^{3}H]SCH23390 binding by the antagonist could be described with inhibition curves corresponding to the presence of single binding sites for these ligands, as the Hill coefficients remained close to unity (Table 1). However, agonists' curves had considerably more shallow slope (Fig. 5) with Hill coefficients of 0.6 – 0.7 (Table 1). Fitting the data to a model predicting two independent binding sites revealed two sets of dissociation constants for all agonists with the proportion of high-affinity binding sites being 44% – 64% (Table 1). Activation of G-proteins (by 100 μM GTPγS) decreased the apparent potencies of agonists to the IC_{50} values of 46 μM, 0.7 μM, and 5 nM for DA, DHX, and A 77636, respectively, while Hill coefficients remained significantly below unity for all these agonists. Analyzing the data with a two-site binding model revealed that GTPγS had no effect on the affinity constants of agonists (listed in Table 1), but decreased the percentage of high-affinity binding sites to the level of 27%, 22%, and 43% for DA, DHX, and A 77636, respectively.

Table 1. Activities of dopaminergic ligands in terms of modulation of adenylate cyclase activity and inhibition of [^{3}H]SCH23390 binding in rat striatum

	Adenylate cyclase	[^{3}H]SCH23390 binding	
	EC_{50} / K_B effect	pIC_{50} (n_H)	K_H, K_L α_H
DA	26 μM (16 – 42)	5.42 ± 0.12	0.34 μM, 16 μM
	100 ± 7%	(0.71 ± 0.13)	63%
DHX	435 nM (235 – 806)	6.58 ± 0.05	8 nM, 383 nM
	122 ± 13%	(0.64 ± 0.05)	44%
A 77636	1.8 nM (1.0 – 3.3)	8.60 ± 0.09	0.28 nM, 6.6 nM
	140 ± 9%	(0.63 ± 0.07)	64%
SCH23390	0.9 nM (0.6 – 1.3)	8.80 ± 0.03	0.46 nM
	inh. 100%	(0.98 ± 0.06)	N.D
SKF83566	1.0 nM (0.6 – 1.7)	8.38 ± 0.04	1.23 nM
	inh. 100%	(1.04 ± 0.09)	N.D

EC_{50} (95% confidence interval in parenthesis) is the concentration of an agonist causing half maximal activation of adenylate cyclase, and K_B (95% confidence interval in parenthesis) is concentration of antagonist inhibiting 50% of the effect caused by 1 mM DA and divided by 1 + ([DA] / EC_{50}). Effect is the level of AC activation in comparison with the effect of DA. pIC_{50} is the negative logarithm of ligand concentration causing 50% inhibition of 1.7 – 2.1 nM [^{3}H]SCH23390 binding, and Hill coefficients (n_H) of the ligand/[^{3}H]SCH23390 displacement curves are presented in parentheses. K_H, K_L, and α_H are dissociation constants of high and low-affinity sites and proportion of high-affinity binding sites of ligand/[^{3}H]SCH23390 displacement curves calculated according to the Cheng-Prusoff equation (35).

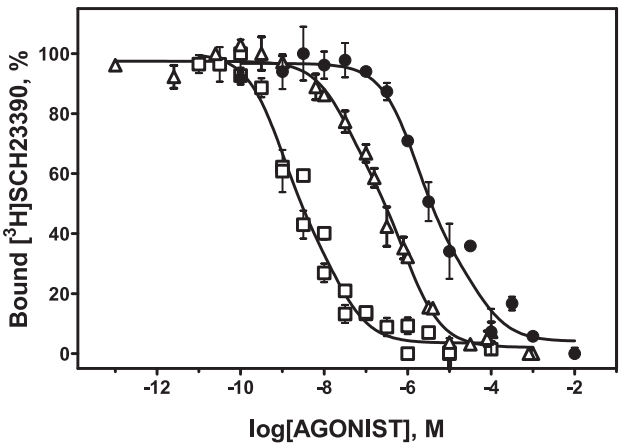

Fig. 5. Displacement of [^{3}H]SCH23390 binding to rat striatal membranes by DA (circle), DHX (triangle), and A 77636 (square). Membrane homogenate of rat striatum (1.9 mg tissue/ml) was incubated with 1.8 nM [^{3}H]SCH23390 in the presence of the indicated concentrations of studied ligands for 45 min at 30°C, and bound radioactivity was measured as described in Materials and Methods. Binding of [^{3}H]SCH23390 was estimated as the percentage of its specific binding in absence of competitive ligand, which was 21.6 ± 0.5 fmol/mg tissue (ww). Data are each presented as the mean ± S.E.M. of two independent experiments carried out in duplicate.

