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Affinity Chromatography of A_1 Adenosine Receptors of Rat Brain Membranes

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SUMMARY

The A_1 adenosine receptor of rat brain membranes has been solubilized with digitonin and purified approximately 150-fold by affinity chromatography. The digitonin-solubilized receptor, which can be labeled with 8-cyclopentyl-1,3-[^{3}H]dipropylxanthine([^{3}H]DPCPX), was adsorbed on xanthine amine congener (XAC)-linked agarose. The interaction of the solubilized receptor activity with the affinity gel was biospecific. Adenosine agents blocked adsorption of solubilized receptor activity to the XAC-agarose with the appropriate A_1 adenosine selectivity. For agonists, 8-cyclopentyladenosine > (*R*)-phenylisopropyladenosine > CV-1808, whereas, for antagonists, 8-cyclopentyltheophylline (CPT) > XAC > isobutylmethylxanthine=theophylline. The same A_1 adenosine receptor specificity was observed for elution of [^{3}H]DPCPX binding activity from the gel. XAC-agarose adsorbed 65–80% of the solubilized [^{3}H]DPCPX binding activity and, after the gel was washed, 30–40% of the adsorbed activity could be eluted with 100 μM CPT, with specific binding activity of approximately 60 pmol/mg of protein. The order of potency of adenosine agonists [8-cyclopentyladenosine > (*R*)-phenylisopropyladenosine > 5′-*N*-ethylcarboxamidoadenosine > (*S*)-phenylisopropyladenosine] and antagonists (DPCPX > XAC > CPT > isobutylmethylxanthine) with the affinity-purified preparation was found to be similar to that of the solubilized adenosine A_1 receptor. This affinity chromatography procedure should prove to be valuable in the isolation and molecular characterization of A_1 adenosine receptors.

Adenosine exerts physiological effects in both the central nervous system and the periphery, and most of these effects are mediated through receptors on the extracellular surface of cell membranes (1, 2). On the basis of both pharmacological and biochemical studies, the adenosine receptors have been classified into two subtypes, A_1 and A_2 (3, 4). The A_1 receptor mediates an inhibition and the A_2 receptor a stimulation of adenylate cyclase activity. A third receptor subtype, termed A_3, that is directly linked to calcium influx is also proposed (5). The ultimate understanding of the molecular events involved in adenosine transduction will require the eventual purification of the receptor as well as the various other components of the adenosine signal transfer system.

Purification of the A_1 adenosine receptor has not yet been accomplished, mainly due to the lack of suitable tools. Several previous reports on the solubilization and characterization of A_1 receptors from brain and testis membranes (6–10) showed that the receptors can be solubilized with retention of their ligand-binding properties and can interact with guanine nucleotides. Previous attempts to purify A_1 receptors using gel filtration (11) or sucrose density gradient centrifugation (8) did not yield any significant increase of the specific binding activity. Only the report of Ku *et al.* (12) showed about 40-fold enrichment of the adenosine binding sites that bind [^{3}H]PIA, by chromatography on adenosine-6-aminocaproyl AH-Sepharose 4B gel.

In this paper, I describe the synthesis of a new affinity gel and its use in the development of a biospecific affinity chromatography procedure for the A_1 adenosine receptor. This affinity matrix yields approximately 150-fold-purified A_1 receptor preparations from rat brain membranes.

Experimental Procedures

Materials. [^{3}H]DPCPX (95–103 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). Other adenosine agonists and antagonists were purchased from Research Biochemicals Incorp. (Richmond, CA). Guanine nucleotides were purchased from Sigma Chemical Co. (St. Louis, MO). Affi-Gel 10 was a product of Bio-Rad (Richmond, CA). Digitonin was from Gallard-Schlesinger. All other chemicals were from commercial sources.

Preparation of XAC-agarose gel. Coupling of XAC to agarose was carried out as follows. Routinely, 10 ml of Affi-Gel 10, *N*-hydroxysuccinimide esters of a derivatized cross-linked agarose, were washed extensively with dimethylsulfoxide and the moist gel cake was resuspended in 20 ml of dimethylsulfoxide/ethyl alcohol (3:1, v/v) that

ABBREVIATIONS: PIA, N^6-phenylisopropyladenosine; CPA, N^6-cyclopentyladenosine; CPT, 8-cyclopentyltheophylline; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; XAC, xanthine amine congener, 8-[4-[[[[(2-aminoethyl)amino]carbonyl]methyl]oxy]phenyl]-1,3-dipropylxanthine; Gpp(NH)p, guanylylimidodiphosphate; NECA,5′-*N*-ethylcarboxamindoadenosine; CHAPS, 3-[(2-cholamidopropyl)dimethyl-ammonio]-1-propanesulfonate; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*,N′,*N*′-tetraacetic acid.

contained 30 mg of XAC. The coupling reaction was carried out at room temperature for 15 hr with continuous gentle rotation. The reaction was stopped by washing the gel extensively with dimethylsulfoxide. The gel was then washed sequentially with water, 1 M Tris, and water. The washed gel was incubated with 200 mM Tris-acetate buffer, pH 8.0, for 24 hr at 4° to block remaining active esters. Finally, after washing with water, the gel was stored at 4° in 0.01% NaN_3 aqueous solution.

