Inhibitory Effects of Sodium and Other Monovalent Cations on Human Platelet Adenylate Cyclase*

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Both basal and prostaglandin E1 (PGE1)-stimulated human platelet adenylate cyclase activity are inhibited by Na+ and other monovalent cations. Inhibition occurs when the cations are present in concentrations of 20 to 120 mM. Inhibition of basal activity by epinephrine requires the presence of GTP but not Na+. In the presence of epinephrine and GTP, Na+ further reduces basal activity, and the combined effects of all three ligands results in a 70% reduction of basal activity. Sodium, however, does not increase the fractional inhibition of basal activity attributable to epinephrine. Sodium increases the concentrations of epinephrine and of PGE1 required for half-maximal inhibition and stimulation of adenylate cyclase but does not alter the apparent \( k_m \) for Mg2+ or ATP. The rate and extent of adenylate cyclase activation by the GTP analog guany1-5'-yl imidodiphosphate is reduced by Na+, although the cation does not prevent the acceleration of activation by guany1-5'-yl imidodiphosphate induced by PGE1. Other monovalent cations also inhibit the platelet cyclase with an order of potency of Na+ > Li+ > K+ > choline+. In addition to demonstrating that Na+ is not required for hormonal inhibition of platelet adenylate cyclase, these studies demonstrate the multiple inhibitory effects which can be induced by Na+ and other monovalent cations. The pattern of these effects suggests that the cations interfere with functional coupling between receptors, guanine nucleotide binding units, and catalytic units of the adenylate cyclase complex.

Hormonal stimulation and inhibition of adenylate cyclase requires the presence of guanine nucleotides such as GTP. Recent reports have noted that hormonal inhibition of adenylate cyclase may also require the presence of sodium ions. These reports prompted the present study which has evaluated the effects of sodium, as well as other monovalent cations, on human platelet adenylate cyclase, an enzyme which can be stimulated by PGE1 and inhibited by \( \alpha \)-adrenergic hormones.

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† The abbreviations used are: PGE1, prostaglandin E1; Gpp(NH)p, guany1-5'-yl imidodiphosphate; EGTA, ethylene glycol bis(\( \beta \)-aminoethyl ether)-\( \mathrm{N},
\mathrm{N},
\mathrm{N}',
\mathrm{N}'\)-tetraacetic acid; \( k_m \), rate constant for activation.

MATERIALS AND METHODS

RESULTS

Effects of NaCl on Epinephrine and GTP-dependent Inhibition—Epinephrine, in the presence of 10 \( \mu \)M GTP, caused 48% inhibition of adenylate cyclase activity, even in the absence of added sodium (Fig. 1). The monovalent cation itself caused a similar, but GTP-independent, inhibition. The combined effects of epinephrine with NaCl and GTP were additive, resulting in a 71% inhibition (Fig. 1). Sodium chloride (40 mM) increased the GTP concentration required for half-maximal inhibition by epinephrine from 1 to 8 \( \mu \)M but did not alter the fractional inhibition attributable to epinephrine in the presence of 0.1 mM GTP. Sodium chloride (40 mM) was also found to increase the epinephrine concentration required for half-maximal inhibition from 0.3 to 3.0 \( \mu \)M (Fig. 2).

Effects of NaCl on Basal Activity—Sodium chloride caused a concentration-dependent decrease in basal adenylate cyclase activity (Fig. 3). The inhibition of basal activity induced by a fixed concentration of epinephrine (10 \( \mu \)M and GTP (10 \( \mu \)M) was only slightly enhanced by NaCl. This, combined with the decrease in basal activity caused by NaCl, resulted in a reduction in the fractional inhibition, induced by epinephrine plus GTP at high NaCl concentrations (Fig. 3). This reduction in fractional inhibition probably results from the salt-induced increases in both epinephrine and GTP concentrations required for maximal inhibition (Figs. 1 and 2).

Effects of NaCl on Mg2+ and ATP Requirements—Although NaCl (40 mM) reduced the activity observed with saturating concentrations of Mg2+ and ATP, the apparent \( k_m \) for both agents (2.5 and 0.06 mM, respectively) was not altered by the salt (data not shown).

Effects of NaCl on PGE1, Stimulation and Gpp(NH)p Activation—Sodium chloride caused a concentration-dependent inhibition of PGE1-stimulated activity in the presence and in the absence of added GTP (Fig. 3). The salt increased the PGE1 concentration required for half-maximal stimulation from 30 nM to more than 500 nM (Fig. 4). The poorly hydrolyzable GTP analog Gpp(NH)p activates platelet adenylate cyclase in a hormone-independent and quasi-irreversible fashion. The rate of Gpp(NH)p activation was noted to be reduced by NaCl (Fig. 5). Prostaglandin E1, as noted previ-
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Effect of NaCl on concentration dependence of GTP-facilitated epinephrine inhibition. Platelet membranes (50 µg) were incubated for 10 min at 30 °C in the presence of epinephrine (10 µM) with or without NaCl (40 mM) and varying concentrations of GTP. In the absence of epinephrine and GTP, the activity was 325 ± 5 pmol/mg/min in the absence and 150 ± 3 pmol/mg/min in the presence of 40 mM NaCl.