Discussion

On a cellular scale, one of the main roles of the dopaminergic system is to regulate the intracellular level of cAMP, which among other effectors is modulated by receptor-coupled G-proteins. In rat striatum, high expressions of both D_1 and D_2 dopaminergic receptors have been found (2, 3). However, using homogenate of rat striatum, we could only detect the effect of D_1 receptors on AC activity. All the agonists of D_1 receptors tested resulted in a concentration-dependent activation of cAMP accumulation with EC_{50} values being in agreement with the potencies reported earlier for striatal membranes (23, 24). In addition, good congruence was found between EC_{50} and K_i values of agonists determined in cAMP accumulation assays and [^{3}H]SCH23390 displacement experiments, respectively (Table 1). The situation was the same for antagonists as the potencies of SCH23390, SKF83566, and butaclamol in inhibiting DA-stimulated cAMP accumulation were comparable to their potencies in inhibiting the binding of [^{3}H]SCH23390. Sulpiride, on the contrary, remained ineffective at up to 1 μM concentrations.

Thus, we have optimized the assay conditions for in vitro characterization of dopamine D_1-receptor–mediated signal transduction in a membrane preparation of rat striatum. This allows us to study biochemical correlations and possible mechanisms of pathologies and behavioral peculiarities that are associated with D_1-receptor signaling in the striatum. The presence of D_2 receptors in these membranes has been detected by specific binding of [^{3}H]raclopride and its displacement by dopaminergic ligands. The essential part of high-affinity binding sites of quinpirole (51%) indicated also their coupling with G-proteins, which in addition to the activation of [^{35}S]GTPγS binding (7) has been used to observe changes in signal transduction of D_2 receptors caused by dopaminergic denervation with 6-OHDA (25) or partial noradrenergic denervation with DSP-4 (26, 27), as well as by manipulation of animals (12).

In the early 80's, it was already discovered that activation of D_2 receptors has an inhibiting and activation of D_1 receptors has a stimulating effect on the activity of AC in rat striatum (28). However, experimental conditions used herein only allowed observation of the effect of D_1-receptor activation, while D_2-receptor agonists had no influence on cAMP accumulation. There may be several reasons for the lack of a D_2-specific signal in the membrane preparation. For one, it has been shown that dopamine D_2 receptors modulate the level of cAMP in mice striatum via isoform 5 of AC. However, D_2 receptors preferentially couple to the G_o subtype of G-proteins (29), which, as shown previously, have practically no influence on AC5 activity in vitro (30). Thus, it is possible that the effect of D_2 receptors on cAMP accumulation is relatively small compared to the basal level of AC activation and therefore remains hidden in a given system. One possibility to decrease the non-dopaminergic signal is to use CGS21680 for the activation of AC via adenosine A_{2A} receptors (20), colocalized with D_2 receptors in rat striatum (31). The antagonistic influence of heterodimerization of these receptors may of course decrease the response, but would not eliminate it (32). However, in our conditions, quinpirole remained ineffective in modulation of cAMP, while being very effective in inhibition of [^{3}H]raclopride binding.

It is also reported, that a treatment of striatal membranes in mildly acidic buffer (pH 4.5) enhances signalling of opiate receptors via $G_{i/o}$-proteins (33). In our case, nevertheless, the method described had no impact on D_2-receptor–mediated cAMP accumulation.

Moreover, striatal D_2 receptors may also be coupled with effectors that do not modulate the activity of AC. It has been shown that if D_2 receptors are co-activated with D_1 receptors in striatum, this will lead to activation of the phospholipase C (PLC) pathway (34), which is not connected with the activation of $G_{i/o}$-proteins. On the other hand, using a [^{35}S]GTPγS binding assay, an effective D_2-specific activation of $G_{i/o}$-proteins in rat striatal membranes has been shown in this kind of membrane

preparation (7). Thus, the reasons why D_2-specific modulation of AC activity in rat striatal membranes did not appear in these studies remain to be studied, but we hope that this does not hinder the usage of the D_1-specific assay.

In summary, we have developed assay conditions for dopamine D_1-specific signal transduction in rat striatal membranes, keeping a possible cross-reactivity with dopamine D_2-receptor signaling fully hidden.

Acknowledgments

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