Biochemical Pharmacology, Vol. 23, pp. 845–855. Pergamon Press, 1974. Printed in Great Britain.

EFFECT OF LITHIUM ON PROSTAGLANDIN E_1-STIMULATED ADENYLATE CYCLASE ACTIVITY OF HUMAN PLATELETS*

YAO-CHUN WANG,† GHANSHYAM N. PANDEY,‡ JOE MENDELS‡§ and ALAN FRAZER‡§

Departments of Pharmacology and Psychiatry, University of Pennsylvania, Philadelphia, Pa., U.S.A.

(*Received* 25 *April* 1973; *accepted* 27 *July* 1973)

Abstract—The effect of lithium (Li) on the stimulation of adenylate cyclase by prostaglandin E_1 (PGE_1) was examined. In platelet sonicates, Li, as well as sodium (Na), potassium (K), and rubidium (Rb) significantly reduced the PGE_1-induced stimulation of adenylate cyclase in a dose-dependent manner. The inhibition due to Rb was significantly less than that produced by the other cations; at a high concentration, 64 mM, Li was a more potent inhibitor than the other cations. In intact platelets, only Li reduced the PGE_1-enhanced accumulation of [^{3}H]cyclic AMP, K and Rb being ineffective. As little as 1 mM Li significantly reduced the stimulatory effect of PGE_1 on [^{3}H]cyclic AMP production in this sytem. The inhibition produced by Li was not blocked by phentolamine, whereas phentolamine did block the inhibition due to norepinephrine (NE). Magnesium enhanced the stimulatory effect of PGE_1 on the production of labeled cyclic AMP in intact platelets and antagonized the inhibition produced by Li on this process. These data show that Li antagonizes PGE_1-induced stimulation of platelet adenylate cyclase at a site distinct from that at which NE acts.

THE increasing use of lithium (Li) in psychiatric practice has stimulated numerous investigations into the possible mechanisms of action of this cation. Much of this research has involved the interaction of Li with biogenic amines, as such compounds have been postulated to play a key role in the pathogenesis of affective illness.[1,2] Recently, Li has been shown to affect a system through which many hormones, and possibly neurotransmitter substances as well,[3,4] exert their effects, namely the adenylate cyclase–adenosine 3′,5′-monophosphate (cyclic AMP) system. In many tissues, Li appears to inhibit hormone-induced activation of adenylate cyclase without lowering basal enzyme activity. For example, high concentrations of Li reduce the increase in brain adenylate cyclase activity produced by fluoride or catecholamines,[5] decrease the stimulation of renal adenylate cyclase by antidiuretic hormone (ADH),[6] inhibit parathyroid hormone stimulation of renal cortical adenylate cyclase in male rats[7] and inhibit corticotrophin- and fluoride-induced stimulation of the enzyme in fat cell ghosts.[8] Lower concentrations of Li diminish the activation of adenylate cyclase in thyroid slices by thyroid-stimulating hormone (TSH)[9,10] and reduce the norepinephrine (NE)-stimulated formation of [^{14}C]cyclic AMP produced from ATP prelabeled with [^{14}C]adenine in rat cerebral cortical slices.[11]

* This investigation was supported in part by research funds from the Veterans Administration and NIH grant HL-01813. A preliminary report of part of this work appeared in *Clin. Res.* **21**, 265 (1973).

‡ The work presented here is in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Department of Pharmacology, University of Pennsylvania.

‡ Affective Diseases Research Unit, Veterans Administration Hospital, Philadelphia, Pa. 19104.

§ Departments of Pharmacology and Psychiatry, University of Pennsylvania, School of Medicine.

In the present paper, we report data which extend these observations by showing that prostaglandin E_1 (PGE_1)-induced stimulation of platelet adenylate cyclase activity is significantly reduced by Li.

MATERIALS AND METHODS

About 35 ml of venous blood was obtained from fasting subjects at 8:30 a.m. and placed in a plastic tube containing 0·5 ml of 150 mM EDTA as an anticoagulant. Adenylate cyclase assays were performed immediately after obtaining the platelet preparations. Subjects were drug-free for at least 2 weeks before blood was obtained.

For experiments in which adenylate cyclase activity was measured in platelet membrane fragments, platelets were isolated and sonicated essentially as reported by Wolfe and Shulman[12] and Marquis *et al.*[13] Before sonication, the platelet pellet contained an average of 1 leukocyte per 20,000 platelets, as determined by light microscopy.[14] The sonication procedure resulted in less than 1 per cent of the total initial platelets remaining intact.

Adenylate cyclase activity of the platelet membrane fragments was estimated by the method of Krishna *et al.*[15] The membrane fractions (0·05 ml) were incubated at 37° in a total volume of 0·10 ml with [^{14}C]ATP, 1 μCi or [^{3}H]ATP, 10 μCi; ATP, 2·5 mM; $MgCl_2$, 4·0 mM; Tris, 40 mM, pH 7·4. PGE_1 or cations were added as indicated. The incubation was started by the addition of labeled ATP with carrier to the other components and terminated after 15 min by the addition of 0·4 ml of 0·75 mM carrier cyclic AMP, and boiling for 3 min. A boiled sample of platelet membrane fragments was run with each assay as a control. All incubations were done in duplicate.