Membrane preparation. Rat frozen whole brains were thawed and homogenized with a Polytron in 3 volumes of 50 mM Tris-acetate buffer, pH 7.2, containing 1 mM EDTA and 1 mM phenylmethanesulfonyl fluoride. The homogenate was centrifuged at 39,000 × *g* for 20 min at 4° and the resulting pellet was washed 3 times in 10 volumes of the same buffer. The washed pellet was resuspended in 3 volumes of the same buffer containing 2 units/ml adenosine deaminase and was incubated for 20 min at 30°, followed by centrifugation at 39,000 × *g* for 20 min at 4°. The final pellet was suspended in 3 volumes of 50 mM Tris-acetate buffer, pH 7.2, and kept frozen at −85° until use.

Membrane solubilization. Frozen brain membrane preparations were thawed and centrifuged at 30,000 × *g* for 20 min at 4°; the pellet was suspended in 10 volumes of 50 mM Tris-acetate buffer, pH 7.2, containing 1% digitonin, 0.1% sodium cholate, 100 mM NaCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol, 0.1 mM phenylmethanesulfonyl fluoride, and 1 μg/ml each of pepstatin A, leupeptin, chymostatin, and antipain. The suspension was gently homogenized by Polytron and stirred on ice for 1 hr. The mixture was then centrifuged at 100,000 × *g* for 1 hr at 4° and the clear supernatant was saved as the solubilized preparation. Longer centrifugations and passage of the solubilized preparation through a 0.22-μm Millipore filter did not decrease receptor binding. The solubilized preparations were used immediately for further purification or stored at −85° until use, without a significant loss of the binding activity.

Affinity chromatography. Routinely, 3 ml of XAC-agarose were packed into a 1 × 4 cm column and equilibrated with 50 mM Tris-acetate buffer, pH 7.2, containing 100 mM NaCl, 10 mM EGTA, and 0.1% digitonin (buffer A). The solubilized preparation (3–5 ml), concentrated by Centricon 10 from 15 ml of the digitonin-solubilized fractions, was loaded at a flow rate of 5 ml/hr, and the column was washed with 5 bed volumes of buffer A at a flow rate of 10 ml/hr. The column was eluted with 5 bed volumes of 100 μM CPT in buffer A at a flow rate of 5 ml/hr. Fractions of 3 ml each were collected. In order to assay the eluted activity, the eluate was desalted on a Sephadex G-50 column (0.6 × 13.5 cm) to separate unbound ligand from the receptor (13). All operations were conducted in a cold room (4–7°). Further details are given in the legend to Fig. 2.

Binding assays. The binding assays for solubilized and affinity-purified preparations were performed as follows, unless otherwise indicated. [^{3}H]DPCPX (2 nM) and receptor preparations were incubated at 0° for approximately 12 hr in 0.25-ml total volume of 40 mM Tris-acetate buffer, pH 7.2, 80 mM NaCl, 4 mM $MgCl_2$, and 0.08% digitonin. Nonspecific binding was determined by the addition of 2 μM XAC. The reaction was terminated by filtration through Whatman GF/B filters pretreated with 0.3% polyethyleneimine (14). The filters were washed three times with 5 ml of cold 50 mM Tris-acetate buffer, pH 7.2, and were counted in 10 ml of Aquasol. All the binding data are given as specific binding unless otherwise stated.

Protein. Protein concentrations were determined by the method of Bradford (15), using bovine serum albumin as a standard. Solutions containing less than 10 μg of protein/ml were assayed by the Amido-Schwarz method (16).

Data analysis. Saturation and displacement curves were analyzed by the computer program EBDA-LIGAND (Elsevier-BIOSOFT).

