Biochemical Pharmacology, Vol. 44, No. 12, pp. 2365–2370, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

EFFECTS OF MONO- AND DIVALENT IONS ON THE BINDING OF THE ADENOSINE ANALOGUE CGS 21680 TO ADENOSINE A_2 RECEPTORS IN RAT STRIATUM

BJÖRN JOHANSSON,* FIONA E. PARKINSON and BERTIL B. FREDHOLM
Department of Pharmacology, Karolinska Institutet, Box 60 400, S-104 01 Stockholm, Sweden

(*Received* 6 *June* 1992; *accepted* 14 *September* 1992)

Abstract—The effect of monovalent and divalent cations on equilibrium binding of the adenosine A_2-selective agonist ligand CGS 21680 (2-[*p*-(2-carbonylethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine) to membranes prepared from rat striatum was examined. Competition experiments with cyclohexyladenosine, 2-chloroadenosine, *N*-ethylcarboxamidoadenosine and CGS 21680 suggest that at 2 nM [^{3}H]CGS 21680 binds to a single site with the pharmacology of an A_{2a} receptor. Magnesium and calcium ions caused a concentration-dependent increase in binding that reached about 10-fold at 100 mM. Manganese ions had a biphasic effect on binding with a maximal increase at 5 mM. Lithium, sodium and potassium ions all caused a concentration-dependent decrease of binding. Sodium was most potent, potassium least. At 200 mM ion concentration, the inhibition of binding was 88% by sodium, 47% by lithium and 29% by potassium ions. The effect of sodium chloride was the same as that of sodium acetate. The effect of sodium ions was essentially similar to that of Gpp(NH)p. However, sodium ions produced a larger effect than even maximally effective concentrations of Gpp(NH)p. The maximal inhibition by Gpp(NH)p was about 55% at 2 nM radioligand concentration irrespective of the magnesium concentration. The maximal effect of sodium ions was reduced by increasing concentrations of magnesium ions. Increasing magnesium ion concentration from 1 to 100 mM increased the half-maximally effective concentration of Gpp(NH)p almost 10-fold and that of sodium ions less than 2-fold. Furthermore, sodium ions and Gpp(NH)p had additive effects. The binding of an agonist to striatal A_{2a} receptors shows an unusually large dependence on both divalent and monovalent cations that can only partly be explained by a change in the coupling to G_s proteins.

This study was undertaken to investigate the dependence of adenosine A_2 receptor binding on ions, since this receptor is coupled to G_s and it is well known that the binding of ligands to other G-protein coupled receptors and the subsequent regulation of cell function is influenced by ions [1].

N-Ethylcarboxamidoadenosine (NECA†) has often been used as the ligand to study adenosine A_2 receptors. However, this ligand binds to other sites in addition to the high-affinity A_2 receptor and in fact has a higher affinity for A_1 receptors [2]. Recently, a derivative of NECA, CGS 21680 (2-[*p*-(2-carbonylethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine), was found to be highly selective for the high-affinity A_2 receptor [3]. Using receptor autoradiography we found the binding of CGS 21680 to be much more Mg^{2+}-sensitive than the binding of A_1 receptor agonists to their receptors [4]. In apparent contrast, Jarvis *et al.* [3] found only a 10% increase in CGS 21680 binding when Mg^{2+} ions were added to their membrane preparation. A first aim of the present study was, therefore, to examine if the magnitude of the Mg^{2+} effect might vary between structurally intact membranes that are examined using quantitative receptor autoradiography, and membrane preparations.

Among divalent cations not only Mg^{2+}, but also e.g. Ca^{2+} and Mn^{2+} often increase agonist binding to receptors, including the adenosine A_1 receptor [5, 6]. Since agonist binding is strongly dependent upon the interactions with G-proteins, whose function is affected by Mg^{2+}, but not by the other divalent cations, we wanted to compare the effect of Mg^{2+}, with that of Ca^{2+} and Mn^{2+}. Guanine nucleotides decrease high-affinity agonist binding, and this is thought to be due to a functional or physical uncoupling of the receptor from the G-protein [7]. The interaction between Mg^{2+} and Gpp(NH)p, a GTP-analogue which is not subject to Mg^{2+}-sensitive hydrolysis, as are GTP and GTPγS [8], was therefore also studied in an attempt to determine whether the magnesium effect is due to an action on G-proteins.

