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Inhibition of Aggregation and Stimulation of Cyclic AMP Generation in Intact Human Platelets by the Diterpene Forskolin

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SUMMARY

The diterpene forskolin, a general activator of adenylate cyclase, is a potent inhibitor of aggregation of human platelets. Complete inhibition of aggregation in platelet-rich plasma occurs at 10–20 μM forskolin. The IC_{50} values for forskolin versus ADP-induced and arachidonate-induced aggregation are 6 μM and 0.6 μM, respectively. Prostaglandins (PG) which activate platelet adenylate cyclase via specific receptors are also more potent versus arachidonate-induced aggregation. Forskolin markedly augments the ability of the prostaglandins PGI_2, PGD_2, and PGE_1 to inhibit platelet aggregation. Forskolin (ED_{50} 4–5 μM) elevates ^{3}H-labeled cyclic AMP levels in [^{3}H]adenine-labeled washed platelets by 30- to 40-fold. This response is near maximal after 2 min and is rapidly reversible. The presence of PGI_2, PGE_1, or PGD_2 increases both the efficacy and the potency of forskolin as an activator of cyclic AMP-generating systems. Conversely, a low concentration of forskolin (0.8 μM) markedly increases both the efficacy and the potency of PGI_2, PGE_1, and PGD_2 as activators of cyclic AMP-generating systems. This concentration of forskolin also augments the elevation of cyclic AMP elicited by $PGF_{1\alpha}$, 6-keto-$PGF_{1\alpha}$, PGE_2, and 2-chloroadenosine. Isoproterenol has no effect on cyclic AMP in the presence or absence of forskolin, whereas norepinephrine inhibits the forskolin-induced elevation of cyclic AMP. There is an excellent correlation between the effect of PGE_1 or forskolin on cyclic AMP levels and their inhibitory effect on platelet aggregation. The physiological effects of forskolin on human platelets appear to be mediated both by direct stimulation of adenylate cyclase and through a marked enhancement of receptor-mediated stimulation of the enzyme.

INTRODUCTION

Forskolin, a diterpene isolated from the roots of *Coleus forskohlii* (1), directly activates adenylate cyclase in membrane preparations and in intact cells from a variety of tissues (2–4). In addition, forskolin has been demonstrated to augment receptor-mediated increases in cyclic AMP (3, 5). Forskolin augments contractile force in guinea pig atria concomitant with activation of adenylate cyclase and cyclic AMP-dependent protein kinase (2). The physiological and biochemical responses of a single cell type to forskolin have now been correlated using human platelets.

The physiological response in platelets, which has appeared to be linked to activation of cyclic AMP systems, is inhibition of aggregation. Thus, platelet aggregation caused by a wide variety of agents can be inhibited in a concentration-dependent manner by PG[4]: PGD_2, PGE_1, and PGI_2 (6). Inhibition of aggregation by prostaglandins is mediated through specific membrane receptors (7, 8) which stimulate the platelet adenylate cyclase (9, 10). Forskolin, like PGI_2, PGE_1, and PGD_2, causes a concentration-dependent inhibition of platelet aggregation which correlates with its ability to stimulate cyclic AMP production in this system. Forskolin significantly augments the response of platelets to these prostaglandins both with respect to cyclic AMP generation and inhibition of platelet aggregation. PGs, in turn, augment the responses of platelets to forskolin.

MATERIALS AND METHODS

Materials. Prostaglandins were a gift from Dr. John Pike, of The Upjohn Company (Kalamazoo, Mich.) and were kept (with the exception of PGI_2) as 10 mM stock solutions in ethanol at −20°. PGI_2 was kept as a 10 mM stock solution in 50 mM Tris-HCl (pH 9.3) at −20°.

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[4] The abbreviation used is: PG, prostaglandin.

