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Activation of Various Subtypes of G-Protein α Subunits by Partial Agonists of the Adenosine A_1 Receptor

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ABSTRACT. The activation of different G protein subtypes by the rat adenosine A_1 receptor initiated by stimulation with the full agonist 2-chloro-N^6-cyclopentyladenosine (CCPA) and by six structurally distinct partial agonists of this receptor was investigated. Endogenous G protein α subunits in rat cortical membranes were inactivated by *N*-ethylmaleimide (NEM). Activation of rat recombinant myristoylated α_o, α_{i1}, α_{i2} and α_{i3} by partial agonists in comparison to the full agonist was assessed by guanosine-5′-(γ-[^{35}S]thio)triphosphate ([^{35}S]GTPγS) binding after reconstitution of G protein α subunits with the adenosine A_1 receptor in *N*-ethylmaleimide-treated membranes. 2-Chloro-N^6-cyclopentyladenosine and 3′-deoxy-N^6-cyclopentyladenosine (3′-d-CPA), the partial agonist with the highest intrinsic activity, were significantly more potent in activation of α_i subtypes than α_o. In contrast, 5′-methylthioadenosine (MeSA), 2′-deoxy-2-chloroadenosine (cladribine), 2′-deoxy-N^6-cyclopentyladenosine (2′-d-CPA), 2-phenylaminoadenosine (CV 1808) and C8-aminopropyl-N^6-cyclopentyladenosine (C8-aminopropyl-CPA) did not exhibit higher potency for G_o or any G_i subtype. All partial agonists, although carrying structurally different modifications, showed higher relative intrinsic activities in activation of G_i than of G_o, indicating that G_i-coupled pathways may be activated selectively via the A_1 receptor by partial agonists, but not G_o-mediated responses. BIOCHEM PHARMACOL **56**;10: 1287–1293, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. adenosine A_1 receptor; partial agonist; reconstitution; G_o; G_i; GTPγS

Partial agonists of adenosine A_1 receptors may be more advantageous therapeutic agents than full agonists due to more specific effects on the target organ and to fewer side effects, owing to the widespread localization of this receptor. For therapeutic purposes, it would be advantageous to minimize the side effects of adenosine A_1 receptor agonists—e.g. on the cardiovascular system—while maintaining the depressant effects on the CNS in the development of adenosine-related psychotropic drugs. Generally, different efficacies of partial agonists of G-protein-coupled receptors in different target organs have been attributed to different levels of spare receptors [1, 2] or to activation of the receptor coupled to specific G-protein subtypes, leading to preferential activation of specific effector systems [3, 4]. The concept that multiple conformational states selective for particular G-protein/effector pathways are induced during activation of a single receptor is supported by the induction of ligand-specific conformational changes in the β_2 -adrenergic receptor [5] and by agonist-specific coupling of α_2 -adrenergic receptors to G_s protein [6]. Although these data may be interpreted to be in agreement with the two-state model of receptor activation, there is additional evidence for agonist-specific states from the reversal of agonist potencies in activation of distinct effector pathways, which is not compatible with the two-state model [3]. Agonists of the *Drosophila* octopamine/tyramine receptor permanently expressed in Chinese hamster ovary cells, which differ by only a single hydroxyl group, may preferentially either inhibit adenylate cyclase through a pertussis toxin-sensitive G protein or elevate intracellular Ca^{2+} levels via a pertussis toxin-insensitive pathway [7]. A reversal in agonist potency was also noted for agonists of the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor expressed in LLC PK1 cells for stimulation of cAMP production and the stimulation of inositol phosphate production [8].

Adenosine A_1 receptors couple to G proteins of the G_i and G_o subtype [9–12]. Bovine and human A_1 receptors exhibit distinct G protein coupling selectivity [13, 14]. Through activation of G_o, A_1 receptors inhibit L-type Ca^{2+} currents in rat frontal cortex [15], whereas the functional role of A_1 receptor/G_o coupling in the heart [16] has not been clarified. Through interaction with the G_i subtype, A_1 receptors induce antiadrenergic effects in the heart [17] and through inhibition of adenylate cyclase via $G_{i\alpha 2}$ an inhibition of lipolysis in adipocytes [18, 19]. If these different effects of adenosine could be mimicked selectively by

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partial agonists, side effects could be greatly reduced. This potential selectivity of partial agonists might be caused either by different degrees of receptor reserve or by selective activation of G-protein subtypes leading to preferential activation of particular effector systems.