Monovalent cations are known to inhibit the binding of agonists to several G-protein coupled receptors [1] including adenosine A_1 receptors [5]. However, some receptors can react differently as exemplified by the angiotensin II receptor, at which binding is markedly increased by sodium [9]. The third aim of the present study was therefore to

* Corresponding author. Tel. (46) 8-728 79 36; FAX (46) 8-33 16 53.

† Abbreviations: 2-CADO, 2-chloroadenosine; CGS 21680, 2-[*p*-(2-carbonylethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine; EGTA, ethyleneglycol-bis-(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; Gpp(NH)p, guanylyl imidodiphosphate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); IBMX, 3-isobutyl-1-methylxanthine; NECA, 5′-*N*-ethylcarboxamidoadenosine; *R*-PIA, *R*-*N*^{6}-phenylisopropyladenosine.

examine the effect of three monovalent cations on the binding of the A_2 receptor agonist.

MATERIALS AND METHODS

This study was carried out on membranes from the striatum of rat brain. Sprague–Dawley rats weighing 180–230 g (ALAB, Sweden) were used. The animals were anesthetized with CO_2, decapitated, and the brain and then the striatum were rapidly dissected out on ice. The striata were weighed and sonicated 3 × 10 sec with an MSE 100 W ultrasonic disintegrator in a buffer that contained 320 mM sucrose, 50 mM Tris, 2 mM EGTA, 1 mM dithiotreitol, pH 7.6 at 4° with HCl. The resulting homogenate was centrifuged at 1000 *g* at 4° for 10 min. The supernatant was transferred to a new tube and centrifuged at 13,000 *g* for 20 min. The pellet was resuspended by vortexing in a new buffer that contained 50 mM Tris (pH 7.4 at 23°), 2 mM EGTA, 1 mM EDTA, 5 U/mL adenosine deaminase from calf intestine (ADA, Boehringer Mannheim, Sweden) and preincubated at 37° for 30 min. The homogenate was then centrifuged at 30,000 *g* for 10 min, and the pellet resuspended by vortexing in the incubation buffer which was Tris–HCl 50 mM, pH 7.4. Incubations were made for 120 min at room temperature and contained 2 nM [^{3}H]CGS 21680 (DuPont NEN) and 2 UY/mL adenosine deaminase (Boehringer Mannheim). Non-specific binding was determined in the presence of 20 μM 2-chloroadenosine (2-CADO; RBI) and sometimes also (in a separate incubation) with 500 μM isobutylmethylxanthine (IBMX; Sigma). None of the ions tested changed the non-specific binding and 20 μM 2-CADO defined the same non-specific level as 500 μM IBMX. The non-specific binding using 2 nM [^{3}H]CGS 21680 was approximately 20% of the total in the presence of 10 mM $MgCl_2$. In the competition experiments, *R*-N^6-phenylisopropyladenosine (*R*-PIA; Boehringer Mannheim), 2-CADO (Sigma), NECA (Sigma) or CGS 21680 (RBI) were included at increasing concentrations in the incubations.

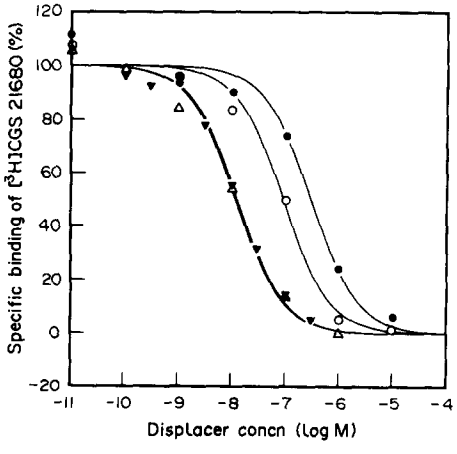

Fig. 1. Concentration-dependent inhibition of [^{3}H]-CGS 21680 binding by various adenosine receptor agonists. Membranes were incubated with 10 mM $MgCl_2$ and increasing concentrations of unlabelled *R*-PIA (●), 2-CADO (○), CGS 21680 (▼) or NECA (△). A one-site competition curve was fitted to the experimental data with a least squares method. Abscissa: concentration of inhibitor in log M. Ordinate: specific binding of [^{3}H]CGS 21680 in per cent of maximal.

