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EFFECTS OF NaCl AND GTP ON THE INHIBITION OF PLATELET ADENYLATE CYCLASE BY 1-*O*-OCTADECYL-2-*O*-ACETYL-*sn*-GLYCERYL-3-PHOSPHORYLCHOLINE (SYNTHETIC PLATELET-ACTIVATING FACTOR)

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We have investigated the effects of NaCl and GTP on the inhibition of platelet adenylate cyclase by 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholine (1-octadecyl-2-acetyl-G-3-PC), using particulate fractions from human and rabbit platelets that had been frozen and thawed in the presence of ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetate to prevent Ca^{2+}-dependent proteolysis. When 10 μM GTP was present, 100 mM NaCl stimulated the activity of the rabbit enzyme 5.6-fold and that of the human enzyme 2.2-fold. Under these conditions, maximum inhibitions of 90% and 64% were obtained on addition of 100 nM 1-octadecyl-2-acetyl-G-3-PC to rabbit and human preparations, respectively. These inhibitions resulted partly from an NaCl-independent inhibition of basal enzyme activity and partly from reversal of the stimulatory effect of NaCl. The relative abilities of the chlorides of different monovalent cations to enhance inhibition of rabbit platelet adenylate cyclase were: NaCl > LiCl > KCl > choline chloride. NaCl also increased the concentrations of 1-octadecyl-2-acetyl-G-3-PC required for half-maximal inhibition of adenylate cyclase but this action of NaCl did not correlate with its stimulatory effect on enzyme activity. After particulate fractions from platelets of either species were washed, 10 μM GTP inhibited basal adenylate cyclase activity in the absence of NaCl but stimulated the enzyme in the presence of NaCl. Inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC was then either enhanced by GTP (rabbit material) or completely dependent on added GTP (human material). Stimulation of the activity of the washed human preparations by NaCl required GTP, but concentrations lower than required for potentiation of the inhibitory effect of 1-octadecyl-2-acetyl-G-3-PC by NaCl were effective.

Introduction

The blood platelet is widely used as a model system for investigation of receptor-mediated inhibition of adenylate cyclase [1–3]. Two inducers of platelet aggregation, epinephrine and ADP, have long been known to inhibit the accelerated rates of cyclic AMP formation observed in intact platelets exposed to prostaglandin E_1 (for review, see Ref. 4). Both have also been shown to inhibit the adenylate cyclase activity of isolated platelet membrane preparations via specific receptors [5,6]. Recently, a study in this laboratory has shown that a third aggregating agent, 1-octadecyl-2-acetyl-G-3-PC (synthetic platelet-activating factor) is on a molar basis a much more potent inhibitor of adenylate cyclase, both in intact rabbit platelets and in particulate fractions from this species [7]. The structural requirements for this effect were

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Abbreviations: 1-octadecyl-2-acetyl-G-3-PC, 1-*O*-octadecyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholine; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

highly specific and, although inhibition of enzyme activity was also observed with human platelet particulate fraction, none was detected with rabbit liver membranes [7]. Thus, it is likely that specific membrane receptors mediate this action of 1-octadecyl-2-acetyl-G-3-PC. In the present investigation, we have explored the factors involved in regulating the inhibition of platelet particulate fraction adenylate cyclase by this compound.

Hormone-mediated inhibition of adenylate cyclase has been shown to depend on the presence of GTP and, in many systems, is facilitated by sodium ions which increased enzyme activity in the absence of hormone [1–3]. In the platelet, the inhibitory effect of epinephrine requires GTP [8,9] but the role of Na^+ is less clear. Thus, NaCl has been reported to inhibit the adenylate cyclase activity of membrane preparations from human platelets, an effect that was merely additive with the GTP-dependent inhibition of activity by epinephrine [10,11]. On the other hand, Michel et al. [12] found that NaCl potentiated the inhibitory action of epinephrine on the prostaglandin E_1-stimulated adenylate cyclase activity of rabbit platelet particulate fraction. We have therefore investigated the effects of NaCl and GTP on the inhibition of platelet adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC using both human and rabbit preparations. Although quantitative differences were observed, NaCl was found to stimulate adenylate cyclase activity and to potentiate the inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC in material from both species. Moreover, these effects of NaCl and of 1-octadecyl-2-acetyl-G-3-PC were GTP-dependent.

