Life Sciences, Vol. 37, pp. 1971-1983 Pergamon Press
Printed in the U.S.A.

A COMPARISON BETWEEN DOPAMINE-STIMULATED ADENYLATE CYCLASE AND ^{3}H-SCH 23390 BINDING IN RAT STRIATUM

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(Received in final form September 6, 1985)

Summary

Methods for measuring ^{3}H-SCH 23390 binding and dopamine (DA) stimulated adenylate cyclase (AC) were established in identical tissue preparations and under similar experimental conditions. Pharmacological characterization revealed that both assays involved interaction with the D1 receptor or closely associated sites. In order to investigate whether the binding sites for ^{3}H-SCH 23390 and DA in fact are identical, the antagonistic effects of a variety of pharmacologically active compounds were examined. Surprisingly, the K_i-values obtained from Schild-plot analysis of the antagonism of DA-stimulated AC, were 80-240 times higher than the K_i-values obtained from competition curves of ^{3}H-SCH 23390 binding. Since both assays were performed under identical conditions, the differences in K_i-values indicate the possibility of different binding sites for DA and ^{3}H-SCH 23390 or, that DA and ^{3}H-SCH 23390 label different states of the same receptor.

Dopamine (DA) receptors have been classified into a D1 and a D2 type (1,2). Previously, D1 receptors have been studied with respect to their function, i.e., DA-stimulation of adenylate cyclase (AC) or in binding assays using the tritiated thioxanthenes, ^{3}H-flupentixol or ^{3}H-piflutixol (3,4,5). These ligands are not ideally suited for this purpose since they bind equally well to both D1 and D2 receptors and since their levels of nonspecific binding are sometime as high as 75 % of the total binding.

Recently, the highly selective D1 antagonist SCH 23390 (6) was introduced as a ligand for D1 receptors (7,8). The binding of this ligand to D1 receptors in rat brain has been characterized extensively (7). At present, ^{3}H-SCH 23390 appears to be the ligand of choice for D1 receptor binding due to its low level of nonspecific binding (4-8%) and extreme selectivity.

However, all previous D1 binding assays (3,4,5,7,8) suffer from one major disadvantage: they include tissue preparations and experimental conditions which are very different from those used for measuring DA-stimulated AC. Consequently, it has been a problem to compare AC-assay and D1-binding data.

In order to avoid the above mentioned difficulties, both assays

0024-3025/85 $3.00 + .00

were established in the same tissue preparation. Initially we selected a previously described "purified" rat striatal membrane preparation (9). This purification can be of importance when studying regulation of AC or ^{3}H-SCH 23390 binding, since both cations and nucleotides are known to affect AC-activity and receptor binding.

In the present study, we have attempted to further examine the biochemical and pharmacological characteristics of the AC-assay in comparison with ^{3}H-SCH 23390 binding. K_i-values obtained in the AC- or binding assay, when run under similar experimental conditions, were expected to be similar if the binding sites for DA and for ^{3}H-SCH 23390 are identical.

Materials and Methods

Drugs. (N-methyl-^{3}H)-SCH 23390 (85 Ci/mmole) was synthesized and purified on HPLC in our laboratories. ^{3}H-spiperone (22 Ci/mmol) and ^{3}H-cAMP (39 Ci/mmol) were from NEN. Cis- and trans-flupentixol were from H. Lundbeck A/S, Copenhagen, Denmark. d- and l-sulpiride were from Ravizza Research Laboratories, SpA, Italy. 3,4-Dihydroxynomifensine (3,4-DHN) was from Hoechst, pergolide and LY 171555 from Eli Lilly, and clozapine from Sandoz. Haloperidol, spiperone, pimozide, domperidone and ketanserin were purchased from Janssen Pharma, Birkeroed, Denmark, (+)- and (-)-butaclamol, ADTN and SKF 38393 from Research Biochemicals Inc., USA and phenothiazine, promethazine, apomorphine and chlorpromazine from Sigma. The drugs were dissolved in various acids or in methanol and diluted further with water.