The salt to be studied was added to the incubation so that the final volume was 0.3 mL. The incubations in the presence of Mn^{2+} also contained 0.01% ascorbic acid. Membranes were freshly prepared before each incubation. The incubations were terminated by filtering the incubation medium through a Whatman filter by means of a Skatron cell harvester, setting 333. Washing solution was 50 mM Tris–HCl, pH 7.4. The filters were soaked in Ready Safe scintillation fluid (Beckman) and counted by an LKB-Wallac liquid scintillation counter. GraphPAD InPlot (GraphPAD Software, San Diego) was used for collection of data and curve fitting. Parameters obtained by curve fitting were compared using Fischer's protected least significant differences. Chemicals were Merck analysis grade, unless otherwise noted. Guanylyl imidodiphosphate (Gpp(NH)p) was from Boehringer Mannheim. Protein concentration was measured by the Bio-Rad assay.

RESULTS

Binding of 2 nM [^{3}H]CGS 21680 was linearly related to the concentration of protein and reached equilibrium at 2 hr incubation (not shown). A typical competition experiment (Fig. 1) showed that the rank order of potency for inhibition of [^{3}H]-CGS 21680 binding was NECA (IC_{50} 12 nM) = CGS (13 nM) > 2-CADO (85 nM) > *R*-PIA (294 nM). This is consistent with an A_2 receptor and is in agreement with previous studies showing that CGS 21680 is an A_2-selective adenosine receptor agonist [3, 10]. A single site model was adequate to describe the data, since a two site model in no case significantly improved the fit ($P > 0.05$).

There was a marked increase of binding of 2 nM [^{3}H]CGS 21680 upon addition of magnesium. The binding without added divalent cations was only about 10% of the binding at 10 mM magnesium (Fig. 2). This finding essentially agrees with the data obtained in a separate series of experiments using quantitative autoradiography, though magnesium appeared to be even more potent in the autoradiographic experiments than in the present experiments (see Ref. 4). The CGS 21680 binding steadily increased when Mg^{2+} was added up to a concentration of at least 100 mM. The influence of Ca^{2+} on binding was very similar to that of Mg^{2+} with the exception that with Ca^{2+} there was a tendency to a plateau at the highest ion concentrations used, possibly indicating a biphasic effect (Fig. 2). The effect of Mn^{2+} was clearly biphasic (Fig. 2), with a maximal stimulation of binding being observed at 5 mM.

The chloride salt of all three monovalent cations studied caused a decrease in binding: NaCl was quite potent, LiCl was considerably less potent, and the effect of KCl was rather small (Fig. 3). The inhibition of binding was best described by a monoexponential

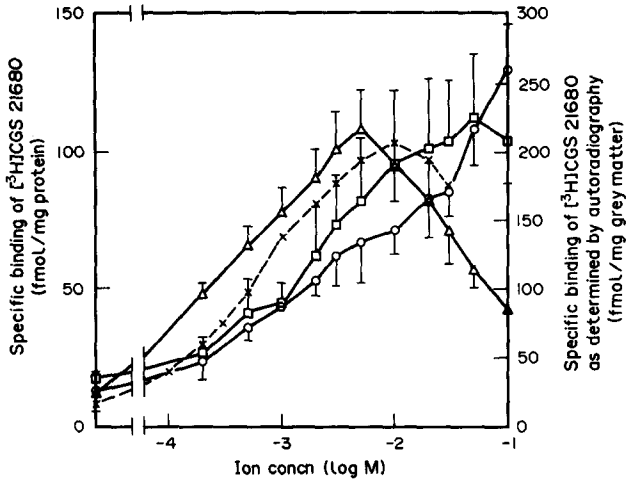

Fig. 2. Influence of divalent cations on [^{3}H]CGS 21680 binding to rat striatum. Membranes were incubated with 2 nM [^{3}H]CGS 21680 and graded concentrations (0.2–100 mM) of $MgCl_2$ (○), $CaCl_2$ (□) or $MnCl_2$ (△). For comparison, the corresponding results obtained with autoradiography [4] are shown. Sections were incubated for 4 hr in the presence of 2.5 nM [^{3}H]CGS 21680 and 0.1–30 mM $MgCl_2$ (×). Points represent mean ± SEM (N = 3 or 4). Error bars were omitted from autoradiography data for the sake of clarity. Abscissa: $MgCl_2$ concentration in log M. Ordinate: specific binding in fmol/mg protein (membranes) or fmol/mg grey matter (sections).