Effect of NaCl on concentration dependence of epinephrine-induced inhibition. Platelet membranes (60 µg) were incubated for 12 min at 30 °C in the presence of 10 µM GTP and varying concentrations of epinephrine either without or with 40 mM NaCl.

Concentration dependence of NaCl effects. Platelet membranes were incubated for 16 min at 30 °C with varying concentrations of NaCl in the absence of other added ligands (A—C) or in the presence of 10 µM GTP (A—C), 10 µM GTP plus 10 µM epinephrine (A—C), 10 µM GTP plus 1 µM PGE, (A—C), and 1 µM PGE, (A—C). Assays with PGE, contained 40 µg of protein, while those without PGE, contained 90 µg of protein.

Discussion

Inhibition of adenylate cyclase by hormones has been demonstrated in several types of broken cell preparations, including adipocytes (4, 5), myocardium (6), NG108-15 cells (7, 8), and platelets (1, 9). In each of these systems, GTP is required for the maximal expression of hormonal inhibition. Several recent reports have indicated that Na⁺ may also be required for cyclase inhibition. For example, Blume et al. (8) noted that opiates had little or no inhibitory effect on basal activity of NG108-15 adenylate cyclase unless both GTP and Na⁺ were present. In addition, opiates reversed the increase in PGE₂-stimulated activity induced by GTP and Na⁺, leading to an apparent 40% reduction of PGE₂-stimulated activity in the presence of both GTP and Na⁺. Aktories et al. (5) found that a-adrenergic as well as PGE₂-induced inhibition of hamster adipocyte adenylate cyclase required both GTP and Na⁺. In this complex system, GTP inhibited the enzyme and Na⁺ reversed the GTP-induced inhibition. The inhibitory hormones appeared to prevent the Na⁺-induced reversal of GTP inhibition, thus leading to an apparent inhibition of activity when both GTP and Na⁺ were present. These and other observations led Jakobs (10) to suggest that a requirement for both Na⁺ and GTP may be a general characteristic of hormonally inhibitable adenylate cyclase systems.

The studies reported in this paper indicate that Na⁺ is not required for α-adrenergic inhibition of human platelet adenylate cyclase, although Na⁺ does have profound inhibitory effects in this system. Sodium inhibits both basal and PGE₂-stimulated activity and augments the inhibition induced by epinephrine in the presence of GTP (Figs. 1–3). The additive effects of maximally effective concentrations of Na⁺, GTP, and epinephrine result in a 70% reduction of basal activity (Fig. 1). The fractional inhibition induced by epinephrine is not increased by Na⁺ (Fig. 1). Indeed, fractional inhibition attributable to epinephrine in the presence of GTP may be decreased by Na⁺ since the cation reduces basal activity more than it lowers the epinephrine-GTP inhibited activity (Fig. 3). Sodium also reduces the apparent affinities with which epinephrine inhibits and PGE₂ stimulates the enzyme (Figs. 1, 2, and 4). Finally, Na⁺ retards the rate of adenylate cyclase activation by Gpp(NH)p (K₉₅) (Fig. 5).

Many of these Na⁺-induced effects are qualitatively similar to those induced by GTP in the platelet system. The nucleotide, at high concentrations, also inhibits basal and PGE₂-stimulated activity and reduces K₉₅ as measured with Gpp(NH)p (1). Furthermore, GTP also reduces the apparent affinity for agonist hormones converting high into low affinity α-receptors (11, 12).

Hormone-stimulated and, perhaps, basal adenylate cyclase activity is believed to be regulated by a guanine nucleotide binding element which couples receptor occupancy with activation of the cyclase catalytic component. Activation, according to this model, reflects GTP binding to this high affinity regulatory component, while deactivation occurs when GTP is hydrolyzed to GDP (13). The reduction in agonist affinity and the facilitation of hormone-induced inhibition of adenylate cyclase require higher GTP concentrations than are needed for hormone stimulation and basal activity, suggesting that one or more lower affinity GTP binding sites also exist in the platelet system (1).

It is tempting to speculate on the mechanisms which underlie Na⁺-induced inhibitory effects in the platelet system in...
terms of our current understanding of the adenylate cyclase complex. Thus, the ability of Na⁺ to inhibit basal and PGE₁-stimulated activity and to reduce the rate of activation by Gpp(NH)p may indicate that the cation uncouples the high affinity guanine nucleotide binding component from the cyclase catalytic unit. Reduction in agonist apparent affinity by Na⁺ suggests that the cation may directly interfere with hormone-receptor binding (as suggested by direct binding studies (12)) or, alternatively, facilitate the conversion of high affinity into lower affinity agonist receptors which is mediated via a lower affinity GTP-binding component.