Adenosine A_1 receptor partial agonists have been characterized *in vitro* by biochemical and pharmacological methods [20, 21], and partial agonism has also been demonstrated functionally in *in vivo* studies [22–24]. Modifications of the adenosine molecule, e.g. removal of the 2′- or 3′-hydroxyl group of the ribose [22, 23], substitution of the 5′-hydroxyl group by a methylthio substituent [21] and substitution of the C2 position [21] and aminoalkyl substituents at the C8 position of the purine ring [24] lead to lower intrinsic activities. MeSA,† CV 1808 and cladribine are not necessarily selective for adenosine A_1 receptors. In rat brain membranes, however, these partial agonists as well as the selective agonist CCPA stimulate [^{35}S]GTPγS binding solely through activation of A_1 receptors [21]. The partial agonists containing an N^6-cyclopentyl substitution (2′-d-CPA, 3′-d-CPA and C8-aminopropyl-CPA) are selective for adenosine A_1 receptors [23, 24]. In order to characterize the effects of adenosine A_1 receptor partial agonists on distinct G-protein subtypes, we investigated the potency and intrinsic activity of six structurally different partial agonists to activate $G_{o\alpha}$, $G_{i\alpha1}$, $G_{i\alpha2}$ and $G_{i\alpha3}$ in comparison to the full agonist CCPA. For this purpose, α subunits of endogenous G_o and G_i proteins in rat forebrain membranes were inactivated by alkylation with NEM, which does not affect the adenosine receptor protein [25]. An identical approach was used previously to compare the species differences on G protein coupling selectivity of the human and bovine adenosine A_1 receptor [13]. Pretreated membranes containing the endogenous adenosine A_1 receptor were reconstituted with individual G-protein α subunits. The activation of the reconstituted α subunits ($G_{o\alpha}$, $G_{i\alpha1}$, $G_{i\alpha2}$ and $G_{i\alpha3}$) by the full and partial agonists was determined as stimulation of [^{35}S]GTPγS binding.

MATERIALS AND METHODS

Materials

[^{35}S]GTPγS (1,000–1,500 Ci/mmol) was obtained from New England Nuclear. Biologically active myristoylated rat recombinant G-protein α subunits (α_o, α_{i1}, α_{i2} and α_{i3}) came from Calbiochem. Alamethicin, BSA, CHAPS, cladribine, GTPγS, MeSA and NEM were purchased from Sigma. Adenosine deaminase (from calf intestine; 200 U/mg), DTT and guanosine diphosphate were from Boehringer Mannheim. Polyethyleneglycol 6,000 was from Serva. CCPA and CV 1808 came from Research Biochemicals. C8-aminopropyl-CPA, 2′-d-CPA and 3′-d-CPA were generous gifts from Dr. A. P. IJzerman, Leiden/Amsterdam Center for Drug Research, Leiden. All other chemicals were of the highest purity commercially available and obtained from standard sources.