Tissue preparation. Male wistar rats (150-200 g, Møllegårds labs., Lille Skensved, Denmark) were decapitated and the striatum or prefrontal cortex were rapidly dissected. The tissue was prepared as previously described (9). Briefly, the tissue was gently homogenized in 100 vol of 10 mM imidazole buffer (pH 7.40 at 25°C) containing 1 mM EDTA, using a glass-teflon homogenizer and centrifuged at 40,000 x g for 20 min. This step was performed three times. The final pellet was resuspended in 100 vol (of the original wet weight) of 2 mM imidazole buffer (pH 7.40 at 25°C) containing 2 mM EGTA. The resulting suspension was used for binding- or AC-assays (below).

Adenylate cyclase assay. A mixture of 50 ul of the above mentioned tissue suspension, 300 ul buffer (16.67 mM imidazole (pH 7.40 at 25°C), 16.67 mM theophylline, 1 mM EGTA and 10 mM $MgSO_4$) 25 µl DA/H_2O (stimulated/unstimulated activity), 50 ul GTP and 25 ul test compound solution were preincubated on ice for 15 min. Fifty µl of ATP (final concentration 1.5 mM) was added and the samples were incubated for 5 min at 30°C. The reaction was terminated by boiling for 3 min followed by centrifugation at 2800 x g for 20 min. Cyclic AMP was measured in the clear supernatant.

^{3}H-SCH 23390 binding One hundred µl of the tissue suspension, 600 µl buffer (16.67 mM imidazole (pH 7.40 at 25°C), 16.67 mM theophylline, 1 mM EGTA and 1 mM $MgSO_4$), 100 µl ^{3}H-SCH 23390 and 200 µl H_2O/test compound solution were incubated at 30° C for 90 min followed by rapid filtration through Whatman GF/B-filters under vacuum (200 mm Hg). The filters were washed twice with 2 x 10 ml 0.9% NaCl and counted in 4 ml Filter-count (Packard) in a scintilation-counter. Nonspecific binding was defined as the amount of ^{3}H-SCH 23390 bound in the presence of 1 µM of cis-flupentixol.

^{3}H-spiperone binding Striatal tissue was homogenized for 20 sec in 2 x 10 ml 50 mM Tris-citrate buffer (pH 7.4 at 30°C) containing 120 mM NaCl and 4 mM $MgCl_2$ using an Ultra-turrax homogenizer. The homogenate was centrifuged at 40,000 x g for 10 min and the pellet was resuspended in 1,000 vol (original wet tissue weight) of the same buffer. Two and a half ml of this suspension, 25 µl ^{3}H-spiperone (final concentration 0.05 nM) and 25 ul test substance solution were incubated for 20 min at 37°C followed by 10 min on ice. Unspecific binding was defined as the amount of ^{3}H-spiperone bound in the presence of 100 nM domperidone, and was routinely 10-18% of total binding.

cyclic AMP assay cAMP was measured as previously described (9). Briefly, 50 µl of the resulting supernatant from the AC-assay was incubated with 50 µl 1.25 pmol ^{3}H-cAMP, 50 µl purified cAMP dependent protein kinase (10) and 50 µl 50 mM Tris-HCl (pH 7.4 at 4°) containing 30 mM EDTA and 200 uM mercaptoethanol for 90 min at 4° C. 1 ml of 70% $(NH_4)_2SO_4$ was added and the incubation continued for further 15 min. Thereafter, the incubates were centrifuged at 2800 x g, and decanted. After adding 0.1 ml H_2O and 1.2 ml scintillation fluid the samples were counted in a scientillation counter. Standards containing 0 - 8 pmol cAMP/50 µl were run in an identical way.