Results

Affinity chromatography of rat brain A_1 receptor. XAC, which displays a dissociation constant of approximately 1 nM in ligand binding assays (17), was immobilized on activated agarose via an amide linkage. The presumed structure of the XAC-linked agarose is shown in Fig. 1. The prepared gel was stable for at least 6 months at 4°. A chromatographic profile of solubilized receptor from rat brain membranes on XAC-agarose is shown in Fig. 2. Approximately 65–80% of the [^{3}H]DPCPX binding activity adsorbed to the XAC-agarose. In contrast, no significant adsorption of [^{3}H]DPCPX binding activity occurred when the solubilized receptor was passed over Affi-Gel 10 in which the activated groups had been blocked with Tris. After the gel was washed with buffer A, [^{3}H]DPCPX binding activity was eluted by addition of the elution buffer containing 100 μM CPT. With this procedure, 30 ± 10% of the adsorbed activity could be recovered, with 150 ± 50-fold purification (60 ± 20 pmol/mg of protein) compared with the starting solubilized preparation (five experiments). A typical purification scheme is summarized in Table 1. In some experiments, the digitonin-solubilized preparation was applied di-

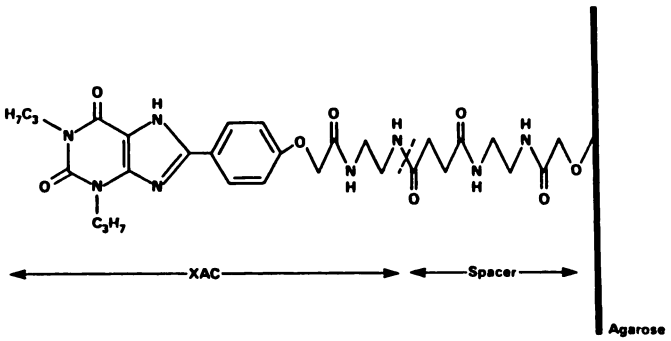

Fig. 1. Presumed structure of the XAC-agarose.

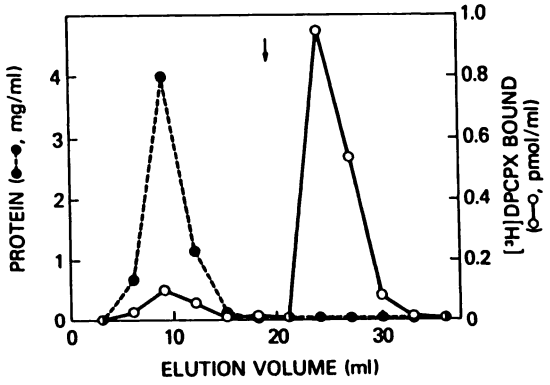

Fig. 2. Affinity chromatography of digitonin-solubilized rat brain A_1 adenosine receptor. The digitonin-solubilized preparation (15 ml) was concentrated by Centricon 10 (Amicon) and the resulting concentrate (3.5 ml) was applied to a 1 × 4 cm column at 4°. The column was washed and eluted as described in Experimental Procedures. The *arrow* indicates the beginning of the elution buffer.

TABLE 1

Purification of A_1 adenosine receptors from rat brain membranes

Results are from one preparation, which is representative of five experiments.

Step	Total Protein	[^{3}H]DPCPX Binding	Specific Binding Activity	Yield
	mg	*pmol*	*pmol/mg of protein*	%
Membranes	87.6	42.0	0.48	100
Solubilized	26.3	10.5	0.40	25
Affinity-purified[a]	0.067	4.0	60	10

[a] Proteins for the affinity-purified fractions were determined by the Amido-Schwarz method (16).

rectly to the affinity column and basically the same results were obtained. However, it was observed that, if the column was extensively washed with more than 10 bed volumes of buffer A before the elution, the yield of [^{3}H]DPCPX binding activity eluted with CPT was always very low (<5%). Addition of dithiothreitol, glycerol, or salts in the washing or elution buffer did not improve the recovery yield of binding activity.

Specificity of XAC-agarose. In order to establish the specificity of XAC-agarose gel, the solubilized preparation of rat brain membranes was incubated with various adenosine receptor agonists and antagonists and applied to the gel. As demonstrated in Fig. 3A, incubation of solubilized preparation with a potent adenosine A_1 agonist, CPA, or potent adenosine A_1 antagonist, CPT and XAC, before exposure to the affinity gel reduced adsorption of [^{3}H]DPCPX binding activity by about 50%. The less active agonists or antagonists such as CV-1808, isobutymethylxanthine, or theophylline, were ineffective at the indicated concentrations.

The specificity of elution from XAC-agarose was also investigated by the elution of bound A_1 receptor with various adenosine receptor agonists and antagonists, as shown in Fig. 3B. The specificity of elution was essentially the same as that for inhibition of adsorption, described above. CPA and CPT were the most effective agents in eluting the bound receptor and CV-1808 and isobutylmethylxanthine were ineffective. About 50% of the bound [^{3}H]DPCPX binding activity was eluted with 10 μM CPT under the conditions employed. The combined results from Fig. 3, A and B, suggest that A_1 adenosine receptor interactions with XAC-agarose are biospecific.

