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Guanine Nucleotide and Cation Regulation of the Binding of [^{3}H]Cyclohexyladenosine and [^{3}H]Diethylphenylxanthine to Adenosine A_1 Receptors in Brain Membranes

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SUMMARY

Guanine nucleotides, divalent cations, and sodium differentially regulate agonist and antagonist binding to adenosine A_1 receptors in brain membranes. Guanine nucleotides decrease the binding of the adenosine A_1 receptor agonist [^{3}H]N^6-cyclohexyladenosine ([^{3}H]CHA) to guinea pig and bovine brain membranes by about 50% at 1–3 μM, while not affecting binding of the antagonist [^{3}H]1,3-diethyl-8-phenylxanthine ([^{3}H]DPX) to A_1 receptors in bovine brain. GTP decreases the potency of agonists competing for [^{3}H]DPX binding by 3–6 times, without altering the potency of antagonists. This effect can be used to grade experimental substances along an adenosine agonist-antagonist continuum. The 66% inhibition of [^{3}H]CHA binding by 1 mM EDTA, with no change in [^{3}H]DPX binding, suggests that endogenous divalent cations may regulate adenosine receptor interactions. Removal of endogenous divalent cations by EDTA treatment greatly increases the enhancement of [^{3}H]CHA binding by divalent cations. Specific binding of [^{3}H]CHA to guinea pig brain is increased 150–170% by 0.3–1.0 mM Mn^{2+}, Mg^{2+}, and Ca^{2+} following EDTA preincubation, secondary to an increase in apparent affinity and receptor number. Sodium ions also selectively regulate the binding of [^{3}H]CHA. Sodium decreases [^{3}H]CHA binding 40%, whereas lithium and potassium are ineffective. Sodium does not affect [^{3}H]DPX binding.

INTRODUCTION

Adenosine affects numerous physiological processes, including platelet aggregation, coronary vasodilation, lipolysis, and neuronal function in brain. Adenosine regulates adenylate cyclase activity via two apparently distinct receptors. The A_1 receptors are responsible for the lowering of adenylate cyclase activity, whereas A_2 receptors mediate augmentation in adenylate cyclase (1–3). The substrate specificity of these two receptors differs, with adenosine having nanomolar potency at A_1 and micromolar potency at A_2 receptors. Moreover, marked stereospecificity for the isomers of PIA[3] occurs at A_1 but not A_2 receptors (4). Recently it has been possible to label adenosine receptors in brain membranes with a variety of ligands. A_1 receptors can be labeled with the adenosine agonists [^{3}H]CHA (5), [^{3}H]PIA (6), and, under certain circumstances, [^{3}H]2-chloroadenosine (7). In bovine brain membranes [^{3}H]DPX, a potent xanthine adenosine antagonist, labels A_1 receptors, whereas in guinea pig brain membranes, [^{3}H]DPX binding has properties suggesting interactions with adenosine A_2 receptors (5). Screens of numerous tissues reveal adenosine receptor binding only in the brain (4, 6, 7), testes (7, 8), and fat tissue (9).

Receptor binding for several hormone and neurotransmitter receptors whose actions are associated with adenylate cyclase are regulated by guanine nucleotides (10–17). Guanine nucleotides selectively decrease the affinity of agonists but not antagonists for receptors. Sodium also decreases the affinity of agonists but not antagonists for histamine H_1 (17), opiate (18), and *alpha*$_2$ (19) adrenergic receptors. By contrast, in numerous instances receptor binding for agonists is enhanced by divalent cations, which do not influence antagonist interactions with receptors (20–24). Earlier we reported preliminary data indicating that [^{3}H]CHA binding is decreased by guanine nucleotides (5). In the present study we characterize in detail the regulation of adenosine A_1 receptor binding in guinea pig and bovine brain membranes by guanine nucleotides, divalent cations, and sodium.

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[3] The abbreviations used are: PIA, N^6-phenylisopropyladenosine; L-PIA, N^6-(L-phenylisopropyl)adenosine; CHA, N^6-cyclohexyladenosine; DPX, 1,3-diethyl-8-phenylxanthine; Gpp(NH)p, 5′-guanylyl imidodiphosphate.

