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Interaction of Full and Partial Agonists of the A_1 Adenosine Receptor with Receptor/G Protein Complexes in Rat Brain Membranes

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SUMMARY

Full and partial agonists of the A_1 adenosine receptor were characterized with respect to their influence on G protein activation and their thermodynamic parameters of receptor binding in rat brain membranes. G protein activation was determined through measurement of [^{35}S]guanosine-5′-(γ-thio)-triphosphate ([^{35}S]GTP[S]) binding, and receptor binding was studied under identical conditions through the displacement of [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) in equilibrium binding studies. The intrinsic activity in stimulating [^{35}S]GTP[S] binding did not correlate with the affinity of the ligands. 5′-Deoxy-5′-methylthioadenosine, 2-phenylaminoadenosine, and 2-chloro-2′-deoxyadenosine were identified as partial A_1 agonists in the G protein activation assay. Depending on the temperature, these ligands showed agonistic and antagonistic properties to varying extents. EC_{50} values for G protein stimulation and K_H and K_L values of the partial agonists decreased when the incubations were performed at lower temperatures, indicating a mainly enthalpy-driven process of interaction with the receptor. Thermodynamic parameters of receptor binding of the partial agonists resembled the characteristics of the antagonist DPCPX more closely than those of the agonist 2-chloro-N^6-cyclopentyladenosine. In addition, partial agonists detected fewer A_1 adenosine receptors in the high affinity state than did full agonists. The lower efficacy in stimulation of the binding of [^{35}S]GTP[S] is probably the consequence of an impaired ability of the partial agonists to release GDP from the G protein, as was shown by an impaired release of prebound [^{35}S]GDP[S] from the membranes.

Agonists and antagonists are defined by their ability or inability to induce a response after binding to a receptor. Partial agonists have aroused increasing interest due to their ambiguous ability to act as either antagonist or agonist, depending on the physiological or experimental settings (1–3). However, intrinsic activity cannot be defined as a characteristic of the ligand alone because it also depends on spare receptors and on the G protein or second messenger system that is evaluated (2–6).

Differentiation of agonist or antagonist properties of a ligand can be performed through investigation of responses to receptor occupancy. In addition, agonists and antagonists show different characteristics in ligand binding to G protein-coupled receptors. Agonists and antagonists can also be differentiated on the basis of their thermodynamic binding parameters, as the changes in entropy or enthalpy in agonist binding are usually ascribed to a conformational change of the receptor protein from an inactive to an active conformation, which, in turn, activates the respective G protein (7–10). Agonists and antagonists of A_1 adenosine receptors induce different conformations of the receptor (11). In a recently developed model (12), receptors may exist in an inactive conformation (R) or in a spontaneously active form (R^*). Although antagonists bind equally well to R and R^*, agonists preferably bind to R* and increase the concentration of R^*. Therefore, thermodynamic parameters would reflect the ability to increase R^* and may, with some precautions, be used for classification of ligands as neutral or inverse antagonists or as full or partial agonists.

Information is relatively scarce about partial agonists at A_1 adenosine receptors in the nervous system, which modulate a great variety of second messenger systems, e.g., adenylyl cyclase (13, 14), potassium channels (15), calcium channels (16), and phospholipase C (17). Intrinsic activities of A_1 adenosine receptor ligands have been measured mainly in adenylyl cyclase studies (18). Thermodynamic studies indicate that agonist binding to A_1 adenosine receptors is an entropy-driven process, whereas antagonist binding is en-

ABBREVIATIONS: GTP[S], guanosine-5′-(γ-thio)-triphosphate; CCPA, 2-chloro-N^6-cyclopentyladenosine; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; CIA, 2-chloroadenosine; CPT; 8-cyclopentyltheophylline; CSC, 8-(3-chlorostyryl)caffeine; CV 1808, 2-phenylaminoadenosine; DPCPX, 8-cyclopentyl-1,3-dipropylxanthine; GDP[S], guanosine-5′-(β-thio)-diphosphate; MeSA, 5′-deoxy-5′-methylthioadenosine; MIG, 1-methylisoguanosine; NECA, 5′-*N*-ethylcarboxamidoadenosine; PIA, N^6-phenylisopropyladenosine.

thalpy or enthalpy and entropy driven (18–21). Partial agonists show thermodynamic binding parameters and efficacies that are intermediate between those of full agonists and antagonists (18). Moreover, it has been suggested that the affinity and efficacy of A_1 adenosine receptors are closely correlated (18), which would mean that molecular mechanisms regulating the affinity of the ligand at adenosine receptors would also play a critical role in ligand efficacy. However, it is generally assumed that ligand affinity and efficacy are not related (4). Results of our previous study of G protein activation by adenosine receptor agonists (22) do not support the correlation between affinity and intrinsic activity of adenosine receptor ligands.

However, as pointed out above, such differences must be considered carefully because differences in experimental settings may be crucial for the classification of the intrinsic activity of a ligand. Therefore, we decided to investigate the binding of A_1 adenosine receptor ligands and the initiation of response under identical conditions. The most important action of adenosine in the central nervous system, the inhibition of transmitter release, proceeds independent of adenylyl cyclase (23), and intrinsic activity of A_1 receptor ligands as determined with adenylyl cyclase studies in brain possibly would not predict efficacies for the physiologically relevant action of adenosine. The A_1 adenosine receptor in brain is coupled to a set of G proteins (24). Therefore, G protein activation determined by [^{35}S]GTP[S] binding (22), which most probably detects a composite response, rather than measurement of adenylyl cyclase was chosen as the parameter to indicate the efficacy of ligands.

Experimental Procedures

Materials. [^{35}S]GTP[S] (1000–1500 Ci/mmol), [^{35}S]GDP[S] (1000–1500 Ci/mmol), [^{3}H]DPCPX (80–120 Ci/mmol), and anti-G protein antisera from rabbits (GC/2, amino terminus of α_o; AS/7, carboxyl terminus of $\alpha_{i1,\ 2}$; EC/2, carboxyl terminus of α_o and α_{i3}) were purchased from New England Nuclear (Bad Homburg, Germany). CCPA, CPT, CSC, CV 1808, MIG, and NECA were obtained from Research Biochemicals (Cologne, Germany). Adenosine deaminase (from calf intestine; 200 units/mg) and GDP were obtained from Boehringer-Mannheim (Mannheim, Germany). GTP[S], ClA, cladribine (2-chloro-2′-deoxyadenosine), MeSA, CHAPS, bovine serum albumin, theophylline, and dithiothreitol were purchased from Sigma (Deisenhofen, Germany). All other chemicals were obtained from standard sources and of the highest purity commercially available.

Preparation of rat brain membranes. Membranes from rat forebrains were prepared as described previously (22). Protein content was determined according to the method of Peterson (25). Bovine serum albumin was used as standard protein.

[^{35}S]GTP[S] binding to rat brain membranes. [^{35}S]GTP[S] binding was measured according to a reported method (22). Briefly, 2 μg of membrane protein was incubated in a total volume of 100 μl for 2 hr at 25°, 2.5 hr at 20°, or 3 hr at 10° in a shaking water bath. [^{35}S]GTP[S] binding in control incubations and in the presence of agonists showed a linear time course up to these incubation times. All incubations were done in triplicate. The reaction mixture contained, if not otherwise indicated, 50 mM Tris·HCl, pH 7.4, 2 mM triethanolamine, 1 mM EDTA, 5 mM $MgCl_2$, 10 μM GDP, 1 mM dithiothreitol, 100 mM NaCl, 0.2 units/ml adenosine deaminase, 0.2 nM [^{35}S]GTP[S], and 0.5% bovine serum albumin. Nonspecific binding was determined in the presence of 10 μM GTP[S]. Incubations were terminated through filtration of the samples over GF/B glass fiber filters (Whatman, Maidstone, UK) presoaked in 50 mM Tris·HCl, pH 7.4, 5 mM $MgCl_2$, and 0.2% CHAPS followed by two 4-ml washes with the same buffer. In some experiments, membranes (1 mg/ml) were preincubated with anti-G protein antisera (diluted 1:20) or nonimmune serum in 50 mM Tris·HCl, pH 7.4, and 2.5% bovine serum albumin for 1 hr at 30° before [^{35}S]GTP[S] binding was assessed.

Release of [^{35}S]GDP[S] from rat brain membranes. [^{35}S]GDP[S] (0.4 nM) was incubated with 2 μg of membranes as described above for [^{35}S]GTP[S] but in the absence of unlabeled GDP for 2 hr at 25°. At this time, [^{35}S]GDP[S] binding had reached equilibrium. Consecutively, agonists or antagonists dissolved in the incubation medium were added to the samples, and incubations were performed for different additional incubation periods. Control incubations were performed without the addition of adenosine receptor ligands. [^{35}S]GDP[S], which remained membrane bound, was measured after filtration of the samples as described for [^{35}S]GTP[S] binding. Released [^{35}S]GDP[S] was calculated as the difference between control incubations and incubations in the presence of receptor ligands.

