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Characterization of the Solubilized A_1 Adenosine Receptor from Rat Brain Membranes

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Abstract: A_1 adenosine receptors from rat brain membranes were solubilized with the zwitterionic detergent 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate. The solubilized receptors retained all the characteristics of membrane-bound A_1 adenosine receptors. A high and a low agonist affinity state for the radiolabelled agonist (*R*)-N^6-[^{3}H]phenylisopropyladenosine([^{3}H]PIA) with K_D values of 0.3 and 12 n*M*, respectively, were detected. High-affinity agonist binding was regulated by guanine nucleotides. In addition agonist binding was still modulated by divalent cations. The solubilized A_1 adenosine receptors could be labelled not only with the agonist [^{3}H]PIA but also with the antagonist 1,3-diethyl-8-[^{3}H]phenylxanthine. Guanine nucleotides did not affect antagonist binding as reported for membrane-bound receptors. These results suggest that the solubilized receptors are still coupled to the guanine nucleotide binding protein N_i and that all regulatory functions are retained on solubilization. **Key Words:** A_1 adenosine receptors—Solubilization—Rat brain membranes. **Klotz K.-N. et al.** Characterization of the solubilized A_1 adenosine receptor from rat brain membranes. *J. Neurochem.* **46**, 1528–1534 (1986).

Adenosine modulates a great variety of physiological functions through membrane-bound receptors coupled to the adenylate cyclase system. Two subtypes of adenosine receptors have been characterized mediating either inhibition (A_1 or R_i) or stimulation (A_2 or R_a) of adenylate cyclase (van Calker et al., 1978; Londos et al., 1980).

A_1 adenosine receptors have been investigated in binding studies with radiolabelled agonists and antagonists in the CNS (Bruns et al., 1980; Schwabe and Trost, 1980) as well as in peripheral tissues (Trost and Schwabe, 1981; Lohse et al., 1985). Agonist affinity for A_1 adenosine receptors is regulated by divalent cations (Goodman et al., 1982; Ukena et al., 1984) and guanine nucleotides (Goodman et al., 1982; Lohse et al., 1984*b*; Ukena et al., 1984), suggesting that a guanine nucleotide binding regulatory protein (N_i) is involved in the coupling to adenylate cyclase (Rodbell, 1980). Elucidation of the molecular mechanism involved in this coupling and biochemical characterization of the structural components will require solubilization of the adenosine receptor system.

There are only few studies on solubilization of A_1 adenosine receptors (Gavish et al., 1982; Nakata and Fujisawa, 1983; Stiles, 1985) with different detergents. Sensitivity of agonist binding to the solubilized receptor to guanine nucleotides was demonstrated by Gavish et al. (1982) and Stiles (1985) whereas enhancement of agonist binding by divalent cations was lost (Gavish et al., 1982). Furthermore, only one affinity state could be demonstrated in the absence of GTP and characterization with an antagonist radioligand has been unsuccessful in all these reports.

In our study we describe A_1 adenosine receptors solubilized with 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) which retained the regulation by guanine nucleotides and divalent cations. The high and the low affinity states of the solubilized receptors were characterized with the agonist [^{3}H]N^6-phenylisopropyladenosine ([^{3}H]PIA). In addition we describe successful labelling of the A_1 adenosine receptor in the soluble state with the antagonist radioligand 1,3-diethyl-8-[^{3}H]phenylxanthine ([^{3}H]DPX).

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Abbreviations used: CHAPS, 3-[3-(cholamidopropyl)dimethylammonio]-1-propanesulfonate; [^{3}H]DPX, 1,3-diethyl-8-[^{3}H]phenylxanthine; GTPγS, guanosine-5′-*O*-(3-thiotriphosphate); NECA, 5′-*N*-ethylcarboxamidoadenosine; PEI, polyethyleneimine; PIA, N^6-phenylisopropyladenosine; [^{3}H]PIA, *R*-N^6-[^{3}H]phenylisopropyladenosine.