Experimental Procedures

Materials. Synthetic 1-octadecyl-2-acetyl-G-3-PC (Ro 14-8161) was a generous gift from Dr. H.R. Baumgartner of F. Hoffmann-La Roche and Co. (Basel, Switzerland). Tris-ATP, disodium ATP (prepared by phosphorylation of adenosine and therefore essentially GTP-free) and Tris-phosphocreatine were obtained from Sigma Chemical Co. (St. Louis, MO). [2,8-^{3}H]ATP (26.6 Ci/mmol) was obtained from ICN (Irvine, CA). This material was diluted with disodium ATP to a specific activity of approx. 20 mCi/mmol and then freed from a labelled impurity that co-purified with [^{3}H]cAMP by elution with water from a small column containing Dowex 50 resin (Bio-Rad AG 50 W-X8). The [^{3}H]ATP obtained was adjusted to pH 7.4 with Tris. All other materials were obtained from sources listed elsewhere [7].

Preparation of platelet particulate fractions. Platelets (rabbit or human) were isolated and washed, as described previously [13], except that they were finally resuspended at 25 mg wet weight/ml in buffer (pH 7.4) containing 150 mM Tris-HCl and 5 mM Tris-EGTA. Suspensions were rapidly frozen in solid CO_2/acetone and stored for up to 4 days at −50°C before thawing in a waterbath with shaking to maintain the temperature of the platelet material at 0°C. The resulting lysate was centrifuged at 37500 × *g* for 40 min at 4°C to give pellets containing about 40% of the platelet protein. In many experiments, these pellets were resuspended using a Dounce homogenizer (A pestle) in a medium containing all the components of the assay mixture except [^{3}H]ATP and the compounds or salts under investigation. However, when the action of added GTP was studied, endogenous guanine nucleotides were removed by homogenization of the pellets in a hypotonic buffer containing 10 mM Tris-HCl and 5 mM Tris-EGTA, pH 7.4. After centrifugation as before and removal of the supernatant, this procedure was repeated. The platelet particulate fraction was then isolated by a final centrifugation and resuspended for assay.

Assay of adenylate cyclase. Approximately 30 min after resuspension of the particulate fraction, 150 μl was mixed with 80 μl of other additions (salts, GTP and 1-octadecyl-2-acetyl-G-3-PC), the mixture was incubated for 1 min at 30°C to permit temperature equilibration and the assay was then started by addition of 20 μl of 5 mM Tris-[^{3}H]ATP (2 μCi). Final assay mixtures (250 μl) all contained 75 mM Tris-HCl (pH 7.4), 5 mM $MgCl_2$, 0.4 mM [^{3}H]ATP, 4 mM Tris-phosphocreatine, 20 units of creatine phosphokinase/ml, 1 mM cAMP, 1 mM 3-isobutyl-1-methylxanthine, 1 mg of crystallized bovine serum albumin/ml, 0.4 mM dithiothreitol and 0.4 mM Tris-EGTA. GTP-free ATP that had been converted to its Tris salt was used in experiments in which the concentration of GTP was varied; otherwise commercial Tris-ATP was used.

After incubation for 10 min at 30°C, assays were terminated and [^{3}H]cAMP isolated and quantitated, as described previously [7]. All assays were carried out in triplicate; mean values ± S.E. are presented. The platelet protein present in assays was measured as described previously [7], using the method of Lowry et al. [14].

Results

Effects of NaCl

When platelets were lysed in the presence of EGTA, the adenylate cyclase activity of the particulate fraction was stimulated by NaCl (Fig. 1). With material from both rabbit and human platelets, 40 mM NaCl caused approx. 2-fold increases in enzyme activity. However, the extent and pattern of stimulation by higher NaCl concentrations was different in the two species. With rabbit platelet particulate fraction, enzyme activity increased linearly between 40 and 200 mM NaCl, reaching about 8-fold higher than the control at the latter concentration (Fig. 1, A). In contrast, the increase in enzyme activity with human platelet particulate fraction was roughly hyperbolic, reaching a maximum of 2.5-fold at 200 mM NaCl (Fig. 1, B). In the presence of 100 mM NaCl, which was used in most experiments, the increases in adenylate cyclase activity amounted to 5.6 ± 0.4-fold and 2.2 ± 0.1-fold in rabbit and human platelet particulate fractions (means ± S.E.; from eleven and four experiments, respectively).