When radioactive cyclic AMP synthesis by platelet membrane fragments was investigated, no phosphodiesterase (PDE) inhibitor was added to the reaction medium. Preliminary experiments indicated that such an inhibitor was unnecessary: neither basal enzyme activity nor PGE_1-stimulated activity was altered significantly when either caffeine (20 mM) or the phosphodiesterase stimulating agent imidazole (2 mM) was added to the reaction medium. Such results are in keeping with the finding of Marquis *et al.*[13] and Song and Cheung[16] that the bulk of platelet phosphodiesterase activity is in the 100,000 *g* supernatant fluid following sonication.

As an index of adenylate cyclase activity in intact platelets, the pulse-labeling technique developed by Kuo and DeRenzo[17] and by Shimizu *et al.*[18] was used. Platelet-rich plasma (PRP) was obtained by centrifuging whole blood at 200 *g* for 15 min at 4°. The PRP was centrifuged again for 5 min to remove contaminating leukocytes. To the PRP (~15 ml) was added 100 μCi [^{3}H]adenine.

At this stage, if either Li or magnesium (Mg) was to be added directly to the platelets, the PRP was immediately divided into aliquots. The aliquot which received neither Li nor Mg, had Tris buffer added to it in a concentration which produced an osmolarity equivalent to that in the aliquots containing Li or Mg.

The platelet suspensions were incubated in a Dubnoff metabolic shaker at 37°. After 60 min, the platelets were collected by centrifugation at 2500 *g* for 10 min at 4° and washed with an iso-osmotic Tris buffer solution of the following composition: $NaHCO_3$, 3·3 mM; KCl, 2·5 mM; Na_2HPO_4, 0·4 mM; glucose, 5·0 mM; EDTA, 6·6 mM; Tris, 35 mM, pH 7·4 at 37°; NaCl was added to this buffer solution such that its final osmolarity was 280 mOsm. The platelets were collected again and resus-

pended in the Tris buffer solution. Then 0·05 ml of the platelet suspension was added to an incubation solution consisting of $MgSO_4$ (10 mM) and caffeine (10 mM) in Tris buffer solution. The final volume of the reaction mixture was 0·10 ml. Drugs were added as indicated. A boiled sample of the labeled platelets was run with each assay as a control. Such control preparations were obtained from all aliquots of labeled platelets, i.e. aliquots to which either Li or Mg was added as well as aliquots to which Tris buffer was added. Incubation proceeded for 2 min at 37° and was terminated by the addition of 0·40 ml of 0·75 mM cyclic AMP and boiling for 5 min. Preliminary experiments showed that the PGE_1-stimulated net synthesis of [^{3}H]cyclic AMP was maximal at 2 min.

The boiled mixture was centrifuged and the supernatant assayed for [^{3}H]cyclic AMP by the method of Krishna *et al.*[15] Each incubation was performed in duplicate. The purity of the radioactive cyclic AMP product was confirmed by ascending paper chromatography. Results were calculated as the per cent conversion of total radioactivity in each incubation tube into labeled cyclic AMP.

To determine the amount of cold ATP and [^{3}H]ATP in the platelets after incubation with radioactive adenine, the following procedure was adopted. After incubation of the PRP with [^{3}H]adenine, the platelets were collected and washed as described previously. The platelet pellet was suspended in 0·8 ml perchloric acid (PCA, 0·625 N) and sonified twice for 15 sec each time. The sonicate was centrifuged for 10 min at 3500 *g* at 4° and an aliquot of the supernatant fluid was neutralized with a solution of K_2CO_3 (1·67 M)–triethanolamine (0·5 M, pH 7·0). The ATP concentration of an aliquot of the neutralized solution was determined enzymatically, using phosphoglycerate kinase and glyceraldehyde-3-phosphate dehydrogenase.[19]

To another aliquot of the neutralized platelet preparation was added carrier ATP solution and the mixture was placed on a column (3 × 0·5 cm) of Dowex 1–X8 resin (chloride form; 200–400 mesh). ATP was eluted from the column with 5 ml HCl (0·25 N) as described by Garrahan and Glynn.[20] Preliminary experiments showed that adenine, adenosine, AMP, cyclic AMP and ADP were clearly separated from ATP by this technique, being eluted by water or lower strength HCl solutions before ATP. The radioactivity of a 0·5-ml aliquot of the 0·25 N HCl eluate was counted by liquid scintillation spectrophotometry.

