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SODIUM AND POTASSIUM REGULATION OF GUANINE NUCLEOTIDE-STIMULATED ADENYLATE CYCLASE IN BRAIN

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Abstract—The present study examines the influence of potassium and sodium ions on guanine nucleotide regulation of adenylate cyclase in various brain regions, including the locus coeruleus (LC), dorsal raphe (DR), ventral tegmentum (VT), hippocampus (HP), frontal cortex (FC), substantia nigra (SN), neostriatum (NS) and cerebellum (CB). Guanine nucleotide regulation of adenylate cyclase was highest in the LC, DR and VT and lowest in NS and CB. Sodium and potassium ions were found to stimulate basal or GTP-activated adenylate cyclase in NS and SN, whereas the cations were found to specifically inhibit guanine nucleotide-stimulated enzyme activity in all other brain regions with the exception of CB, where there was no effect. With regard to stimulation of adenylate cyclase, lithium was more potent than sodium which was more potent than potassium in SN and NS. With regard to inhibition of the enzyme, potassium was equipotent to lithium which was greater than sodium in the other brain regions examined. Both stimulatory and inhibitory effects of cations in the different regions were significant ($P < 0.05$) at 30 mM and were maximal at 90–120 mM. Sodium ion inhibition of GTP-stimulated adenylate cyclase in LC and DR was partially blocked by pertussis toxin treatment, whereas cation stimulation in NS was not affected by the toxin. The results demonstrate marked region-specific effects of sodium and potassium on adenylate cyclase, which could occur at either G-proteins or the catalytic unit of the enzyme. The possibility that ion fluxes alter G-protein function is discussed.

Guanine nucleotide binding proteins (G-proteins) comprise a family of membrane bound proteins that mediate the transduction of neurotransmitter or hormone receptor signals in cells. Receptors coupled with adenylate cyclase either stimulate or inhibit cAMP formation by coupling with stimulatory (G_s) or inhibitory (G_i) G-proteins [1, 2]. A third class of G protein (G_o) found in large quantities in brain may mediate the action of neurotransmitters on other effector systems such as phospholipase C [2]. Recently, regulation of potassium channels by neurotransmitter receptors, including α_2-adrenergic, opiate, muscarinic-2 cholinergic, $GABA_B$, serotonin-1, and dopamine-2 receptors, has also been shown to be mediated by G-proteins [3–6]. Although the precise G-protein involved remains unknown, such receptor-activation of potassium channels is sensitive to pertussis toxin, which implicates the involvement of G_i and/or G_o. Interestingly, all of these same receptors have also been shown to be negatively coupled to adenylate cyclase and their inhibition of the enzyme is sensitive to pertussis toxin [1, 2]. These results suggest that receptor regulation of cAMP formation and ion channels may be mediated by a common pool of pertussis toxin sensitive G-proteins.

Receptor-activated second messenger systems are exquisitely regulated by feedback mechanisms as well as other modulating signals [7]. For example, there are numerous reports of receptor desensitization, some of which may be mediated through receptor phosphorylation [7–9]. More recently, the regulation of G-proteins and the catalytic unit of adenylate cyclase by calcium-dependent protein kinases has been demonstrated [10–12]. Therefore, it is conceivable that receptor-activation of ion channels and the consequent changes in submembrane ion concentrations may regulate G-proteins coupled to second messenger systems such as adenylate cyclase or to ion channels themselves. Such actions would represent feedback mechanisms for ion channel regulation.

In the present study, the influence of cations on guanine nucleotide stimulated-adenylate cyclase was examined in different brain regions. The results indicate that physiological concentrations of sodium or potassium cations inhibited adenylate cyclase only in the presence of GTP in some brain regions, but stimulated cAMP production, independent of GTP, in other brain regions.