MATERIALS AND METHODS

Materials

CHAPS and polyethyleneimine (PEI) were purchased from Sigma, Taufkirchen (F.R.G.). All other materials were purchased as reported previously (Lohse et al., 1984*b*) and were of the highest quality available.

Preparation of rat brain membranes

Rat brain membranes were prepared as described previously by Lohse et al. (1984*a*). To remove endogenous Mg^{2+}, membranes were treated with EDTA as described in the solubilization procedure. After centrifugation membranes were resuspended in 50 m*M* Tris-HCl, pH 7.4 (abbreviated in the following as Tris-HCl) to a final protein concentration of 0.5–1 mg/ml.

Solubilization procedure

Rat brain membranes (about 10 mg/ml protein) were diluted with 10 volumes of Tris-HCl containing 11 m*M* EDTA to remove endogenous Mg^{2+}. After centrifugation (30 min at 39,000 *g*) the supernatant was discharged and the pellet was resuspended in ice-cold Tris-HCl containing 1% CHAPS (solubilization buffer) at a protein concentration of 5–15 mg/ml. After 30 min on ice the extract was diluted with four volumes of ice-cold Tris-HCl and centrifuged at 50,000 *g* for 1 h. The clear supernatant (0.2% CHAPS) was immediately used for binding assay. About 35–40% of membrane protein was extracted by this procedure. If EDTA treatment was omitted the extract could be frozen in liquid nitrogen and stored at −80°C for several months. Protein was determined according to Peterson (1977).

Binding assays

[^{3}H]PIA binding to membranes was measured by a procedure similar to that used in previous studies (Lohse et al., 1984*b*). In a total volume of 250 μl 50–100 μg protein were incubated with 1 n*M* [^{3}H]PIA at different temperatures. After the indicated times incubation was terminated by filtration of a 200-μl aliquot through a Whatman GF/B filter, followed by two washes with 4 ml ice-cold Tris-HCl buffer.

Binding of [^{3}H]PIA to the solubilized receptors was carried out in the same way using 30–50 μg of protein. The incubation was terminated by filtration of a 200-μl aliquot through Whatman GF/B filters treated with 0.3% PEI according to Bruns et al. (1983). Filters were immediately washed twice with ice-cold Tris-HCl buffer as with membranes. The same protocol was used in binding studies with [^{3}H]DPX (10 n*M*) except that the amount of protein was increased to 60–100 μg and only 3 ml of ice-cold buffer were used for washing the filters.

Nonspecific binding was determined in the presence of 1 m*M* theophylline for both [^{3}H]PIA and [^{3}H]DPX binding and amounted to about 5% and 15%, respectively. All binding data are given as specific binding. Experiments were performed in triplicate unless stated otherwise. All incubations were carried out in the presence of 0.2 U/ml adenosine deaminase to remove endogenous adenosine.

Data analysis

Equilibrium binding data were analyzed by nonlinear curve-fitting using the program SCTFIT described by De Lean et al. (1982). Two affinity states were assumed when the corresponding fit was significantly better at the $p < 0.001$ level.

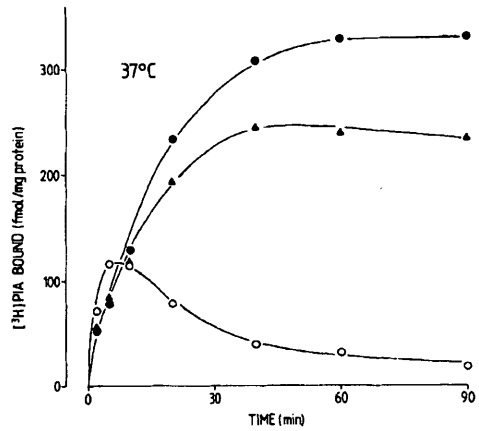

FIG. 1. Association of [^{3}H]PIA to solubilized adenosine receptors at 37°C. The association was measured in the absence (○) and in the presence of 1 m*M* (▲) and 10 m*M* (●) $MgCl_2$.