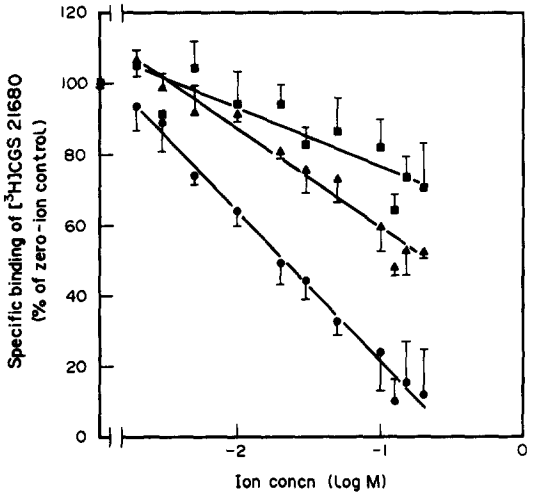

Fig. 3. Concentration-dependent inhibition of [^{3}H]-CGS 21860 binding by monovalent cations. Membranes were incubated with 10 mM Mg^{2+} and graded concentrations (2–200 mM) of LiCl (▲), NaCl (●) or KCl (■). Points represent mean ± SEM (N = 3). The lines were fitted to data (excluding the control value) with a least squares method. The slopes of the lines correspond to a loss of 28, 43 and 17% of the control binding for LiCl, NaCl and KCl, respectively, for each 10-fold increase of concentration. Control values were 90.7 ± 0.8, 85.9 ± 4.4 and 97.7 ± 1.3 fmol/mg protein. Abscissa: salt concentration in log M. Ordinate: specific binding of [^{3}H]CGS 21680 in % of zero-ion control.

curve. In agreement with this, the inhibition of binding did not reach a clear-cut maximum for any of the monovalent ions used, even at 200 mM ion concentration. However, with 200 mM NaCl only about 12% specific binding remained. With 200 mM LiCl or KCl, the remaining binding was 53% and 71%, respectively. Over most of the concentration range, the inhibition by LiCl and KCl was roughly 40% and 25% of the inhibition by NaCl. NaCl caused a consistent inhibition even at very low concentrations (a few mM) whereas LiCl and KCl had no clear effect in the low millimolar range.

To see if the anion or osmolarity contributed to the effect of the salt, we did experiments to compare the effects of the chloride and acetate salts of sodium. The inhibition was essentially the same regardless of the anion over the concentration range 1–50 mM (not shown). In these experiments, the osmolarity was kept constant at 116 mOsM by addition of an appropriate amount of Tris. Altering the ionic strength or osmolarity by adding Tris buffer or sucrose did affect binding to some extent, but only at higher osmolarities than that used in this study (data not shown).

To investigate if the effects of mono- and divalent cations acted at the same site, we studied the influence of Na^+ on binding in the presence of three different Mg^{2+} concentrations. The inhibition by Na^+ was essentially the same regardless of the Mg^{2+} concentration (Fig. 4; IC_{50} at 1, 10 and 100 mM $MgCl_2$ was 11, 12 and 18 mM, respectively) but the inhibition by Na^+ appeared less complete at 100 mM Mg^{2+} than at 1 mM Mg^{2+}.

For comparison, we examined the effect of a stable GTP analogue, Gpp(NH)p. Gpp(NH)p caused

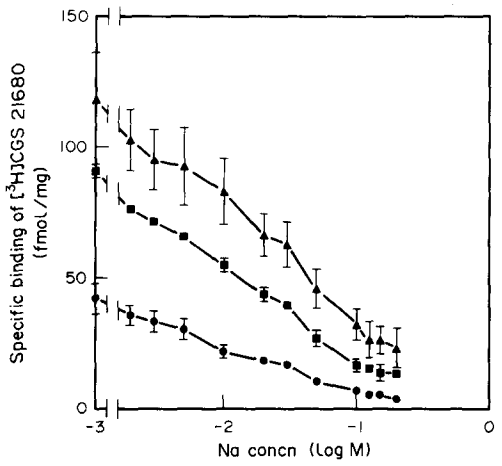

Fig. 4. Inhibition of CGS 21680 binding by NaCl in the presence of various concentrations of $MgCl_2$. Incubations were made in the presence of graded concentrations of NaCl (2–200 mM) and 1 mM $MgCl_2$ (▲), 10 mM $MgCl_2$ (■) or 100 mM $MgCl_2$ (●). Abscissa: concentration of NaCl in mM. Ordinate: specific binding of [^{3}H]CGS 21680 in fmol/mg protein. Data were fitted to a sigmoid curve using non-linear regression. Maximal inhibition of binding was assumed at 200 mM NaCl. For 1, 10 and 100 mM $MgCl_2$, the best fit parameters were as follows. Remaining binding in presence of NaCl: 10, 15, 20%. IC_{50} for NaCl: 11, 12, 18 mM. Hill slope −1.04, −1.08, −1.16. No parameter differed significantly among the groups ($P > 0.05$).