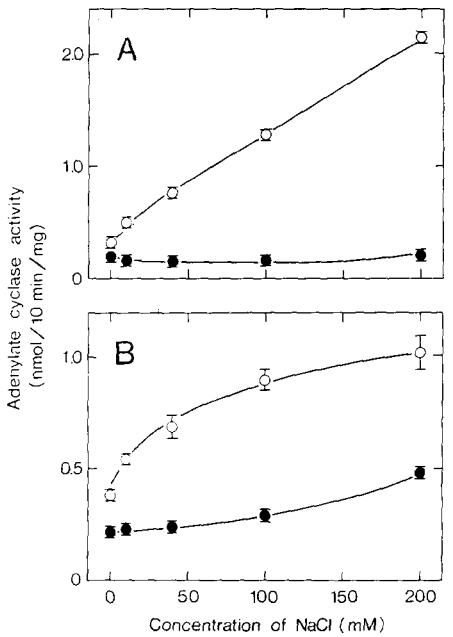

Fig. 1. Effects of different concentrations of NaCl on the adenylate cyclase activities of platelet particulate fractions and on the inhibitory action of 1-octadecyl-2-acetyl-G-3-PC. Assays were carried out with particulate fractions from both rabbit platelets (A) and human platelets (B); all contained 10 μM GTP. Other additions consisted of the indicated concentrations of NaCl without (○) or with (●) 100 nM 1-octadecyl-2-acetyl-G-3-PC.

Addition of 100 nM 1-octadecyl-2-acetyl-G-3-PC inhibited the adenylate cyclase activities of rabbit and human platelet particulate fractions to similar extents in the absence of NaCl (35 ± 2% and 39 ± 2%, means ± S.E. from eight and three experiments, respectively). In the case of rabbit material, 100 nM 1-octadecyl-2-acetyl-G-3PC also prevented the increases in adenylate cyclase activity attributable to NaCl (Fig. 1, A), with the result that the inhibition reached a maximum of 90 ± 1% (mean ± S.E., six experiments) in the presence of 100 mM NaCl. With the human enzyme, 1-octadecyl-2-acetyl-G-3-PC almost completely blocked the effects of NaCl concentrations up to 100 mM, at which a maximum inhibition of 64 ± 3% (mean ± S.E., four experiments) was achieved (Fig. 1, B).

The specificity of the actions of NaCl on rabbit platelet adenylate cyclase was investigated by comparison with the effects of the chlorides of other monovalent cations. In the absence of 1-octadecyl-2-acetyl-G-3-PC, LiCl caused only a weak activation of adenylate cyclase, KCl was slightly inhibitory and choline chloride had no effect (Table I). These results show that the activation of adenylate cyclase by NaCl is not due to changes in the ionic strength of the medium or to any action of Cl^- but is a relatively specific effect of Na^+. The effectiveness of these salts in enhancing the inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC decreased in the order NaCl > LiCl > KCl > choline chloride (Table I) but all showed some activity. Thus, the effects of these salts on the inhibition of adenylate cyclase could not be fully accounted for by the ability of 1-octadecyl-2-acetyl-G-3-PC to block salt enhancement of enzyme activity. In addition, there were in the presence of these salts, but particularly the first three,

TABLE I

EFFECTS OF DIFFERENT MONOVALENT CATIONS ON THE ADENYLATE CYCLASE ACTIVITY OF RABBIT PLATELET PARTICULATE FRACTION; MODIFICATION OF THE INHIBITORY ACTION OF 1-OCTADECYL-2-ACETYL-G-3-PC (OAGPC)

Adenylate cyclase activities were assayed in the presence of 10 μM GTP and the indicated salt concentrations. Values are means ± S.E. from triplicate assays.