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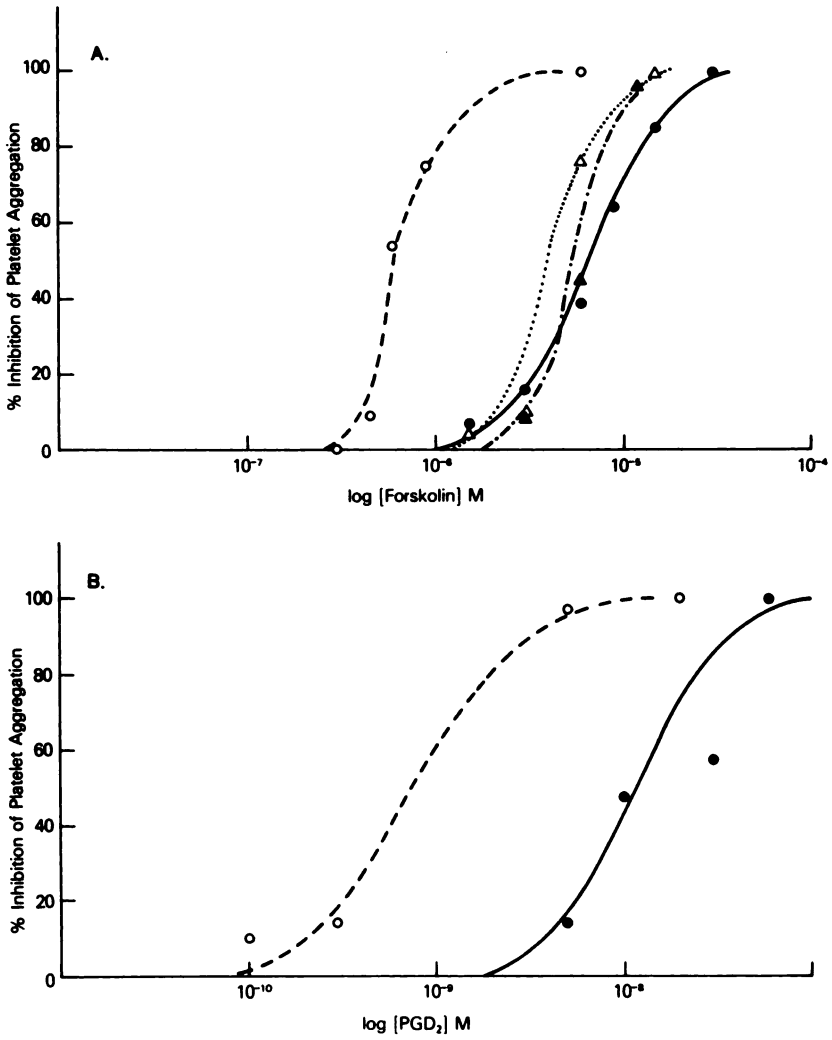

FIG. 1. *Forskolin-induced (A) and PGD_2-induced (B) inhibition of platelet aggregation*

Aggregation was determined as described under Materials and Methods. Forskolin or PGD_2 was added 30 sec before the aggregating agent. Results were calculated as the percentage inhibition of full aggregation using the maximal extent of aggregation for each curve. Aggregations were induced by 1 mM arachidonic acid (○- - -○), 5 μM ADP (●——●), 20 μM epinephrine (△- - - △), and collagen (5 μg/ml) (▲···▲). Data shown are from a single representative experiment (one of three). Similar results were obtained in the two other experiments, each experiment with a different batch of platelets.

Forskolin (7β-acetoxy-8,13-epoxy-1α-6β-9α-trihydroxy-labd-14-en-11-one, mol wt 410) was generously provided by Hoechst Pharmaceuticals Ltd. (Bombay, India). Forskolin is now available from Calbiochem-Behring Corp. (La Jolla, Calif.). Forskolin (10 mM) was dissolved in 95% ethanol and was stable for at least 6 months in solution as determined by bioassay of effects on cyclic AMP-generating systems. [2,8-^{3}H]Adenine (16 Ci/mmole) was obtained from New England Nuclear Corporation (Boston, Mass.). All other compounds were obtained from standard commercial sources.