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MATERIALS AND METHODS

CHA and L-PIA were obtained from Boehringer Mannheim (Mannheim, Federal Republic of Germany); theophylline was obtained from Sigma Chemical Company (St. Louis, Mo.). [^{3}H]CHA (11.5 Ci/mmole) and [^{3}H]DPX (11.5 Ci/mmole) were obtained from New England Nuclear Corporation (Boston, Mass.). Nucleotides were obtained from Sigma Chemical Company. All other material was obtained from standard sources.

Preparation of membranes. Initial preparation of crude guinea pig brain and bovine forebrain membranes was similar to that reported previously (5). Briefly stated, fresh or thawed frozen (Pel-Freez) guinea pig brains or fresh bovine forebrains were disrupted with a Polytron in 5 volumes of 50 mM Tris-Hcl buffer at pH 7.7. The homogenate was centrifuged at 50,000 × *g* for 10 min, and the pellet was suspended in Tris, centrifuged, and resuspended in Tris containing 2 IU of adenosine deaminase (Sigma, Type III) per milliliter (to eliminate endogenous deaminase). After a 30-min incubation at 37°, the membranes were recentrifuged and either resuspended in 100 volumes (10 mg of tissue per milliliter) of Tris and used for binding assay (normal preparation) or were treated further. Membranes were resuspended in 5 volumes of Tris containing 0, 1, 10, 100, or 500 mM EDTA and incubated at 25° for 45 min, then centrifuged and resuspended in 10 volumes of Tris four times, incubating at 25° for 10 min between centrifugations. The final pellets were then resuspended in 100 volumes (10 mg of tissue per milliliter) of Tris containing 0.2 IU of adenosine deaminase per milliliter and used in binding assays.

Incubation and filtration conditions. Incubations were carried out in 50 mM Tris-HCl at pH 7.7. Standard conditions were as follows: [^{3}H]CHA or [^{3}H]DPX at concentrations specified under Results, 1.9 ml (19 mg) of tissue homogenate and nucleotides, ions or unlabeled CHA, L-PIA, or theophylline added to a total volume of 2 ml. Incubations were carried out at 25° for 2 hr unless otherwise specified, and terminated by rapid filtration through a 25-mm Whatman GF/B filter under reduced pressure; 4 ml of ice-cold buffer were added to the sample tube and the mixture was poured onto the filter; the filters were then washed twice with 4 ml of ice-cold buffer. Specific binding was defined as total binding minus binding in the presence of 5 μM L-PIA. All results are means of triplicates from one or more experiments as indicated.

Protein determinations were made by the method of Lowry *et al.* (25).

RESULTS

Regulation of [^{3}H]CHA binding by guanine nucleotides and cations. [^{3}H]CHA binding to bovine brain membranes is decreased potently and selectively by guanine nucleotides (Table 1). GTP, GDP, and the nonmetabolized GTP analogue Gpp(NH)p exert this effect, whereas GMP and adenine nucleotides are inactive. These three guanine nucleotides have similar potencies with about 50% inhibition of binding at 1–3 μM.

These results resemble preliminary data reported earlier with [^{3}H]CHA binding to guinea pig brain membranes, fitting with other data indicating that [^{3}H]CHA interactions with A_1 receptors are quite similar in bovine and guinea pig membranes (5).

Numerous neurotransmitter receptors are regulated by divalent cations which selectively enhance agonist binding interactions. Endogenous divalent cations may be normal modulators of receptor activity since, with receptors such as the opiate receptor, removal of endogenous divalent cations with EDTA diminishes agonist binding (20). In preliminary experiments, we found only about a 10–40% enhancement of [^{3}H]CHA binding with magnesium, manganese, or calcium. To explore whether this small and inconsistent effect may derive from the fact that endogenous divalent cations were already augmenting receptor binding, we evaluated the effects of EDTA (Table 2A). Exposure of guinea pig brain membranes to 10 mM and 100 mM EDTA, followed by washing, lowers specific binding 19 and 54%, respectively, as compared with control (washed) membranes (data not shown). Exposure to 100 mM EDTA before washing also lessens the inhibition of [^{3}H]CHA binding by subsequently added 1 mM EDTA from a 32% decrease in control (washed) membranes to a 10% decrease. Washing alone decreases the inhibition by 1 mM EDTA from 66% to 32% (data not shown). No significant difference in the enhancement of [^{3}H]CHA binding by divalent cations oc-

TABLE 1

Nucleotide regulation of specific [^{3}H]CHA binding in bovine brain membranes

Various concentrations of nucleotides were added to tubes containing 0.5 nM [^{3}H]CHA and bovine brain membranes prepared as described under Materials and Methods. Specific binding was determined as described under Materials and Methods and results are expressed as the percentage of specific binding in the absence of nucleotides. The results are means of three or more triplicate determinations which varied by less than 10%. Typical binding values were 1250 total cpm and 75 blank cpm, giving 1175 specific cpm.