Protein concentrations were determined by the method of Lowry *et al.*[21]

Statistical evaluations were by Student's or paired *t*-test[22] or analysis of variance.[23] Dose–response regression lines were calculated by the method of least squares.[24]

RESULTS

In the initial experiments, varying concentrations of the chlorides of Li, as well as of Na, K and Rb, were added *in vitro* to sonicated platelet preparations whose adenylate cyclase activity was then measured. As the stimulatory effect of 1×10^{-6} M PGE_1 varied somewhat with each experiment (producing on the average a 7-fold increase in adenylate cyclase activity), results with the cations are expressed as the percentage of maximal PGE_1-induced stimulation in the absence of any drugs. Basal enzyme activity was not affected by any of the cations at any concentration used (2, 8, 16 or 64 mM); for example, in the presence of 64 mM Li,

basal adenylate cyclase activity was 0·16 ± 0·02 nmole/mg/10 min (mean ± S.E.M.) vs 0·15 ± 0·01 without Li (N = 4, P > 0·5; paired *t*-test).

All the cations, however, in the presence of PGE_1 (1×10^{-6} M) lowered enzyme activity as shown by the log dose–response lines, calculated by the method of least squares (Fig. 1). To evaluate the statistical significance of these data, an analysis of variance was performed. This was a mixed-type model with between-subjects (drugs) and within-subject (dose and drug × dose) effects. All terms in the analysis of variance were significant (P < 0·001). The significant drug effect obtained in the analyses indicates that the various cations had different overall inhibitory effects. Furthermore, as is evident in Fig. 1, increasing the concentrations of all the cations produced greater inhibition that that observed at lower concentrations. Some of the dose–response curves for the different cations were also different, as demonstrated by the significant drug × dose term.

Both Li and K produced significantly greater inhibition of PGE_1-stimulated enzyme activity than did Rb at every dose (Student's *t*-test, P < 0·02). In general, this was true for Na as compared with Rb as well; only at a concentration of 16 mM was the inhibition produced by Na and by Rb not significantly different. The inhibition produced by Li increased most sharply as the concentration was raised, so that at 64 mM this ion produced significantly greater inhibition of PGE_1-stimulated adenylate cyclase activity than that observed with any other cation (P < 0·001).

It should be mentioned that the inhibition produced by these monovalent cations on PGE_1-stimulated adenylate cyclase is not the result of an ionic strength effect. Addition of Tris buffer (pH 7·4) to the incubation medium to raise its final concentration to 104 mM (as compared to its normal concentration of 40 mM) caused no effect on the stimulation of adenylate cyclase produced by PGE_1.

As Na, K and Rb produced effects qualitatively similar to those of Li (if quantitatively different) in the platelet membrane system, it was decided to evaluate the effects

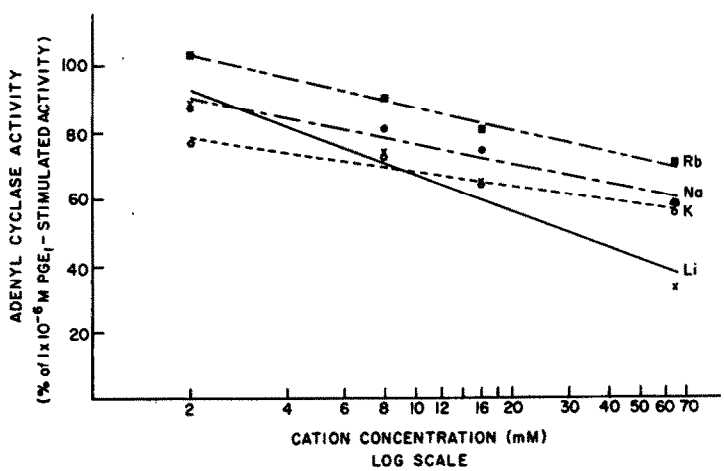

FIG. 1. Inhibitory effect of Li, K, Na and Rb on PGE_1-stimulated adenylate cyclase activity in platelet sonicates. PGE_1 (1×10^{-6} M)-stimulated enzyme activity averaged 1·09 ± 0·06 nmoles/mg/10 min, which represents a 7-fold increase over basal values. The response to PGE_1 has been taken as 100 per cent. Each point represents the mean of seven determinations. Regression lines were calculated by the method of least squares. Li, (×); K, (○); Na, (●); Rb, (■).