Computer fitting Equation (1) was fitted to the data for the concentrations of specifically bound (B) and unbound (U) ^{3}H-SCH 23390 from the saturation studies (fig. 2) using the least squares programme of the (VB01AD) from a HARWELL library.

$$B = (B_{max} \times U)/(K_d + U) \quad (1)$$

B_{max} indicates maximal number of binding sites and K_d is the ligand affinity for the receptor. As weights were used U^{-2}

The model shown in equation (2) (two classes of binding sites (R_1 and R_2) with equal affinities for the ligand (L) and different affinity for the displacer (D)) was used to describe the displacement of ^{3}H-SCH 23390 by cis-flupentixol.

$$LR_1 \overset{K_d(L)}{\rightleftharpoons} \underset{\underset{DR_1}{\downarrow K_{d1}(D)}}{R_1} + L + \underset{\underset{DR_2}{\downarrow K_{d2}(D)}}{R_2} \overset{K_d(L)}{\rightleftharpoons} LR_2 \quad (2)$$

The fitting was carried out using the least squares method mentioned above. The value was calculated by numerical solution until equilibrium of three coupled differential equations gave the change per unit time in (LR_1+LR_2), DR_1 and DR_2 when a certain

concentration of the displacer was added. The initial value of LR_1+LR_2 was calculated from L_{total}, B_{max} and $K_{d(L)}$ and the numerical solution was carried out using another HARWELL subroutine (DC01AD).

Results

Kinetics of ^{3}H-SCH 23390 binding The binding of ^{3}H-SCH 23390 to striatal membrane receptors was stable and reversible. At the selected incubation temperature (30°C), the binding reached an equilibrium after 90 min and was stable for at least 30 min longer. The association rate constant k_{on}, was 0.14 $nM^{-1} \times min^{-1}$ (for further details, see legend to Fig 1).

When dissociation was initiated after incubation for 90 min by addition of excess of cis-flupentixol (10 µM), a k_{off} value of 0.06 min^{-1} was estimated from a semi-log plot. Thus, the half-life of the ligand was 16.7 min. From the relation k_{off}/k_{on}, a kinetic K_d-value of 0.43 nM was obtained.

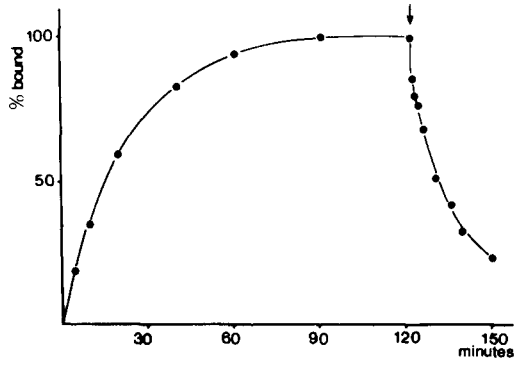

FIG. 1

Association and dissociation of specific ^{3}H-SCH 23390 binding. Association: The tissue preparation was incubated at 30°C with 0.2 nM ^{3}H-SCH 23390 for increasing periods before filtration. Assuming first-order kinetics, K_{obs} of the association process was determined from a plot of $\ln (B_{eq})/(B_{eq}-B_t)$ vs time, where B_{eq} is bound ^{3}H-SCH 23390 in dpm at equilibrium and B_t at the time t. The on-rate was computed from the following relation:

$$k_{on} = (K_{obs} \times B_{eq})/(B_{max} \times L)$$

where B_{max} is the maximal number of binding sites obtained by Scatchard analysis and L is the concentration of ^{3}H-SCH 23390 used. Dissociation: the tissue was incubated for 90 min at 30°C. Bound ^{3}H-SCH 23390 was measured at increasing times following addition (indicated by the arrow) of cis-flupentixol (10 µM). Off-rate was estimated from a plot of $\ln(B_t/B_{eq})$ vs time.

TABLE I

Kinetic constants for ^{3}H-SCH 23390 binding.

Constant	Unit	Value
k_{on}	$(nM \times min)^{-1}$	0.14
k_{off}	min^{-1}	0.06
K_d (kinetic)	nM	0.43
K_d (Scatchard)	nM	0.14

For details concerning the constants, see legend to Fig. 1.