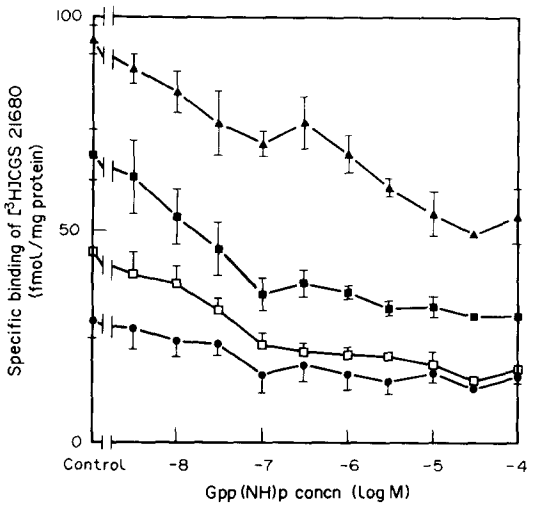

Fig. 5. Inhibition of CGS 21680 binding by Gpp(NH)p in the presence of various ion concentrations. Incubations were made in the presence of graded concentrations of Gpp(NH)p (3 nM–100 μM) and 1 mM $MgCl_2$ (●), 10 mM $MgCl_2$ (■), 100 mM $MgCl_2$ (▲) or 10 mM $MgCl_2$ and 20 mM NaCl (□). Abscissa: concentration of Gpp(NH)p in log M. Ordinate: specific binding of [^{3}H]CGS 21680 in fmol/mg protein. Data were fitted to a sigmoid curve with non-linear regression. For 1, 10 and 100 mM $MgCl_2$ and 10 mM $MgCl_2$ + 20 mM NaCl, the best fit parameters were as follows. Remaining binding in presence of Gpp(NH)p: 53, 41, 46, 42%. IC_{50} for Gpp(NH)p: 42, 23, 330, 26 nM. Hill slope: −0.92, −1.0, −0.33, −1.0. The IC_{50} for Gpp(NH)p in the presence of 100 mM $MgCl_2$ was significantly different ($P < 0.01$) from all other groups. No other parameter was significantly different at any of the ion concentrations, although the slope tended to be less negative at 100 mM $MgCl_2$.

a concentration-dependent inhibition of CGS 21680 binding (Fig. 5). Irrespective of the Mg^{2+} concentration used, a maximal inhibition (at 100 μM) of about 55% was found. The potency of Gpp(NH)p was somewhat reduced when the Mg^{2+} concentration was increased from 1 to 100 mM (IC_{50} at 1 mM, 10 mM, 100 mM $MgCl_2$, 10 mM $MgCl_2$ + 20 mM NaCl was 42, 23, 330, 26 nM, respectively). The potency of Gpp(NH)p was thus unaffected by the addition of Na^+ ions in a concentration that caused an inhibition of CGS 21680 binding that was equivalent to that of a maximally effective concentration of Gpp(NH)p.

DISCUSSION

The magnesium dependence of the binding to A_2 receptors in rat striatal membranes is very high. Increasing Mg^{2+} concentration from 0 to 100 mM increased the binding of 2 nM CGS 21680 10–20-fold. This effect is considerably larger than that observed for several other G-protein coupled receptors including the A_1 receptor [5, 6, 11], the α_2 receptor [12], the formyl peptide receptor [13] and the opiate receptor [14]. All the above-mentioned receptors activate the G_i family of G-proteins rather than the G_s, which is activated by the A_2 receptor. The effect of Mg^{2+} that we find is, however, also much larger than that observed with the G_s-coupled β-adrenoceptor [15]. The effect is also much larger than the effect of Mg^{2+} on [^{3}H]CGS 21680 reported by Jarvis *et al.* [3]. The apparent discrepancy with their finding is probably explained by the fact that Jarvis *et al.* did not include any chelator to remove divalent cations and that even their basal binding might have been stimulated by e.g. calcium or magnesium ions as has been shown to be the case for A_1 receptors [5].