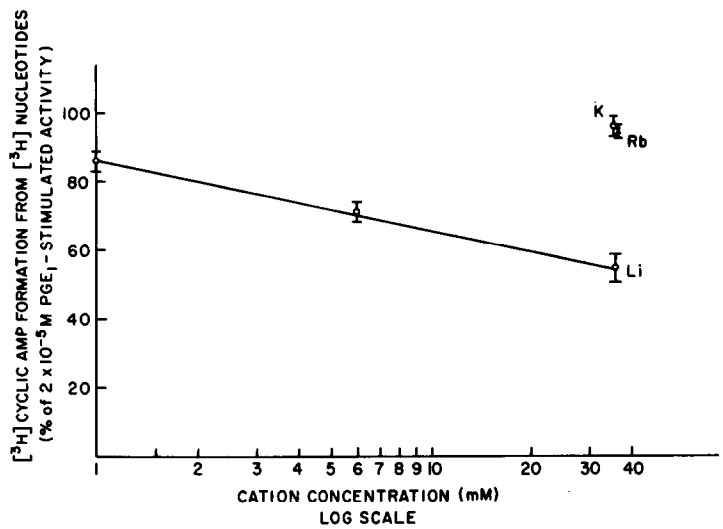

FIG. 2. Inhibitory effect of Li, K and Rb on 2×10^{-5} M PGE_1-stimulated production of [^{3}H]cyclic AMP in intact platelets. The average per cent conversion of [^{3}H]nucleotides to [^{3}H]cyclic AMP with PGE_1 was 1·30 ± 0·10, which represents a 32-fold increase over basal values. Response to PGE_1 has been taken as 100 per cent. The cations were added during the labeling incubation with [^{3}H]adenine. Each point on the dose–response curve for Li represents the mean of five experiments; the Rb point represents the mean of five experiments, and the K point the mean of four experiments. Bars around each mean show the S.E.M. The regression line was calculated by the method of least squares.

of these cations on adenylate cyclase activity using intact platelets. One disadvantage of such a system is that it yields a more indirect measure of adenylate cyclase activity than the membrane fragment system, in which exogenous ATP is the substrate. However, in several different experimental situations, changes in the amount of radioactive cyclic AMP produced were shown to reflect changes in the endogenous content of the cyclic nucleotide.[17,18] Furthermore, this system has the advantage of being more physiological than the use of platelet membrane fragments, since the intact platelets are incubated in a medium of high Na and low K concentration.

Of the three cations (Li, Rb and K) tested for their effects on the production of radioactive cyclic AMP (Na was not tested as it was already present in the medium in high concentration, ~ 100 mM), only Li inhibited significantly the increased production of the labeled nucleotide produced by 2×10^{-5} M PGE_1 (Fig. 2). In this system, 1 mM Li inhibited the stimulatory effect of PGE_1 by 14 per cent ($P < 0{\cdot}02$, paired *t*-test) with higher concentrations of Li producing even greater inhibition. As shown in Fig. 2, Rb and K were ineffective in this system.

In these experiments, the cations tested were added at the beginning of the 60-min incubation with [^{3}H]adenine, and were not present during the 2-min reaction. If the cations were present only during the 2-min reaction, considerably less inhibition was produced. For example, 6 mM Li added just to the reaction mixture and not to the incubation with [^{3}H]adenine produced only an 8 per cent decrease in the stimulatory effect of PGE_1 (compared with the 29 per cent inhibition shown in Fig. 2).

It is unlikely that the inhibitory effect of Li on PGE_1-stimulated adenylate cyclase activity of intact platelets can be attributed to Li producing a diminshed uptake of [^{3}H]adenine by the platelets. There was a 2 per cent decrease in the uptake of

[^{3}H]adenine in the presence of 36 mM Li, which was statistically significant. However, 6 mM Li produced no significant alteration in [^{3}H]adenine uptake, whereas it did significantly inhibit the stimulatory effect of PGE_1 on radioactive cyclic AMP production (Fig. 2). [^{3}H]Adenine uptake in the presence of 6 mM Li was 64·6 ± 0·6 per cent vs 64·0 ± 0·4 per cent in the absence of Li ($N = 8$; $P > 0·4$).

Furthermore, as will be demonstrated, elevation of the magnesium concentration during the incubation with [^{3}H]adenine allowed for a greater than normal stimulation of adenylate cyclase by PGE_1. However, the uptake of [^{3}H]adenine in the presence of a high concentration of magnesium (64·5 ± 0·4 per cent) was not different from that observed with a normal concentration of magnesium (63·8 ± 0·4 per cent; $N = 6$, $P > 0·2$).

It appears, then, that an effect of Li or Mg on the uptake of [^{3}H]adenine cannot be correlated with their subsequent effects on PGE_1-stimulated accumulation of radioactive cyclic AMP.

Most of the radioactivity in the platelets incubated with labeled adenine was [^{3}H]ATP. In four experiments, the percentage of total radioactivity present as [^{3}H]ATP was 74·6 ± 1·2; when 36 mM Li was present during the incubation with [^{3}H]adenine, 75·9 ± 1·9 per cent of the total radioactivity was [^{3}H]ATP ($P > 0·3$, paired *t*-test). In these experiments, the total ATP content of the platelets was not altered if Li was present during the labeling incubation such that the specific activity of ATP in the platelets in the absence of Li was 81·4 ± 6·6 mCi/m-mole and it was 82·4 ± 7·3 mCi/m-mole when Li was present ($P > 0·7$, paired *t*-test). Thus, the inhibitory effect of Li on the stimulation of [^{3}H]cyclic AMP net synthesis produced by PGE_1 is not due to the cation decreasing [^{3}H]adenine uptake or decreasing the conversion of [^{3}H]adenine into [^{3}H]ATP.