Saturation experiments When ^{3}H-SCH 23390 binding was evaluated as a function of increasing ligand concentration (Fig. 2A), saturation of specific sites was clearly achieved; in contrast, nonspecific binding increased almost linearly over the concentration range studied (8-12% of total binding). Scatchard analysis (fig. 2B) indicated the presence of only a single binding site and fitting of equation (1) resulted in a K_d of 0.14 ± 0.05 nM and a B_{max} of 85.6 ± 6.0 fmol/mg tissue (means ± SD, N=2). Similar experiments performed on prefrontal cortex tissue also revealed a single binding site with an identical K_d-value (0.17 ± 0.06 nM) and a B_{max} of 13.4 ± 2.1 fmol/mg tissue (means ± SD, N=4). The level of non-specific binding in cortical tissue was similar to that observed in striatal tissue.

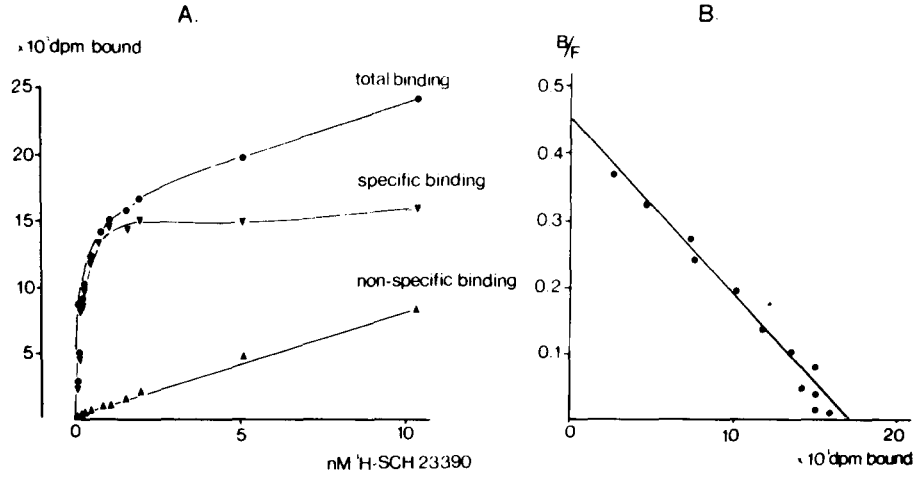

FIG. 2

A. Saturation of ^{3}H-SCH 23390 binding in rat striatum as a function of increasing concentrations of ^{3}H-SCH 23390 (0.01-10 nM). Non-specific binding was defined as the amount of binding in the presence of 1 µM cis-flupentixol.

B. Scatchard analysis of specifically bound ^{3}H-SCH 23390.

Stability of the preparation. Both ^{3}H-SCH 23390 binding, unstimulated and DA-stimulated AC-activity was fully stable after 24 hours on ice. When incubated at 30°C, both unstimulated and DA-stimulated AC-activity increased linearly for more than 2 hours. Furthermore, freezing the preparation at -20° C for 4 weeks had no effect on ^{3}H-SCH 23390 binding. AC-activity was stable after freezing for more than two months.

Within a pH interval of 6.5-8.0, the binding was stable; only a minor decrease in the total binding at the extreme ends of the interval was observed. Ascorbic acid (0.1%), which is used when preparing solutions of unstable compounds, had no effect on the binding or AC-activity.

Effect of DA. DA dose-dependently increased cAMP formation in the present preparation. From the dose-response relationship shown in Fig. 3, the EC_{50} for DA was calculated to 43 ± 22 μM (mean ± SD, N=10). However, the ability of DA to stimulate AC was highly dependent upon the presence of guanylnucleotides, (i.e., GTP or GDP(NH)P). As shown in Fig. 4, optimal stimulation was obtained with GTP concentrations between 10 and 100 μM. Outside of this range, the DA-stimulation was lower or, unstimulated activity increased. Similar effects were obtained with the nonhydrolysable analog GDP(NH)P (not shown).