Nucleotide	Specific [^{3}H]CHA binding (% of control) at nucleotide concentrations of						
	10^{-7} M	3×10^{-7} M	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M
Gpp(NH)p	90	74	68	54	45	40	38
GTP	80	76	53	46	36	36	34
GDP	82	64	52	42	37	36	32
GMP	100	102	98	102	94	96	88
App(NH)p[a]	90	94	85	92	95	96	96
ATP	105	106	106	102	100	94	98
ADP	100	100	98	99	96	98	93

[a] App(NH)p, 5′-adenylyl imidodiphosphate.

TABLE 2

Cation regulation of [^{3}H]CHA binding to guinea pig brain

A. Various concentrations of ions or EDTA were added to tubes containing 3.0 nM [^{3}H]CHA and guinea pig brain membrane preparations with preincubation in the presence or absence of EDTA.

B. Guinea pig brain membranes were prepared as described under Materials and Methods with a 100 mM EDTA preincubation. Specific binding was determined as described under Materials and Methods, and results are expressed as the percentage of the specific binding in the absence of added ions or EDTA. The results are means of three or more triplicate determinations which varied by less than 10%. Typical control binding was 1100 total cpm and 200 blank cpm, giving 900 specific cpm.

A. Incubation condition	Specific [^{3}H]CHA binding (% of control) at preincubation EDTA concentrations of				
	0	1 mM	10 mM	100 mM	Preparations with 5 mM EDTA[a]
1 mM EDTA	68	60	67	90	60
0.1 mM $CaCl_2$	117	94	130	206	146
1 mM $CaCl_2$	138	123	154	270	201
1 mM $MgCl_2$	130	145	169	264	232
0.1 mM $MnCl_2$	105	114	124	207	212

B. ION	Specific [^{3}H]CHA binding (% of control) at ion concentration of								
	3×10^{-6} M	10^{-5} M	3×10^{-5} M	10^{-4} M	3×10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M	1.5×10^{-1} M
$MnCl_2$	97	126	179	207	248	192			
$MgCl_2$	103	107	126	177	219	264			
$CaCl_2$	100	104	117	161	208	270			
LiCl							107	122	122
NaCl							85	70	60
KCl							104	124	120

[a] An experiment with 5 mM EDTA present from the initial step is described under Results, and these results represent means of triplicates with standard errors less than 10%.

curs in washed membranes as compared with control unwashed membranes (data not shown). However, exposure to increasing concentrations of EDTA prior to washing causes large increases in the enhancement. For example, 1 mM $MgCl_2$ causes 30, 69, and 164% increases in specific binding in control membranes (washed) and those treated with 10 mM and 100 mM EDTA, respectively.

Two or three treatments with 5 mM EDTA, followed by washings to remove the EDTA and associated divalent cations, results in a 50% loss of receptor binding (data not shown). A similar loss can be obtained using single treatments with 100 mM EDTA in guinea pig and 500 mM EDTA in bovine brain membranes. The requirement for a substantially higher concentration of EDTA to reduce binding with only a single treatment presumably reflects the dilution of the EDTA during the washing procedure and an associated failure to remove a majority of endogenous divalent cations. To minimize the number of washes required before receptor binding we have routinely provided a single treatment with the higher concentrations of EDTA.

We were also concerned about the possibility that these higher concentrations of EDTA might be removing more than just divalent cations, such as endogenous nucleotides and loosely associated membrane proteins. To investigate this possibility, we examined guinea pig brain membranes prepared with a low EDTA concentration (5 mM) present from the initial Polytron step to chelate divalent cations before they bind tightly to exposed membrane components. The buffer included 5 mM EDTA until the centrifugation following the 37° incubation, and then the pellet was resuspended and centrifuged four times and assayed as described under Materials and Methods. The effects of divalent cations on [^{3}H]CHA binding to this tissue (Table 2) are almost identical with, but slightly less than, those following the preincubation with the higher concentrations of EDTA. The enhancement of binding is increased from 30–40% to 100–130%, with manganese still being most potent. In contrast, 1 mM EDTA still inhibits the binding, indicating that the higher EDTA concentrations more completely remove endogenous divalent cations.