It should be emphasized that whereas Li did inhibit the increased production of [^{3}H]cyclic AMP caused by PGE_1, it was without effect on the basal net synthesis of [^{3}H]cyclic AMP. For example, the per cent conversion of [^{3}H]nucleotides to [^{3}H]cyclic AMP in the absence of Li was 0·04 ± 0·01, whereas it was also 0·04 ± 0·01 when 36 mM Li was added to the labeling incubation ($N = 5$, $P > 0·9$; paired *t*-test). Similar results were obtained with 6 mM and with 1 mM Li. This lack of an effect of Li on the basal net synthesis of [^{3}H]cyclic AMP does not appear to be due to the fact that the basal net synthesis of [^{3}H]cyclic AMP was so low that it could not be reduced further since, in four experiments, NE (2×10^{-5} M) lowered [^{3}H]cyclic AMP production from 0·05 ± 0·005 to 0·03 ± 0·005 per cent ($P < 0·02$, paired *t*-test).

This inhibitory effect of Li was compared with the known inhibitory effect of catecholamines such as epinephrine or NE on PGE_1-stimulated adenylate cyclase activity.[25–27] In confirmation of these reports, NE was found to inhibit the enhanced production of [^{3}H]cyclic AMP produced by PGE_1 and this inhibition was reversed by the alpha-adrenergic blocking agent, phentolamine (Fig. 3). Regardless of whether it was present throughout the labeling incubation or the 2-min reaction or throughout both, phentolamine had no effect on the inhibition produced by Li, nor did it alter the production of labeled cyclic AMP in the absence of PGE_1 (Fig. 3).

If two activators compete for the same binding site of an enzyme, their effect when combined at concentrations near their K_m of activation is approximately equal to the arithmetic mean of their effects tested separately at twice this concentration.[28]

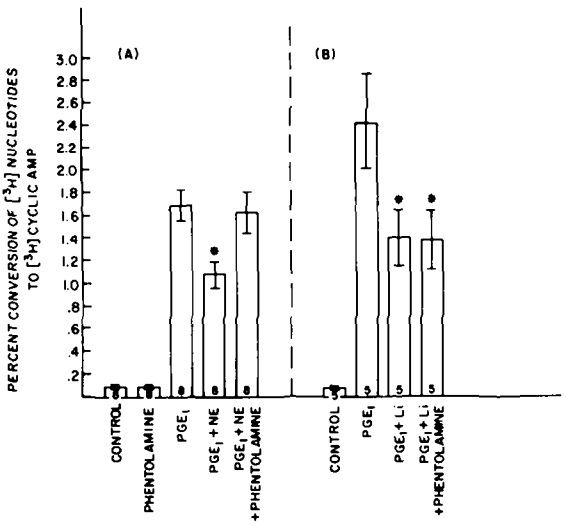

FIG. 3. Effect of phentolamine on the inhibition produced by NE (A) and by Li (B) on PGE_1-induced accumulation of [^{3}H]cyclic AMP in intact platelets. The bars and brackets represent the mean and S.E.M. respectively; the number of experiments is indicated in each bar. Drugs were used in the following concentrations: PGE_1, 2×10^{-5} M; Li, 36 mM; NE, 2×10^{-5} M; phentolamine, 2×10^{-4} M. Li was added during the incubation with [^{3}H]adenine; the other drugs were added during the 2-min reaction. Asterisks indicate values significantly different from the corresponding PGE_1 mean value ($P < 0{\cdot}01$, paired t-test).

This is true for inhibitors as well. To compare further the inhibitory effects of Li and NE, such experiments were done. The results are shown in Table 1. In these experiments, PGE_1 produced more than a 40-fold stimulation in the production of [^{3}H]cyclic AMP. Either NE (4×10^{-5} M) or Li (72 mM), tested separately, significantly reduced the PGE_1 stimulation by about 60 per cent. When Li (36 mM) and NE (2×10^{-5} M) were added together, they produced about 75 per cent inhibition

TABLE 1. INHIBITORY EFFECT OF LITHIUM AND NE ON THE PGE_1-INDUCED INCREASE IN [^{3}H]CYCLIC AMP PRODUCTION IN INTACT PLATELETS

Pretreatment*	Drug	[^{3}H]cyclic AMP production (% conversion)	P† (compared to PGE_1 with no pre-treatment)
None (7)	None	0·05 ± 0·01‡	< 0·001
None (7)	PGE_1§	2·14 ± 0·28	
None (7)	PGE_1 and NE‖	0·91 ± 0·11	< 0·001
Li (72 mM) (7)	PGE_1	0·83 ± 0·04	< 0·005
Li (36 mM) (7)	PGE_1 and NE¶	0·58 ± 0·05	< 0·005
			< 0·02**

* Indicates addition made only during incubation with [^{3}H]adenine. Number in parentheses is the number of observations.

† Paired t-tests.

‡ Mean ± S.E.M.

§ PGE_1, 2×10^{-5} M.

‖ NE, 4×10^{-5} M.

¶ NE, 2×10^{-5} M.