RESULTS

The time course of [^{3}H]PIA binding to solubilized adenosine receptors from rat brain at 37°C showed that equilibrium binding could be reached in the presence of 10 m*M* Mg^{2+} but not in the absence of Mg^{2+} (Fig. 1). Mg^{2+} appeared to have a stabilizing effect on binding of the radioligand. To achieve equilibrium binding also in the absence of Mg^{2+} we lowered the temperature to 12°C. As shown in Fig. 2 stable [^{3}H]PIA binding was achieved at this temperature after about 2 h for at least 6 h. After 24 h binding was decreased to about 70% of the equilibrium level. Thus, after long incubation times a small decrease in binding was also observed at

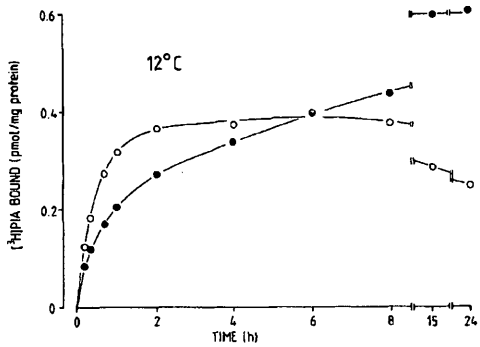

FIG. 2. Association of [^{3}H]PIA to solubilized adenosine receptors at 12°C. The association was measured in the absence of (○) and in the presence of (●) of 10 m*M* $MgCl_2$.

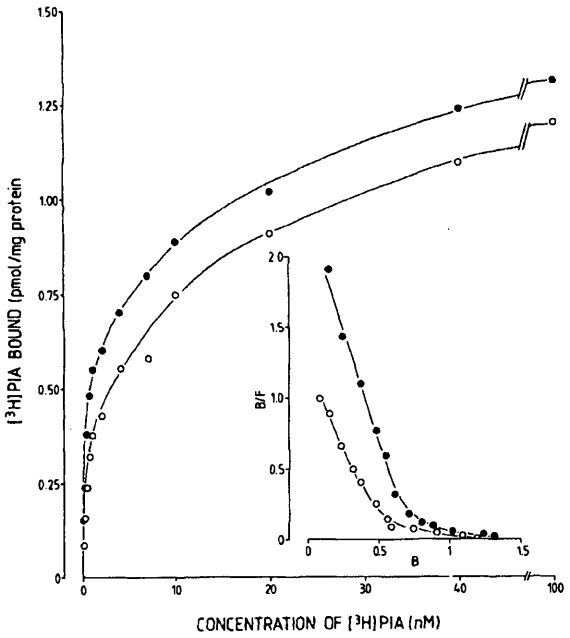

FIG. 3. Saturation of [^{3}H]PIA binding to solubilized A_1 receptors. Specific [^{3}H]PIA binding was determined after incubation for 3 h in the absence (○) and for 15 h in the presence (●) of 10 m*M* $MgCl_2$ at 12°C. Values are the means of two experiments performed in quadruplicate. The Scatchard plots of the data are shown in the **inset**. K_D and B_{max} values obtained from nonlinear curve-fitting were: in the absence of $MgCl_2$ 0.34 n*M* and 0.44 pmol/mg protein for the high-affinity state and 12.4 n*M* and 0.83 pmol/mg protein for the low-affinity state, respectively. In the presence of 10 m*M* $MgCl_2$ the values were 0.20 n*M* and 0.56 pmol/mg protein for the high-affinity state and 13.6 n*M* and 0.76 pmol/mg protein for the low-affinity state, respectively.

12°C. Mg^{2+} increased [^{3}H]PIA binding at 12°C both by stabilization of the solubilized receptors and a regulatory enhancement. Mg^{2+} reduced the association rate at both temperatures which was not observed with membranes.