Salt	Concn. (mM)	Adenylate cyclase activity (nmol/10 min per mg protein)		Inhibition by OAGPC (%)
		without OAGPC	with 100 nM OAGPC	
None	—	0.196 ± 0.005	0.146 ± 0.001	26 ± 2
NaCl	30	0.405 ± 0.002	0.105 ± 0.003	74 ± 1
	150	1.155 ± 0.027	0.121 ± 0.003	90 ± 0
LiCl	30	0.240 ± 0.006	0.135 ± 0.005	44 ± 3
	150	0.358 ± 0.005	0.131 ± 0.004	63 ± 1
KCl	30	0.184 ± 0.002	0.123 ± 0.001	33 ± 1
	150	0.170 ± 0.001	0.107 ± 0.000	37 ± 0
Choline-Cl	30	0.197 ± 0.002	0.134 ± 0.006	32 ± 2
	150	0.207 ± 0.004	0.138 ± 0.003	33 ± 2

small decreases in enzyme activity below the level observed in the presence of 1-octadecyl-2-acetyl-G-3-PC alone (see also Table II). With human platelet particulate fraction, neither NaCl nor choline chloride consistently exerted this effect and no increase in the inhibitory activity of 1-octadecyl-2-acetyl-G-3-PC was seen in the presence of the latter salt (data not shown).

The effectiveness of different concentrations of 1-octadecyl-2-acetyl-G-3-PC in inhibiting platelet adenylate cyclase was studied using both rabbit and human platelet particulate fractions (Fig. 2). In both preparations, increasing concentrations of agonist overcame the stimulatory effects of 40 mM, 100 mM and 200 mM NaCl on adenylate cyclase activity, but the higher concentrations of

TABLE II

EFFECTS OF GTP ON THE ADENYLATE CYCLASE ACTIVITY OF RABBIT PLATELET PARTICULATE FRACTION IN THE PRESENCE OF DIFFERENT NaCl CONCENTRATIONS; POTENTIATION OF THE INHIBITORY EFFECT OF 1-OCTADECYL-2-ACETYL-G-3-PC (OAGPC)

The particulate fraction was washed twice with hypotonic buffer prior to resuspension for assay (see Experimental Procedures). Values are means ± S.E. from triplicate assays.

Additions		Adenylate cyclase activity (nmol/10 min per mg protein)		Inhibition by OAGPC (%)
NaCl (mM)	GTP (μM)	without OAGPC	with 100 nM OAGPC	
0	0	0.590 ± 0.014	0.490 ± 0.021	17 ± 4
	10	0.366 ± 0.011	0.290 ± 0.003	21 ± 3
30	0	0.736 ± 0.018	0.483 ± 0.016	34 ± 3
	10	0.630 ± 0.008	0.187 ± 0.006	70 ± 1
150	0	1.222 ± 0.002	0.746 ± 0.024	39 ± 2
	10	1.927 ± 0.035	0.243 ± 0.010	87 ± 1

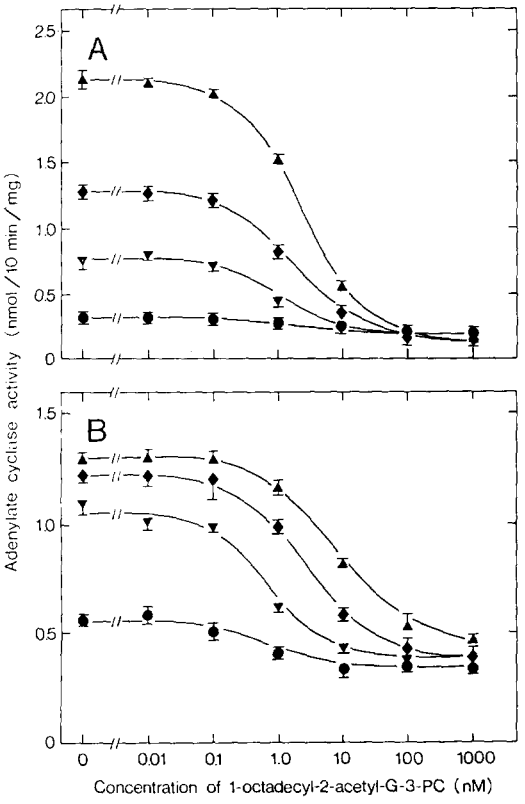

Fig. 2. Dose-response curves for the inhibition of platelet adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC in the presence of different concentrations of NaCl. The adenylate cyclase activities of particulate fractions from both rabbit platelets (A) and human platelets (B) were assayed in the presence of 10 μM GTP, 1-octadecyl-2-acetyl-G-3-PC as indicated and NaCl at the following concentrations: ●, none; ▼, 40 mM; ◆, 100 mM; ▲, 200 mM.