** Compared to either PGE_1 with 4×10^{-5} M NE or PGE_1 with 72 mM lithium.

TABLE 2. EFFECT OF MAGNESIUM AND LITHIUM ON PGE_1-STIMULATED PRODUCTION OF RADIOACTIVE CYCLIC AMP

Pretreatment*	Drug	[^{3}H]cyclic AMP production (% conversion)	P† (compared to PGE_1 with no pre-treatment)
None (6)	None	0·12 ± 0·02‡	< 0·001
Mg (6 mM) (6)	None	0·13 ± 0·02	< 0·001
None (6)	PGE_1§	2·94 ± 0·36	
Li (6 mM) (6)	PGE_1	2·23 ± 0·17	< 0·02
Mg (6 mM) (6)	PGE_1	3·60 ± 0·46	< 0·01
Li (6 mM) + Mg (6 mM) (6)	PGE_1	3·27 ± 0·40	< 0·01 < 0·02‖

* Indicates addition made only during incubation with [^{3}H]adenine. Number in parentheses is the number of observations.
† Paired *t*-test.
‡ Mean ± S.E.M.
§ PGE_1, 2×10^{-5} M.
‖ Compared to PGE_1 stimulation with 6 mM Mg.

of the PGE_1 effect, which was significantly greater than the inhibition produced by either drug tested alone at twice the concentration ($P < 0·02$ in both cases). These results, as well as those obtained with phentolamine, suggest that Li and NE act at separate sites.

It was of interest to determine whether the inhibitory effect of Li on PGE_1-stimulated activity could be influenced by changing the components of the medium in which the platelets were labeled. To test this, the Mg concentration of the labeling medium was elevated, since comparison of the physical properties of the lithium ion and the magnesium ion suggests the possibility of an interaction between them. When the concentration of Mg in the labeling incubation was raised by 6 mM, the basal production of [^{3}H]cyclic AMP was not altered but the stimulatory effect of PGE_1 was significantly enhanced (Table 2). Li (6 mM) produced its usual degree of inhibition in the normal Mg medium. In the presence of additional Mg, Li still produced a significant inhibition of the PGE_1 stimulation, but the magnitude of this inhibition was reduced. In other words, Li (6 mM) reduced the stimulatory effect of PGE_1 by 22 per cent when Mg was not added to the labeling incubation, but by only 8 per cent when the Mg concentration was elevated (Table 2). This Mg-produced decrease in the per cent inhibition seen with Li was significant ($P < 0·02$, paired *t*-test). In this system, then, elevation of the Mg concentration had two effects—it increased the stimulatory effect of PGE_1 and it reduced the inhibitory effect of Li on PGE_1-induced stimulation.

DISCUSSION

Since the classic observations of Sutherland and Rall[29] on cyclic AMP metabolism, distribution and function, it has been established that cyclic AMP serves as an intracellular messenger in many biological systems. Recent evidence indicates that the cyclic nucleotide is important in platelet function.[30] Substances that inhibit platelet aggregation produce an increase in cyclic AMP, whereas agents that cause platelet clumping result in a decrease.

For example, PGE_1, an extremely potent inhibitor of platelet aggregation[31,32] increases platelet cyclic AMP concentration[25,33] by stimulating adenylate cyclase.[12,13,27,34] In contrast, epinephrine and NE, which induce platelet aggregation,[35,36] have been reported to lower basal levels of platelet cyclic AMP[37] and certainly depress the increased formation of cyclic AMP produced by PGE_1.[25–27] This antagonism by catecholamines toward the stimulatory effect of PGE_1 on platelet cyclic AMP concentration can be prevented by alpha-adrenergic blocking agents such as phentolamine.[25–27]

Data presented here confirm these observations on the interaction of PGE_1 and NE in the regulation of platelet adenylate cyclase activity. In addition, it was shown that Li antagonizes the stimulatory effect of PGE_1 on both adenylate cyclase activity of platelet sonicates and on the production of [^{3}H]cyclic AMP from [^{3}H]nucleotides in intact platelets. Li produces this effect at a site distinct from that at which NE acts, since the inhibitory effect of Li was not blocked by phentolamine as was the effect of NE.

Also, the degree of inhibition produced by Li appears to be time-dependent, since considerably more inhibition was observed when the platelets were in contact with Li for 60 min as compared to being in contact with the cation for only 2 min. The lesser amount of inhibition seen during the short incubation with Li may be due to the fact that the cation is not incorporated into the membrane properly during the 2-min period.

These data extend the numerous reports that Li inhibits hormone activation of adenylate cyclase in various tissues in animals.[5–11] Inhibition of hormone-stimulated adenylate cyclase by Li appears to be involved in several effects of this drug in humans as well. For example, the polyuria which can occur as a side effect of Li[38,39] appears to result from its antagonizing the renal effects of ADH[40,41] by, perhaps, inhibiting the response of adenylate cyclase to ADH.[6] Similarly, the anti-thyroid effects of Li observed in psychiatric patients receiving the drug,[42,43] and which are sufficiently potent to suggest its use clinically in thyrotoxicosis,[44,45] may result, in part, from lithium inhibiting the stimulatory effect of TSH on thyroid adenylate cyclase[9,10] in addition to its other inhibitory effects on thyroid hormone release.[46,47] Whether the pharmacological effect of Li on platelet adenylate cyclase observed in the present report has similar functional significance, namely antagonizing the inhibitory effect of PGE_1 on platelet aggregation, has yet to be investigated.