Binding of [^{3}H]DPCPX to rat brain membranes. Equilibrium binding to A_1 adenosine receptors was performed in an identical incubation medium as used for [^{35}S]GTP[S] binding with the addition of 0.02% CHAPS, which did not change the affinities of the ligands. Membrane protein (40 μg) was incubated in a total volume of 2 ml at 25° for 2 hr (0.8 nM [^{3}H]DPCPX), 20° for 2.5 hr (0.6 nM [^{3}H]DPCPX), 10° for 3 hr (0.4 nM [^{3}H]DPCPX), or 0° for 8 hr (0.3 nM [^{3}H]DPCPX), according to previous time course experiments. Nonspecific binding was measured in the presence of 10 μM *R*-PIA. Reactions were terminated as described above.

Data analysis. K_d values, K_H and K_L values (K_i values for the high and low affinity states of the receptor for agonists), and B_{max} values were calculated through nonlinear curve fitting with the use of the program SCTFIT (26). EC_{50} values for stimulation of [^{35}S]GTP[S] binding were calculated with SigmaPlot. Affinity and EC_{50} values are shown as geometric mean values with 95% confidence limits derived from three to nine independent experiments. B_{max} values are given as arithmetic mean ± standard error. Standard free energy was calculated as $\Delta G° = -RT\ln K_A$, where T = 298.15 K and R = 8.314 J/K/mol); standard enthalpy (ΔH°) was calculated from van't Hoff plots ($\ln K_A$ versus $1/T$, where the slope = $-\Delta H°/R$); and standard entropy (ΔS°) was calculated as $(\Delta H° - \Delta G°)/T$.

Results

G protein activation by A_1 adenosine receptors was determined in the presence of several adenosine receptor agonists (Fig. 1). The level of maximum stimulation of [^{35}S]GTP[S] binding induced by CCPA, NECA, ClA, and MIG was identical for all compounds regardless of their affinities. Therefore, these agonists were considered to be full agonists. Of the three anti-G protein antibodies tested, only GC/2, directed against the amino terminus of α_o, significantly inhibited the stimulation by CCPA on [^{35}S]GTP[S] binding. GC/2 reduced the agonist effect to 77.9 ± 4.9% compared with stimulation in the presence of nonimmune serum.

In additional experiments, three adenosine derivatives were identified as partial agonists. MeSA, CV 1808, and cladribine stimulated [^{35}S]GTP[S] binding to a lesser degree than the full agonist CCPA (Fig. 2). At 25°, maximum concentrations of MeSA induced ~40% of the stimulation of [^{35}S]GTP[S] binding obtained in the presence of CCPA. The efficacies of CV 1808 and cladribine at 25° were ~50% and ~20% compared with CCPA as a full agonist. Moreover, the relative efficacies of the partial agonists to induce G protein activation were dependent on temperature (Fig. 2). At 20°, relative efficacies were only ~25% for MeSA and CV 1808, respectively, and ~10% for cladribine. In contrast to CCPA,

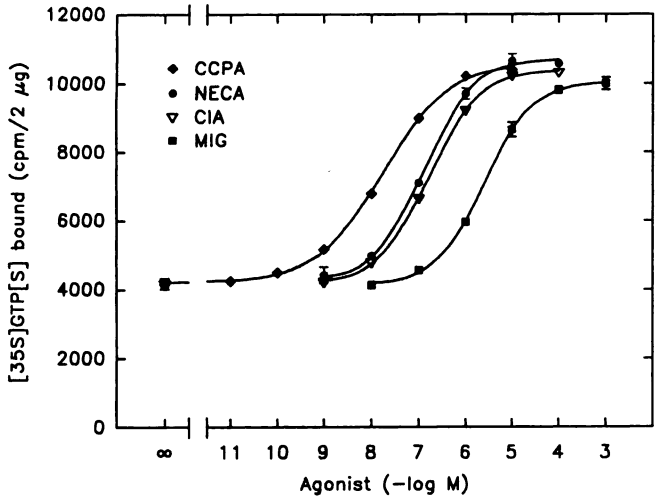


Fig. 1. Stimulation of G protein activation in rat brain membranes by adenosine A_1 receptor agonists. [^{35}S]GTP[S] binding was performed at 25° for 2 hr in the presence of increasing concentrations of CCPA, NECA, CIA, and MIG, essentially as described in Experimental Procedures. Data are mean ± standard deviation from one experiment performed in triplicate. Four to nine experiments were performed for each agonist.

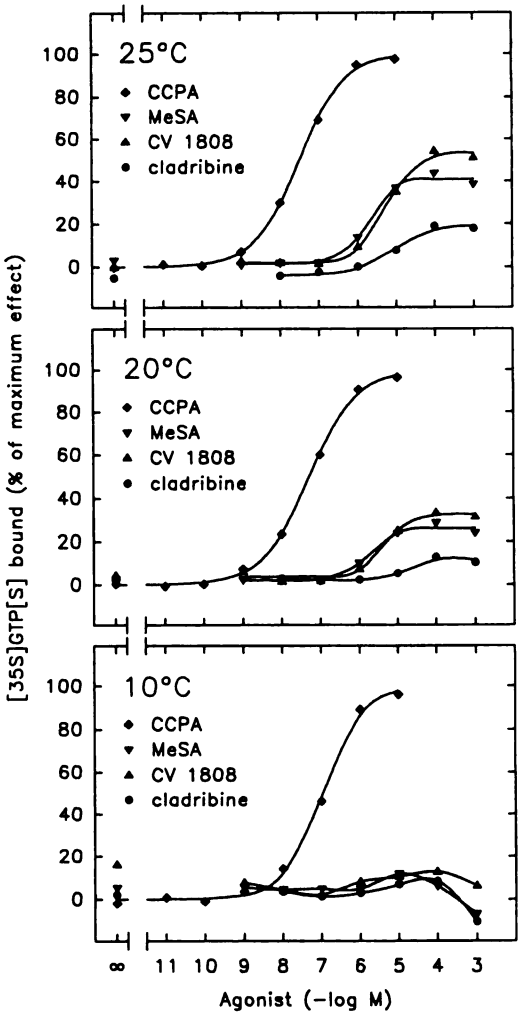


Fig. 2. Stimulation of [^{35}S]GTP[S] binding to rat brain membranes at 25°, 20°, and 10° in the presence of CCPA, MeSA, CV 1808, or cladribine. Incubation periods were 2 hr at 25°, 2.5 hr at 20°, and 3 hr at 10°. Data are from one representative experiment with triplicate samples. Three to nine experiments were performed for each agonist. EC_{50} values are given in Table 1. Basal binding values were 4410 ± 242 cpm at 25°, 3355 ± 240 cpm at 20°, and 2363 ± 132 cpm at 10°. Maximum binding levels induced by CCPA were 9975 ± 313 cpm at 25°, 7725 ± 481 cpm at 20°, and 4351 ± 464 cpm at 10°. The agonist effect was calculated as the difference between maximum and basal binding levels and decreased from 5565 cpm at 25° to 4370 cpm at 20° and to 1988 cpm at 10°.

no partial agonist stimulated [^{35}S]GTP[S] binding at 10° (Fig. 2). The affinities of full and partial agonists were influenced differently by incubation temperature. EC_{50} values of the full agonists increased for CCPA (from 25.1 to 75.8 nM) and for MIG (from 2.62 to 8.35 μM) when the incubations were carried out at lower temperatures (Table 1). Similarly, the EC_{50} values for NECA and ClA were higher when the incubation temperatures were decreased (Table 1). In contrast, MeSA was significantly more potent at 20° (EC_{50} = 2.30 μM) than at 25° (EC_{50} = 942 nM; Table 1). The EC_{50} values obtained for CV 1808 and cladribine did not differ significantly between 20° and 25° (Table 1).

Further experiments were carried out to investigate whether the partial agonists acted at the same receptor site as the A_1 receptor-selective agonist CCPA. Therefore, [^{35}S]GTP[S] binding was determined in the absence or presence of maximum concentrations of CCPA or MeSA, and increasing concentrations of CCPA were combined with MeSA to test whether the stimulatory effects of these compounds were additive (Fig. 3). Increasing concentrations of CCPA in incubations in the presence of 100 μM MeSA induced additional stimulation of [^{35}S]GTP[S] binding. However, the highest levels of G protein stimulation in the presence of both agonists never exceeded the maximum stimulation that was induced by CCPA alone (Fig. 3). When CV 1808 was used instead of MeSA and combined with CCPA, the results were identical (data not shown). Combinations of MeSA and CV 1808 were not additive with respect to stimulation of [^{35}S]GTP[S] binding (data not shown).

The stimulatory effects of CCPA, MeSA, and CV 1808 were compared with respect to inhibition by adenosine receptor antagonists. The pharmacological profiles obtained (Fig. 4) demonstrate that the effects of full and partial agonists were inhibited by antagonists in the same rank order of potency: DPCPX > theophylline; the A_2 adenosine receptor-selective antagonist CSC did not inhibit [^{35}S]GTP[S] binding (data not shown). Because this rank order of potency is typical for A_1 adenosine receptors and the actions of the full agonist CCPA and the partial agonists MeSA and CV 1808 were inhibited by the antagonists in the same rank order of potency, we conclude that CCPA as well as MeSA and CV 1808 acted at the A_1 adenosine receptor.