Characterization of the ligand binding properties of the affinity-purified A_1 adenosine receptor of rat brain membranes. Time courses of [^{3}H]DPCPX binding to solubilized and affinity-purified adenosine receptors from rat brain membranes at 25° or 0° are shown in Fig. 4. The specific binding at 25° occurred rapidly and reached its maximum at about 20 min for both solubilized and affinity-purified preparations. However, the binding gradually decreased for both preparations and reached 50% of the maximum level after 2 and 6 hr for the solubilized and affinity-purified preparations, respectively. The specific binding of [^{3}H]DPCPX at 0° occurred relatively slowly and reached equilibrium at about 2 hr for the solubilized preparation and at about 1 hr for the affinity-purified preparation. In contrast to the incubation at 25°, the binding at 0° was stable for at least 12 hr (data for the longer incubation were not shown). When the temperature of the incubation mixture was quickly changed, at 2 hr of incubation, from 0° to 25° or from 25° to 0°, a rapid decrease or an increase, respectively, of the binding was observed at 3 hr, nearly to the same level of binding obtained after incubation at the second temperature initially (Fig. 4B).

The saturation binding of [^{3}H]DPCPX with solubilized and affinity-purified receptor is shown in Fig. 5. Scatchard analysis of [^{3}H]DPCPX binding to the affinity-purified receptor showed a linear relationship and the K_D and B_{max} were approximately 0.57 nM and 56 pmol/mg of protein, respectively. The K_D value was in close agreement with the K_D value obtained for the solubilized receptor under the same conditions (K_D = 0.65 nM and B_{max} = 0.49 pmol/mg of protein for the solubilized receptor). Fig. 6 shows the binding specificity of the affinity-purified preparation. The rank order of potency for agonists in competing for [^{3}H]DPCPX binding agrees with the known specificity

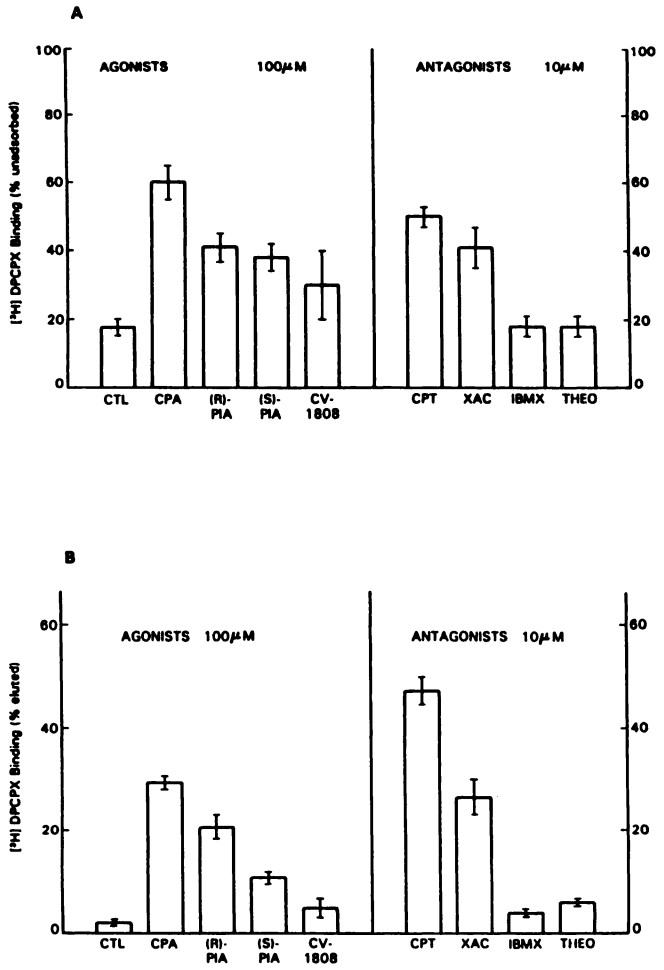

Fig. 3. Biospecific characteristics of XAC-agarose. A, Biospecificity of adsorption. A solubilized preparation (0.5 ml, 0.5 pmol of [^{3}H]DPCPX binding activity) from rat brain membranes was incubated with the indicated concentrations of agonists or antagonists or with buffer A (control, *CTL*) for 3 hr at 0° and then was applied to a 1-ml column. The column was washed with 1.5 ml of buffer A, and the eluate was desalted on Sephadex G-50 for [^{3}H]DPCPX binding. % *unadsorbed* is the amount of specific [^{3}H]DPCPX binding present in the flow-through fraction and the wash as a percentage of the specific [^{3}H]DPCPX binding applied to the column. B, Biospecificity of elution. A solubilized preparation (0.5 ml, 0.5 pmol of [^{3}H]DPCPX binding activity) from rat brain membranes was loaded on a 1-ml column and washed with 2.5 ml of buffer A. The column was then eluted with 2.7 ml of the same buffer containing the indicated concentrations of adenosine agents. The eluate was desalted and assayed for [^{3}H]DPCPX binding. % *eluted* is the specific [^{3}H]DPCPX binding present in the eluate as a percentage of the specific [^{3}H]DPCPX binding applied to the column minus the specific [^{3}H]DPCPX binding that appeared in the wash. Controls (*CTL*) were obtained by elution with buffer A only. The results shown are the average of two experiments. *THEO*, theophylline; *IBMX*, isobutylmethylxanthine.