Not only Li, but Na, K and Rb as well, inhibited PGE_1 stimulation of adenylate cyclase in platelet sonicates. That this was not a non specific ionic effect is suggested by the following: (1) the different monovalent cations produced different degrees of inhibition; and (2) raising the ionic strength by addition of Tris to the reaction medium had no effect on the stimulation due to PGE_1.

Although the monovalent cations tested produced qualitatively similar effects on PGE_1 stimulation of platelet adenylate cyclase, the divalent cations, Mg and calcium (Ca), appear to produce opposite effects on PGE_1-stimulated activity in platelets. In contrast to what we observed upon raising the Mg concentration, Ca at an elevated concentration in the medium inhibits the stimulatory effect of PGE_1 on platelet adenylate cyclase.[48] Also, the TSH-induced increase in enzyme activity in sheep thyroid homogenates is inhibited by Ca.[10] Lithium has effects similar to those of Ca in these tissues.

In the present report, we did not examine whether the inhibitory effect of Li resulted from some interaction with Ca, as has been shown in other systems.[49,50] We did observe, however, that raising the Mg concentration during the incubation with [^{3}H]adenine antagonized the inhibitory effect of Li, in addition to enhancing the stimulation produced by PGE_1. These effects of Mg occurred without its producing any change in the basal per cent conversion of [^{3}H]nucleotides to [^{3}H]cyclic AMP. In many tissues, basal adenylate cyclase activity of homogenates is increased in the presence of high concentrations of Mg.[8,51,52] This has been attributed to its increasing the affinity of adenylate cyclase for ATP through its activation of the enzyme at a site different from the substrate binding site.[8,52]

The antagonism of the inhibitory effect of Li by Mg suggests that Li might compete with Mg for this secondary site on the enzyme. The stimulatory effect of both fluoride and ACTH on adipose cell adenyl cyclase has been attributed to these agents increasing the affinity of the enzyme for Mg.[8] If the stimulatory effect of PGE_1 on the platelet enzyme is due to the same mechanism, and the enhanced stimulation produced by PGE_1 in the presence of high concentrations of Mg is suggestive in this regard, then a competition between Li and Mg might account for the present results.

Acknowledgements—The encouragement and advice of Drs. Ella and Niels Haugaard (Department of Pharmacology, University of Pennsylvania) throughout the course of this investigation are gratefully acknowledged. Dr. Carl Cochrane (Behavioral Sciences Center, Bowman Gary School of Medicine) advised with the statistical evaluation of the data. The assistance of Elaine Serlick with the ATP determinations is appreciated.

REFERENCES

1. J. J. SCHILDKRAUT, *Am. J. Psychiat.* **122,** 509 (1965).
2. J. MENDELS and J. STINNETT, in *Biological Psychiatry* (ED. J. MENDELS), p. 65. Wiley, New York (1973).
3. B. J. HOFFER, G. R. SIGGINS and F. E. BLOOM, *Adv. Biochem. Psychopharmac.* **3,** 349 (1970).
4. D. A. MCAFEE, M. SCHORDERET and P. GREENGARD, *Science, N.Y.* **171,** 1156 (1971).
5. T. DOUSA and O. HECHTER, *Lancet* **I,** 834 (1970).
6. T. DOUSA and O. HECHTER, *Life Sci.* **9,** 765 (1970).
7. R. MARCUS and G. D. AURBACH, *Biochim. biophys. Acta* **242,** 410 (1971).
8. L. BIRNBAUMER, S. L. POHL and M. RODBELL, *J. biol. Chem.* **244,** 3468 (1969).
9. J. WOLFF, S. C. BERENS and A. B. JONES, *Biochem. biophys. Res. Commun.* **35,** 462 (1969).
10. G. BURKE, *Biochim. biophys. Acta* **220,** 30 (1970).
11. J. FORN and F. G. VALDECASAS, *Biochem. Pharmac.* **20,** 2773 (1971).
12. S. M. WOLFE and N. R. SHULMAN, *Biochem. biophys. Res. Commun.* **35,** 462 (1969).
13. N. R. MARQUIS, R. L. VIGDAHL and P. A. TAVORMINA, *Biochem. biophys. Res. Commun.* **36,** 945 (1969).
14. I. DAVIDSOHN and D. A. NELSON, in *Clinical Diagnosis by Laboratory Methods* (Eds. I. DAVIDSOHN and J. B. HENRY), p. 120. Saunders, Philadelphia (1969).
15. G. KRISHNA, B. WEISS and B. B. BRODIE, *J. Pharmac. exp. Ther.* **163,** 379 (1968).
16. S.-Y. SONG and W. Y. CHEUNG, *Biochim. biophys. Acta* **242,** 593 (1971).
17. J. F. KUO and E. C. DERENZO, *J. biol. Chem.* **224,** 2252 (1969).
18. H. SHIMIZU, C. R. CREVELING and J. W. DALY, *Adv. Biochem. Psychopharmac.* **3,** 135 (1970).
19. H. U. BERGMEYER, *Methods of Enzymatic Analysis*, p. 539. Academic Press, New York (1965).
20. P. J. GARRAHAN and I. M. GLYNN, *J. Physiol., Lond.* **192,** 237 (1967).
21. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
22. G. W. SNEDECOR, *Statistical Methods*, 5th Edn., p. 59. Iowa State College Press, Ames (1956).
23. H. M. WALKER and J. LEV, *Statistical Inference*, p. 196, Holt, Rinehart & Winston, New York (1953).
24. H. L. ALDER and E. B. ROESSLER, *Introduction to Probability and Statistics*, 4th Edn, p. 175. Freeman, San Francisco (1968).
25. G. A. ROBISON, A. ARNOLD and R. C. HARTMANN, *Pharmac. Res. Commun.* **1,** 325 (1969).
26. N. R. MARQUIS, J. A. BECKER and R. L. VIGDAHL, *Biochem. biophys. Res. Commun.* **39,** 783 (1970).
27. J. MOSKOWITZ, J. P. HARWOOD, W. D. REID and G. KRISHNA, *Biochim. biophys. Acta* **230,** 279 (1971).
28. M. J. REINER, *Behavior of Enzymes*, p. 120. Burgess. Minneapolis (1959).