NaCl caused shifts to the right in the dose-response curves. For example, addition of 200 mM NaCl increased the concentration of 1-octadecyl-2-acetyl-G-3-PC causing half-maximal inhibition from 0.6 to 2.3 nM in rabbit platelet particulate fraction and from 0.7 to 7.4 nM with human material. The ability of different concentrations of NaCl to increase that of 1-octadecyl-2-acetyl-G-3-PC required for half-maximal inhibition did not correlate with the stimulation of basal adenylate cyclase activity by this salt. Thus, NaCl increased rabbit platelet adenylate cyclase activity more than human, but caused a larger shift in the dose-response curves with enzyme from the latter species. Moreover, NaCl concentrations above 100 mM had little effect on human platelet adenylate cyclase activity but caused marked shifts in the dose-response curve (Fig. 2). Addition of choline chloride (100–200 mM) had no effects on the dose-response curves for inhibition of rabbit or human platelet adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC in either the presence or absence of NaCl (data not shown).

Effects of GTP

To demonstrate effects of GTP on inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC, it was first necessary to remove endogenous guanine nucleotides from the particulate fractions used. It was found that after two washes of the human platelet material with either an isotonic buffer (150 mM Tris-HCl + 5 mM Tris-EGTA, pH 7.4) or a hypotonic buffer (10 mM Tris-HCl + 5 mM Tris-EGTA, pH 7.4), inhibition of enzyme activity was completely dependent on added GTP (Fig. 3). With rabbit material, isotonic washes were ineffective and only a partial dependence of inhibition on GTP could be demonstrated after two hypotonic washes carried out as described under Experimental Procedures (Table II).

Addition of 10 μM GTP to rabbit platelet particulate fraction that had been washed with hypotonic buffer inhibited adenylate cyclase activity by about 40% in the absence of NaCl but stimulated the enzyme by about 60% in the presence of 150 mM NaCl (Table II). As a result, the increase in basal enzyme activity caused by NaCl was considerably enhanced by added GTP. Inhibition of the rabbit platelet enzyme by 1-octadecyl-2-acetyl-G-3-PC was unaffected by added GTP in the absence of NaCl (about 20% inhibition) but was markedly enhanced by GTP in the presence of 150 mM NaCl (from about 40% to 90%).

Qualitatively similar observations were made with washed human platelet particulate fraction but, probably because GTP was removed more effectively, no significant stimulation of enzyme activity by NaCl or inhibition by 1-octadecyl-2-acetyl-G-3-PC was observed in the absence of added GTP (Fig. 3). Comparison of the effects of different concentrations of GTP showed that this nucleotide had biphasic effects on enzyme activity, tending to stimulate at low concentrations, an effect that was potentiated by NaCl, and to inhibit

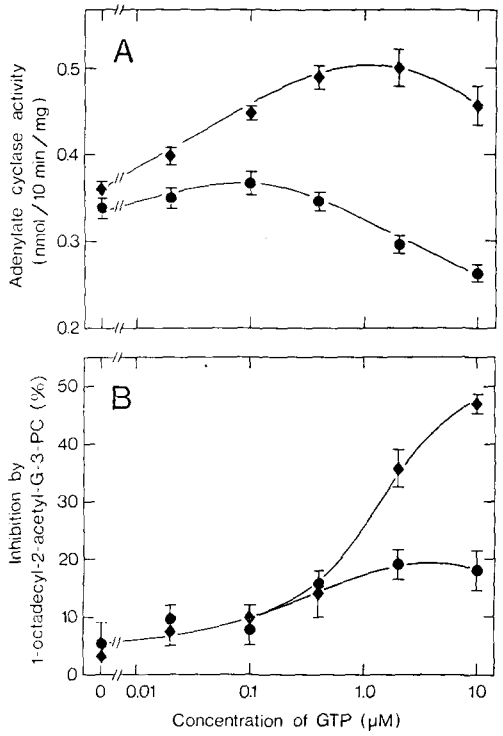

Fig. 3. Effects of different concentrations of GTP with and without NaCl on the adenylate cyclase activity of human platelet particulate fraction; potentiation of the inhibitory action of 1-octadecyl-2-acetyl-G-3-PC. The particulate fraction was washed twice with hypotonic buffer prior to resuspension for assay (see Experimental Procedures). (A) Adenylate cyclase activities at the indicated concentrations of GTP in the absence (●) and presence (◆) of 100 mM NaCl. (B) Inhibition of these adenylate cyclase activities by 100 nM 1-octadecyl-2-acetyl-G-3-PC.