In addition to agonistic properties, the partial agonists MeSA and CV 1808 showed partial antagonistic behavior. Both compounds partially inhibited the stimulation of [^{35}S]GTP[S] binding in the presence of CCPA. CCPA-stimulated G protein activation was decreased dose-dependently by MeSA (Fig. 5). At all three temperatures (25°, 20°, and 10°), MeSA inhibited the effect of CCPA to the level of [^{35}S]GTP[S] binding in the presence of this partial agonist alone, which was above basal binding at 25° and 20°. At 10°, MeSA alone did not influence [^{35}S]GTP[S] binding but decreased the stimulatory effect of CCPA to basal levels, which

TABLE 1

EC_{50} values of full and partial A_1 adenosine receptor agonists for G protein activation as determined with [^{35}S]GTP[S] binding

Membranes (2 μg) were incubated for 2 hr (25°), 2.5 hr (20°), or 3 hr (10°) with increasing concentrations of CCPA, NECA, ClA, MIG, MeSA, CV 1808, or cladribine. EC_{50} values were calculated from three to nine independent experiments for each agonist. Data are given as geometric mean values with 95% confidence limits (in parentheses). Significance of differences in affinity versus 25° was tested with the Student's *t* test for unpaired samples.

	EC_{50}		
	25°	20°	10°
		nM	
CCPA	25.1 (20.5–30.8)	39.8 (28.6–55.5) $p < 0.05$	75.8 (65.5–87.7) $p < 0.0001$
NECA	140 (117–169)	233 (160–339) $p < 0.05$	536 (393–732) $p < 0.0005$
ClA	186 (146–236)	227 (192–268) NS	387 (343–436) $p < 0.0005$
MIG	2,620 (2,270–3,020)	3,500 (2,570–4,760) NS	8,350 (6,320–11,040) $p < 0.0005$
MeSA	2,300 (2,100–2,500)	942 (763–1,163) $p < 0.001$	No stimulation
CV 1808	3,720 (3,160–4,380)	3,030 (2,340–3,940) NS	No stimulation
Cladribine	9,320 (5,930–14,600)	7,110 (3,470–14,600) NS	No stimulation

is typical for a neutral antagonist. Similar results were obtained for CV 1808 (data not shown).

Taken together, these results from [^{35}S]GTP[S] binding experiments indicate that MeSA, CV 1808, and cladribine are partial agonists of the A_1 adenosine receptor. As may be expected for partial agonists, they also showed partial antagonist characteristics. Their affinities and efficacies were regulated by temperature in a way that is distinct from full agonists. Therefore, we investigated whether partial agonistic properties could also be detected in radioligand binding experiments of the A_1 adenosine receptor. It has been reported previously (18–21) that binding of A_1 receptor agonists is entropy driven, whereas binding of antagonists is enthalpy driven or driven by both enthalpy and entropy. Therefore, we investigated the binding of the A_1-selective antagonist [^{3}H]DPCPX in saturation studies and the binding of the full agonists CCPA, NECA, ClA, and MIG in competition studies with [^{3}H]DPCPX. All [^{3}H]DPCPX binding experiments were performed under conditions identical to those used for the determination of [^{35}S]GTP[S] binding. The binding data of the partial agonists MeSA, CV 1808, and cladribine at different temperatures were compared with the data for the antagonists and agonists, and their thermodynamic parameters were determined.

Fig. 6 shows representative Scatchard plots at 0°, 10°, 20°, and 25° for saturation experiments with [^{3}H]DPCPX of the A_1 adenosine receptor in rat brain membranes. The plots are slightly curvilinear at higher incubation temperatures. This may hint at different pools of receptors. Because A_1 receptors interact with a variety of G proteins (24), their affinities for [^{3}H]DPCPX could differ slightly depending on the G protein coupling state. Alternatively, different pools of receptors

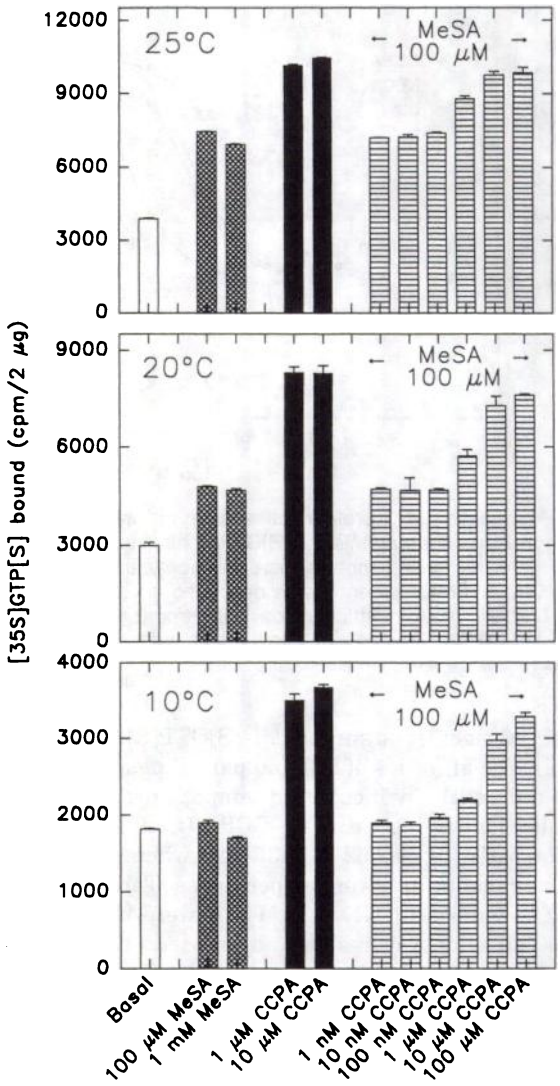


Fig. 3. G protein activation by combinations of CCPA and MeSA. Data are mean ± standard deviation from triplicate samples from one of two experiments. [^{35}S]GTP[S] binding was determined at 25°, 20°, and 10° under basal conditions (*open bars*), in the presence of MeSA (*cross-hatched bars*), in the presence of CCPA (*solid bars*), and in the presence of 100 μM MeSA and increasing concentrations of CCPA (*striped bars*).

might display different accessibilities to the radioligand. However, because the pore-forming antibiotic alamethicin (10 μg/tube) did not change levels of [^{3}H]DPCPX binding at either incubation temperature (data not shown), we assume that the slightly curvilinear form of the plots may reflect different pools of receptors with respect to G protein interaction. Clearly, more detailed investigations are necessary to characterize the interaction between A_1 antagonists and receptors coupled to different G proteins. Although some evidence suggested different pools of receptors, fitting of the data to a two-site model was not successful. No changes in the maximum binding capacities were observed between the various incubation temperatures. The affinity of [^{3}H]DPCPX increased with decreasing temperature, from a K_d value of 0.78 nM at 25° to 0.24 nM at 0°, pointing to a mainly enthalpy-driven process.

In contrast, binding of the full agonists CCPA, NECA, and MIG was favored at the higher incubation temperatures (Ta-

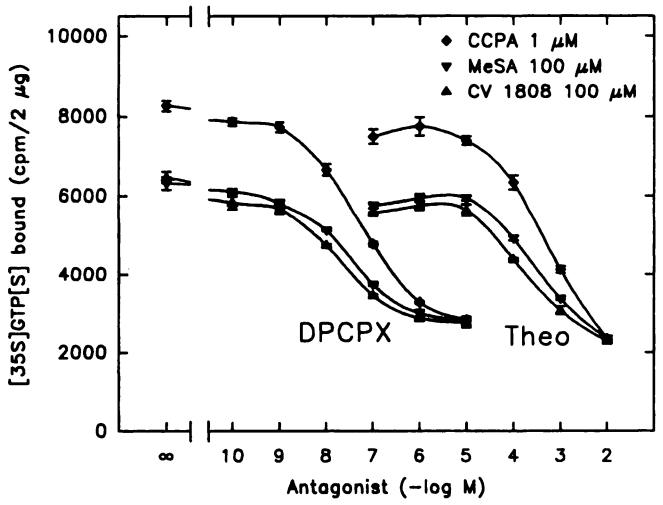


Fig. 4. Inhibition of the G protein activation induced by CCPA (1 μM), MeSA (100 μM), and CV 1808 (100 μM) in the presence of adenosine receptor antagonists. Data are mean ± standard deviation from triplicate samples for one of three experiments. [^{35}S]GTP[S] binding was performed at 25° for 2 hr in the presence of increasing concentrations of DPCPX or theophylline. CSC did not affect [^{35}S]GTP[S] binding (not shown).