Divalent cations enhance [^{3}H]CHA binding to both guinea pig (Table 2B) and bovine brain membranes (data not shown). Manganese appears to be somewhat more potent than magnesium and calcium, which have similar effects. Significant enhancement of binding is apparent with as little as 10 μM manganese, whereas comparable effects of magnesium and calcium occur at 3–10 times higher concentrations. Maximal enhancement of [^{3}H]CHA binding in the guinea pig occurs at 0.3 mM manganese and 1 mM magnesium or calcium. Divalent cations are less effective in augmenting binding in bovine brain than in guinea pig brain. Whereas the maximal increase of binding is similar for all three divalent cations in guinea pig brain (150–170%), in bovine brain manganese elicits only a 30% maximal augmentation of binding while magnesium and calcium produce a 50% maximal increase. In both guinea pig and bovine brain, binding tends to decrease at higher concentrations of manganese.

Unlike the increase in binding with divalent cations, the monovalent cation sodium decreases binding. About a 40% reduction of [^{3}H]CHA binding occurs at 150 mM sodium. This effect is selective and is not apparent at concentrations as high as 150 mM lithium or potassium.

Saturation and kinetic analysis of guanine nucleotide and divalent cation influences on [^{3}H]CHA binding.

With several neurotransmitter and hormone receptors the reduction of agonist binding interactions by guanine nucleotides is related to a decreased affinity for binding sites with no change in the maximal number of binding sites (10–16). This decreased affinity is often associated with an accelerated dissociation rate (10, 12, 13, 15, 16). In some instances, as with the opiate receptor (16), there is some augmentation by guanine nucleotides of the association rate.

As observed earlier, [^{3}H]CHA binding is saturable in both bovine and guinea pig brain membranes with dissociation constants (K_D) of about 0.50 and 5.0 nM and maximal numbers of binding sites (B_{max}) of about 410 and 206 fmoles/mg of protein (Fig. 1 for bovine brain results; guinea pig data not shown). Scatchard analysis of these experiments indicates that GTP decreases the affinity of [^{3}H]CHA with no pronounced influence on B_{max} values (Fig. 1B). By contrast, divalent cations enhance binding. In bovine brain this occurs almost exclusively by a 40% reduction of the K_D (Fig. 1B), whereas in guinea pig brain there is primarily a reduction of the K_D (80% decrease) but also a significant increase in the number of binding sites (65%) (data not shown).

The differential K_D and B_{max} alterations in guinea pig and bovine brain are apparent in comparisons of the divalent cation effect on binding at concentrations of [^{3}H]CHA far below and above its apparent K_D value. Incubation with 0.1 nM [^{3}H]CHA in bovine brain membranes yields increases of 43, 62, and 68% with 0.1 mM $MnCl_2$, 1 mM $MgCl_2$, and 1 mM $CaCl_2$, respectively, whereas no significant increases are found at 10 nM [^{3}H]CHA. By contrast, at 1.0 nM [^{3}H]CHA in guinea pig brain membranes these ion concentrations cause increases of 220, 266, and 271%, respectively, whereas much smaller, but significant increases (63, 52, and 38%) occur at 50 nM [^{3}H]CHA. These results confirm the Scatchard analysis described above, that the primary effect of the divalent cations is to increase the affinity of the adenosine A_1 receptors for [^{3}H]CHA.

To evaluate interactions between guanine nucleotides and divalent cations, we measured [^{3}H]CHA binding in the presence of various concentrations of manganese. Manganese does reverse the GTP depression of [^{3}H]CHA binding (Fig. 2). However, it is much less effective in reversing inhibition of binding by Gpp(NH)p. Similar effects are observed with magnesium and calcium. Reversal of the GTP effect is not seen with the addition of 50 mM ATP (Fig. 2).