Platelet aggregations. Blood from healthy drug-free volunteers was drawn into 0.1 volume of 3.8% sodium citrate and centrifuged at 180 × *g* for 15 min to obtain platelet-rich plasma. Aggregations were performed within 3 hr of venipuncture with a Chronolog aggregometer (Chronolog Inc., Havertown, Pa.), using standard techniques (6). IC_{50} values were obtained by visual inspection of dose-response curves.

Platelet cyclic AMP. Fresh platelet concentrates, treated with anticoagulant citrate dextrose solution, were obtained from the National Institutes of Health Blood Bank and were prepared as follows: Platelet concentrates (25–50 ml in plasma) were chilled on ice for 10 min before the addition of 0.05 volume of 0.1 M EDTA (pH 7.4). This suspension was then centrifuged at 1475 × *g* for 15 min at 4° in a precooled Sorvall centrifuge. The pellet was then resuspended in 15 ml of ice-cold buffer [15 mM Tris-HCl, 134 mM NaCl, 5 mM glucose, and 1 mM EDTA (pH 7.4)]. To remove contaminating red cells, this suspension was centrifuged at 100 × *g* for 9 min and the supernatant (containing primarily platelets) was decanted; the pellet was resuspended in an additional 15 ml of buffer and centrifuged, and the supernatant was decanted. To obtain the final platelet suspension, the combined supernatants (30 ml) were centrifuged at 1000 × *g* for 10 min and resuspended in 20 ml of buffer. This suspension was labeled with 150 μCi [^{3}H]adenine (specific activity 0.63 Ci/mmole) for 40 min at 37° in a shaker bath. The labeling procedure was terminated by centrifuging the suspension at 1000 × *g* for 10 min, washing with 30 ml of ice-cold buffer, and centrifuging. The platelet pellet was then resuspended in 12–24 ml of buffer and kept on ice until used.

To measure changes in platelet cyclic AMP levels, 0.2 ml of the platelet suspension was added to Eppendorf Microfuge tubes (1.5 ml) and allowed to equilibrate at 37° for 5 min. The stimulant was added in 50 μl of buffer solution and the incubation was continued for 5 min with mild shaking. The incubation was terminated by the addition of 0.5 ml of 10% trichloroacetic acid. An additional 0.25 ml of a 1.5 mM solution of cyclic AMP was added to monitor sample recovery. Platelet ^{3}H-labeled cyclic AMP was isolated by the method of Salomon *et al.* (11). Data were calculated as percentage conversions, i.e., the percentage of total radioactive adenine taken up by the platelets that was converted to radioactive cyclic AMP. For most experiments these data have been expressed as multiples of basal cyclic AMP percentage conversion. The range of basal conversions in these experiments was 0.025–0.05%. This prelabeling technique has afforded results which are consistent with data based on measurements of endogenous cyclic AMP by radioimmunoassay (12, 13). EC_{50} values were obtained by calculator-assisted linear regression analysis of double-reciprocal plots of the data.

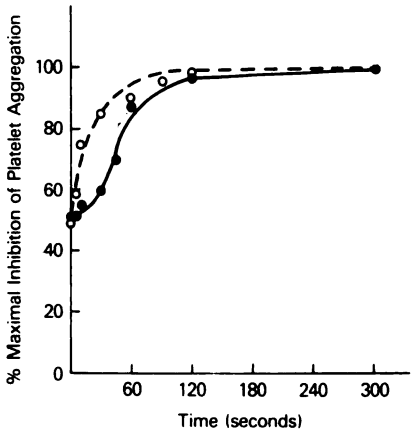

FIG. 2. *Time course for onset of inhibition of ADP-induced aggregation*

Aggregation was induced by 5 μM ADP as described under Materials and Methods. Either 9 μM forskolin (●——●) or 9 nM PGI_2 (○- - -○) was added either simultaneously with ADP (zero time point) or 5–300 sec before the addition of ADP. The 100% maximal inhibition was set equal to the degree of inhibition after 300 sec. Data shown are from a single representative experiment (one of three).