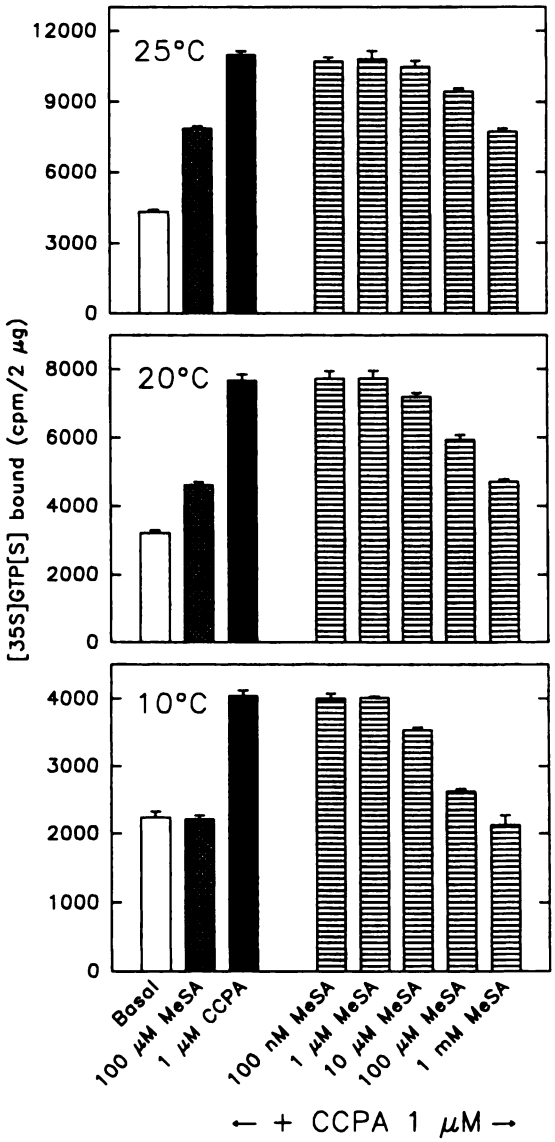


Fig. 5. Inhibition of CCPA-induced G protein activation by MeSA. [^{35}S]GTP[S] binding was measured after incubation periods of 2 hr at 25°, 2.5 hr at 20°, and 3 hr at 10° in the absence of agonists (*open bars*), in the presence of 100 μM MeSA (*cross-hatched bars*), and in the presence of 1 μM CCPA without (*solid bars*) or with increasing (*striped bars*) concentrations of MeSA. Data are mean ± standard deviation from triplicate samples from one of two experiments.

ble 2). High and low affinity binding sites were detected at all temperatures. The proportion of A_1 adenosine receptors in the high affinity state decreased with decreasing temperature, from 45% at 25° to 14% at 0° (Fig. 7, *top*, and Table 2). The affinities for both the high and the low affinity states of the receptor increased with temperature, indicating that agonist binding to the G protein-coupled and -uncoupled state is entropy driven. However, at 0°, the affinity of MIG was higher than that at 10 or 20°; the reason for this is not known. The affinity of ClA for both the high and the low affinity state was only marginally affected by incubation temperature (Table 2).

Like full agonists, the partial agonists MeSA, CV 1808, and cladribine generally detected a lower proportion of A_1 adenosine receptors in the high affinity state (Fig. 7, *middle* and *bottom*). At 10° and 0°, no partial agonist showed biphasic inhibition curves, indicating that for this class of ligands all A_1 adenosine receptors seemed to be in the low affinity state. This result is in agreement with the inability of these ligands to stimulate G protein activation at 10°. However, at 10°, these ligands were competitive antagonists in the [^{35}S]GTP[S] binding assay, which is in accordance with monophasic displacement of [^{3}H]DPCPX at low temperatures. In contrast to full agonists, the affinities of partial agonists increased at lower incubation temperatures (Table 2). In this respect, the partial agonists displayed a characteristic of the antagonist [^{3}H]DPCPX. In general, the results concerning temperature dependence of inhibition of [^{3}H]DPCPX binding were in good agreement with the temperature dependence of EC_{50} values for stimulation of [^{35}S]GTP[S] binding: both affinity and EC_{50} were shifted to lower concentrations for full agonists at increasing temperatures, and the reverse was found for the partial agonists MeSA, CV 1808, and cladribine.

Fig. 8 shows the van't Hoff plots for [^{3}H]DPCPX, CCPA, NECA, ClA, MIG (10–25°), MeSA, CV 1808, and cladribine. The plot for the full adenosine receptor agonists showed negative slopes for binding to the high as well as to the low affinity state of the A_1 receptor. In contrast, the van't Hoff plot slopes were positive for the antagonist [^{3}H]DPCPX and for the partial agonists MeSA, CV 1808, and cladribine. Thermodynamic parameters (Table 3 and Fig. 9) show that binding of full agonists to both the high and the low affinity state of the receptor was entropy driven, whereas binding of [^{3}H]DPCPX was both enthalpy and entropy driven. Binding of the partial agonist CV 1808 more closely resembled the characteristics of antagonist binding than those of agonist binding, with relatively small positive values for standard entropy ($\Delta S°$) and large negative values for standard enthalpy ($\Delta H°$). In contrast, the second partial agonist, MeSA, displayed an exclusively enthalpy-driven binding. Thermodynamic parameters for cladribine were intermediate between those of MeSA and CV 1808 (Table 3 and Fig. 9). The rela-

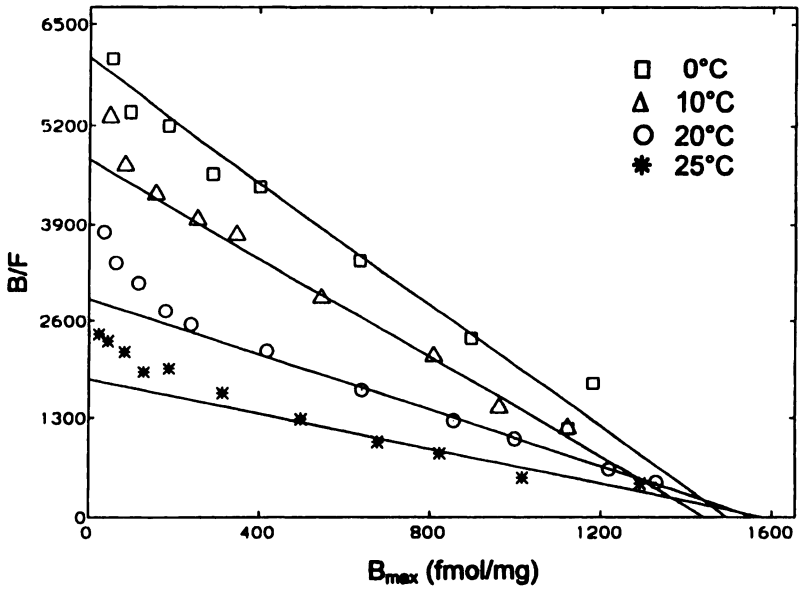


Fig. 6. Saturation of A_1 adenosine receptors with [^{3}H]DPCPX at 0°, 10°, 20°, and 25°. Incubations were performed as described in Experimental Procedures. Representative Scatchard plots are shown from one of three experiments. The following K_d (with 95% confidence limits) and B_{max} (± standard error) values were calculated: 25°, 0.78 (0.58–1.04) nM and 1325 ± 135 fmol/mg; 20°, 0.53 (0.41–0.68) nM and 1347 ± 108 fmol/mg; 10°, 0.36 (0.30–0.42) nM and 1359 ± 72 fmol/mg; and 0°, 0.24 (0.23–0.26) nM and 1460 ± 134 fmol/mg.

TABLE 2

Agonist competition for [^{3}H]DPCPX binding at 0–25°

Rat brain membranes (40 μg) were incubated for 2 hr (25°), 2.5 hr (20°), 3 hr (10°), or 8 hr (0°) with [^{3}H]DPCPX and increasing concentrations of CCPA, NECA, CIA, MIG, MeSA, CV 1808, or cladribine. Inhibition curves were fitted to a one-site model if fitting to two sites did not improve the fit significantly ($p < 0.05$). K_H and K_L are the K_i values for the high and low affinity states of the receptor, respectively, and are given as geometric mean values with 95% confidence limits. B_{max} values are given as arithmetic mean ± standard error. B_{maxH} and B_{maxL} represent the amount of receptors in the high or low affinity state, respectively. % R_H indicates the percentage of A_1 receptors in the high affinity state ± standard error.