METHODS

Male Sprague–Dawley rats (140–160 g) were used for all experiments and were housed under a 12-hr light–dark cycle with food and water *ad lib*. The brains were removed rapidly and placed in ice-cold artificial cerebral spinal fluid for dissection of frontal cortex (FC), neostriatum (NS), cerebellum (CB), and hippocampus (HP). The locus coeruleus (LC), dorsal raphe (DR), ventral tegmentum (VT), and substantia nigra (SN) were isolated by taking 15 gauge punches from 0.5 to 1 mm coronal cross sections of brain. For adenylate cyclase analysis, the tissue was homogenized using a loose-fitting teflon

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on glass homogenizer in approximately 250 vol. of 20 mM Tris–HCl, pH 7.4, 1 mM dithiothreitol (DTT), and 1 mM ethyleneglycolbis(amino-ethyl-ether)-tetraacetate (EGTA) and centrifuged at 10,000 *g* for 15 min. The pellet was resuspended and washed once more in the same buffer. Finally, the pellet was resuspended in 10 mM Tris–HCl, pH 7.4 for the assay. Adenylate cyclase assays were conducted essentially according to a previously described method [13], with the final mixture of 100 μl containing 50 mM triethanolamine HCl, pH 7.4, 0.05 mM [α-^{32}P]ATP (2×10^6 cpm), 1 mM EDTA, 0.5 mM isobutylmethylxanthine, 0.1 mM cAMP, 2 mM $MgCl_2$, 1 mM phosphoenolpyruvate, 5 mg/ml creatine phosphokinase and 5–10 μg of brain homogenate protein per tube. The reaction was initiated with the addition of [α-^{32}P]ATP and after 12 min was terminated with 0.1% sodium dodecyl sulfate containing 100 μM cAMP. Some experiments were carried out in the presence of neurotransmitter receptor agonists or antagonists including: yohimbine HCl, naloxone HCl, and atropine sulfate, all purchased from Sigma Pharmaceutical (St Louis, MO). Levels of [α-^{32}P]cAMP were analyzed by the double column method of Salomon *et al.* [14].

Pertussis toxin treatments were carried out as previously described [15]; tissue was homogenized in buffer containing 50 mM Tris–HCl, pH 7.6, 5% sucrose, 6 mM $MgCl_2$, 1 mM EDTA, 3 mM benzamidine, 1 mM DTT and 1.25 μg/100 μl soybean trypsin inhibitor, and the homogenate was pelleted as before. The pellet was resuspended in buffer containing 100 mM Tris–HCl, pH 7.6, 1 mM thymidine, 1 mM isoniazid, 4% sucrose, 5 mM $MgCl_2$, 1 mM EDTA, 2.4 mM benzamidine, 1 mM DTT, 50 μM NAD, 2.5 mM ATP, 2.0 mM GTP and 0.8/ml soybean trypsin inhibitor and incubated either without or with pertussis toxin (List Laboratories, Campbell, CA) (2.5 μg toxin/mg tissue in 200 μl of buffer) for 45 min at room temperature. The mixture was centrifuged and the pellet resuspended and washed two times in 20 mM Tris–HCl, pH 7.4, 1 mM EGTA and 1 mM DTT. The final pellet was resuspended in 10 mM Tris–HCl, pH 7.4, and used for adenylate cyclase analysis. These conditions have been shown to result in the incorporation of 30 pmol of [^{32}P]ADP-ribose per mg protein into G_i:G_o at a ratio of about 1:2 ([15] and unpublished observations). While this incubation either with or without pertussis toxin did not alter significantly adenylate cyclase activity, pertussis toxin treatment does largely block opiate receptor inhibition of adenylate cyclase [13].

Protein levels were determined by the method of Lowry *et al.* [16]. Punches of brain nuclei were found to contain approximately 50 μg of protein.

RESULTS

Effects of NaCl *on basal and forskolin-stimulated adenylate cyclase.* Basal adenylate cyclase was approximately the same in all brain regions studied (15–35 pmol/mg protein/min), with the exception of the NS, where enzyme activity was substantially higher (240 pmol/mg/min) (Fig. 1). The addition of 120 mM NaCl significantly increased basal activity in the NS and SN by 80% (Fig. 1), but had little effect in the other brain regions studied. Forskolin-stimulated adenylate cyclase varied from approximately 5- to 9-fold of basal in all regions except the NS and SN, where forskolin caused a 13- to 15-fold activation. The addition of NaCl had no effect on forskolin-activated adenylate cyclase in any of the brain regions examined (Fig. 1).