Reconstitution of G-Protein α Subunits in Rat Forebrain Membranes

Preparation of membranes from rat forebrain was performed as previously described [26]. Protein content was assessed according to Peterson [27], using BSA as standard. Endogenous α subunits of the G_i and G_o subtype were inactivated by treatment of the membranes at a protein concentration of 1 mg/mL with NEM (1 mM) as described [26]. NEM-pretreated membranes (1 mg/mL) were resuspended in 1 mL of 50 mM Tris-HCl, pH 7.4, 1 mM EDTA, 2 mM DTT, 2% (w/v) CHAPS, and 20% (v/v) glycerol and were incubated for 30 min on ice. Thereafter, 5 μg of either α_o, α_{i1}, α_{i2} or α_{i3} were added in 1 mL of the same buffer with the omission of CHAPS and incubated for a further 30 min. Samples were applied to prepackaged PD-10 columns (Pharmacia; 2 mL sample/column) equilibrated in 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 1 mM EDTA and 2 mM DTT. After application of the sample, 1 mL of column equilibration buffer was added, and the eluate was discarded. 2.5 mL of column equilibration buffer were added, and this eluate was collected. After 20 min on ice, an equal volume of polyethyleneglycol 6,000 (final concentration: 12%, w/v) in 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ and 1 mM EDTA was added. After 10 min at room temperature, samples were centrifuged at 100,000 *g* (37,000 rpm in a Beckman Ti 60 rotor) at 4° for 120 min. The pellets were resuspended in 50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$ and 1 mM EDTA, frozen in liquid nitrogen and stored at −70° until use. Control membranes were subjected to identical treatment, but with the omission of NEM in the pretreatment and without addition of α subunits in the reconstitution. For the control of the effect of pretreatment with NEM, an aliquot of membranes which had received pretreatment with this compound was also subjected to reconstitution, but buffer was substituted for G-protein α subunits.

Stimulation of [^{35}S]GTPγS Binding

G-protein activation by full and partial agonists of the adenosine A_1 receptor was assessed in a total volume of 100 μL containing 50,000 cpm (approx. 0.2 nM) of [^{35}S]GTPγS, 50 mM Tris-HCl pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, 1 mM DTT, 100 mM NaCl, 1 μM guanosine diphosphate, 0.2 U/mL of adenosine deaminase, 0.5 mg of BSA and 5 μg of alamethicin/tube. Incubations were performed with 0.5–2.5 μg of reconstituted membranes in the absence or presence of agonists for 75 min at 37° and terminated by filtration over nitrocellulose filters (BA 85, Schleicher and Schuell), followed by two 4 mL washes with

† *Abbreviations*: C8-aminopropyl-CPA, C8-aminopropyl-N^6-cyclopentyladenosine; CCPA, 2-chloro-N^6-cyclopentyladenosine; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]propanesulfonate; cladribine, 2-chloro-2′-deoxyadenosine; CV 1808, 2-phenylaminoadenosine; d-CPA, deoxy-N^6-cyclopentyladenosine; DTT, dithiothreitol; GTPγS, guanosine-5′-(γ-thio)triphosphate; MeSA, 5′-methylthioadenosine; and NEM, *N*-ethylmaleimide.

ice-cold buffer (50 mM Tris-HCl, pH 7.4, 5 mM $MgCl_2$, 0.02% (w/v) CHAPS). Data were fitted to sigmoid dose-response curves using the SigmaPlot program. EC_{50} values are given as geometric means with 95% confidence limits from four to seven experiments. For partial agonists, the intrinsic activity (±SEM) to stimulate [^{35}S]GTPγS binding is given as a percentage in relation to the reference full agonist CCPA, the efficacy of which was set as 100%. Comparisons were performed after ANOVA followed by the multiple comparison Newman–Keuls test. Differences were considered significant when $P < 0.05$.

RESULTS

Myristoylated rat recombinant G protein α subunits were reconstituted with adenosine A_1 receptors in rat brain membranes after inactivation of endogenous α subunits by alkylation with NEM. In control membranes, CCPA induced an approximately two-fold increase in [^{35}S]GTPγS binding over nonstimulated binding. Pretreatment of the membranes with NEM reduced the A_1 receptor-dependent G protein activation from 1,391 ± 138 fmol/mg protein in control membranes to 60 ± 12 fmol/mg (4.3% of control; Fig. 1). Successful reconstitution of α_o, α_{i1}, α_{i2} and α_{i3} with the adenosine A_1 receptor in pretreated membranes was demonstrated by significant enhancement of the maximum stimulation induced by the full agonist CCPA (Fig. 1). After reconstitution with α_o, α_{i1}, α_{i2} or α_{i3}, CCPA stimulated [^{35}S]GTPγS binding approximately two-fold compared to basal binding, a signal-to-noise ratio almost identical to that observed in control membranes. Therefore, the extent of stimulation of [^{35}S]GTPγS binding was sufficient to investigate the relative intrinsic activities of partial agonists.