		K_H	K_L	K_L/K_H	B_{max}	B_{maxH}	B_{maxL}	R_H
		nM	nM		fmol/mg	fmol/mg	fmol/mg	%
CCPA	25°	0.79 (0.59–1.05)	67.3 (46.7–97.0)	85.2	1,786 ± 54	808 ± 41	978 ± 18	45 ± 1
	20°	1.02 (0.81–1.28)	64.4 (55.0–75.4)	63.1	1,619 ± 93	597 ± 60	1,022 ± 40	37 ± 2
	10°	2.80 (2.04–3.85)	118 (103–135)	42.1	1,824 ± 74	426 ± 13	1,398 ± 66	24 ± 1
	0°	7.19 (4.50–11.5)	138 (114–169)	19.2	1,593 ± 55	211 ± 40	1,382 ± 94	14 ± 3
NECA	25°	10.4 (7.24–15.0)	448 (325–617)	43.1	1,280 ± 35	519 ± 54	761 ± 37	40 ± 3
	20°	12.5 (7.50–20.9)	559 (439–710)	44.7	1,292 ± 79	556 ± 43	736 ± 64	43 ± 3
	10°	53.7 (24.8–116)	1,360 (960–1,930)	25.3	1,385 ± 51	594 ± 95	791 ± 61	42 ± 6
	0°	48.1 (24.9–93.2)	994 (860–1,150)	20.7	1,203 ± 66	260 ± 33	943 ± 71	22 ± 4
CIA	25°	14.9 (10.1–22.0)	811 (427–1,538)	54.4	1,379 ± 78	628 ± 46	751 ± 115	47 ± 6
	20°	12.8 (7.18–22.9)	610 (445–836)	47.7	1,414 ± 65	572 ± 53	842 ± 78	41 ± 4
	10°	20.6 (11.6–36.5)	723 (576–906)	35.1	1,498 ± 34	390 ± 30	1,108 ± 54	26 ± 2
	0°	19.9 (6.07–65.5)	702 (611–806)	35.3	1,354 ± 38	188 ± 38	1,166 ± 65	14 ± 3
MIG	25°	146 (88.5–240)	6,520 (5,080–8,370)	44.7	1,345 ± 45	591 ± 38	754 ± 60	44 ± 3
	20°	382 (262–558)	9,170 (7,580–11,100)	24.0	1,377 ± 118	605 ± 26	772 ± 99	44 ± 3
	10°	536 (303–948)	13,100 (10,400–16,600)	24.4	1,639 ± 65	506 ± 50	1,133 ± 103	31 ± 4
	0°	227 (140–368)	8,740 (8,460–9,020)	38.5	1,559 ± 92	191 ± 16	1,368 ± 105	13 ± 2
MeSA	25°	367 (244–551)	7,670 (4,830–12,200)	20.9	1,486 ± 39	748 ± 154	738 ± 159	51 ± 10
	20°	184 (112–302)	2,710 (2,210–3,310)	14.7	1,456 ± 99	409 ± 92	1,047 ± 27	27 ± 4
	10°	NA	1,140 (1,050–1,220)	NA	1,625 ± 131	NA	1,625 ± 131	0
	0°	NA	420 (370–476)	NA	1,551 ± 100	NA	1,551 ± 100	0
CV 1808	25°	232 (131–411)	7,510 (6,620–8,520)	32.4	1,421 ± 55	380 ± 43	1,041 ± 38	27 ± 2
	20°	207 (128–334)	7,420 (6,630–8,310)	35.8	1,413 ± 101	332 ± 55	1,081 ± 55	23 ± 2
	10°	NA	4,950 (3,760–6,520)	NA	1,583 ± 152	NA	1,583 ± 152	0
	0°	NA	3,960 (3,110–5,050)	NA	1,528 ± 134	NA	1,528 ± 134	0
Cladribine	25°	2,020 (972–4,200)	34,500 (26,900–44,200)	18.8	1,387 ± 96	384 ± 42	1,003 ± 119	28 ± 4
	20°	1,600 (880–2,930)	28,300 (22,400–35,700)	18.9	1,386 ± 90	321 ± 87	1,065 ± 204	25 ± 6
	10°	NA	13,400 (12,800–14,100)	NA	1,486 ± 49	NA	1,486 ± 49	0
	0°	NA	7,190 (6,120–8,450)	NA	1,271 ± 62	NA	1,271 ± 62	0

NA, not applicable.

tionship between observed ΔH° and ΔS° changes is depicted in Fig. 9, which can be summarized as a linear regression line with the following equation: $-T\Delta S° = -1.07\Delta H° - 38.52$ ($r = 0.99$).

To consider the mechanism of partial agonism, we investigated the influence of adenosine receptor ligands on the GDP/GTP exchange of the G protein by [^{35}S]GDP[S] and [^{35}S]GTP[S] binding experiments. Time course experiments of [^{35}S]GTP[S] association at 25° showed a linear association of the radioligand for ≤2 hr under control conditions as well as in the presence of CCPA and MeSA (Fig. 10). In addition, they revealed that the binding proceeded faster in the presence of CCPA than of MeSA.

Because the differences in binding of [^{35}S]GTP[S] in the

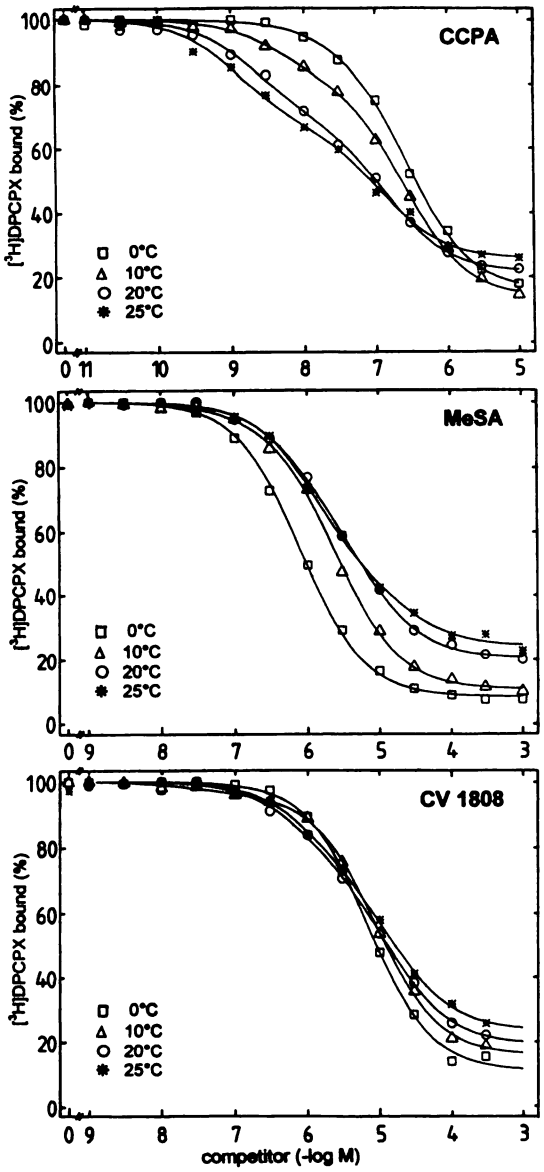


Fig. 7. Competition of adenosine receptor agonists for [^{3}H]DPCPX binding. [^{3}H]DPCPX (0°, 0.3 nM; 10°, 0.4 nM; 20°, 0.6 nM; 25°, 0.8 nM) was incubated with 40 μg of membranes and increasing concentrations of CCPA, MeSA, or CV 1808. Data are from one of three to five independent experiments. K_H and K_L values and A_1 adenosine receptors in the high and low affinity states are summarized in Table 2.

presence of a full or a partial agonist might be either due to a direct effect on [^{35}S]GTP[S] binding or secondary to a diminished rate of GDP release from G proteins induced by partial agonists, which would lead to a slower binding of [^{35}S]GTP[S], we investigated the role of GDP in greater detail.

When concentration-response curves for stimulation of [^{35}S]GTP[S] binding for CCPA and MeSA were determined at increasing GDP concentrations (10, 30, and 100 μM GDP), a shift of responses to CCPA to significantly higher EC_{50} values at higher GDP concentrations was noted, whereas EC_{50} values for the partial agonist MeSA were not changed (Fig. 11). The GDP dependence of the stimulatory effects to these ligands was determined in greater detail over a wider range of GDP concentrations at maximally active concentrations of CCPA (10 μM) or MeSA (1 mM) at 10°, 20°, and 25°. Even when the GDP concentrations were varied, the stimulatory effects of MeSA never exceeded the stimulation produced by CCPA. The results presented in Fig. 12 show that at 25°, the maximum stimulation of [^{35}S]GTP[S] binding (stimulated minus control binding) by CCPA is found at 10 μM GDP, whereas the maximum stimulation obtained in the presence of MeSA occurs at a slightly lower GDP concentration of ~3 μM. At 20°, the difference in GDP dependence was more evident: although maximum CCPA effects were observed at 10 μM GDP, the peak stimulation by MeSA was determined at 1 μM GDP. At 10°, the CCPA maximum effect was detected at 1 μM GDP, whereas MeSA was inactive with respect to stimulation of [^{35}S]GTP[S] binding over the entire range of GDP concentrations.