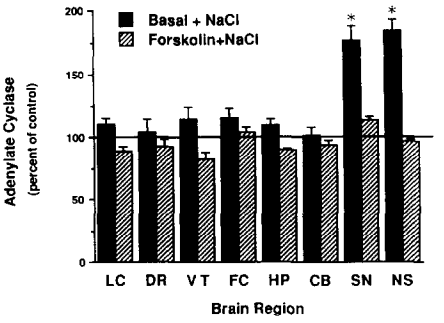

Fig. 1. Influence of NaCl on basal and forskolin-stimulated adenylate cyclase. The effect of NaCl on adenylate cyclase was examined as described under Methods in locus coeruleus (LC), dorsal raphe (DR), ventral tegmentum (VT), frontal cortex (FC), hippocampus (HP), cerebellum (CB), substantia nigra (SN) and neostriatum (NS). Results are expressed as percent of control, which represented either no addition (basal) or forskolin (5 μM) in the absence of NaCl (120 mM). The level of basal [^{32}P]cAMP production (pmol/mg protein/min) in the various brain regions was: LC, 25 ± 3 (mean ± SE); DR, 15 ± 3; VT, 23 ± 3; FC, 34 ± 5; HP, 26 ± 4; SN, 32 ± 3; NS, 239 ± 40; and CB, 28 ± 4. Each bar represents the mean ± SE of three to six separate experiments, each performed in duplicate. Key: (*) $P < 0.05$ compared to control (Student's *t*-test).

Effect of NaCl *on GTP- and GTPγS-stimulated adenylate cyclase.* Guanine nucleotide-stimulated adenylate cyclase varied dramatically among the brain regions examined (Fig. 2). GTP stimulation was highest in the LC, DR, and VT (approximately 300% above basal) and lowest in the FC, HP, SN and CB (25–75% above basal); as reported by others [17], GTP at higher concentrations inhibited adenylate cyclase in the NS. The addition of NaCl (120 mM) resulted in region-specific effects. In the LC, DR and VT, NaCl caused a 40–50% inhibition of adenylate cyclase in the presence of GTP, whereas in the SN and NS, NaCl stimulated adenylate cyclase in the presence of GTP (Fig. 2A). NaCl did not affect GTP-stimulated adenylate cyclase significantly in FC, HP or CB (Fig. 2A).

The regional profile of GTPγS-stimulated adenylate cyclase was different from that of GTP. The response to GTPγS was approximately 600% above basal in LC, DR, VT and FC; 400% above basal in HP and SN; 200% above basal in NS (with no inhibition seen at higher concentrations); and lowest in cerebellum. The addition of NaCl resulted in 50% inhibition of GTPγS-stimulated adenylate cyclase in LC, DR and VT as well as the FC and HP, while having no effect in CB, SN and NS (Fig. 2B).

The ability of NaCl to inhibit adenylate cyclase in LC was dependent on the presence of at least 1 μM GTP, as no inhibition of enzyme activity was