Saturation experiments with [^{3}H]PIA resulted in biphasic curves both in the presence and the absence of 10 m*M* Mg^{2+} (Fig. 3). Scatchard plots from the saturation data were curvilinear, suggesting two affinity states. In the absence of Mg^{2+} the dissociation constants at 12°C for the high- and low-affinity state were 0.34 n*M* and 12.4 n*M*. The corresponding B_{max} values were 0.44 pmol/mg protein and 0.83 pmol/mg protein. Determination in the presence of 10 m*M* Mg^{2+} gave K_D values of 0.20 n*M* and 13.6 n*M*, with B_{max} values of 0.56 and 0.76 pmol/mg protein for the high- and low-affinity states, respectively. Thus, magnesium induced an increase of the portion of the high-affinity state with only minor effects on the K_D values.

Further characterization of the solubilized adenosine receptors was achieved in competition experiments. Various compounds known as agonists and antagonists at adenosine receptors were used to compete for [^{3}H]PIA binding. The K_i values in Table 1 demonstrate the typical features of an A_1 subtype with the high stereoselectivity of PIA, with the *R*-isomer being the more potent, and 5′-*N*-ethylcarboxamidoadenosine (NECA) being less potent than *R*-PIA. The K_i values for agonists obtained with solubilized A_1 receptors agree well with those determined in membranes; for antagonists there was a slight difference, with the antagonists being less potent at solubilized receptors.

TABLE 1. *Comparison of* K_i *values for [^{3}H]PIA binding to solubilized and membrane-bound adenosine receptors*

Compound	K_i (n*M*)	
	Solubilized	Membrane-bound
R-PIA	0.59 (0.48–0.73)	0.40 (0.19–0.84)
CHA	0.65 (0.57–0.74)	0.48 (0.12–1.92)
NECA	2.13 (1.98–2.30)	3.09 (2.39–4.00)
2-Chloroadenosine	2.62 (1.84–3.75)	4.77 (3.14–7.25)
S-PIA	31.5 (21.2–46.8)	61 (27–136)
8-Phenyltheophylline	72 (63–82)	25 (15–40)
3-Isobutyl-1-methylxanthine	2,060 (946–4,478)	428 (280–654)
Theophylline	2,800 (2,090–3,750)	1,640 (1,440–1,870)

Solubilized and membrane-bound adenosine receptors were incubated with 1 n*M* [^{3}H]PIA at 12°C for 3 h as described under Materials and Methods. Seven concentrations of the various agonists and antagonists were used for competition. At the [^{3}H]PIA concentration used binding was detected to the high-affinity state only, and therefore, data were fitted assuming a one-site model. K_i values are shown as geometric means of three experiments performed in triplicate with the 95% confidence limits in parentheses. CHA, cyclohexyladenosine.

To evaluate the regulation of [^{3}H]PIA binding by divalent cations we compared the effects of Mg^{2+}, Ca^{2+}, and Mn^{2+} on agonist binding to solubilized and membrane-bound receptors at 12°C and 37°C. Figure 4 shows that Mg^{2+} and Ca^{2+} raised binding in exactly the same manner under all conditions, whereas Mn^{2+} had a different effect. In membranes Mg^{2+} and Ca^{2+} up to 10 m*M* enhanced [^{3}H]PIA binding at both temperatures whereas Mn^{2+} induced a similar increase of binding at concentrations up to 0.3 m*M* but then decreased it. Mn^{2+} was slightly more potent than the other two cations at both temperatures. As already shown in Fig. 1, higher concentrations of Mg^{2+} stabilized [^{3}H]PIA binding to solubilized receptors at 37°C. Therefore, at 37°C a greater enhancement of [^{3}H]PIA binding by Mg^{2+} was detected for solubilized than for membrane-bound receptors (Fig. 4). Mn^{2+} enhanced binding in a similar manner but decreased it above 0.2 m*M*. At 12°C all cations tested enhanced [^{3}H]PIA binding with similar potencies. Mg^{2+} and Ca^{2+} had a maximal effect already at 100 μ*M*. Mn^{2+} decreased [^{3}H]PIA binding at concentrations above 50 μ*M*.