The stimulatory effect of magnesium ions on the binding of [^{3}H]CGS 21680 to membranes is of the same magnitude as that observed using quantitative autoradiography [4]. However, in sections of rat striatum magnesium appeared somewhat more effective at lower concentrations and the concentration–effect curve was biphasic. We do not know the reason for the small differences, which also have been observed in the case of A_1 receptors: in that case magnesium ions markedly increase binding in a membrane preparation [5] but has only minor effects in experiments with tissue sections [4]. This could be related to the fact that membrane preparations may be composed of a mixture of inside-out and outside-out sacs, whereas the membrane orientation in thin sections is more uniform. There may also be a difference in the "integrity" of the two types of preparations. The important result is, however, that two different

experimental techniques show that magnesium ions cause a dramatic increase in binding of agonist high-affinity to A_2 receptors.

For many receptors, magnesium is more potent than calcium ion at increasing binding (e.g. β-adrenergic receptor [15]; α-adrenergic receptor [16]) but there are receptors, such as the adenosine A_1 receptor, at which binding is increased to an equal extent by magnesium and calcium. It seems likely that this difference relates to whether the effect of divalent cations is exerted at the G-protein level or not. There is an effect of micromolar concentrations of Mg^{2+}, but not of the other divalent cations, promoting the effect of receptor agonists to stimulate GTP hydrolysis [1]. In addition to this effect, which is selective for GTP, but not GDP analogues, there are magnesium effects on the ability of GDP analogues to decrease high affinity binding at least in some receptor systems such as the A_1 receptor [4]. This suggests that there are strongly magnesium-dependent interactions between receptor and G-protein that will influence agonist-binding in the A_1 receptor system. In the present study of A_2 agonist binding the effects of magnesium were shared by calcium and manganese ion. There were minor differences between the cations, particularly at high concentrations. However, the essential similarity between the three divalent cations indicates that the stimulatory effect is not exerted at the level of the G-protein, but rather at the receptor itself.

This conclusion is also supported by the finding that the stimulatory effect of Mg^{2+} was essentially independent of the presence of a maximally active concentration of the stable GTP analogue Gpp(NH)p. Conversely, the GTP-analogue did not reduce the binding of CGS 21680 by more than some 50% irrespective of the Mg^{2+} concentration. Since magnesium increased binding 10- or 20-fold it is clear that the effect cannot be entirely explained by an increase in a high-affinity form associated with G-protein.

The incomplete inhibition by Gpp(NH)p of CGS 21680 binding contrasts with results obtained with quantitative autoradiography, where only 1.2% of the specific binding remained at 100 μM GTP [10] and very little also with 100 μM Gpp(NH)p [4] but agrees rather well with a previous result with membranes [3]. A similar degree of inhibition of agonist binding by GTP analogues has been found also in studies of other receptors, e.g. α_2-adrenergic [17] and dopamine receptors [18, 19 and references therein]. In the case of dopamine receptors, it has been suggested that the limited effects of the GTP analogues can be explained by GTP-sensitive and -insensitive dopamine receptors that have different distributions among anatomical regions and cell structures [18, see discussion in 19]. It is also possible, as noted above, that structural integrity is higher in sections than in membranes. Recent kinetic studies of A_2 receptors have indicated that diffusion of receptors and/or G-proteins in the plane of the membrane is rate-limiting [20]. It is conceivable that in disrupted membranes such diffusion is even more limited.

A_2 binding was also highly sensitive to sodium. Agonist binding to A_2 receptors is more sensitive to monovalent cations than some other receptors including the A_1 receptor [5]. The shape of the inhibition curves is not explained simply by a single monovalent cation site with different affinities for lithium, sodium and potassium ions, since this model would predict that the curves would have similar shapes but be shifted along the horizontal axis. Instead the ions may differ in their efficacy at a single site or there may be different binding sites for the different monovalent cations.

The effect of sodium ions was essentially the same irrespective of the magnesium ion concentration, indicating that the two types of cations acted at different sites, as reported for e.g. α-adrenoceptors [16]. Furthermore, the effects of sodium and Gpp(NH)p were additive. This is in agreement with results on α-receptors in platelets [21, 22] and β-adrenoceptors in lymphoma cells [23]. The latter study conclusively showed that the sodium effect was independent of G-proteins and that seems likely to be the explanation also for the present data.