for A_1 receptors, CPA > (*R*)-PIA > 5′-*N*-ethylcarboxamidoadenosine > (*S*)-PIA. The rank order of potency for antagonists was DPCPX > XAC > CPT ≫ isobutylmethylxanthine, which also agrees with the known ligand specificity for A_1 receptors. The binding specificities of adenosine agonists and antagonists for the affinity-purified and solubilized receptor are compared in Table 2. The IC_{50} values for various antagonists were in close agreement for the purified and solubilized preparations. However, IC_{50} values for agonists were higher for the affinity-purified receptor than for the solubilized receptor, which may

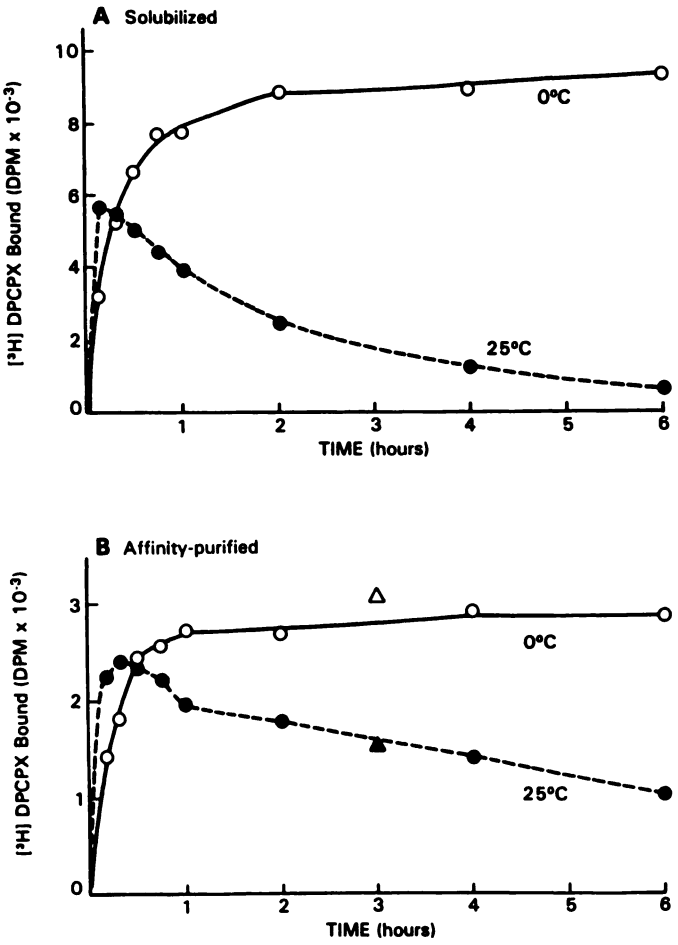

Fig. 4. The time courses of specific [^{3}H]DPCPX binding to solubilized (A) and affinity-purified (B) preparations from rat brain membranes. The solubilized preparation (140 μg of protein) or the affinity-purified preparation (0.3 μg of protein) were incubated with 1.5 nM [^{3}H]DPCPX for the indicated times at the indicated temperatures. △,▲, [^{3}H]DPCPX binding where the temperature of the assay was changed from 0° to 25° (▲) or 25° to 0° (△) at 2 hr.