One reason for enhanced [^{3}H]PIA binding to A_1 adenosine receptors in the presence of Mg^{2+} (or Ca^{2+}) could be the activation of a GTPase by these

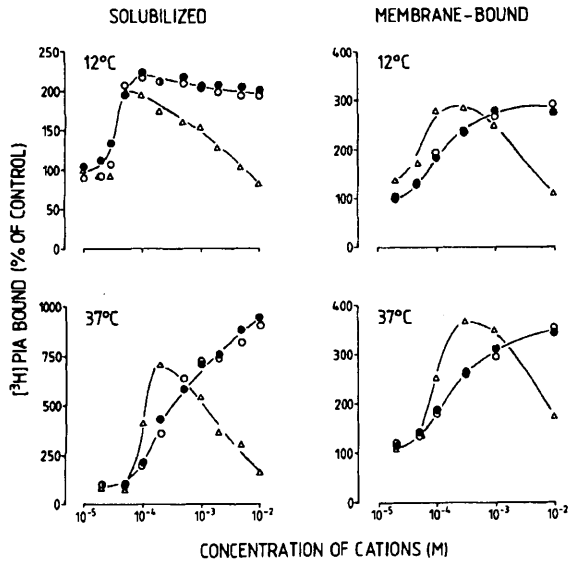

FIG. 4. Effects of divalent cations on [^{3}H]PIA binding to membrane-bound and solubilized A_1 adenosine receptors. [^{3}H]PIA was incubated in the presence of $MgCl_2$ (●), $CaCl_2$ (○), and $MnCl_2$ (△) under the following conditions (control binding is given in parentheses): Solubilized receptors were incubated for 15 h at 12°C (300 fmol/mg protein) and for 45 min at 37°C (25 fmol/mg protein); membrane-bound receptors were incubated for 4 h at 12°C (100 fmol/mg protein) and for 1 h at 37°C (80 fmol/mg protein).

cations (Childers and Snyder, 1980). As GTP decreased agonist binding (see below) activation of a GTPase would increase it by removing endogenous GTP. To test this hypothesis we did the kinetic experiment shown in Fig. 5. As the effects of GTPases can be expected to be more prominent in membranes, we performed this experiment with membrane-bound receptors. It shows that the effect of Mg^{2+} was also seen when Mg^{2+} was added after establishment of equilibrium in the absence of Mg^{2+}. EDTA had no effect of its own, but the enhancement of binding by Mg^{2+} was almost completely reversed by addition of EDTA. This reversibility would not have occurred if Mg^{2+} had induced the breakdown of endogenous GTP.

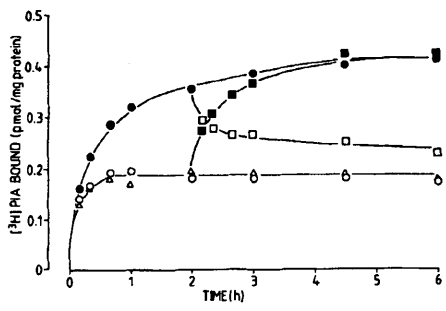

FIG. 5. Effects of Mg^{2+} and EDTA on [^{3}H]PIA binding to rat brain membranes. [^{3}H]PIA binding was determined in a control time course (○), in the presence of 10 m*M* EDTA (△), and in the presence of 1 m*M* $MgCl_2$ (●) at 25°C. After 2 h $MgCl_2$ was added to a second control incubation to a final concentration of 1 m*M* (■). At the same time EDTA was added to a final concentration of 10 m*M* to an incubation started with 1 m*M* $MgCl_2$ (□).

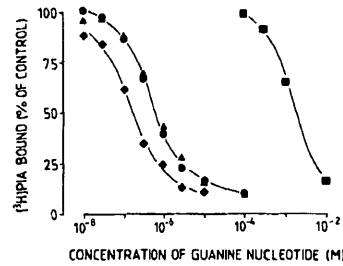

FIG. 6. Regulation of agonist binding to solubilized adenosine receptors by guanine nucleotides. Solubilized receptors were incubated with [^{3}H]PIA and various concentrations of GTPγS (◆), GTP (●), GDP (▲), and GMP (■) for 3 h at 12°C.