MeSA, cladribine, 2′-d-CPA, 3′-d-CPA, CV 1808 and C8-aminopropyl-CPA have been characterized previously as partial agonists of the adenosine A_1 receptor [21–24]. These adenosine derivatives were investigated with respect to possible differences in activation of G-protein α subunits. All compounds tested showed potencies and intrinsic activities lower than CCPA (Table 1). Ligand potencies for activation of different G-protein α subunits did not grossly differ between the different subtypes activated, except for the two ligands with highest intrinsic activity. CCPA showed a significantly (3- to 4-fold) higher potency in activation of G_i subtypes compared to G_o activation (Fig. 2). Similarly, 3′-d-CPA, the partial agonist of highest intrinsic activity, also displayed a preference for G_i rather than G_o activation (Table 1). In contrast, all other partial agonists were equipotent in activation of the different α subunits. An example is given in Fig. 2, illustrating the stimulation of [^{35}S]GTPγS binding to α_o and different α_i subtypes by MeSA. Thus, at a given ligand concentration, CCPA and 3′-d-CPA, due to higher affinities, but not partial agonists of lower intrinsic activity, preferentially activated adenosine A_1 receptors coupled to G_i rather than G_o-coupled receptors.

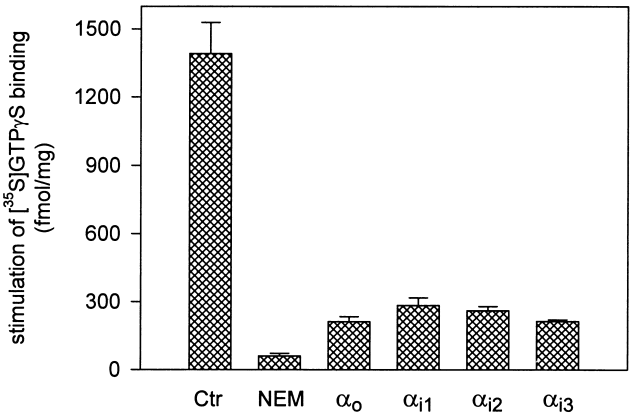

FIG. 1. G protein activation by the full agonist CCPA. Maximum stimulation of [^{35}S]GTPγS binding was assessed in the presence of the full adenosine A_1 receptor agonist CCPA in rat forebrain membranes not treated (Ctr) or pretreated with NEM, and NEM-treated membranes reconstituted with rat recombinant G protein α_o, α_{i1}, α_{i2} or α_{i3} subunits. The assay of [^{35}S]GTPγS binding was carried out as described in Materials and Methods. Membranes were incubated with increasing concentrations of CCPA, and the maximum stimulation of [^{35}S]GTPγS binding by the agonist was calculated as the difference between the asymptotic minima and maxima of sigmoid dose-response curves. [^{35}S]GTPγS binding amounted to 1,453 ± 227 fmol/mg (basal) and 2,844 ± 342 fmol/mg (CCPA-stimulated) in control membranes, and 210 ± 23 fmol/mg (basal) and 270 ± 34 fmol/mg (CCPA) in NEM-pretreated membranes. After reconstitution of NEM-pretreated membranes with different α subunits, [^{35}S]GTPγS binding was increased to 333 ± 35 fmol/mg (basal) and 545 ± 81 fmol/mg (CCPA; reconstitution with α_o), 326 ± 33 fmol/mg (basal) and 609 ± 64 fmol/mg (CCPA; α_{i1}), 326 ± 24 fmol/mg (basal) and 586 ± 42 fmol/mg (CCPA; α_{i2}) and 282 ± 8 fmol/mg (basal) and 495 ± 12 fmol/mg (CCPA; α_{i3}). The data are means ± SEM from five to seven experiments for each preparation.

A further selectivity for α subunits of the G_i subtype was noted for all other partial agonists tested. Although MeSA, cladribine, 2′-d-CPA, CV 1808 and C8-aminopropyl-CPA showed identical potencies in stimulation of [^{35}S]GTPγS binding to all α subunits, the intrinsic activities determined for activation of α subunits were significantly different. The rank order in intrinsic activity for all partial agonists was identical with $(\alpha_{i1} \approx \alpha_{i2} \approx \alpha_{i3}) > \alpha_o$ (Table 1). Figure 3 illustrates the differences in intrinsic activity; the maximum stimulation of [^{35}S]GTPγS binding to α subunits was taken from Table 1. Because the same difference in intrinsic activity was noted for all partial agonists irrespective of their chemical structures, we assume that *in vivo* as well adenosine A_1 receptor partial agonists will preferentially activate G_i-mediated responses rather than G_o-coupled pathways.