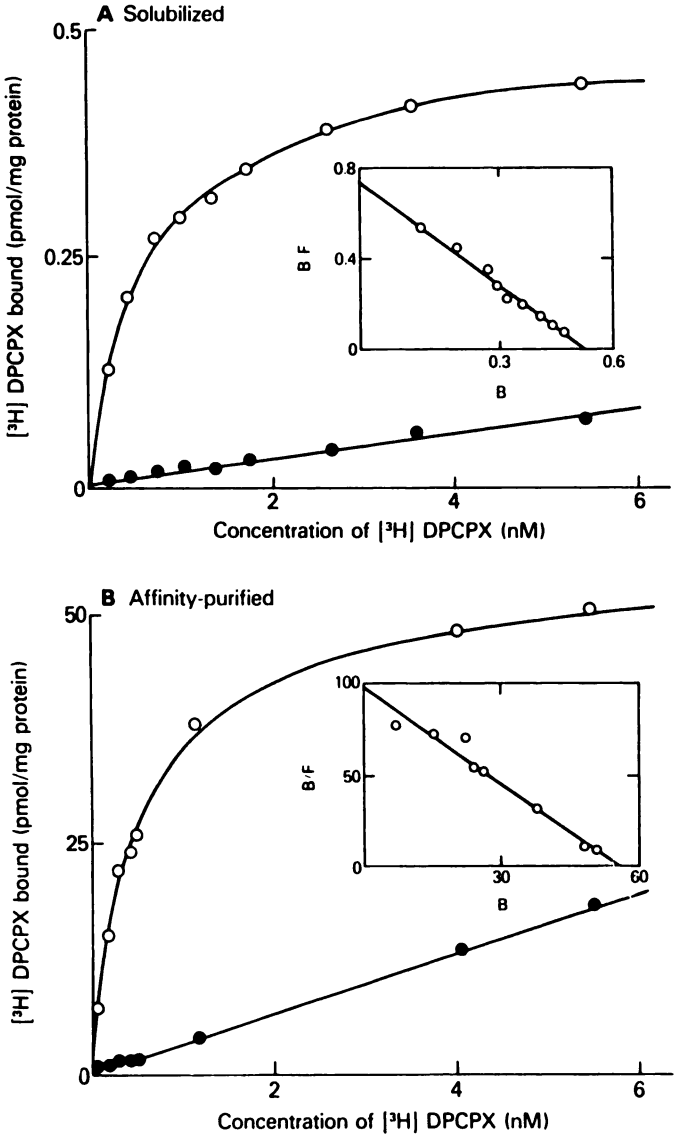

Fig. 5. Saturation isotherms for [^{3}H]DPCPX binding with solubilized (A) and affinity-purified (B) rat brain A_1 adenosine receptors. The receptor binding assay was performed as described in Experimental Procedures. The solubilized A_1 receptors (70 μg of protein) and affinity-purified A_1 receptors (0.4 μg of protein) were assayed with various concentrations of [^{3}H]DPCPX. The K_D and B_{max} values were 0.65 ± 0.07 nM and 0.49 ± 0.09 pmol/mg of protein, respectively, for solubilized preparations (three experiments). The K_D and B_{max} values for affinity-purified preparations were 0.57 ± 0.10 nM and 56 ± 11 pmol/mg of protein, respectively (three experiments). ○, Specific binding; ●, nonspecific binding. *Inset*, Scatchard plots of the same data.

be due to an increased number of low affinity sites in the purified preparation.

Guanine nucleotides, such as GTP and its stable analogue Gpp(NH)p, increased [^{3}H]DPCPX binding by approximately 2-fold and GMP decreased the [^{3}H]DPCPX binding to the solubilized preparations by half, at the concentration of 0.1 mM, as shown in Fig. 7A. Gpp(NH)p was more potent than GTP in the activation effect. EC_{50} values for Gpp(NH)p and GTP were approximately 0.2 and 0.5 μM, respectively. In contrast, neither an increase nor a decrease of [^{3}H]DPCPX binding to the affinity-purified preparations by guanine nucleotides was observed, as shown in Fig. 7B.

[^{3}H]DPCPX binding activity of the affinity-purified preparation was found to be relatively stable. After storage at 4° in the presence of 100 μM CPT/buffer A for 7 days at the protein concentration of 0.01 mg/ml, more than 70% of the original activity remained. When stored for 1 month at −85°, approximately 50% of the original activity was lost.

It was observed that high concentrations of NaCl (0.5–1 M) in the assay mixture caused a significant increase (about 200%) in [^{3}H]DPCPX binding with the affinity-purified preparation, although only a slight increase (about 30%) in [^{3}H]DPCPX binding in the presence of high concentrations of NaCl was observed with the solubilized preparation.

Discussion

The purification of A_1 adenosine receptors has been hampered by the lack of a suitable affinity chromatography matrix. We report here the partial purification of A_1 adenosine receptors from a central nervous tissue (brain) using biospecific affinity chromatography on a newly synthesized agarose matrix that contains an immobilized adenosine antagonist, XAC. Approximately 150-fold purification was obtained by use of a single affinity chromatography step, with an overall recovery