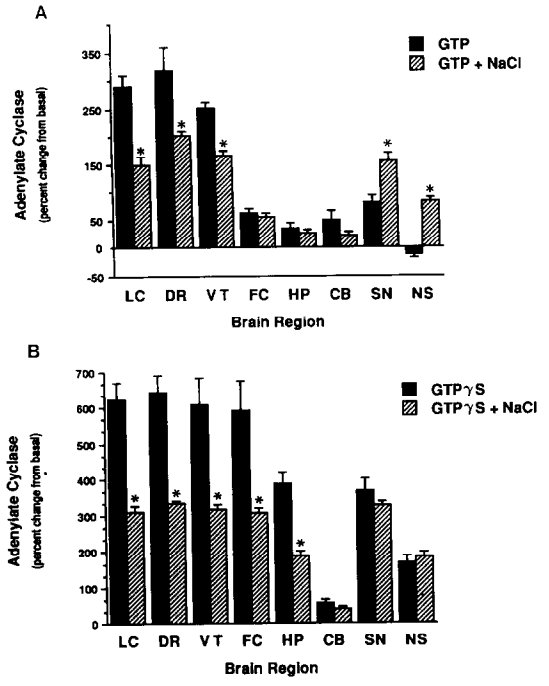

Fig. 2. NaCl regulation of GTP (A)- or GTPγS (B)-stimulated adenylate cyclase. GTP (100 μM, A) or GTPγS (10 μM, B)-stimulated adenylate cyclase, in the absence or presence of 120 mM NaCl, was analyzed as described under Methods. Results are expressed as percent change from basal. Each bar represents the mean ± SE of three to six separate experiments, each performed in duplicate. Abbreviations of brain regions are the same as those given in the legend to Fig. 1. Key: (*) $P < 0.05$ compared to GTP (A) or GTPγS (B) (Student's *t*-test).

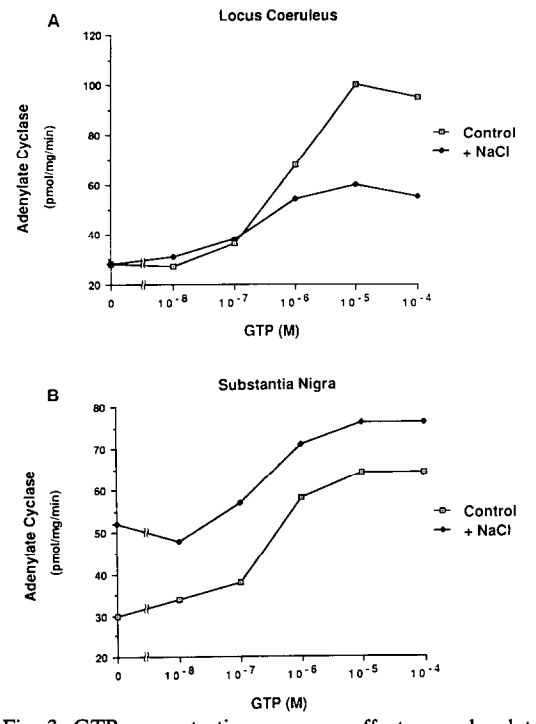

Fig. 3. GTP concentration–response effects on adenylate cyclase plus or minus NaCl. The influence of various concentrations of GTP on adenylate cyclase in the absence or presence of NaCl (120 mM) was analyzed in LC (A) or SN (B) as described under Methods. Results are expressed in pmol of [^{32}P]cAMP formed per mg protein per min. Each point represents the mean of two experiments, each performed in duplicate.

observed at lower concentrations of GTP or in its absence (Fig. 3A, also Fig. 1). The inhibition by NaCl reduced the maximum stimulation without affecting the potency of GTP to stimulate adenylate cyclase (Fig. 3A). In contrast, NaCl activation of adenylate cyclase in the SN (Fig. 3B) or NS (data not shown) was observed at all concentrations of GTP, as well as in its absence (see Fig. 1).

Concentration–response effects of NaCl, KCl, *and* LiCl. The lowest concentrations of NaCl examined (30 mM) significantly influenced GTP (100 μM) -stimulated cAMP formation in the LC (where it produced inhibition) and SN (where it produced stimulation), and the effect plateaued at 90–120 mM (data not shown).

With regard to inhibition of GTP-activated cAMP production in the LC, LiCl and KCl were equipotent, and both were more potent than NaCl at all concentrations tested (Fig. 4B). With regard to stimulation of GTP-activated cAMP production in the SN, LiCl was greater than NaCl which was greater than KCl (Fig. 4A).