The normal intracellular sodium ion concentration in neurons is roughly 10 mM and can increase several-fold on addition of a depolarizing agonist [24] or on inhibition of Na^+,K^+-ATPase by ouabain [25]. Parts of a cell that have a high surface-to-volume ratio, such as thin dendritic processes, may be expected to experience especially large fluctuations of ion concentration. Thus, the binding of adenosine might be influenced by physiologically or pharmacologically induced variations in the intracellular sodium concentration brought about by receptor- or voltage-activated ion channels or by receptor-mediated regulation of Na^+,K^+-ATPase [26]. It will be of interest to decide whether the Na^+ site is intracellular or extracellular. For the α_2-adrenergic and the dopamine D_2 receptor there is evidence that the sodium site faces the cytoplasm [27–29], and it is situated at a conserved amino acid residue. We suggest that such an intracellular sodium site might mediate some receptor–receptor interactions.

In conclusion, we have shown that both divalent and monovalent cations strongly influence high-affinity agonist binding to A_2 receptors. Both effects are probably exerted at the receptor itself, but the two ions apparently interact with the receptor at different sites. The cation effect reported for the A_2 receptor is larger than those found in several other receptor systems. It will be interesting to determine whether they can in fact play a role in determining the functional responses to A_2 receptor stimulation.

Acknowledgements—These studies were supported by the Swedish Medical Research Council (04X-2553), the Promotion Fund of the International Coffee Organization, AB Astra and by Karolinska Institutet. F.E.P. was a recipient of a Medical Research Council of Canada/CIBA-Geigy fellowship.

REFERENCES

1. Gierschik P, McLeish K and Jakobs KH, Regulation of G-protein-mediated signal transduction by ions. *J Cardiovasc Pharmacol* **12**(Suppl 5): S20–S24, 1988.
2. Bruns RF, Lu GH and Pugsley TA, Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in