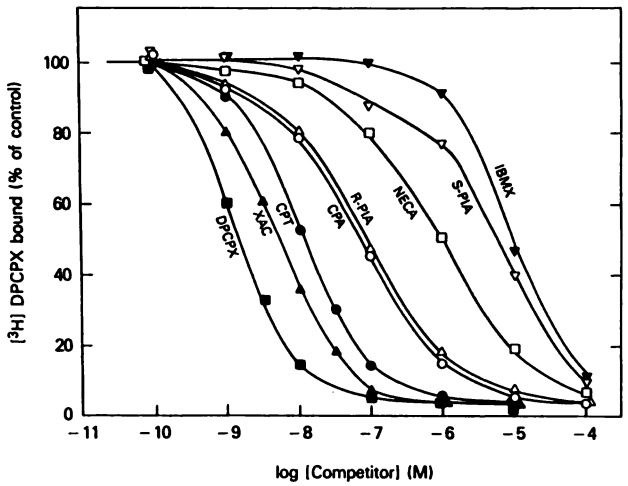

Fig. 6. Specificity of binding of adenosine receptor agonists and antagonists to affinity-purified preparations. Affinity-purified receptor preparations were incubated in the presence of various concentrations of adenosine agents with 1 nM [^{3}H]DPCPX for 12 hr at 0° as described in Experimental Procedures. The data shown are representative of two or three experiments. Total binding activity of these preparations (100% controls) represented approximately 6000 dpm. *IBMX*, isobutylmethylxanthine.

TABLE 2

Inhibition of [^{3}H]DPCPX binding to A_1 adenosine receptors by adenosine agonists and antagonists

The IC_{50} and Hill coefficient (n_H) values were obtained from the computer program EBDA and are means of two or three separate experiments.

Agents	Solubilized		Affinity-purified	
	IC_{50}	n_H	IC_{50}	n_H
	nM		nM	
Agonists				
CPA	3.8 ± 1.0	0.81	71 ± 30	0.78
(*R*)-PIA	4.7 ± 0.7	0.77	87 ± 46	0.82
NECA	18 ± 5.5	0.71	980 ± 320	0.98
(*S*)-PIA	240 ± 53	0.65	6100 ± 2500	0.78
Antagonists				
DPCPX	1.3 ± 0.2	1.0	1.3 ± 0.2	1.1
XAC	4.2 ± 0.5	1.0	4.9 ± 0.9	0.92
CPT	12 ± 5	1.0	9.6 ± 2.3	1.0
IBMX[a]	7100 ± 1400	1.0	7900 ± 1200	0.98

[a] IBMX, isobutylmethylxanthine.

of 10% calculated from rat brain membranes. Although it was demonstrated that this affinity chromatography is biospecific, as shown in Fig. 3, it was noticed that the efficacy of (*R*)-PIA is significantly lower than that of CPA in prohibiting the A_1 receptor binding to the affinity gel after preincubation with these adenosine ligands, as shown in Fig. 3A, in spite of the similarities of the affinity constants for (*R*)-PIA and CPA (Table 2).

The affinity-purified receptor fulfills the following criteria for being an A_1 adenosine receptor. (a) The rank order of potency for adenosine agonists (CPA > (*R*)-PIA > NECA > (*S*)-PIA) and antagonists (DPCPX > XAC > CPT > isobutylmethylxanthine) are similar to those reported for membrane-bound A_1 adenosine receptors (18, 19). (b) The purified receptor retains a stereospecificity with respect to (*R*)- and (*S*)-PIA. (c) The equilibrium dissociation constant (K_D) is in a range similar to that of the membrane-bound and solubilized receptor (Refs. 18 and 19 and this study).

It should be pointed out that the recovery of the receptor binding activity was very low when the affinity column was washed extensively before the specific elution with CPT. Therefore, further purification of A_1 adenosine receptor could not be achieved in this study. One possible reason for the low yields of the receptor activity after the extensive washing was that the purified A_1 receptor was extremely labile. Attempts to stabilize the receptor during and after the elution by including stabilizing agents (dithiothreitol, glycerol, salts), by replacing digitonin with CHAPS, by adding protease inhibitors, or by eluting with other selective agonists and antagonists were all unsuccessful. In fact, the affinity-purified receptor was found to be relatively stable in the presence of its antagonist, CPT, as described in RESULTS, suggesting that the loss of binding activity occurred while the receptor was bound to the affinity gel.

In the time-course experiments shown in Fig. 4B, it was observed that the time-dependent decrease of the [^{3}H]DPCPX binding at 25° was reversed by a rapid change in the temperature from 25° to 0°. This result suggests that the decrease of the binding activity is not due to the instability of the receptor at 25°. It may be explained by conformational changes of the receptors or unknown artifacts of the assay methodology.