Influence of neurotransmitter receptor antagonists on NaCl *regulation of adenylate cyclase.* Neurotransmitter receptor inhibition of adenylate cyclase is, at least in part, dependent on the presence of sodium ions [17–19]. Consequently, it is possible that the cation inhibition of GTP-stimulated adenylate cyclase observed in this study was due to endogenous inhibitory neurotransmitters remaining in the isolated brain membranes. To test this hypothesis, antagonists of three neurotransmitter receptors known to inhibit adenylate cyclase were added individually to the assay in the presence of NaCl; the antagonists used were yohimbine, naloxone, and atropine for α_2-adrenergic, opiate, and muscarinic cholinergic receptors respectively. None of these antagonists reversed the NaCl inhibition of GTP-stimulated adenylate cyclase in LC (Table 1); similar results were observed in the DR and HP (data not shown). The addition of these receptor-antagonists to homogenates of NS (Table 1) and SN (data not shown) did not influence cation enhancement of adenylate cyclase.

Influence of pertussis toxin treatment. The influence of sodium and potassium ions on GTP regulation of adenylate cyclase could theoretically be mediated through effects on G_s and/or G_i. To examine if the effects are mediated by G_i, membranes were treated with pertussis toxin and the influence of NaCl on GTP-stimulated activity was examined. Conditions used for pertussis toxin treatment have been shown to block opiate-receptor inhibition of adenylate cyclase, indicating that G_i is uncoupled from the receptor under these conditions [13]. It was found that such pertussis toxin treatment had no effect on NaCl activation of adenylate cyclase in the presence

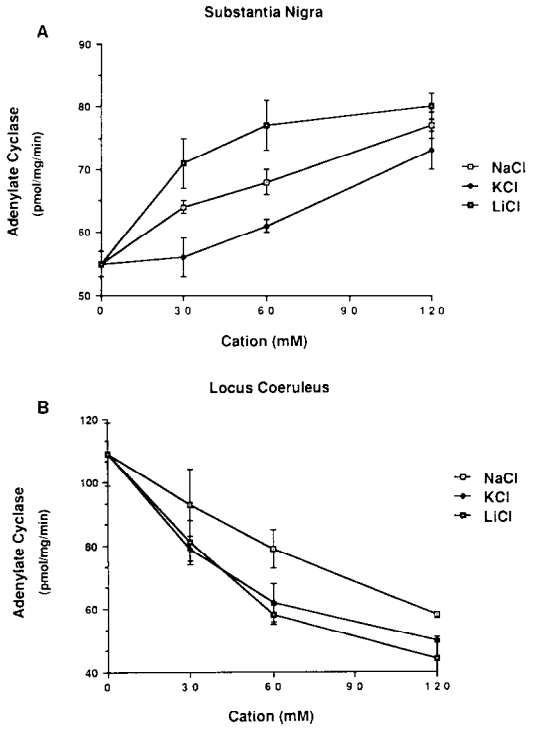

Fig. 4. NaCl, KCl and LCl concentration–response effects on GTP-stimulated adenylate cyclase in SN and LC. The influence of the chloride salts of sodium, potassium and lithium (30–120 mM) on GTP (100 μM)-stimulated adenylate cyclase in SN and LC was examined as described under Methods. Results are expressed as [^{32}P]cAMP (pmol/mg protein/min). Each point represents the mean ± SE of three or four separate experiments, each analyzed in duplicate.

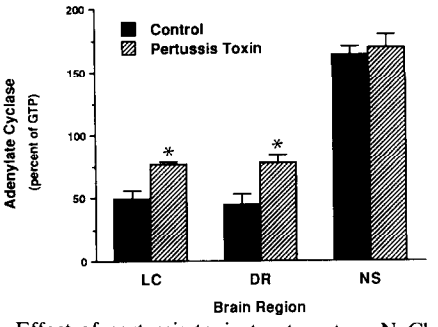

Fig. 5. Effect of pertussis toxin treatment on NaCl regulation of adenylate cyclase. The influence of NaCl (120 mM) on GTP (100 μM)-stimulated cAMP production in control or pertussis toxin-treated membranes from LC, DR and NS was examined as described under Methods. Membranes were incubated with pertussis toxin (1 μg/100 μl) for 45 min at 20° and analyzed for GTP-stimulated adenylate cyclase in the absence or presence of NaCl (120 mM). Results represent the inhibition (LC and DR) or stimulation (NS) of adenylate cyclase by NaCl and are expressed as percent of GTP. Each bar represents the mean ± SE of three separate experiments, each analyzed in duplicate. Key: (*) $P < 0.05$ compared to control (Student's t-test).

of GTP in the NS, but partially blocked the inhibition of GTP-stimulated adenylate cyclase by NaCl in the LC and DR (Fig. 5).