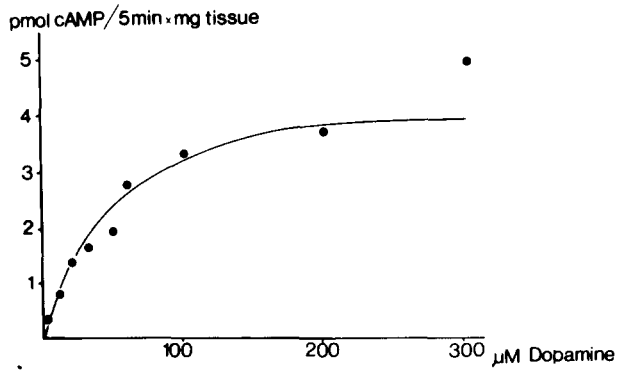

FIG. 3

Dose-response relationship for DA-stimulated AC in rat striatum. Shown as increase in activity. The concentration of DA ranged from 1 to 300 μM. Unstimulated activity was 3.45 ± 0.75 pmol cAMP/5 min x mg tissue. The results represent means of 10 separate experiments.

Analysis of the DA competition curves for bound ^{3}H-SCH 23390 revealed an IC_{50}-value of 780 nM and a Hill-coefficient (n_H) of 0.7 (Fig. 5). When adding 10, 30 or 300 μM of GTP; both the IC_{50}- and the n_H increased: IC_{50} = 3.0 μM, n_H = 0.8, IC_{50} = 3.4 μM, n_H = 0.9,

IC_{50} = 3.9 µM, n_H = 1.0 respectively. (Both IC_{50}-and n_H-value were estimated from Hill-plots: log $((V_{max}/V)-1)$ vs log s where V_{max} is the amount of ^{3}H-SCH 23390 displaced by 1 µM of cis-flupentixol, V is the amount of ^{3}H-SCH 23390 binding displaced by the test compound and s is the concentration of the test compound).

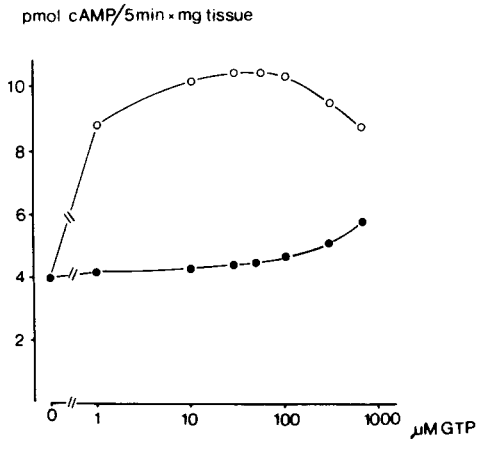

FIG. 4

GTP-dependency of DA-stimulated AC in rat striatum. The concentration of GTP varied from 1 to 500 µM. o---o DA-stimulated (300 µM) AC-levels and •---• unstimulated AC-levels.

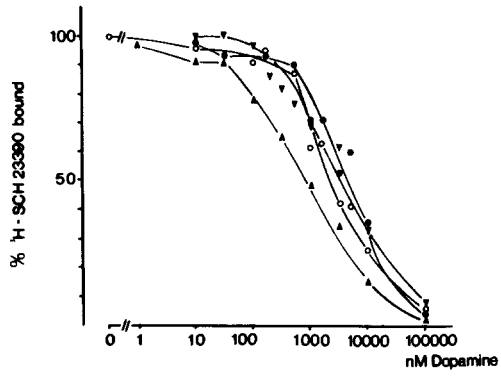

FIG. 5

Influence of GTP on DA displacement of specifically bound ^{3}H-SCH 23390. Increasing concentrations of DA (1-100.000 nM) were allowed to compete with 0.2 nM ^{3}H-SCH 23390 in the absense (▲---▲) or presence of different concentrations of GTP (▼---▼ 10 µM, o---o 30 µM and •---• 300 µM, respectively).

Pharmacological characterization. In order to determine the receptor specificity of the DA-stimulation and ^{3}H-SCH 23390 binding, various pharmacologically active compounds were tested in the assays.

It was readily apparent that the pharmacological characteristics of ^{3}H-SCH 23390 binding and the DA-stimulation were similar (Table II). D2-compounds (e.g. pimozide, domperidone and l-sulpiride) were inactive as displacers or antagonists, while compounds previously known to exhibit D1 or mixed D1-D2 activity, were potent as displacers or antagonists in the ^{3}H-SCH 23390 and AC-assays, respectively. An opposite rank order of potencies was obtained with ^{3}H-spiperone binding (Table II). However, the K_i-values obtained in the AC-assay or from ^{3}H-SCH 23390 binding was apparantly very dif-

Table II

Inhibition of DA-stimulated adenylate cyclase (AC), ^{3}H-SCH 23390 and ^{3}H-spiperone binding.