The potency of adenosine antagonists in competing for [^{3}H]DPCPX binding was comparable among membrane-bound (18, 19), digitonin-solubilized and affinity-purified preparations, as shown in Fig. 6 and Table 2. In contrast, the potency of adenosine agonists in competing for [^{3}H]DPCPX binding was significantly reduced in the affinity-purified preparation. This is probably due to disruption of the receptor-guanine nucleotide-binding protein interactions, responsible for high affinity receptor-agonist interactions, upon the affinity purification of adenosine receptors. The possibility that the purification by affinity chromatography destabilizes the receptor-guanine nucleotide-binding protein (presumably G_i) interaction was further examined by studying the effects of guanine nucleotides on [^{3}H]DPCPX binding with solubilized and affinity-purified receptors, as shown in Fig. 7. It has already been observed that the binding of radiolabeled adenosine antagonists such as [^{3}H]diethylphenylxanthine and [^{3}H]XAC, with A_1 adenosine receptors of rat hippocampus and adipocyte membranes, respectively, is enhanced by Gpp(NH)p and GTP (20, 21). It has also been shown that this enhancement was dependent on a pertussis toxin-sensitive guanine nucleotide-binding protein (21). In this study, a significant increase in [^{3}H]DPCPX binding by the addition of GTP and Gpp(NH)p was observed with solubilized preparations but neither an increase nor a decrease was observed with affinity-purified preparations. These findings suggest that the influence of GTP and Gpp(NH)p on [^{3}H]DPCPX binding with the solubilized A_1 receptors is likely related to the presence of an endogenous adenosine-liganded A_1 receptor-guanine nucleotide-binding protein complex and that purification of A_1 receptor by the affinity chromatography results in a physical separation, or at least a functional uncoupling, of receptor-guanine nucleotide-binding protein interactions.

The enhancement of [^{3}H]DPCPX binding by high concentrations of NaCl (>0.5 M) with the affinity-purified A_1 receptors was observed in this study. Recently, a similar activation effect of NaCl on antagonist binding with solubilized brain A_1 receptors was reported (22), although it is known that moderate concentrations of sodium ions decrease agonist affinities with brain membrane A_1 receptors (23).

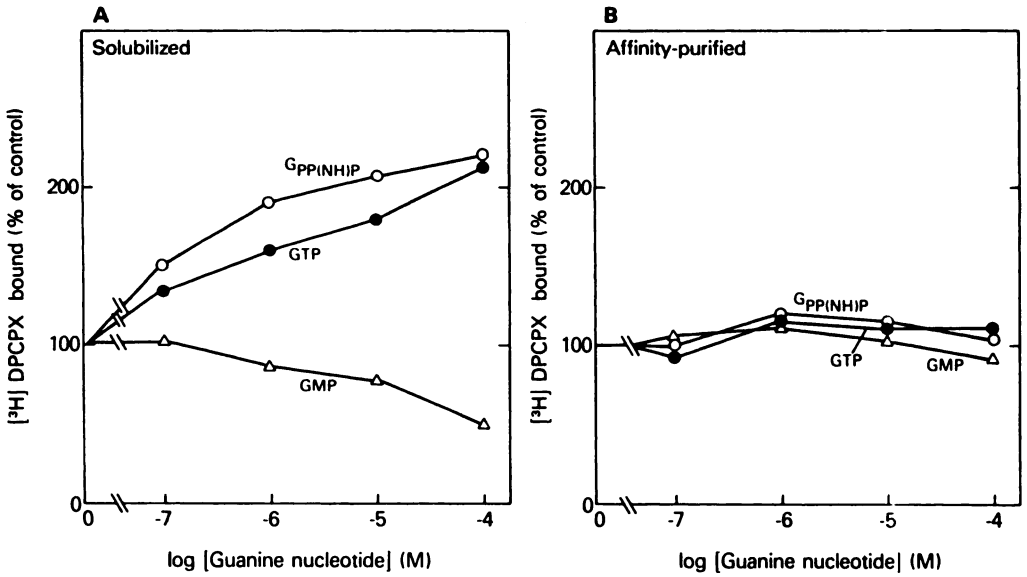

Fig. 7. Effects of guanine nucleotides on the binding of [^{3}H]DPCPX to A_1 adenosine receptors. Solubilized (A) and affinity-purified (B) preparations of A_1 adenosine receptors from rat brains were incubated with [^{3}H]DPCPX and various concentrations of Gpp(NH)p, GTP, or GMP for 12 hr at 0°. Specific [^{3}H]DPCPX binding activity of control (100%) in solubilized and affinity-purified receptors represented approximately 7000 and 5000 dpm, respectively.

The affinity chromatography procedure described here should prove to be a key tool in the eventual purification of the A_1 adenosine receptor. The process by which purification was obtained appears to be biospecific, because interaction of the receptor with the affinity matrix demonstrated appropriate specificity. This method was successfully applied to the partial purification of A_1 adenosine receptors from brain membranes. Because the theoretical specific binding activity of pure A_1 adenosine receptor will be 29 nmol/mg of protein, on the basis of the assumption that the ligand-binding subunit of the receptor resides on a peptide of molecular weight of approximately 35,000 (24–26), an additional 500-fold purification will be necessary to yield homogeneous preparations of A_1 receptor. Other procedures such as lectin chromatography [because it has been suggested that the A_1 adenosine receptor is a glycoprotein (27, 28)] and various types of high performance liquid chromatography procedures should represent a good strategy to obtain pure receptor preparations.

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