To evaluate kinetic mechanisms accounting for guanine nucleotide and divalent cation effects, we measured association and dissociation rates (Fig. 3). EDTA, manganese, and Gpp(NH)p fail to change the association rate of [^{3}H]CHA (Fig. 3A). However, manganese does augment and Gpp(NH)p decreases the plateau level of binding, via their effects on the dissociation rate. The dissociation of [^{3}H]CHA is biphasic, with a $t_{1/2}$ of about 18 min for the initial phase and about 180 min for the second phase (Fig. 3B and C). Gpp(NH)p and preincubation with EDTA markedly accelerate the initial phase of dissociation (with $t_{1/2}$ values of about 3 min and 5 min, respectively), whereas manganese slows this initial dissociation rate ($t_{1/2}$ of 60 min). The second dissociation

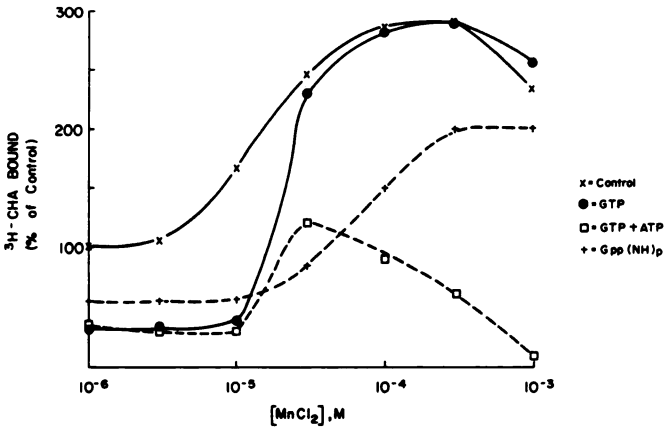

FIG. 2. *Concentration-dependent interactions of manganese and guanine nucleotides at [^{3}H]CHA binding sites in guinea pig brain membranes*

Results are means of three triplicate determinations that varied by less than 15%. Control specific binding (100%) was approximately 900 cpm (1100 cpm total and 200 cpm blank). Conditions were 10 μM GTP (●), 10 μM GTP plus 50 μM ATP (□), 10 μM Gpp(NH)p (+), and no nucleotide addition (×).

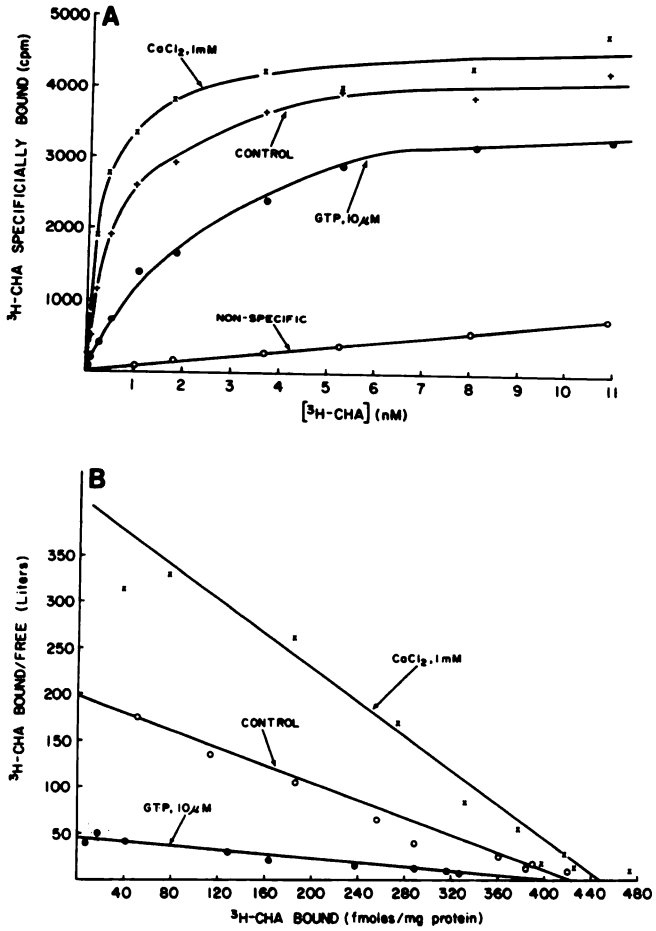

FIG. 1. *[^{3}H]CHA binding in bovine brain*

A. Saturation of [^{3}H]CHA binding in bovine brain with or without 1 mM $CaCl_2$ or 10 μM GTP. Results presented are means of triplicate determinations from a typical experiment.