As in membranes, guanine nucleotides inhibited [^{3}H]PIA binding to solubilized adenosine receptors (Fig. 6). The metabolically stable GTP analogue guanosine-5′-*O*-(3-thiotriphosphate) (GTPγS) was the most potent with an IC_{50} value of 0.2 μ*M;* GTP and GDP showed equal IC_{50} values of 0.6 μ*M* whereas GMP was considerably less potent (IC_{50} at 2.1 m*M*). The inhibitory effect of guanine nucleotides on [^{3}H]PIA binding in membranes was reversed by addition of Mg^{2+} (Goodman et al., 1982; Ukena et al., 1984). This effect was also observed with receptors in the soluble state. Mg^{2+} shifted the IC_{50} values for guanine nucleotide inhibition of [^{3}H]PIA binding to higher values (Table 2).

Recent studies on solubilized adenosine receptors (Gavish et al., 1982; Stiles, 1985) were not successful in binding a radiolabelled antagonist. Therefore, we tried binding of the antagonist [^{3}H]DPX in our system. Saturation experiments were carried out in the absence and in the presence of GTP. Figure 7 shows that the solubilized receptors bound [^{3}H]DPX in a saturable manner which was not affected by GTP. In each case Scatchard plots were linear (Fig. 7, inset), indicating a homogeneous population of binding sites. In the

TABLE 2. *IC_{50} values for guanine nucleotide inhibition of [^{3}H]PIA binding to solubilized A_1 adenosine receptors*

Guanine nucleotide	IC_{50} (μ*M*)	
	Control	10 m*M* $MgCl_2$
GTPγS	0.2	12
GTP	0.6	140
GDP	0.6	170

[^{3}H]PIA was incubated with CHAPS extracts at 12°C in the presence of various concentrations of guanine nucleotides and in the absence and presence of 10 m*M* $MgCl_2$ until the respective equilibrium was reached (15 h in the presence of $MgCl_2$ and 3 h in control incubations; an incubation time of 15 h in control incubations did not alter the IC_{50} values).

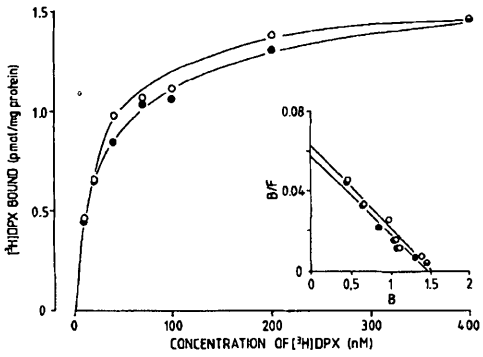

FIG. 7. Saturation of [^{3}H]DPX binding to solubilized A_1 receptors. Specific [^{3}H]DPX binding was determined after incubation for 1 h in the absence (○) and in the presence of 100 μM GTP (●) at 12°C. Each curve represents the means of two separate experiments performed in quadruplicate. The Scatchard plot **(inset)** of the same data gave K_D values of 28 nM in the absence and 25 nM in the presence of GTP with a B_{max} value of 1.5 pmol/mg protein for both curves.

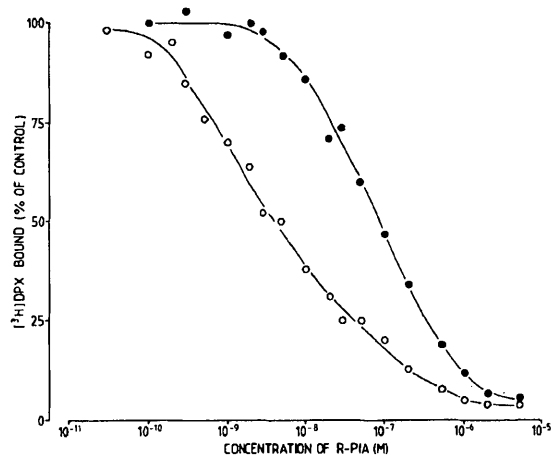

FIG. 8. Competition for [^{3}H]DPX binding to solubilized adenosine receptors by *R*-PIA. Competition of *R*-PIA for [^{3}H]DPX binding was determined in the absence (○) and in the presence (●) of 100 μM GTP. CHAPS extracts were incubated for 3 h at 12°C. In the absence of GTP, analysis of the curves (see Materials and Methods) gave two affinity states with K_D values of 0.34 nM and 27 nM (B_{max} 0.63 pmol/mg protein and 0.65 pmol/mg protein). In the presence of GTP only one affinity state could be detected with a K_D value of 46 nM (B_{max} 1.35 pmol/mg protein).