	K_i(nM)		
Compound	AC	^{3}H-SCH 23390	^{3}H-Spiperone
SCH 23390	39.9[1]	0.14	895
SKF 38393[2] (Km)	190[3]	18	9,300
cis-Flupentixol	40.0[1]	0.32	0.34
trans-Flupentixol	1,200	474	100
Thiothixene		1,520	0.66
Chlorpromazine	463	25	4.6
Phenothiazine		15,630	>20,000
Promethazine		1,780	260
Fluphenazine	212	4.5	0.84
Perphenazine		44	0.95
Thioridazine		21	9.5
Haloperidol		76	2.6
Spiperone	1,720	355	0.11
Pimozide	>5,000	1,420	0.38
Domperidone	>5,000	10,520	0.72
l-Sulpiride	>5,000	>15,000	34
d-Sulpiride		10,900	1,300
Dopamine[2] (Km)	43,000[3]	380	660
Apomorphine[2] (Km)	2,700[3]	87	98
ADTN[2]		5,120	1,370
Pergolide[2]		400	7.8
LY 171555[2]		>5,000	720
3,4-DNH[2] (Km)	57,000[3]	1,140	16,700
Miscellaneous			
(+)-Butaclamol	81.8[1]	0.95	0.90
(-)-Butaclamol	>5,000	>9,000	6,700
Clozapine		55	90
Serotonin	>5,000	>5,000	12,500
Ketanserin		200	400

K_i-values were measured using the relationship $K_i = IC_{50}/(1+(L/K_d))$ where IC_{50} is the drug concentration able to inhibit or displace 50% of the DA-stimulation or specifically bound ^{3}H-SCH 23390/^{3}H-spiperone, respectively. L is the DA or the radioligand concentration (300 µM, 0.19 and 0.05 nM, respectively) used, while K_d is the dissociation constant of DA (43 µM), ^{3}H-SCH 23390 (0.14 nM) or ^{3}H-spiperone (0.08 nM). Displacements studies with agonists were done in the absence of GTP.

[1] values obtained from Schild-plot analysis (Fig. 7).

[2] dopaminergic agonists

[3] efficacy of the agonists were: DA = 1, SKF 38393 = 0.45, apomorphine = 0.7 and 3,4-DHN = 0.3.

ferent. In order to analyze this finding in further detail, competition curves with SCH 23390, cis-flupentixol and (+)-butaclamol were generated for ^{3}H-SCH 23390 binding and Schild-plot analysis of the same three antagonists were performed in the AC-assay.

The Schild-plot analysis of the three antagonists (Fig. 7) revealed K_i-values of 39.9, 40.0 and 81.8 nM, respectively. The slopes were near unity for SCH 23390 and (+)-butaclamol (0.91 ± 0.17, N=6 and 1.09 ± 0.12, N=9) but below 1 for cis-flupentixol (0.77 ± 0.09, N=3), means ± SD).

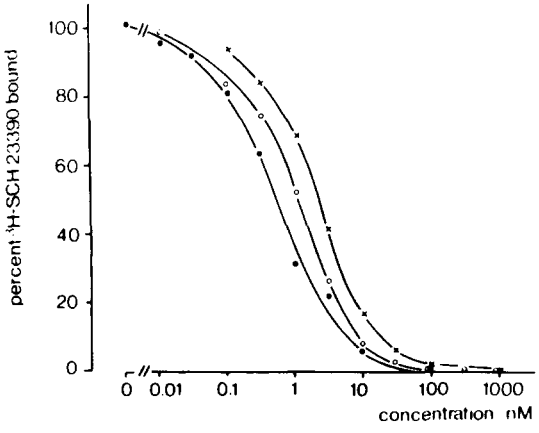

FIG. 6

Displacement of specifically bound ^{3}H-SCH 23390 by SCH 23390 (●---●) cis-flupentixol (o---o) and (+)-butaclamol (x---x). The results represent means of 2-8 separate experiments.