B. Scatchard plot of results from A.

rate is not markedly affected by these incubation variations.

Effects of guanine nucleotides and cations on [^{3}H]DPX binding. Guanine nucleotides fail to influence [^{3}H]DPX binding in bovine brain membranes. Interestingly, some reduction of [^{3}H]DPX binding occurs with manganese (Table 3). In contrast to the inhibition of [^{3}H] agonist binding by sodium, [^{3}H]antagonist binding is minimally affected by sodium (Table 3).

For several neurotransmitter receptors the agonist-an-

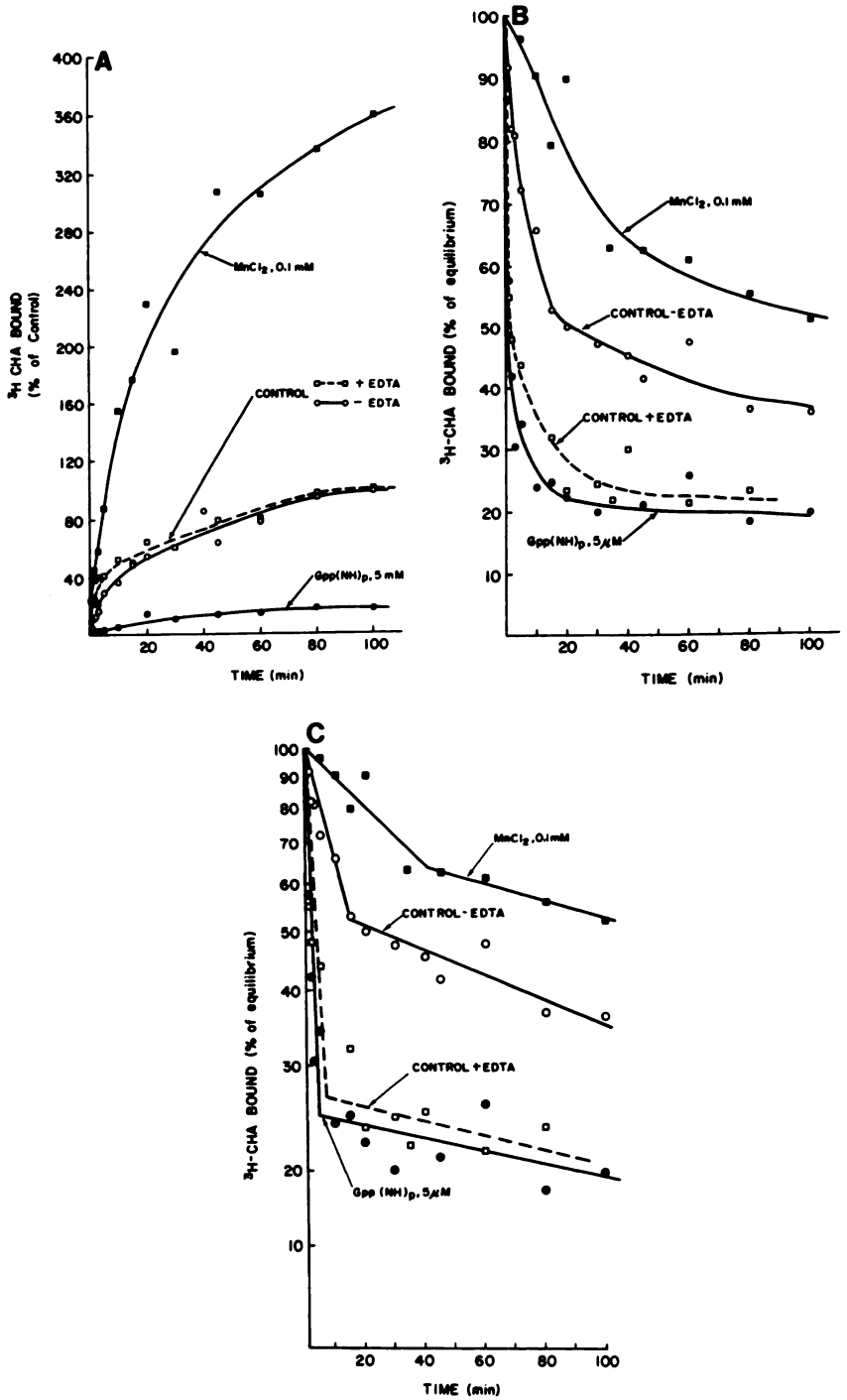

FIG. 3. *Guanine nucleotide and manganese effects on kinetics of [^{3}H]CHA binding*