RESULTS

Inhibition of platelet aggregation. Forskolin elicits a concentration-dependent inhibition of aggregation

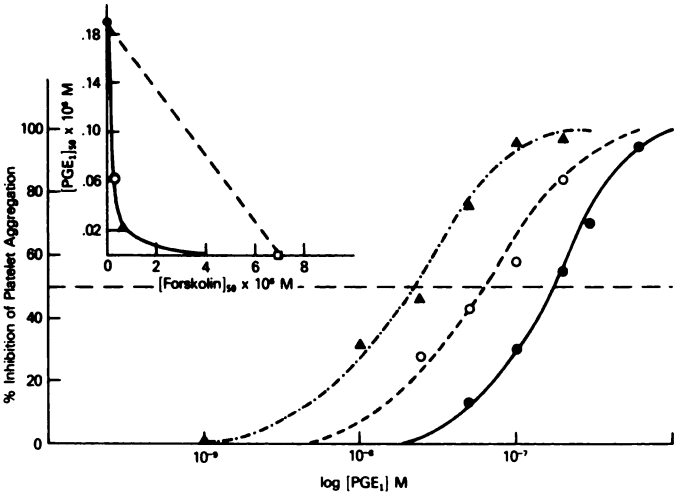

FIG. 3. *Effects of forskolin on inhibition of aggregation by PGE_1*

Aggregations were performed as described under Materials and Methods. PGE_1 or forskolin + PGE_1 were added 30 sec before the addition of 5 μM ADP. ●——●, PGE_1; ○- - -○, PGE_1 plus 0.3 μM forskolin; ▲··▲, PGE_1 plus 0.6 μM forskolin. Data shown are from a single representative experiment (one of two). *Inset*, Concentrations of PGE_1 and forskolin required to produce a 50% inhibition of platelet aggregation are plotted. The value for forskolin alone is obtained from ED_{50} for forskolin inhibition of ADP-induced aggregation shown in Fig. 1. The *broken line* represents plot if effects were additive.

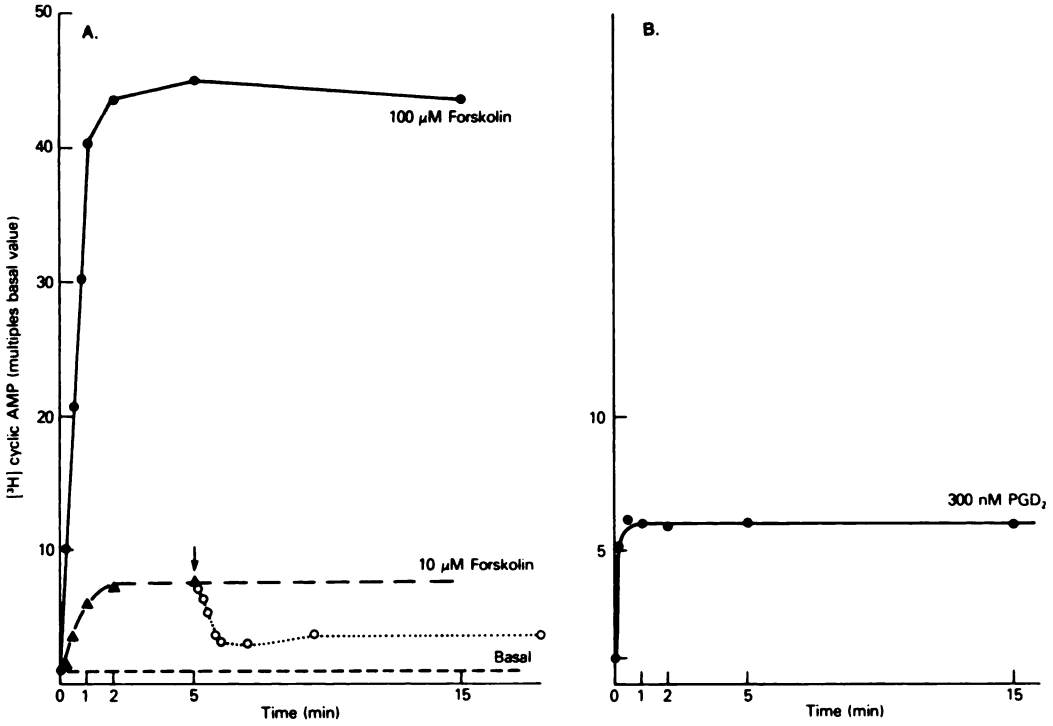

FIG. 4. *Time course for elevation of platelet cyclic AMP by forskolin (A) and PGD_2 (B)*