absence of GTP a K_D value of 28 nM and in the presence of 100 μM GTP of 25 nM was determined. The estimation of B_{max} values gave about 1.5 pmol/mg protein in each case.

Agonist binding was further characterized by measuring the competition of *R*-PIA for [^{3}H]DPX binding (Fig. 8). From the biphasic competition curve a K_D value for *R*-PIA for the high-affinity state of 0.34 nM with a B_{max} of 0.63 pmol/mg protein was estimated. The respective values for the low-affinity site were 27 nM and 0.65 pmol/mg protein.

When the competition experiment was carried out in the presence of 100 μM GTP, monophasic curves were obtained, indicating a single affinity state with a K_D value of 46 nM and a B_{max} value of 1.35 pmol/mg protein.

DISCUSSION

Adenosine receptors solubilized with the zwitterionic detergent CHAPS have been characterized in the present study. They clearly showed all the characteristics typical for the A_1 subtype: first, the stereoselectivity for PIA isomers; second, the higher potency of *R*-PIA compared to NECA; and third, a high affinity K_D value for *R*-PIA in the nanomolar range. The pharmacological characteristics of the solubilized receptors agreed well with those of membrane-bound receptors.

In the absence of divalent cations [^{3}H]PIA binding is not stable at 37°C. This result is in accordance with a previous report from Nakata and Fujisawa (1983). There are two ways to achieve stable receptor solutions, either to reduce incubation temperature to 12°C, or to add Mg^{2+}, which exerts beside its regulatory function (see below) a stabilizing effect on [^{3}H]PIA binding.

In membranes there is evidence for two affinity states of the A_1 adenosine receptor (Lohse et al., 1984*b*; Ukena et al., 1984). These could also be detected in our CHAPS extracts. Gavish et al. (1982) and Nakata and Fujisawa (1983) reported only a high-affinity state for the solubilized receptors. The different methods for separating bound and free radioligand could be the reason for this discrepancy. We used the filtration assay with PEI-treated GF/B filters developed by Bruns et al. (1983), which is superior to precipitation by polyethyleneglycol, resulting in the detection also of the low-affinity state.

For membrane-bound A_1 adenosine receptors a selective inhibition of agonist binding by GTP, GDP, and nonmetabolized analogs of GTP such as GTPγS has been demonstrated in contrast to antagonist binding, which is not affected (Goodman et al., 1982; Lohse et al., 1984*b*; Ukena et al., 1984). Solubilization with CHAPS preserves the sensitivity to guanine nucleotides as reported for sodium cholate (Gavish et al., 1982) and digitonin extracts (Stiles, 1985). The IC_{50} values for inhibition of agonist binding in the range of 1 μM resemble those reported by Gavish et al. (1982).

Agonist binding to membrane-bound A_1 adenosine receptors is regulated by divalent cations (Goodman et al., 1982; Ukena et al., 1984). Two reasons appeared to be responsible for the enhanced [^{3}H]PIA binding to the solubilized receptors in the presence of Mg^{2+} or Ca^{2+}. First, these cations prevented loss of binding activity, and