A. Association: [^{3}H]CHA (2 nM) binding to guinea pig brain membranes was measured with various times of incubation at 25°. Tissue was either prepared as described under Materials and Methods (normal preparation) and incubated in the absence (○) or presence (●) of 5 μM Gpp(NH)p or prepared with exposure to 100 mM EDTA and incubated in the absence (□) or presence (■) of 0.1 mM $MnCl_2$. Binding is expressed as the percentage of control binding at equilibrium for the means of triplicates from a typical experiment.

B. Dissociation: Tissue preparations and incubation conditions were the same as described in A. Following a 2-hr incubation, 5 μM L-PIA was added and homogenates were filtered at various times. Results are reported as for A.

C. Logarithmic transformation of the results in B.

TABLE 3

Nucleotide and cation effects on [^{3}H]DPX binding in bovine brain membranes

Various concentrations of nucleotides and ions were added to tubes containing 0.75 nM [^{3}H]DPX. Specific binding was determined as described under Materials and Methods. Results are averages of three triplicate determinations that varied by less than 10%. Typical specific cpm was 2250 cpm, with 2400 cpm total and 150 cpm blank.

Agent	Binding (% of control) at concentrations of						
	10^{-6} M	10^{-5} M	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M	1.5×10^{-1} M
GTP	102	100	101				
Gpp(NH)p	100	103	100				
ATP	102	102	98				
$MnCl_2$	103	95	92	85			
$MgCl_2$	96	98	98	102			
$CaCl_2$	102	99	101	99			
LiCl			105	106	109	119	121
NaCl			98	92	89	91	93
KCl			102	100	102	108	108

tagonist properties of drugs can be evaluated by determining the extent to which GTP affects their ability to compete for [^{3}H]antagonist binding. The agonists L-PIA and CHA become 3–6 times less potent in competing for [^{3}H]DPX binding in the presence of GTP. The IC_{50} of L-PIA shifts from about 1 nM to 3 nM, whereas the IC_{50} of CHA shifts from 1.5 nM to 9 nM. By contrast, the antagonist theophylline has the same potency (3 μM IC_{50}) in the presence and absence of GTP (Fig. 4). Interestingly, the agonist displacements are somewhat shallow, as indicated by their low Hill coefficients (about 0.7 for L-PIA and 0.6 for CHA), whereas the antagonist displacements (± GTP) are steep (about 0.9–1.0 Hill coefficient for theophylline).

DISCUSSION

The major finding of the present study is that adenosine receptor interactions are regulated by guanine nucleotides and cations. Physiological concentrations of sodium markedly decrease binding of [^{3}H]CHA. This effect is selective, since sodium is substantially more effective than lithium or potassium. In terms of approximate potency and selectivity this effect resembles that of sodium at histamine H_1, opiate, and *alpha*$_2$-adrenergic receptors (17–19). The physiological relevance of this sodium effect is unclear.

The selective reduction of agonist affinity for adenosine receptors by guanine nucleotides resembles results with numerous neurotransmitter and hormone receptors (10–17). As at other receptors the effect is observed with GTP, its nonmetabolized analogue Gpp(NH)p, and GDP, but not with GMP or adenine nucleotides. Because the GTP effect can be monitored by evaluating influences on the potencies of agents in competing for [^{3}H]DPX binding, one can grade experimental substances along an agonist-antagonist continuum by observing effects on adenosine receptor binding. The fact that the agonist displacements, but not those of the antagonist, are shallow is of interest. This is most likely due to one of two possibilities, that [^{3}H]DPX is labeling two or more receptor populations with different agonist affinities or, alternatively, that agonists (but not antagonists) have more than one possible type of binding interaction with the receptor (e.g., high/low affinity agonist states that have no antagonist affinity differences). It is also of interest that we find parallel shifts of the displacements by GTP, rather than the more steep curves found for *beta*- and *alpha*$_2$-receptor binding (26, 27). These results were consistent with the receptors existing in a 50:50% mixture of their high- and low-affinity states prior to the addition of GTP, which converts all to the low-affinity state. Our results with the adenosine receptor would be consistent with a shift from 100% high- to 100% low-affinity state with the addition of GTP, suggesting that all of the receptors are precoupled to the nucleotide binding components.