rat striatal membranes. *Mol Pharmacol* **29**: 331–346, 1986.
3. Jarvis MF, Schulz R, Hutchison AJ, Do UH, Sills MA and Williams M, [^{3}H]CGS 21680, a selective A_2 adenosine receptor agonist directly labels A_2 receptors in rat brain. *J Pharmacol Exp Ther* **251**: 888–893, 1989.
4. Parkinson FE and Fredholm BB, Differential effect of magnesium on receptor-G-protein coupling of adenosine A_1 and A_2 receptors: a quantitative autoradiographical study. *Mol Neuropharmacol* **1**: 179–186, 1992.
5. Goodman RR, Cooper MJ, Gavish M and Snyder SH, Guanine nucleotide and cation regulation of the binding of [^{3}H]-cyclohexyladenosine and [^{3}H]-diethylphenylxanthine to adenosine A_1 receptors in brain membranes. *Mol Pharmacol* **21**: 329–335, 1982.
6. Ukena D, Poeschla E and Schwabe U, Guanine nucleotide and cation regulation of radioligand binding to R_i adenosine receptors of rat fat cells. *Naunyn Schmiedebergs Arch Pharmacol* **326**: 241–247, 1984.
7. Birnbaumer L, Abramowitz J and Brown AM, Receptor–effector coupling by G proteins. *Biochim Biophys Acta* **1031**: 163–224, 1990.
8. Parkinson FE and Fredholm BB, Magnesium-dependent enhancement of endogenous agonist binding to adenosine A_1 receptors: a significant source of error in quantitative autoradiography. *J Neurochem* **48**: 941–950, 1992.
9. Glossman H, Baukal A and Catt KJ, Cation dependence of high-affinity angiotensin II binding to adrenal cortex receptors. *Science* **185**: 281–283, 1974.
10. Parkinson FE and Fredholm BB, Autoradiographic evidence for G-protein coupled A_2-receptors in rat neostriatum using [^{3}H]-CGS 21680 as a ligand. *Naunyn Schmiedebergs Arch Pharmacol* **342**: 85–89, 1990.
11. Stiles GL, A_1 adenosine receptor-G protein coupling in bovine brain membranes: effects of guanine nucleotides, salt and solubilization. *J Neurochem* **51**: 1592–1598, 1988.
12. Tsai BS and Lefkowitz RJ, Agonist-specific effects of monovalent and divalent cations on adenylate cyclase-coupled alpha adrenergic receptors in rabbit platelets. *Mol Pharmacol* **14**: 540–548, 1978.
13. Gierschik P, Stisslinger M, Sidiropoulos D, Herrmann E and Jakobs KH, Dual Mg^{2+} control of formyl-peptide-receptor–G-protein interaction in HL 60 cells. Evidence that the low-agonist-affinity receptor interacts with and activates the G-protein. *Eur J Biochem* **183**: 97–105, 1989.
14. Pasternak GW, Snowman AM and Snyder SH, Selective enhancement of [^{3}H]opiate agonist binding by divalent cations. *Mol Pharmacol* **11**: 735–744, 1975.
15. Bird SJ and Maguire ME, The agonist-specific effect of magnesium ion on binding by β-adrenergic receptors in S49 lymphoma cells. Interaction of GTP and magnesium in adenylate cyclase activation. *J Biol Chem* **253**: 8826–8834, 1978.
16. Glossman H and Presek P, Alpha noradrenergic receptors in brain membranes: sodium, magnesium and guanyl nucleotides modulate agonist binding. *Naunyn Schmiedebergs Arch Pharmacol* **306**: 67–73, 1979.
17. Elliott JM, Payvandi N and Heal DJ, Characterisation of a high-affinity binding site for UK 14304 in rat brain membranes which is insensitive to GTP. *Br J Pharmacol* **102** (Proceedings suppl): 6P, 1991.
18. Creese I, Usdin T and Snyder SH, Guanine nucleotides distinguish between two dopamine receptors. *Nature* **278**: 577–578, 1979.
19. De Keyser J, Walraevens H, Ebinger G and Vauquelin G, In human brain two subtypes of D_1 dopamine receptors can be distinguished on the basis of differences in guanine nucleotide effect on agonist binding. *J Neurochem* **53**: 1096–1102, 1989.
20. Gross W and Lohse MJ, Mechanism of activation of A_2 adenosine receptors. II. A restricted collision–coupling model of receptor–effector interaction. *Mol Pharmacol* **39**: 524–530, 1991.
21. Limbird LE, Speck JL and Smith SK, Sodium ion modulates agonist and antagonist interactions with the human platelet α_2-adrenergic receptor in membrane and solubilized preparations. *Mol Pharmacol* **21**: 609–617, 1982.
22. Michel T, Hoffman BB and Lefkowitz RJ, Differential regulation of the α_2-adrenergic receptor by Na^+ and guanine nucleotides. *Nature* **288**: 709–711, 1980.
23. Minuth M and Jakobs KH, Sodium regulation of agonist and antagonist binding to β-adrenoceptors in intact and N_s-deficient membranes. *Naunyn Schmiedebergs Arch Pharmacol* **333**: 124–129, 1986.
24. Brown DA and Scholfield CN, Changes of intracellular sodium and potassium ion concentrations in isolated rat superior cervical ganglia induced by depolarizing agents. *J Physiol (Lond)* **242**: 307–319, 1974.
25. Deitmer JW and Schlue WR, Intracellular Na^+ and Ca^{2+} in leech Retzius neurones during inhibition of the Na^+-K^+ pump. *Pflügers Arch* **397**: 195–201, 1983.
26. Bertorello AM, Hopfield JF, Aperia A and Greengard P, Inhibition by dopamine of ($Na^+ + K^+$)ATPase activity in neostriatal neurons through D_1 and D_2 dopamine receptor synergism. *Nature* **347**: 386–388, 1990.
27. Motulsky HJ and Insel PA, Influence of sodium on the α_2-adenergic receptor system of human platelets. *J Biol Chem* **258**: 3913–3919, 1983.
28. Horstman DA, Brandon S, Wilson AL, Guyer CA, Cragoe EJ and Limbird LE, An aspartate residue conserved among G-protein receptors confers allosteric regulation of α_2-adrenergic receptors by sodium. *J Biol Chem* **265**: 21590–21595, 1990.
29. Neve KA, Cox BA, Henningsen RA, Spanoyannis A and Neve RL, Pivotal role for aspartate-80 in the regulation of dopamine D_2 receptor affinity for drugs and inhibition of adenylate cyclase. *Mol Pharmacol* **39**: 733–739, 1991.