DISCUSSION

In order to characterize the activation of different subtypes of G-protein α subunits by agonists of the adenosine A_1 receptor, we reconstituted individual rat recombinant G protein α_o, α_{i1}, α_{i2} and α_{i3} subunits with the adenosine A_1

TABLE 1. Potencies and relative intrinsic activities of partial agonists in stimulation of [^{35}S]GTPγS binding to different G protein α subunits reconstituted with the adenosine A_1 receptor in rat forebrain membranes after inactivation of endogenous α subunits with NEM

	α_o		α_{i1}		α_{i2}		α_{i3}	
	R.I.A. (% of CCPA)	EC_{50} (nM)	R.I.A. (% of CCPA)	EC_{50} (nM)	R.I.A. (% of CCPA)	EC_{50} (nM)	R.I.A. (% of CCPA)	EC_{50} (nM)
CCPA	100	183 (144–232)	100	41.2* (25.5–66.5)	100	62.4* (37.8–103)	100	68.3* (44.2–105)
MeSA	42.7 ± 7.5	10,400 (8,990–12,100)	72.0 ± 2.9*	10,200 (7,380–14,200)	58.1 ± 2.6*	10,200 (8,050–13,000)	61.5 ± 4.3*	9,800 (6,890–13,900)
Cladribine	6.1 ± 3.8	ND	29.8 ± 2.8*	29,600 (19,300–45,200)	26.0 ± 1.3*	30,700 (17,200–55,100)	27.7 ± 4.1*	41,500 (24,600–70,100)
2′-d-CPA	27.5 ± 4.3	12,200 (10,400–14,400)	63.6 ± 4.4*	10,500 (9,200–11,900)	53.6 ± 2.6*	11,200 (8,990–13,990)	52.0 ± 4.7*	10,200 (9,670–10,800)
3′-d-CPA	76.6 ± 6.0	2,310 (1,640–3,260)	94.1 ± 3.2	927* (747–1,150)	93.6 ± 4.9	1,380* (1,210–1,570)	89.7 ± 2.8	1,290* (1,160–1,440)
CV 1808	40.7 ± 8.3	9,450 (7,510–11,900)	66.0 ± 3.8*	8,600 (6,970–10,700)	52.9 ± 2.7	9,370 (7,410–11,800)	54.6 ± 3.6	10,300 (9,910–10,600)
C8-aminopropyl-CPA	48.0 ± 5.8	1,540 (1,300–1,850)	79.0 ± 6.0*	1,620 (1,180–2,230)	68.7 ± 7.5*	1,600 (1,300–1,990)	68.8 ± 3.8	1,530 (1,150–2,040)

Relative intrinsic activities (R.I.A.) in comparison to the full agonist CCPA were calculated as outlined in the experimental section and are given as means ± SEM. Potencies to stimulate [^{35}S]GTPγS binding are as geometric means with 95% confidence limits from $N = 4–7$ experiments for each agonist. ND: due to the very low degree of stimulation by cladribine, an EC_{50} value could not be determined for this compound in α_o activation.

*Significant difference in comparison to α_o ($P < 0.05$).