second, there is a regulatory enhancement of agonist binding. At 37°C the stabilizing effect of Mg^{2+} dominates. At 12°C, where inactivation plays only a minor role, the regulatory enhancement could be demonstrated. Assuming that the loss of [^{3}H]PIA binding between 8 and 24 h incubation time at 12°C represents an exponential decay (Fig. 2) one can estimate an equilibrium binding of about 0.44 pmol/mg protein if there were no decay (compared to about 0.40 pmol/mg protein measured in the binding assay). Thus, there remained an enhancement by 10 m*M* Mg^{2+} of about 40% above the estimated equilibrium binding. This demonstrates that regulation by Mg^{2+} was maintained during solubilization. In another report about solubilized adenosine receptors this regulation was lost (Gavish et al., 1982). It is thought that divalent cations augment agonist binding through interactions with the N_i protein (Rodbell, 1980). Because some receptors retain on solubilization only their regulation by guanine nucleotides but not by cations (Gavish et al., 1982; Matsui et al., 1985) it was assumed that distinct subunits of N_i bind guanine nucleotides and cations and that some receptors retain only the guanine nucleotide binding but not the cation binding subunit. In our CHAPS extract the entire regulation by both guanine nucleotides and cations was retained, suggesting that functional receptor–N_i complexes were solubilized from rat brain. In experiments with solubilized bovine brain adenosine receptors at 12°C we were not able to detect enhancement of [^{3}H]PIA binding with Mg^{2+} (1 m*M*) in accordance with the results of Gavish et al. (1982) (data not shown). However, Mg^{2+} reduced the association rate compared to Mg^{2+}-free control associations as shown for CHAPS extract from rat brain (Fig. 2). After equilibrium was reached (about 5 h without and 15 h with Mg^{2+}) there was no difference in the extent of [^{3}H]PIA binding to solubilized receptors from bovine brain in the presence and absence of Mg^{2+}. The slight inhibition using the conditions of Gavish et al. (1982) (2 h incubation at 25°C) could be reproduced and results presumably from the fact that Mg^{2+}-free controls are already in equilibrium whereas samples containing Mg^{2+} are not. There is no explanation for the slower association rate of [^{3}H]PIA to soluble receptors in the presence of Mg^{2+}.

Divalent cations were reported to reverse the GTP-induced inhibition of agonist binding to membrane-bound receptors (Goodman et al., 1982; Ukena et al., 1984). The shift of IC_{50} values for GTP inhibition of [^{3}H]PIA binding by Mg^{2+} in solubilized receptors was in accordance with these results. Reversal of the GTP effect on agonist binding to opiate receptors by divalent cations was attributed to the action of divalent cation-stimulated phosphatases (Childers and Snyder, 1980). The same argument could be used to explain the Mg^{2+}-induced enhancement of agonist binding in the absence of exogenous GTP, namely that Mg^{2+} acted by removing endogenous GTP. There are two lines of evidence against this hypothesis being true in our system. First, Mg^{2+}-induced enhancement of [^{3}H]PIA binding could be reversed by removing Mg^{2+} by EDTA addition (Fig. 5). Second, Mg^{2+} shifted not only IC_{50} values for GTP and GDP inhibition of [^{3}H]PIA binding but also that for the metabolically stable GTP analogue GTPγS (Table 2).

Earlier studies of solubilized A_1 adenosine receptors did not succeed in binding the radiolabelled antagonist [^{3}H]DPX (Gavish et al., 1982; Stiles, 1985). In contrast to these findings, with our solubilization procedure we observed binding of this antagonist. Again, the PEI-filter assay enables measurement of radioligand binding in spite of a relatively high K_D of 28 n*M*. As with membrane-bound receptors this K_D is not affected by GTP (25 n*M* in the presence of 100 μ*M* GTP).

With the use of [^{3}H]DPX binding we could evaluate further the effect of GTP on agonist binding. In the absence of GTP a high- and a low-affinity state for the agonist was detected as in saturation experiments. Addition of GTP converted all receptors into the low-affinity state. These are further results in close agreement with the data from membrane-bound receptors (Goodman et al., 1982; Lohse et al., 1984*b*).

In summary, the present study demonstrates A_1 adenosine receptors solubilized with CHAPS from rat brain membranes that retain the characteristic features reported for membrane-bound receptors. Agonist binding was regulated both by guanine nucleotides and divalent cations. In addition, this is the first report of successful labelling of solubilized adenosine receptors with an antagonist radioligand.

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