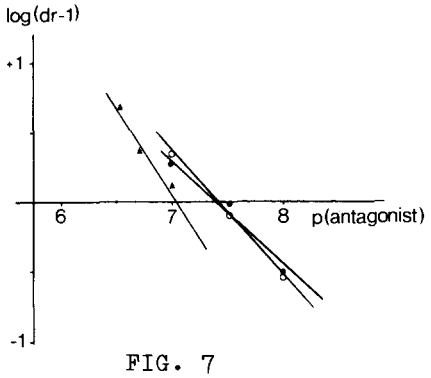

FIG. 7

Schild-plot of the inhibitory action of SCH 23390 (o---o), cis-flupentixol (●---●) and (+)-butaclamol (▲---▲) on DA-stimulated AC in rat striatum. The results represent means of 3-9 separate experiments.
The pA_2-values for the three antagonists were 7.399, 7.398 and 7.087, respectively.

The displacement curves of SCH 23390, (+)-butaclamol and cis-flupentixol (Fig. 6) yielded K_i-values of 0.14, 0.95 and 0.32 nM, respectively. Hill-plot analysis of these data gave n_H-values near unity for the SCH 23390 and (+)-butaclamol displacement curves while the n_H-value for the cis-flupentixol curve was only 0.6, suggesting the presence of two or more binding sites. As observed for other antagonists, the displacement curve of cis-flupentixol was unaffected by GTP.

Computer assisted analysis (equation (2)) of the cis-flupentixol displacement curve yielded the apparent existence of two binding sites with K_d values of 0.02 and 1.51 nM, respectively.

In order to investigate whether exchange of the buffer substance could affect the pharmacological specificity of the binding, displacement of ^{3}H-SCH 23390 binding by (+)-butaclamol and cis-flupentixol were examined when imidazole was substituted with Tris. Interestingly, the IC_{50}-values measured in Tris-buffer were substantially higher than those found when using imidazole-buffer: 10.4 nM vs 0.7 nM (cis-flupentixol) and 5.1 nM vs 2.2 nM ((+)-butaclamol). On the other hand the stereoselectivities of these compounds were unaltered by the buffer substitution.

When substituting imidazole with Tris in the AC-assay, the magnitude of stimulation was decreased but the characteristics were maintained.

Discussion

In agreement with previously published studies, the present data confirm that ^{3}H-SCH 23390 binds specifically to DA D1 receptors (7,8) and, further, that DA stimulation of the AC also occurs via this receptor (3,4). Several facts support that the binding of ^{3}H-SCH 23390 and the DA-stimulation occurs to/or by the D1 receptor. First of all, the rank order of potencies of the presently studied compounds in competing with bound ^{3}H-SCH 23390 or antagonising DA stimulation, indicates that the most potent compounds are also those that have been described previously to possess the highest affinities for D1 receptors (e.g. the thioxanthenes, phenothiazines and (+)-butaclamol). On the converse, the least potent compounds are also those which have been reported to have low affinity for the D1 receptor (e.g., diphenylbutylpiperidines, benzamides and butyrophenones). Secondly, a comparison of the potencies of the compounds in competing for ^{3}H-SCH 23390 binding sites or ^{3}H-spiperone binding sites (D2 receptors) indicates that the binding site for ^{3}H-SCH 23390 is very different from that of ^{3}H-spiperone. Further, the fact that both serotonin and, to a certain extent, ketanserin, were inactive as displacers of bound ^{3}H-SCH 23390 excludes the possibility that the binding of ^{3}H-SCH 23390 was occurring to serotonin receptors.

Scatchard analysis of ^{3}H-SCH 23390 revealed a single binding site in both the striatum and in the prefrontal cortex with B_{max} values of 85.9 $\pm$ 6.0 and 13.4 $\pm$ 2.1 fmol/mg tissue, respectively. The B_{max}

for striatal tissue was within the range previously obtained with the tritiated thioxantines (3,4,5) and with ^{3}H-SCH 23390 itself (7,8).