receptor in rat forebrain membranes after inactivation of the endogenous α subunits by alkylation of sulfhydryl groups with NEM. This treatment uncouples the receptor from G proteins, but does not affect the adenosine A_1 receptor protein [25]. Similarly, Jockers *et al.* [13] have characterized the G protein selectivity of the human and bovine adenosine A_1 receptor after inactivation of G protein α subunits in brain membranes with NEM and consecutive reconstitution with G_i/G_o α subunits. Receptor-G protein coupling was demonstrated by high affinity agonist binding. A similar approach has been taken in the reconstitution of γ-amino-butyric acid B ($GABA_B$) [28] and μ opioid receptors [29] with G proteins of the G_o and G_i subtype, where recovery of high affinity agonist binding and low-K_m GTPase activity were used as indicators of functional reconstitution of receptor/G protein complexes. Generally, functional reconstitution of receptor G-protein complexes can be assessed by measurement of high affinity agonist binding, by characterizing agonist/receptor effects on guanine nucleotide binding to G proteins, or as ligand-stimulated GTPase activity [30]. For assessment of functional interaction of adenosine A_1 receptors with G proteins and for determination of intrinsic activities of partial agonists in relation to the full agonist CCPA, we chose to measure stimulation of [^{35}S]GTPγS binding in the absence or presence of agonists rather than agonist effects on GTPase, because the adenosine A_1 receptor-induced stimulation of GTPase in cortical [31] and hippocampal [32] membranes is only 10 to 30% above basal levels for full agonists. Reconstitution efficiency ranged from 15% to 20% compared to control. This is in reasonable agreement with the reconstitution efficiency of bovine brain adenosine A_1 receptors in human platelet membranes, where 20% of the receptors used were functionally reconstituted with G proteins [11].

The coupling selectivity of the human and bovine adenosine A_1 receptor has been investigated previously. When purified from bovine brain, A_1 receptors are coupled rather indiscriminately to α_{i1}, α_{i2} and α_o [9]. Recombinant bovine A_1 receptors have been shown to couple equally well to all G_i α subtypes and G_o α [12]. On the other hand, Freissmuth *et al.* [10] have found a ten-fold higher affinity of the purified bovine A_1 receptor for recombinant $G_{i\alpha\text{-}3}$ compared with recombinant $G_{i\alpha\text{-}1}$, $G_{i\alpha\text{-}2}$ and $G_{o\alpha}$. Human A_1 receptors showed a preference for G_i over G_o α subunits [13]. These species differences between bovine and human receptors are possibly attributable to a distinct membrane protein, which has been named coupling cofactor, in bovine and rat, but not in human brain membranes [14]. This protein also stabilizes the interaction between the rat adenosine A_1 receptor and $G_{i\alpha}$ subunits [14, 33]. In the present study, we found a higher potency of CCPA to activate rat brain A_1 receptor-$G_{i\alpha}$ complexes over complexes containing $G_{o\alpha}$ (Fig. 2, Table 1). Since in the present study we used receptors in native rat brain membranes and not purified adenosine A_1 receptors for reconstitution, this difference in coupling selectivity between the