Because these results indicated that the ability of partial agonists to stimulate G protein activation was depressed in the presence of high GDP concentrations, we examined the ability of CCPA and MeSA to release the GDP analogue [^{35}S]GDP[S] from G proteins. When [^{35}S]GDP[S] binding had reached equilibrium, the antagonist CPT, the full agonist CCPA, or the partial agonist MeSA was added to the incubation, and [^{35}S]GDP[S], which remained membrane associated, was compared with control incubations without adenosine receptor ligands. The agonists decreased the amount of membrane-associated [^{35}S]GDP[S] but did so to varying extents and with different time courses (Fig. 13). CCPA induced a rapid release of the GDP analogue, which had almost reached maximum after 10 min. Compared with the effect of CCPA, the release of [^{35}S]GDP[S] by MeSA was significantly impaired after 10 and 30 min. After 45 min, there was no difference in the release of [^{35}S]GDP[S] between the full and the partial agonist. The impaired release of the GDP analogue induced by the partial agonist is in accordance with the reduced efficacy of MeSA to stimulate [^{35}S]GTP[S] binding.

Discussion

In the current study, we investigated the interaction of full and partial agonists with the A_1 adenosine receptor and the initiation of response through measurement of G protein activation. G protein activation was determined through measurement of [^{35}S]GTP[S] binding and [^{35}S]GDP[S] release. Because the A_1 adenosine receptor in brain is coupled to at least three different G proteins (24), a method was chosen that allows measurement of responses independent of the various second messenger systems. The agonist response assessed in [^{35}S]GTP[S] binding assays reflects the sum of the activation of various types of receptor/G protein complexes. The activation of G_o by A_1 receptors was shown through the pretreatment of the membranes with an antiserum specific for α_o. In addition, receptors coupled to different G protein subtypes may exhibit different affinities for ligands. When A_1 receptors were saturated with the antagonist [^{3}H]DPCPX, the Scatchard plots were slightly curvilinear at higher incubation temperatures (Fig. 6). Although these results suggested some heterogeneity of the receptors, the data could not be fitted to a two site-model. We attribute these results to the presence of different receptor/G protein complexes rather than to different accessibilities for the radioligand because all receptor binding experiments were performed under equilibrium conditions, as assessed by previous

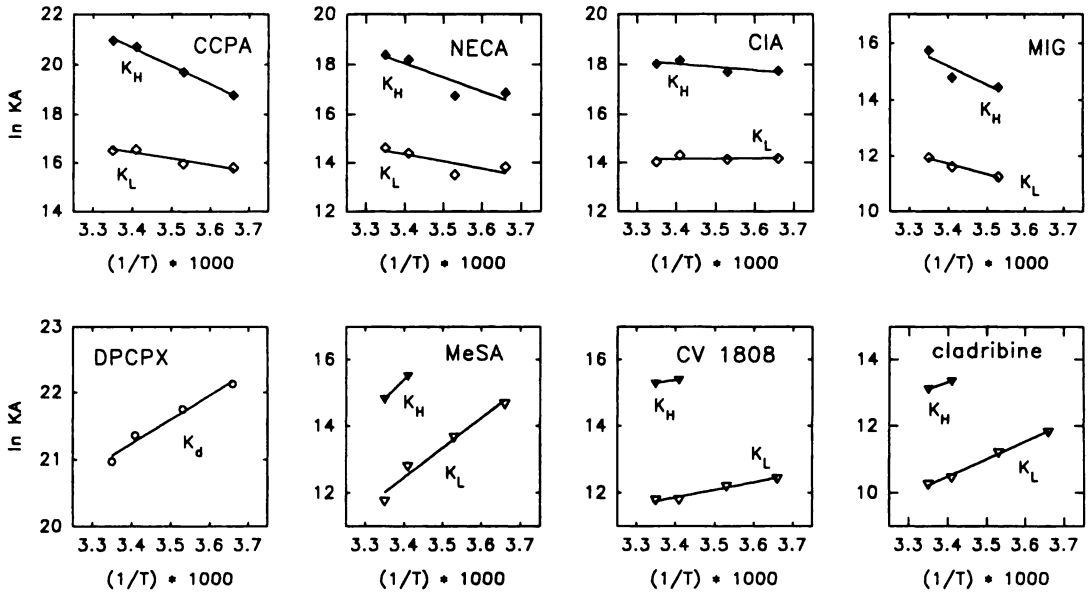


Fig. 8. van't Hoff plots for the antagonist [^{3}H]DPCPX; the full agonists CCPA, NECA, CIA, and MIG; and the partial agonists MeSA, CV 1808, and cladribine. K_d values for [^{3}H]DPCPX were obtained from saturation studies, and K_H and K_L values (K_i values for the high and low affinity states of the A_1 adenosine receptor) were calculated from [^{3}H]DPCPX competition curves. Linear regressions are shown of $\ln K_A$ from three to five independent experiments for each ligand versus (1/T).

TABLE 3

Thermodynamic parameters for ligand binding to A_1 adenosine receptors

Parameters were calculated for [^{3}H]DPCPX binding to rat brain membranes from saturation experiments (0–25°) and for agonist binding from competition experiments with [^{3}H]DPCPX (CCPA, NECA, CIA, MeSA, CV 1808, and cladribine: 0–25°; MIG: 10–25°). ΔG°, ΔH°, and ΔS° values are given in kJ/mol at T = 298.15 K.

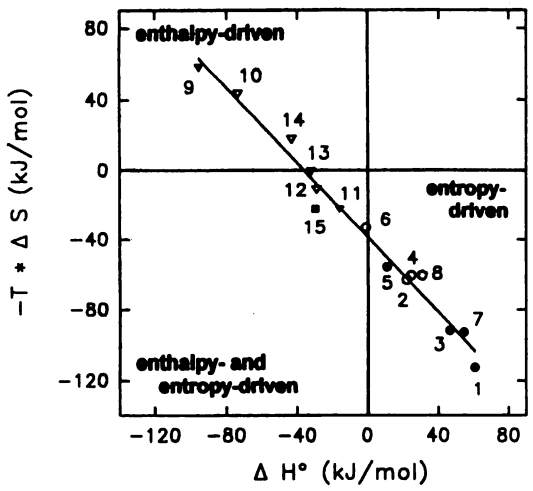
		Standard free energy ΔG°	standard enthalpy ΔH°	standard entropy ΔS°
		kJ/mol	*kJ/mol*	*J/mol/K*
DPCPX	K_d	−51.99	−29.75	74.6
CCPA	K_H	−51.84	60.81	377.8
	K_L	−40.87	22.15	211.4
NECA	K_H	−45.15	46.60	307.7
	K_L	−35.75	24.64	202.5
CIA	K_H	−44.62	11.03	186.7
	K_L	−34.17	−1.05	111.1
MIG	K_H	−38.22	54.59	311.3
	K_L	−29.32	31.01	202.3
MeSA	K_H	−36.68	−95.24	−196.4
	K_L	−29.75	−73.49	−146.7
CV 1808	K_H	−37.72	−15.73	73.8
	K_L	−18.64	−29.03	34.9
Cladribine	K_H	−32.39	−32.15	0.81
	K_L	−25.30	−43.29	−60.31

time course experiments. In addition, the pore-forming antibiotic alamethicin, which has been used to increase [^{3}H]NECA and [^{3}H]CGS 21680 (2-[4-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine) binding to a high-affinity adenosine binding protein and to A_{2A} adenosine receptors (27, 28), did not change the levels of [^{3}H]DPCPX binding.

The first experiments were designed to test a possible relationship between agonist affinity and efficacy. As clearly depicted in Fig. 1, CCPA, NECA, CIA, and MIG were full agonists, regardless of their potencies to stimulate [^{35}S]GTP[S] binding. These results are in agreement with the results of Pirovano *et al.* (29), who found no correlation between agonist affinity and efficacy of N^6-(ω-aminoalkyl)adenosines. However, contrasting findings have been reported by Borea *et al.* (18), who found a correlation between agonist potency and agonist efficacy in the stimulation of adenylyl cyclase of rat adipocytes. However, the direct comparison of results is hampered by the use of different methods for determination of the response. A correlation between agonist affinity and efficacy is not normally observed. For example, it has been shown through site-directed mutagenesis of β_2-adrenergic receptors that activation of adenylyl cyclase can be impaired without attenuating high affinity agonist binding to the receptor (30, 31).

In this study, we chose CCPA and MIG, which were the full agonists with the highest and the lowest potency, respectively, for further comparisons with partial agonists. MeSA, CV 1808, and cladribine showed lower efficacy in the G protein activation assay than did the agonists mentioned above. None of these ligands are selective for the A_1 adenosine receptor (32–37). Therefore, we investigated the site of action of partial agonists with respect to which adenosine receptor was involved. The stimulatory actions of MeSA and CCPA or of CV 1808 and CCPA, respectively, were only additive when CCPA was present in low concentrations; at maximally stimulatory concentrations of CCPA, the partial agonist could not enhance the effect of CCPA, which suggests a common site of action (Fig. 3). Adenosine receptor antagonists inhibited the stimulation of [^{35}S]GTP[S] binding by the selective full A_1 receptor agonist CCPA and the nonselective ligands MeSA and CV 1808 with the same rank order of potency; the A_2 adenosine receptor-selective antagonist CSC was inactive (Fig. 4). Therefore, we assume that the partial agonists exert their actions on G proteins at the same site of action as CCPA, at the A_1 adenosine receptor.