DISCUSSION

The results of this study demonstrate a regional variation of guanine nucleotide stimulated adenylate cyclase which may reflect the regional distribution of G-protein subunits [20]. Moreover, the results demonstrate that physiological concentrations of sodium and potassium ions regulated guanine nucleotide-stimulated adenylate cyclase and that the regulation varied dramatically among different brain regions. Thus, these ions caused activation of GTP-stimulated adenylate cyclase in NS and SN, but inhibited the guanine nucleotide response in LC, DR, VT, HP and FC. The activation of adenylate cyclase in NS and SN appeared to be more sensitive to sodium, whereas the inhibition of GTP-stimulated activity in the other brain regions preferred potassium. These results suggest that monovalent cations may act at two or more distinct sites to regulate adenylate cyclase activity in brain.

Sodium ion inhibition of adenylate cyclase in LC,

Table 1. Influence of neurotransmitter receptor antagonists on NaCl regulation of adenylate cyclase in LC or NS

	Adenylate cyclase (pmol [^{32}P]cAMP/mg protein/min)	
	LC	NS
GTP (10 μM)	168 ± 6	136 ± 7
GTP (10 μM) + NaCl (100 mM)	117 ± 3*	292 ± 13*
+ Yohimbine (10 μM)	120 ± 5*	289 ± 15*
+ Naloxone (10 μM)	119 ± 4*	318 ± 30*
+ Atropine (10 μM)	117 ± 5*	291 ± 19*

The ability of neurotransmitter receptor antagonists to reverse the effects of NaCl on GTP regulation of adenylate cyclase was examined. Washed membranes of LC or NS were prepared, and adenylate cyclase was assayed as described in Methods in the presence of GTP (10 μM) plus or minus NaCl (100 mM) and the receptor antagonists at the concentrations indicated. Each point is the mean ± SE of three experiments, each analyzed in duplicate.

* $P < 0.05$ compared to GTP alone (Student's t-test).

DR and VT was dependent on the presence of GTP, since the cation had no effect on basal or forskolin-stimulated adenylate cyclase. The degree of sodium inhibition in these regions was greater when the nonhydrolyzable analogue of GTP, GTPγS, was used to activate the enzyme (35 vs 50% inhibition respectively). Interestingly, in FC and HP, where GTP stimulation was low relative to LC, DR and VT, the effect of sodium on GTP stimulation was minimal and not significant. However, GTPγS-stimulated adenylate cyclase in FC and HP was much greater than the GTP response and was inhibited approximately 50% by sodium ion. These results suggest that the sodium inhibition of adenylate cyclase in these various brain regions was dependent on the degree of guanine nucleotide stimulation of the enzyme: the inhibition was enhanced when guanine nucleotide stimulation was increased. This was supported by the observation in the LC, DR and VT that the degree of inhibition was greater at higher concentrations of GTP, and was not observed at concentrations below 1 μM.

In contrast, these cations were stimulatory with respect to adenylate cyclase in SN and NS, and this stimulation was not dependent on GTP, as activation of basal enzyme activity was also observed. In fact, sodium ions had no effect on GTPγS-stimulated adenylate cyclase in these two brain regions.

Concentration–response studies showed that 30 mM sodium (the lowest concentration tested) inhibited GTP-stimulated adenylate cyclase in the LC and that the effect of sodium was maximal at 90–120 mM. Inhibition by different monovalent cations exhibited a rank order of potassium equal to lithium, which was greater than sodium, suggesting that potassium may be the preferred cation for this effect. Activation of the enzyme by sodium in the SN and NS also occurred at a concentration of 30 mM and reached a plateau at concentrations of 90–120 mM. However, cation stimulation exhibited a rank order of potency different from inhibition: lithium was greater than sodium, which was greater than potassium, suggesting that sodium may be the preferred cation for the stimulatory effects observed in the nigrostriatal system. The concentrations of cations at which inhibition or stimulation of adenylate cyclase was observed are well within the physiological range of potassium in the intracellular fluid and sodium in the extracellular fluid. These various results also raise the possibility that some clinical actions of lithium may be mediated by its effects on G-proteins. Although the concentrations studied here (30–120 mM) are well above the therapeutic range of lithium (i.e. 1 mM), lithium has been shown at clinically relevant concentrations (2–4 mM) to inhibit isoproterenol- or forskolin-stimulated adenylate cyclase in FC [21].