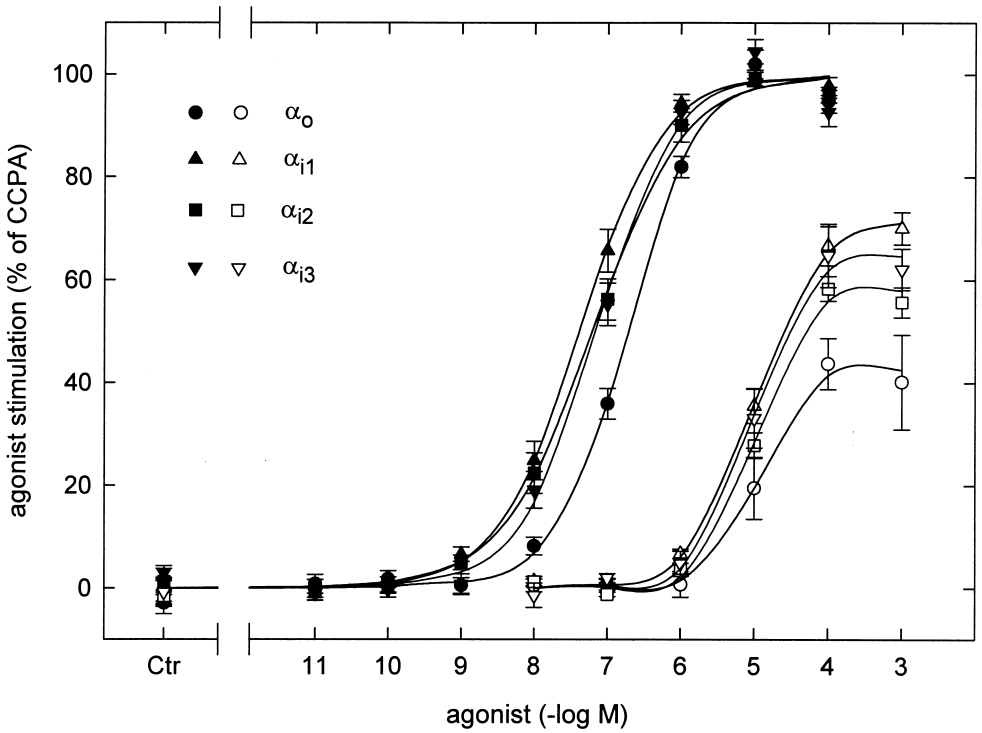

FIG. 2. Activation by CCPA and MeSA of different G protein α subunits reconstituted with the adenosine A_1 receptor in rat brain membranes. Dose-response curves from [^{35}S]GTPγS binding experiments (75 min, 37°) in the absence or presence of increasing concentrations of CCPA (closed symbols) or MeSA (open symbols) were obtained after reconstitution of NEM-treated rat brain membranes with rat recombinant α_o (●, ○), α_{i1} (Δ, ▲), α_{i2} (■, □) and α_{i3} (▽, ▼) as outlined in Materials and Methods. Data are given as percentages of maximum effect ± SEM in the presence of maximally activating concentrations of CCPA and are from four to seven experiments with duplicate samples.

rat A_1 receptor and the more intensely studied bovine receptor may be due either to species differences or to the absence or presence of the coupling cofactor protein.

We observed higher potencies to activate α_i proteins compared to α_o only for the full agonist CCPA and 3′-d-CPA, which was the partial agonist of highest intrinsic activity in relation to CCPA (Table 1). These results indicate that G-protein heterotrimers containing α_o interact more weakly with A_1 receptors than heterotrimers containing α_i. Partial agonists displayed identical potencies in activation of all α subunit types investigated. There was, however, also a preference for G_i over G_o, because the relative efficacy to stimulate [^{35}S]GTPγS binding to α_o was significantly lower than the efficacy in α_{i1-3} activation (Fig. 2, Table 1). Although the partial agonists tested were structurally different, the rank order of efficacy was identical for all compounds ($\alpha_{i1} \approx \alpha_{i2} \approx \alpha_{i3} > \alpha_o$).

The results are in agreement with the notion that G-protein activation through adenosine A_1 receptors proceeds through different activation states, which do not greatly differ qualitatively, but rather quantitatively with respect to the G proteins activated. Apparently, higher agonist intrinsic activity is necessary to activate G_o than G_i. There was no evidence for selective activation of distinct G proteins by structurally different adenosine analogs, as has been reported previously for agonists of the *Drosophila* octopamine/tyramine receptor [7] or the pituitary adenylate cyclase-activating polypeptide (PACAP) receptor [8].

We have shown previously that full agonists detect a higher proportion of A_1 receptors in the high affinity state than partial agonists [21]. The results in the present study are also in accordance with the notion that the extent of receptor reserve determines the intrinsic activity of agonists. For the full agonist CCPA, there may be sufficient receptor G-protein complexes to fully activate the G proteins, whereas the decreased efficacy of partial agonists may be due to the lack of spare receptors. Consequently, due to the relative selectivity of the A_1 receptor for G_i over G_o, the dose-response curve for activation of α_o by CCPA was shifted to the right compared to activation of α_i subtypes, and the dose-response curves of partial agonists were shifted down. At low concentrations of agonists which are likely to prevail *in vivo* when maintenance doses are applied, there is no practical difference between a right shift and a down shift of dose-response curves, and therefore no practical advantage of partial agonists over full agonists. At present, we cannot discriminate if the selectivity for G_i subtypes is due to induction or stabilization of different active states of the receptor, or if the selectivity is merely due to differences in receptor reserve.