at higher concentrations, an effect that was diminished by NaCl. Optimal stimulation was observed with about 0.1 μM GTP in the absence of NaCl and with 2 μM GTP in the presence of 100 mM NaCl (Fig. 3, A). As a result, the increase in enzyme activity caused by NaCl grew progressively larger with 0.02–2 μM GTP. However, the inhibition of adenylate cyclase activity by 1-octadecyl-2-acetyl-G-3-PC was unaffected by NaCl at GTP concentrations ≤ 0.4 μM and was optimal with 10 μM GTP (Fig. 3, B). Thus, the effects of NaCl on basal enzyme activity and on the inhibitory action of 1-octadecyl-2-acetyl-G-3-PC were expressed over different ranges of GTP concentration.

Discussion

Our results on the effects of NaCl differ markedly from those obtained in previous studies with platelet adenylate cyclase. With both human and especially rabbit platelet particulate fractions, we observed a marked stimulation of basal adenylate cyclase activity by NaCl that was dependent on or potentiated by GTP. In contrast, others [10,11] have found that NaCl caused a GTP-independent inhibition of the basal enzyme activity of platelet membrane preparations. Although inhibition of adenylate cyclase by NaCl has also been observed with membranes from neuroblastoma × glioma hybrid cells [15], activation has been a much more frequent observation with enzyme from a variety of cell types [16–19]. It has been suggested that stimulation is encountered with crude but not washed membrane preparations and is a non-specific salt effect [15]. However, no effect of washing platelet particulate fractions on the stimulation of adenylate cyclase by NaCl was observed in the present study provided GTP was added (compare Fig. 1 and Table II). Moreover, this action of Na^+ was quite specific when compared with other monovalent cations. Our results agree well with those of others who have observed a moderately specific and GTP-dependent activation of adenylate cyclase by Na^+ in adipocyte ghosts [18,19]. Recent work in this laboratory [20] has shown that inhibition of adenylate cyclase by NaCl is only seen with particulate fractions from platelets lysed in the absence of 5 mM EGTA and is prevented by lysis of the platelets in the presence of leupeptin, an inhibitor of Ca^{2+}-dependent proteolysis. We believe, therefore, that stimulation of platelet adenylate cyclase by NaCl may be a more physiological response than inhibition.

The importance of chelating calcium ions during lysis of platelets in order to obtain inhibition of adenylate cyclase by high GTP concentrations has been recognized previously [21]. In the present studies, GTP had distinct biphasic effects on the adenylate cyclase activity of washed particulate fraction from human platelets. In the absence of NaCl, low concentrations of GTP were weakly stimulatory and higher concentrations were inhibitory. Previous reports [9,21] have only described inhibition of platelet adenylate cyclase by GTP in

the absence of a stimulatory hormone, though biphasic effects of GTP have been observed in several other tissues [2,17,22,23] and with the platelet enzyme on addition of prostaglandin E_1 [9]. In the presence of NaCl, we found that the stimulatory effect of GTP was enhanced and the inhibitory phase markedly reduced. This has also been observed with membranes from rat adipocytes [23] and cerebral cortex [17]. These biphasic effects are readily interpreted in terms of the different GTP requirements of the N_s and N_i proteins that couple stimulatory and inhibitory receptors to adenylate cyclase [2]. Although we did not investigate the effects of a full range of GTP concentrations on washed particulate fraction from rabbit platelets because of the difficulty in completely removing endogenous guanine nucleotides, comparable results were obtained on addition of 10 μM GTP.