It is likely that the manifold inhibitory effects of monovalent cations described in this paper are of functional significance, as the Na⁺ and K⁺ concentrations required to elicit these effects are well within the physiological range of extra or intracellular fluid. Indeed, Blume et al. (8) noted that the opiate-induced reduction in basal and PGE₁-stimulated intracellular cyclic AMP concentrations in intact viable NG108-15 cells is dependent on the presence of extracellular Na⁺.

REFERENCES
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SUPPLEMENTARY MATERIAL TO

Laboratory Effects of Iodo and Other Nonradioactive Cations on Human Platelet Adenylate Cyclase

BY MICHAEL L. HINES AND ARTHUR WOOD

This supplementary material consists of the Materials and Methods section, Figures 4, 5, 6 and 7, and Table I of this manuscript.

MATERIALS AND METHODS

Creating phosphatidyl, creatine phospho, (t.R:S)adrenaline, ATP (mM), and phosphoCreatine (mM), and phosphoCreatine (mM) were prepared from Nissens' solution: All other chemicals were reagent grade, and all experiments were performed using water doubly distilled in glass.

Preparation of all experiments were performed using platelet membranes prepared from blood of 10 volunteers who had nothing to drink for at least 2 weeks prior to blood donation. Membranes were prepared as described in detail previously (1). Briefly, washed platelets were centrifuged through a 0.2 to 0.1 g glass gradient and disrupted by sonication in hypotonic buffer. The particulate fraction was washed 3 times by repeated centrifugation and resuspension in 140 mM Tris-EDTA (pH 8.0) buffer containing 1 mM EDTA, and adenylate cyclase activity measured immediately after membrane preparation.

Adenylate cyclase assay. Adenylate cyclase activity was measured in a final volume of 0.1 ml containing 50 mM Tris-EDTA (pH 8.0), 114 mM creatine, 21 mM NaCl, 0.1 mM CaCl2, 0.1 mM creatine phospho, and 10 to 20 mg of membrane protein. The samples were incubated at 30°C for 2 to 10 minutes. Under these conditions, AMP production is a linear function of time. The [3H]AMP was isolated and quantitated, as described by Bellet (11). For experiments quantitating the activation of adenylate cyclase by [GTPyS], the membranes were pre-incubated with 100 mM Tris-EDTA (pH 8.0) buffer containing 1 mM MgCl2 and 110 mM Tris-EDTA (pH 8.0). Activity was assayed by transferring an aliquot of this preincubation mixture into one containing 110 mM Tris-EDTA (pH 8.0) buffer 2 minutes prior to stopping the activation and measuring the membrane as described in the text, assay was performed over an 11 min period using 15 μg of membrane protein in the absence of NaCl and [GTPyS].

Table I

<table>
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<tr>
<th>Hormone</th>
<th>Cation</th>
<th>Specific Activity (pmol/min/mg)</th>
<th>Percent Inhibition</th>
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<td>675 ± 4</td>
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<td>ChE</td>
<td>Li+</td>
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Adenylate cyclase activity was measured over 16 minutes at 37°C in the presence of 10 μg cyclic AMP (100x) or 1 μg cyclic GMP (100x). The cyclic nucleotides were present at a concentration of 0.1 mM. Results represent mean ± SEM of triplicate measurements.

Figure 4. Effect of NaCl and other monovalent cations on basal activity. Platelet membranes were pre-incubated with 0.5-10 mM LiCl alone (ΔΔ) or combined with 120 mM NaCl (ΔΔΔ), 1.1 mM KCl (ΔΔΔ), or 120 mM NaCl plus 1-10 mM MgCl2 (ΔΔΔ). After stopping the activation and washing the membranes as described in the text, assay was performed over an 11 min period using 15 μg of membrane protein in the absence of NaCl and [GTPyS].

Figure 7. Effect of monovalent cations on basal activity. Platelet membranes (15 μg) were incubated at 30°C for 10 minutes in the presence of varying concentrations of NaCl (坐着 sitting, KCl (坐着 sitting, LiCl (坐着 sitting, MgCl2 (坐着 sitting, or Na2SO4 (坐着 sitting). The concentration of MgCl2 is expressed as that of the Mg component (i.e., 2 × [MgCl2]).

Figure 5. Dependence of GTPyS activation on NaCl concentration. Platelet membranes were pre-incubated at 20°C for 24 hours in the presence of 1 μM GTPyS and 5 mM NaCl (坐着 sitting) or 1 μM GTPyS and 10 mM NaCl (ΔΔΔ). After stopping the activation and washing the membranes as described in the text, assay was performed over an 11 min period using 15 μg of membrane protein in the absence of NaCl and [GTPyS].