The K_i-values obtained in ^{3}H-SCH 23390 assay are close to the values previously reported for the D1 receptor binding (3,5,7). On the other hand, the K_i-values obtained in the AC-assay are somewhat higher than those reported previously (3,5,12,13). Further, the observed differences in K_i-values obtained in the two assays were higher (80-240 times) than reported previously (6-10 times) when the assays were run under very different assay conditions (3,5,12). In this connection, it can be noted that the conditions used for ^{3}H-flupentixol/^{3}H-piflutixol binding (3,4,5,13,17) abolish DA-stimulated AC and generally decrease tissue cAMP level as compared to the classical method used for DA-stimulated AC-activity (14), or the method used in the present study (unpublished observations).

The previously reported differences in K_i-values (above) could be explained simply by methodologic differences. However, in the present study, using identical experimental conditions, the differences in K_i-values cannot be explained by differences in methodology, but could be explained by the existence of both a D1-receptor and a SCH 23390 receptor. On the other hand, on the basis of the almost identical pharmacological characteristics of the DA-stimulated AC-activity and the ^{3}H-SCH 23390 binding, it seems unlikely that SCH 23390 and DA could bind to different receptors. More plausible, however, is the possibility that SCH 23390 and DA recognize different states of the same receptor, or that SCH 23390 and DA bind to different sites on, or subunits of, the D1-receptor moiety.

Differences between K_i-values obtained in binding and AC-assays are not a common phenomenon for AC-coupled receptors since this has not been observed for beta-receptor coupled AC's (15,16).

The finding that cis-flupentixol antagonized the DA-stimulation of AC, and displaced bound ^{3}H-SCH 23390 from more than one site, is very surprising. Futher research is needed to clarify the characteristics and location of these sites.

DA dose-dependently increased the AC-activity in the present assay preparation provided that GTP (10-100 μM) was added. These GTP concentrations potently shifted and steepened the DA-displacement curves of ^{3}H-SCH 23390 in agreement with previous reports. However, quite high concentrations of guanine nucleotides have been used previously (17,18) (100-500 μM) to change the agonist competition curve from a shallow two site curve to a steep one site curve (from $n_H<1$ to $n_H=1$). The reason for the higher sensitivity to GTP in the present preparation is "purification" of the preparation (which makes the tissue free of GTP) and thereby abolishing DA-stimulation of the AC. Thus, in conclusion, future work on the regulation of agonist binding should be performed on partially "purified" membrane preparations in order to avoid masking of regulatory effects of added substances by endogeneous factors.

In recently published papers (17,18,19) GTP concentrations as high as 300 μM (or 100 μM of GDP(NH)P) were reported to be unable to

shift the two-site DA-displacements curves of ^{3}H-flupentixol/^{3}H-SCH 23390 to a low affinity one-site curve.

Observations from this laboratory indicate that this shift is both GTP- and Mg^{2+} -dependent. Thus, the Mg^{2+} concentrations (4 mM + 1 mM EDTA) used in these reports seem to be insufficient to promote a full shift in agonist displacement curves, (unpublished observations). The Mg^{2+} concentration used in this study (6 mM + 0.8 mM EGTA; approximately 5.8-5.9 mM free Mg^{2+}) allow a full shift of the curve. Thus, future work with agonist-induced GTP shifts, should include a careful determination of the free concentrations of various cations.

In summary, the present data confirm that ^{3}H-SCH 23390 exhibit pharmacological characteristics similar to DA-stimulated AC by virtue of its binding to D1 receptors.

Acknowledgements

The expert technical assistance of H.F. Bech, L. Michelsen, E. Gottfriedsen and N.Sørensen is highly appreciated. The generous gift of drugs from Dr. J. Arnt, H. Lundbeck A/S, professor P. Fresia, Ravizza Research Laboratories and from Hoechst, Eli Lilly and Sandoz is highly appreciated. We would like to thank Dr. P. Langballe for HPLC purification of ^{3}H-SCH 23390 and Drs. E.B. Nielsen and C. Braestrup for commenting on the manuscript. Finally the secretarial assistance of G. Skou is highly appreciated.

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