The maximum inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC in rabbit platelet particulate fraction (90%) was larger than has generally been observed with hormonal inhibitors of adenylate cyclase [2]. However, with human preparations the maximum inhibition (64%) was comparable with that caused by epinephrine [5]. This difference in the effectiveness of 1-octadecyl-2-acetyl-G-3-PC in the two species is readily explained. Thus, the inhibitory effect consisted of two major components, firstly, an inhibition of the basal activity seen in the absence of NaCl, which was similar in the human and rabbit platelet particulate fractions and, secondly, a suppression of the stimulatory effect of NaCl. The overall contribution of this latter effect was greater in rabbit preparations because of the larger increases in enzyme activity caused by NaCl. LiCl was found to potentiate inhibition of adenylate cyclase by the same mechanism in rabbit preparations. These results are in marked contrast with those obtained by others working on human platelet adenylate cyclase [10,11], who have found no effect of NaCl on the fractional inhibition of enzyme activity by epinephrine. This can largely be explained by the failure of these workers to obtain activation of adenylate cyclase by NaCl. Another group [12], using rabbit material, observed that NaCl did potentiate the inhibition of prostaglandin E_1-stimulated activity by epinephrine but did not indicate whether or not NaCl increased basal activity.

A further effect of NaCl was seen in its ability to shift the dose-response curves for the inhibition of adenylate cyclase by 1-octadecyl-2-acetyl-G-3-PC to the right. As this phenomenon did not correlate with the activation of adenylate cyclase by NaCl, it is possible that NaCl exerts these two actions through different sites on the platelet membrane. Several workers have detected a decreased affinity of receptors for inhibitory agonists in the presence of NaCl [2], including decreased binding of epinephrine to α_2-adrenergic receptors on the platelet membrane [12,24–26]. Limbird et al. [25] have also shown that NaCl decreases the affinity of solubilized platelet α_2-adrenergic receptors for epinephrine, implying that Na^+ can modulate receptor affinity via binding sites distinct from any associated with the GTP-binding proteins. The shift in dose-response curves for 1-octadecyl-2-acetyl-G-3-PC could reflect a similar mechanism of action of sodium ions, whereas the activation of adenylate cyclase by NaCl could be due to an action on the N_s and/or N_i proteins, as this effect requires GTP.

Although complete dependence of the action of 1-octadecyl-2-acetyl-G-3-PC on GTP was observed with washed particulate fraction from human platelets, only a partial dependence on GTP was detected with rabbit material. In previous studies on the inhibitory action of epinephrine on platelet adenylate cyclase, a GTP requirement was readily demonstrated with washed human preparations [8,10,11] but was not observed at all with rabbit material [12]. Several workers have explained the roles of GTP and NaCl in the hormonal inhibition of adenylate cyclase in terms of the ability of the former to inhibit basal enzyme activity and of the latter to reverse this effect. Thus, inhibitory hormones have often appeared to act by blocking this GTP-dependent activation of the enzyme by NaCl [3,17,18]. Such a mechanism could account for the Na^+-dependent part of the inhibitory action of 1-octadecyl-2-acetyl-G-3-PC on human and rabbit platelet adenylate cyclase. Potentiation by NaCl of the inhibition of the human enzyme required a relatively high concentration of GTP (> 0.4 μM). This is consistent with many reports that hormonal inhibition of adenylate

cyclase mediated by N_i requires a higher GTP concentration than hormonal activation of the enzyme mediated by N_s [2]. However, a stimulation of basal enzyme activity by NaCl was clearly observed with 0.02–0.4 μM GTP in the present study (see Fig. 3, A). This suggests that NaCl has an additional action on platelet adenylate cyclase at low GTP concentrations that is unrelated to the NaCl-dependent inhibition of the enzyme. A direct or indirect stimulatory effect of NaCl on N_s activity would be consistent with our observations. In contrast, recent experiments with adipocyte membranes have suggested that NaCl may inhibit both N_s and N_i proteins [27]. Further studies are clearly required before the actions of NaCl on adenylate cyclase activity can be fully understood. However, the present work establishes for the first time that NaCl stimulates platelet adenylate cyclase and that the inhibition of this enzyme by 1-octadecyl-2-acetyl-G-3-PC requires GTP and is potentiated by NaCl. As 1-octadecyl-2-acetyl-G-3-PC is an exceptionally potent inhibitor of adenylate cyclase, further analysis of its actions in the platelet system may provide additional insight into the mechanisms involved in the hormonal inhibition of this enzyme.

Acknowledgements

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