Thermodynamics of A_1 adenosine receptor binding have been described in relation to antagonist, partial agonist, or full agonist activities of ligands (18–21). However, incubation conditions for measurement of affinity and efficacy were not identical, and for only one agonist, *R*-PIA, are thermody-



1 CCPA KH
2 CCPA KL

3 NECA KH
4 NECA KL

5 ClA KH
6 ClA KL

7 MIG KH
8 MIG KL

9 MeSA KH
10 MeSA KL

11 CV 1808 KH
12 CV 1808 KL

13 cladribine KH
14 cladribine KL

15 [^{3}H]DPCPX Kd

Fig. 9. Plot of $-T\Delta S°$ versus $\Delta H°$ for the ligands tested in this study: [^{3}H]DPCPX (K_d, ■); the full agonists CCPA, NECA, ClA, and MIG (K_H, ●; K_L, ○); and the partial agonists MeSA, CV 1808, and cladribine (K_H, ▼; K_L, ▽). The line is the linear regression line, which is expressed in the equation $-T\Delta S° = -1.07\Delta H° - 38.52$ ($r = 0.99$).

namic binding data available of the high and low affinity states (20). In this study, we determined the thermodynamic parameters for full and partial agonists to the high and low affinity states of the A_1 adenosine receptor by inhibition of equilibrium binding of the antagonist [^{3}H]DPCPX. In agreement with other studies on the thermodynamics of A_1 adenosine receptor binding (18–21), we found that the binding of full agonists (CCPA, NECA, ClA, and MIG) to the high affinity state of the receptor was entropy driven, whereas binding of the antagonist was enthalpy and entropy driven. The same pattern has been found for the interaction of ligands with the γ-aminobutyric acid$_A$ receptor (38). At β-adrenergic receptors (7, 8, 10) and at rat muscarinic M_2 receptors (39), antagonist binding was driven primarily by entropy, whereas agonist binding was enthalpy driven. In contrast to a previous report (20), we found that under the conditions applied in this study, agonist binding to the low affinity state of this receptor was also entropy driven. Thermodynamic parameters for the partial agonists were strikingly different from those for full agonists (Table 3 and Fig. 9). Although CV 1808 showed characteristics very similar to those of [^{3}H]DPCPX in the plot of $-T\Delta S°$ versus $\Delta H°$, MeSA displayed an exclusively enthalpy-driven binding. Cladribine showed a thermodynamic behavior that was intermediate between that of MeSA and CV 1808. Compared with ClA, it became apparent that the 2′-deoxy modification of cladribine caused fundamental changes in the binding thermodynamics of this ligand. Although binding of the parent compound ClA was mainly entropy driven, binding of cladribine was predominantly enthalpy driven (Table 3 and Fig. 9). In contrast, ribose-modified periodate-oxidized adenosine analogues, which act as full agonists at A_1 receptors, retain the thermodynamic bind-

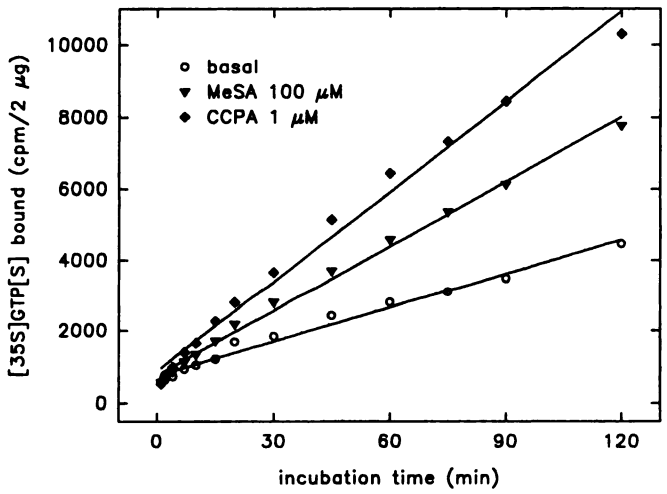


Fig. 10. Time course of [^{35}S]GTP[S] binding to rat brain membranes in the absence of agonists (*basal*) and in the presence of 1 μM CCPA and 100 μM MeSA. Incubations were performed at 25° for varying time intervals as described in Experimental Procedures. This experiment was repeated once with identical results.

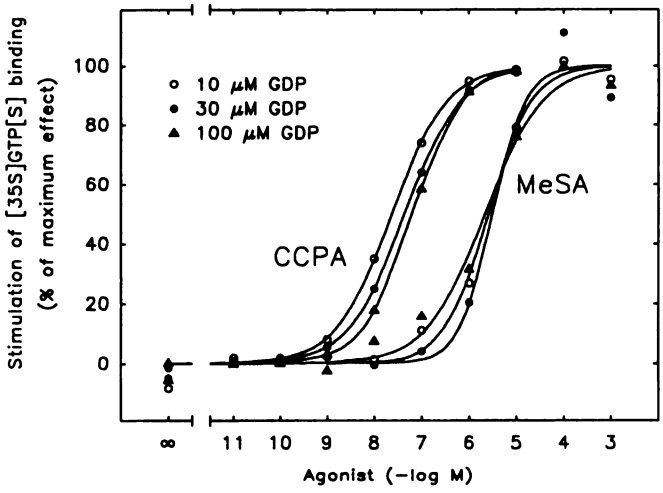


Fig. 11. Influence of GDP on EC_{50} values for stimulation of [^{35}S]GTP[S] binding by CCPA and MeSA. Membrane protein (2 μg) was incubated at 25° for 2 hr with increasing concentrations of agonist in the presence of 10, 30, or 100 μM GDP. Data are given as percentage of maximum response at these GDP concentrations in the incubation and are from one representative experiment with triplicate samples. Four to nine concentration-response experiments were performed for each agonist. Nonlinear curve fitting yielded the following EC_{50} values (with 95% confidence limits): for CCPA: 10 μM GDP, 25.1 (20.5–30.8) nM; 30 μM GDP, 40.3 (36.8–44.1) nM ($p < 0.005$ versus 10 μM GDP); and 100 μM GDP, 67.0 (60.7–73.8) nM ($p < 0.0001$ versus 10 μM GDP); for MeSA: 10 μM GDP, 2.30 (2.10–2.50) μM; 30 μM GDP, 2.54 (2.06–3.15) μM; and 100 μM GDP, 2.67 (2.33–3.06) μM.

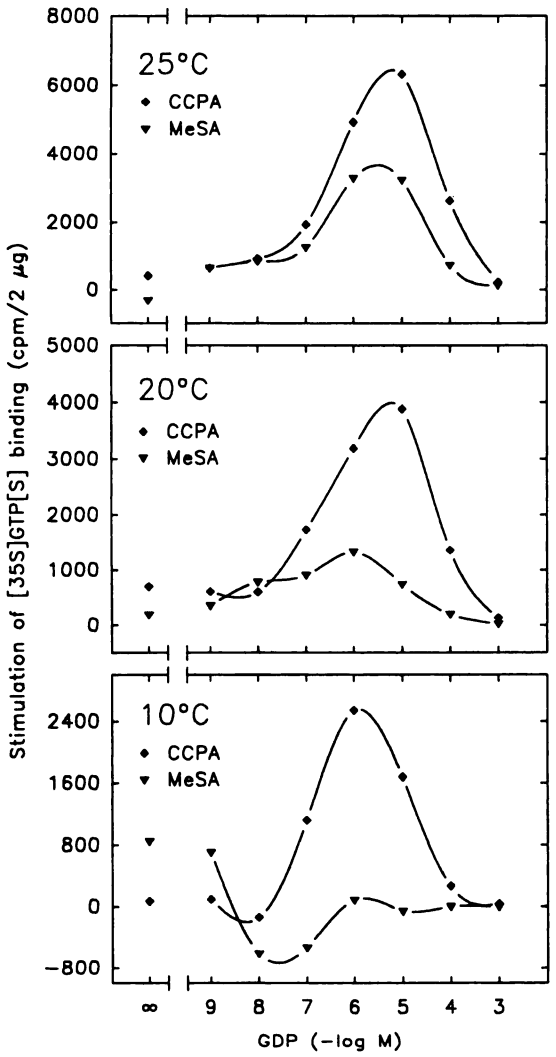


Fig. 12. GDP dependence of the stimulatory effects of CCPA (10 μM) and MeSA (1 mM) at 25°, 20°, and 10° on [^{35}S]GTP[S] binding. Incubations were performed as described in Experimental Procedures for 2 hr (25°), 2.5 hr (20°), or 3 hr (10°). Results are given as the difference between agonist-stimulated binding of [^{35}S]GTP[S] and control binding. Data are from one of three experiments performed with triplicate samples.