29. E. W. SUTHERLAND and T. W. RALL, *Pharmac. Rev.* **12,** 265 (1960).
30. E. W. SALZMAN, *New Engl. J. Med.* **286,** 358 (1972).
31. J. KLOEZE, in *Proc. Second Nobel Symposium, Stockholm* (Eds. S. BERGSTRÖM and B. SAMUELSSON), p. 241. Interscience, New York (1967).
32. J. F. MUSTARD and M. A. PACKHAM, *Pharmac. Rev.* **22,** 97 (1970).
33. R. E. SCOTT, *Blood* **35,** 514 (1970).
34. P. D. ZIEVE and W. B. GREENOUGH, *Biochem. biophys. Res. Commun.* **35,** 462 (1969).
35. J. R. A. MITCHELL and A. A. SHARP, *Br. J. Haemat.* **10,** 78 (1964).
36. J. R. O'BRIEN, *J. clin. Path.* **17,** 275 (1964).
37. E. W. SALZMAN and L. L. NERI, *Nature, Lond.* **224,** 601 (1969).
38. R. V. LEE, L. M. JAMPOL and W. V. BROWN, *New Engl. J. Med.* **284,** 93 (1971).
39. T. A. RAMSEY, J. MENDELS, J. W. STOKES and R. G. FITZGERALD, *J. Am. med. Ass.* **219,** 1446 (1972).
40. C. A. HARRIS and F. A. JENNER, *J. Physiol., Lond.* **200,** 59p (1969).
41. I. SINGER, D. ROTENBERG and J. B. PUSCHETT, *J. clin. Invest.* **51,** 1081 (1972).
42. M. SCHOU, A. AMIDSEN, S. E. JENSEN and T. OLSEN, *Br. med. J.* **3,** 710 (1968).
43. G. SEDVALL, B. JÖNSSON and U. PETTERSON, *Acta psychiat. scand.* **207,** 59 (1969).
44. G. N. BURROW, W. R. BURKE, J. M. HIMMELHOCH, R. P. SPENCER and J. M. HERSHMAN, *J. clin. Endocr. Metab.* **32,** 647 (1971).
45. R. TEMPLE, M. BERMAN, J. ROBBINS and J. WOLFF, *J. clin. Invest.* **51,** 2746 (1972).
46. S. C. BERENS, R. S. BERNSTEIN, J. ROBBINS and J. WOLFF, *J. clin. Invest.* **49,** 1357 (1970).
47. J. A. WILLIAMS, S. C. BERENS and J. WOLFF, *Endocrinology* **88,** 1385 (1971).
48. R. L. VIGDAHL, W. R. MARQUIS and P. A. TAVORMINA, *Biochem. biophys. Res. Commun.* **37,** 409 (1969).
49. L. DEMEIS, *J. biol. Chem.* **244,** 3733 (1969).
50. R. I. KATZ, T. N. CHASE and I. J. KOPIN, *J. Neurochem.* **16,** 961 (1969).
51. M. HIRATA and O. HAYAISHI, *Biochim. biophys. Acta* **149,** 1 (1967).
52. G. I. DRUMMOND and L. DUNCAN, *J. biol. Chem.* **245,** 976 (1970).

Note added in proof—Since this report was accepted for publication, Murphy *et al.* (*Clin. Pharmac.* **14,** 810. 1973) reported that PGE_1-induced stimulation of [^{3}H]cyclic AMP was significantly less than normal in platelets of patients who were treated with Li.