Cation stimulation of adenylate cyclase has been reported previously in fat cells [22], neostriatum [18] and liver [23], and it has been suggested that this effect was due to a stabilization of G_i such that the G_i heterotrimer cannot dissociate and thereby inhibit adenylate cyclase. To test this hypothesis, neostriatal membranes were treated with pertussis toxin which catalyzes the ADP-ribosylation of G_i [24, 25] and thereby inactivates G_i [1]. It was found that sodium ions stimulated adenylate cyclase equally in control and toxin-treated membranes, suggesting that this stimulation does not depend on functional G_i, i.e. that it is not mediated via the inhibition or stabilization of G_i. This conclusion was supported by the findings here and by others that sodium and other cations stimulated adenylate cyclase in the absence of GTP. These findings suggest that the cations act directly at the catalytic site of the enzyme.

Cations have also been shown to inhibit adenylate cyclase in platelets [26, 27] and adipocytes [28, 29], and it has been suggested that this effect is mediated through the activation of G_i [30]. To study the involvement of G_i in sodium inhibition of GTP-activated adenylate cyclase in LC and DR, the effect of pertussis toxin treatment was examined. In contrast to the lack of effect of pertussis toxin in NS, toxin treatment significantly reduced the inhibition by sodium in the LC and DR, although it did not reverse completely the effects of the cation. These findings suggest that cation inhibition was at least partially mediated by pertussis toxin-sensitive G-proteins (G_o/G_i).

Receptor-mediated inhibition of adenylate cyclase is, in part, dependent on the presence of sodium ions which enhance ligand binding to the receptor as well as coupling to G_i [12, 19, 20]. Therefore, the ability of sodium ions to inhibit GTP-stimulated adenylate cyclase could result from the activation of endogenous inhibitory neurotransmitters not removed during the tissue preparation. However, the addition of the receptor-antagonists yohimbine, naloxone, and atropine, at concentrations sufficient to block relatively high concentrations of exogenous agonist, did not reverse the ability of sodium ion to inhibit GTP-stimulated adenylate cyclase in LC, DR, or HP. These results indicate that the NaCl inhibition was not due to activation of α-adrenergic, opiate, or muscarinic cholinergic inhibitory receptors. However, it is possible that other inhibitory agonists are present that could account for the cation inhibition.

The mechanisms by which these cations regulate G-proteins is unknown, although it has been suggested that monovalent cations, particularly lithium, influence adenylate cyclase by competing at a magnesium binding site located on the catalytic unit [21, 29, 31]. There are also regulatory magnesium binding sites on G-proteins [1, 2], and it is possible that the effects of cations observed in this study are mediated by action at these sites.

The results of the present study demonstrate that sodium and potassium, at physiological concentrations, inhibited guanine nucleotide-stimulated cAMP production in a number of brain regions. In addition, these ions also activated adenylate cyclase in the absence of GTP in the NS and SN. The results indicate that these cations have at least two sites of action for regulating cAMP formation and that the two sites of action show a marked region-specific distribution in rat brain. Cation regulation of G-proteins and adenylate cyclase may represent important mechanisms by which the frequency of neuronal firing and ion fluxes regulate the cAMP second messenger system as well as other intracellular second messenger systems regulated by G-proteins (e.g. calcium, phosphatidylinositol). Moreover, sodium and

potassium regulation of G-protein function may represent a feedback mechanism by which ion channels that are activated by G-proteins can then regulate the same G-proteins via their permeant cations.

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