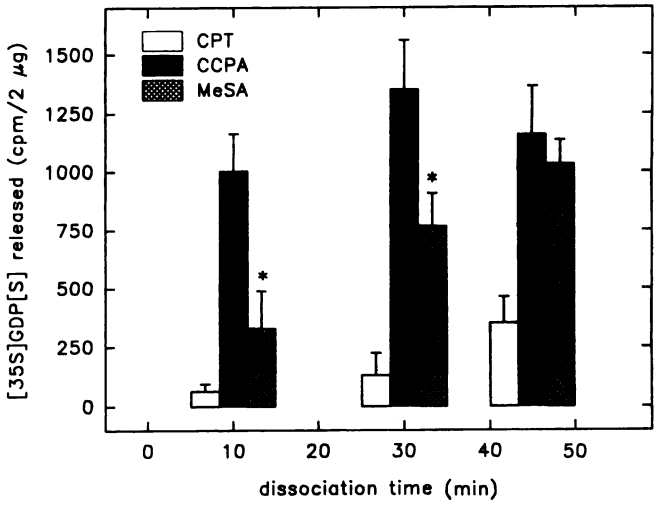


Fig. 13. Time course of release of prebound [^{35}S]GDP[S] from rat brain membranes. Membranes (2 μg) were preincubated for 2 hr at 25° until [^{35}S]GDP[S] binding had reached equilibrium. [^{35}S]GDP[S] binding at equilibrium was 5699 ± 642 cpm. Subsequently, CPT (10 μM), CCPA (10 μM), or MeSA (1 mM) dissolved in incubation medium or incubation medium without ligand was added to the samples, and incubations were continued for different time intervals. [^{35}S]GDP[S] bound to membranes after the addition of the antagonist or agonist and in control incubations was determined after filtration of the samples as described. The addition of incubation medium did not induce release of [^{35}S]GDP[S]. The amount of released [^{35}S]GDP[S] was calculated as the difference between [^{35}S]GDP[S] bound in control incubations and in ligand-containing samples. Data are amount ± standard error of [^{35}S]GDP[S] released by CCPA or MeSA from five independent experiments. *, Significance of difference between the release induced by MeSA in comparison to the effect of CCPA.

ing characteristics of the parent compound (40). Our results suggest that the hydroxyl groups of the ribose rather than the ribose moiety itself of A_1 receptor agonists may be responsible for the entropy-driven mechanism of agonist binding. This apparent paradox may be explained by the possibility that the presence of two vicinal hydroxyl groups, which may interact with each other by intramolecular hydrogen bonds, prevents the formation of intermolecular hydrogen bonds between the agonist ribose and the receptor protein. After removal of one hydroxyl group, formation of an intermolecular hydrogen bond may be facilitated.

The binding characteristics of partial agonists were unexpected because a previous study (18) showed that partial agonists have thermodynamic parameters intermediate between those of antagonists and full agonists. However, in contrast to the current study, determination of efficacy and affinity had not been performed under identical conditions.

When compared with other receptor systems, the classification of ligands on the basis of binding thermodynamics seems to be of ambiguous value. For β-adrenergic receptors and γ-aminobutyric acid$_A$ receptors, a good correlation seems to exist between binding energetics and agonist efficacy (8, 10, 38). On the other hand, structural features may be more relevant than agonistic or antagonistic properties (39, 41–43). At A_1 adenosine receptors, thermodynamic properties have been determined primarily for N^6-substituted agonists. All antagonists tested were xanthine derivatives. For a more complete overall view, a greater number of structurally diverse ligands should be investigated, e.g., nonxanthine adenosine receptor antagonists.

Regardless of intrinsic activities, the entropy and enthalpy changes were closely correlated, in accordance with the general theory of enthalpy/entropy compensation (44). The relation between these two parameters in the current study can be described by the equation $-T\Delta S° = -1.07\Delta H° - 38.52$, which is not very different from the regression obtained by Borea *et al.* (21): $-T\Delta S° = -1.20\Delta H° - 39.8$.

When the incubation temperature in receptor binding assays was lowered, all agonists detected fewer receptors in the high affinity state (Table 2). Compared with full agonists, the partial agonists detected fewer receptors in the high affinity state. Because A_1 receptors interact with different G proteins, partial agonists may preferably bind to the receptor coupled to a particular G protein subtype. The dependence of the proportion of receptors in the high affinity state on temperature has also been reported for β-adrenergic receptors (8). Partial agonists at β-adrenergic and muscarinic receptors also detect a lower percentage of receptors in the high

affinity state (8, 45). Different results were obtained in a reconstituted system of muscarinic acetylcholine receptors and G_i (46): the full agonist carbachol and the partial agonist pilocarpine showed the same occupancy of high and low affinity sites. The reduced efficacy of pilocarpine was not due to a diminished occupancy of receptors in the high affinity state but rather to a reduced affinity of the receptor/ligand complex for the G_i protein.

The reduced ligand binding to A_1 adenosine receptors in the high affinity state at lower temperatures in the current study is in agreement with the results from [^{35}S]GTP[S] binding experiments (Fig. 2). The reason for the decrease in relative intrinsic activity of partial agonists at lower temperatures is not known, and we are presently unaware of comparable results from other investigators, as the efficacies, e.g., to modulate adenylyl cyclase activity, were not investigated at different temperatures.

In a previous study of A_1 receptor-mediated stimulation of [^{35}S]GTP[S] binding in bovine brain membranes (22), we found that agonist concentrations for induction of response and for occupation of the high affinity state of the receptor were identical. However, in this study, the agonist EC_{50} values for stimulation of [^{35}S]GTP[S] binding to rat brain membranes were ~5–40 fold higher than the K_H values, although both sets of experiments were performed under identical conditions. The reason for these divergent results from two species is not known. These results seem to question whether the high affinity state detected in receptor binding studies is responsible for the induction of the G protein response. Although K_L values show a closer correlation with EC_{50} values, we assume that the high affinity state is a necessary precondition for G protein activation rather than the low affinity state because no stimulation of [^{35}S]GTP[S] binding is observed in the absence of high affinity binding. At 10°, the partial agonists MeSA and CV 1808 were devoid of agonistic properties, probably because they were unable to bind to receptors in the high affinity state. The divergence between results obtained in rat and bovine brain membranes might be caused by different levels of spare receptors in the two species as spare receptors modify ligand potency in functional assays (6). A lack of correlation between receptor occupancy of δ-opioid receptors and the potency to activate adenylyl cyclase or G proteins has also been reported by Prather *et al.* (47).

Consecutive experiments were performed to investigate the mechanism of action of the partial agonists with respect to the GDP/GTP exchange. Because the release of prebound GDP is the rate-limiting step for GTP binding (46), we examined the influence of GDP on the actions of CCPA and MeSA. GDP lowers guanine nucleotide exchange in the absence of added ligands (22), thereby facilitating the detection of agonist effects. In the presence of increasing GDP concentrations, the potency of CCPA decreased, whereas the potency of MeSA was unchanged. GDP has been shown to increase the affinity of the antagonist [^{3}H]DPCPX and to decrease the affinity of *R*-PIA in bovine brain membranes (22). In this respect, the partial antagonist showed a characteristic intermediate between that of a full agonist and an antagonist. When the intrinsic activities of CCPA and MeSA were determined at GDP concentrations between 1 nM and 1 mM, it was evident that the optimum GDP concentrations for stimulation by the partial agonist were lower than those for the full agonist (Fig. 12), which is in agreement with the diminished ability of MeSA to release [^{35}S]GDP[S] (Fig. 13). In this study, we found that the release of membrane-bound [^{35}S]GDP[S] by the partial agonist occurred more slowly compared with release by the full agonist (Fig. 13). Murayama and Ui (49) have shown that partial agonists of α- and β-adrenergic receptors induced a smaller release of prebound [^{3}H]GDP from adipocyte membranes than did full agonists. We assume that the velocity of the GDP/GTP exchange may also determine the extent of signal amplification by agonists of different intrinsic activities. Taken together, the GDP dose-response curves and the differences in the ability to release prebound [^{35}S]GDP[S] indicate that receptor/G protein complexes occupied by full agonists have a lower affinity for GDP than do complexes occupied by partial agonists.

In summary, full and partial agonists of the A_1 adenosine receptor have been characterized with respect to their receptor binding characteristics, their efficacies, and possible mechanisms of partial agonism. MeSA, CV 1808, and cladribine were identified as partial agonists of the A_1 receptor. We found no correlation between agonist affinity and intrinsic activity to stimulate G protein activation. The partial agonists detected fewer receptors in the high affinity state. They showed unexpected thermodynamic binding parameters as these parameters were not intermediate between those of antagonists and full agonists. Therefore, thermodynamic parameters should be used with caution for the classification of A_1 receptor ligands with respect to their intrinsic activities. MeSA and CV 1808 showed agonistic as well as antagonistic properties in the G protein activation assay. As a consequence of a slower release of membrane-bound [^{35}S]GDP[S], partial agonists were less efficient in inducing G protein activation as determined with [^{35}S]GTP[S] binding. The underlying differences in the molecular interactions between the A_1 adenosine receptor and its ligands are not clear. Several types of partial agonism are possible from a kinetic point of view (50), and each may appear as a diminished ability to release GDP from G proteins. Future studies might address whether partial agonists display, in addition to quantitative differences, divergent qualitative aspects.

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