The fact that EDTA markedly reduces basal levels of [^{3}H]CHA binding suggests that, as at the opiate receptor (20), endogenous divalent cations may regulate adenosine receptor interactions. Whether divalent cation and guanine nucleotide effects are related is unclear. Since reversal of the effects of GTP but not the nonmetabolized Gpp(NH)p by divalent cations is blocked by ATP, this reversal is probably due to activation of membrane-bound phosphatases, as suggested for opiate receptors (16). This result differs from findings at *alpha*$_2$-adrenergic receptors, where marked interactive effects are observed when divalent cations and guanine nucleotides are combined (23). The combination of the nonmetabolized analogue Gpp(NH)p and divalent cations at adenosine receptors seems to be the algebraic sum of their individual effects. The nature of the combined effects of guanine nucleotide and divalent cations at adenosine receptors resembles results at opiate and histamine receptors (16, 17).

The relative potencies of divalent cations vary at different neurotransmitter receptors. At adenosine receptors manganese is most potent, while magnesium and calcium have similar potencies. This contrasts with results at the opiate receptor, where manganese is most potent, magnesium has moderate activity, and calcium is virtually inactive (20). If the divalent cation effects reflect

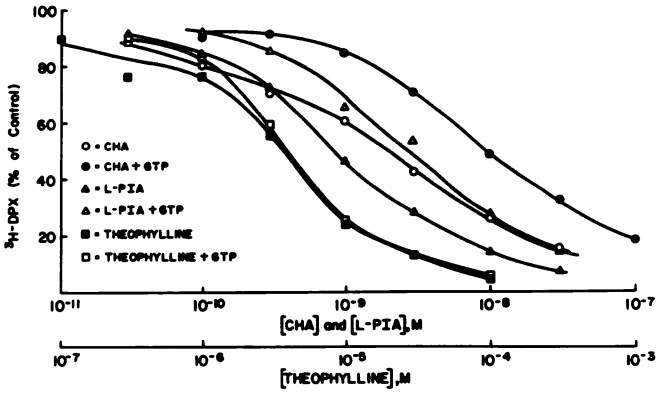

FIG. 4. *Guanine nucleotide effects on displacement of [^{3}H]DPX binding in bovine brain by CHA, L-PIA, and theophylline*

Data presented are means of triplicates determined in the absence and presence of 100 μM GTP and are expressed as the percentage of control specific binding. Control specific binding was approximately 2250 cpm (2400 cpm total and 150 cpm blank), determined as described under Materials and Methods.

influences of endogenous cations, it is conceivable that manganese, calcium, and magnesium are physiological regulators of adenosine receptors. The endogenous brain concentrations of manganese, calcium, and magnesium (0.03, 1.3, and 6.0 mM, respectively) are in the range of potencies observed here (28).

The fact that guanine nucleotides affect the affinity of agonists for adenosine receptors, whereas divalent cations influence affinity as well as the number of sites, suggests that different actions are involved for the cations and the nucleotides, at least in guinea pig brain. Such a conclusion is supported by our recent observations that regulation of adenosine receptor binding by guanine nucleotides is maintained in the soluble state, whereas the influence of divalent cations cannot be demonstrated with soluble adenosine receptors.[4]

Regulation by guanine nucleotides is most frequently apparent for receptors that are linked to adenylate cyclase. Both adenosine A_1 and A_2 receptors are associated with adenylate cyclase. At A_1 receptors, adenosine reduces cyclic AMP formation, while enhancement of adenylate cyclase activity is apparent at A_2 receptors (1–3). All of the binding interactions studied here involve adenosine A_1 receptors. In both guinea pig and bovine brain membranes, [^{3}H]CHA binding displays properties of A_1 receptors (5), and in bovine brain membranes, used exclusively for [^{3}H]DPX studies here, [^{3}H]DPX binding involves only A_1 receptors (5). Opiate receptors, associated in some tissues with reductions in adenylate cyclase activity (29), also are regulated in binding properties by guanine nucleotides. The influence of guanine nucleotides on receptor binding appears similar regardless of whether a receptor is associated with increases or decreases in adenylate cyclase.

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