Based on these results, it may not be possible via adenosine A_1 receptors to activate primarily G_o-dependent effector pathways, such as inhibition of Ca^{2+} channels in

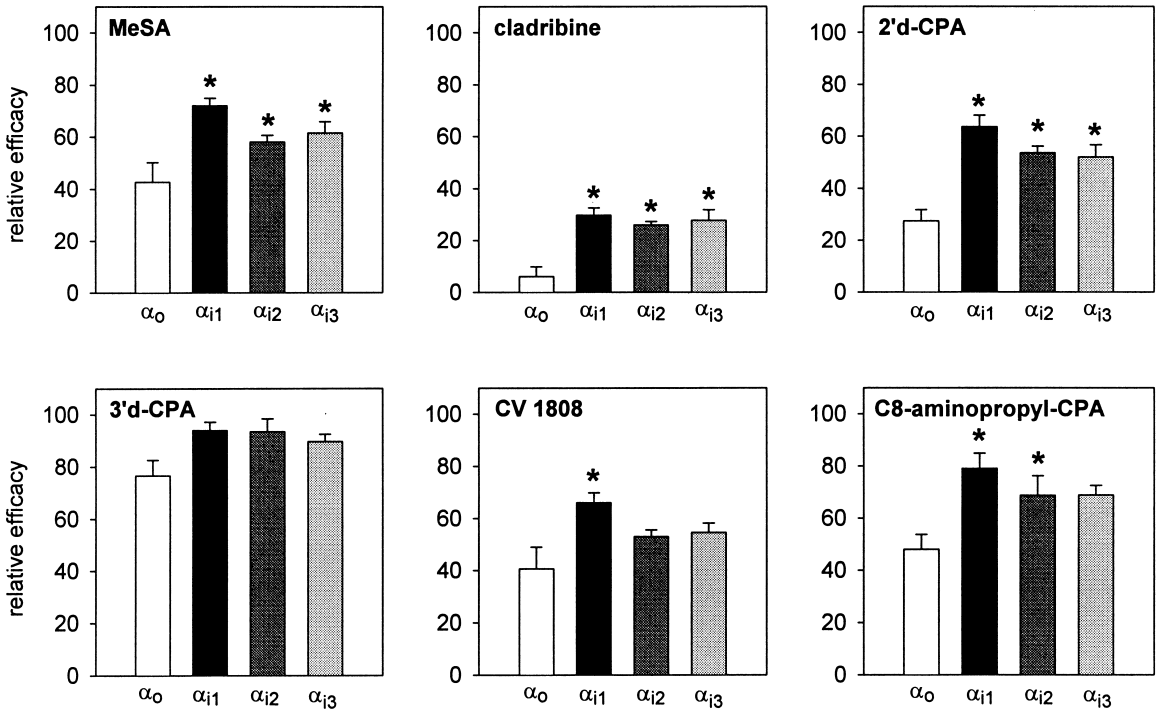

FIG. 3. Maximum activation by different partial agonists of the adenosine A_1 receptor of [^{35}S]GTPγS binding to NEM-pretreated rat forebrain membranes reconstituted with rat recombinant α_o, α_{i1}, α_{i2} and α_{i3}. Radioligand binding was performed as described in Materials and Methods. Basal and maximally stimulated levels of [^{35}S]GTPγS binding were calculated from dose-response curves established for each partial agonist and for the reference full agonist CCPA. The intrinsic activity of partial agonists is given as the percentage of maximum G protein activation induced by the partial agonist in relation to the full agonist CCPA. The data in the figure show mean values ± SEM from four to seven experiments for each compound. *Significant difference versus α_o activation.

brain cortex [15], while G_i-dependent pathways such as the antiadrenergic action through A_1 receptors in the heart [17] are unaffected. This imposes certain limitations on the selectivity of partial agonists of the adenosine A_1 receptor as therapeutic agents. The same selectivity for either G_i- or G_o-mediated responses could be achieved by nonmaximal doses of full agonists (due to the right shift of dose-response curves, as pointed out above) as with partial agonists. At maximally stimulating concentrations of full agonists, the selectivity for G_i over G_o will be reduced or lost. The main advantage of partial agonists would be that their selectivity for G_i over G_o is not abolished at high concentrations. Because all partial agonists exhibited an identical preference for G_i- over G_o-coupled A_1 receptors, the real response would depend on the relative abundance of different receptor/G protein complexes rather than on the type of partial agonist used. In principle, however, the same selectivity as with partial agonists could be achieved by nonmaximal concentrations of a full agonist.

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