Platelets were washed and labeled with [^{3}H]adenine as described under Materials and Methods.

A. Samples of the final platelet suspension (0.2 ml) were equilibrated for 5 min at 37° before the addition of either 100 μM forskolin (●——●) or 10 μM forskolin (▲- - -▲) (final concentration). Incubations were terminated after the given time by the addition of 0.5 ml of 10% trichloroacetic acid. To determine the rate of washout of the effect of 10 μM forskolin (O····O), 0.1 ml of a concentrated platelet suspension was equilibrated and then incubated with 10 μM forskolin for an additional 5 min. At the end of that period 0.9 ml of buffer was added. The washout period was terminated by the addition of 0.25 ml of 20% trichloroacetic acid. Each data point represents the mean of two experiments performed in triplicate.

B. Experiments were performed as described above except for the addition of 300 nM PGD_2. Each point is the mean of two experiments performed in triplicate.

caused by a wide variety of agents. The IC_{50} for inhibition of full aggregation caused by ADP, epinephrine, and collagen was approximately 6 μM whereas the IC_{50} for inhibition of arachidonic acid-induced aggregation was only 0.6 μM (Fig. 1A). However, this shift was not specific for forskolin, since PGD_2-induced inhibition of aggregation showed the same pattern (Fig. 1B). The rate of onset of forskolin action was rapid; 50% of the maximal inhibition was manifest when forskolin and ADP were added simultaneously. This was nearly as rapid as the effect of PGI_2 (Fig. 2).

Unlike PGI_2, PGE_1, and PGD_2, whose effects on platelet aggregation are additive (6), forskolin significantly augmented the inhibition of aggregation due to each of these prostaglandins. For example, 0.6 μM forskolin, a concentration which causes only a small inhibition of ADP-induced platelet aggregation, shifted the dose-response curve for PGE_1 approximately 7-fold (Fig. 3). When an isobar was drawn across Fig. 3 at the 50% level and the concentrations of forskolin and PGE_1 required to produce this effect were plotted against one another (Fig. 3, *inset*), a hyperbola was formed. This indicates an intensely synergistic relationship between forskolin and PGE_1, since an additive relationship would yield a straight line (see Fig. 3, *inset*). Similar results were seen when the experiment was repeated with either PGD_2 or PGI_2 and forskolin (data not shown).

Stimulation of platelet cyclic AMP levels. The time-dependent increase in platelet cyclic AMP due to a large (100 μM) dose of forskolin is shown in Fig. 4A. The rate of onset of forskolin action was rapid, being essentially complete within 2 min, and was sustained, with cyclic AMP remaining at approximately maximal levels for the next 13 min. However, it was not quite as rapid as PGD_2-stimulated responses, which were maximal within 30 sec (Fig. 4B). The time course for activation of adenylate cyclase by forskolin correlated well with the onset of inhibition of platelet aggregation. The effects of forskolin on cyclic AMP were readily reversible. Platelets preincubated with 10 μM forskolin and then diluted 10-fold showed a rapid decrease in the level of platelet cyclic AMP which was complete within 60 sec (Fig. 4A).

The dose-response curves for forskolin stimulation of platelet cyclic AMP-generating systems in both buffer and plasma-buffer could be fitted to a simple sigmoidal plot (Fig. 5) using double-reciprocal analysis of the data. The maximal response was reflected by increases of 34-fold and 33-fold over basal for platelets in buffer alone

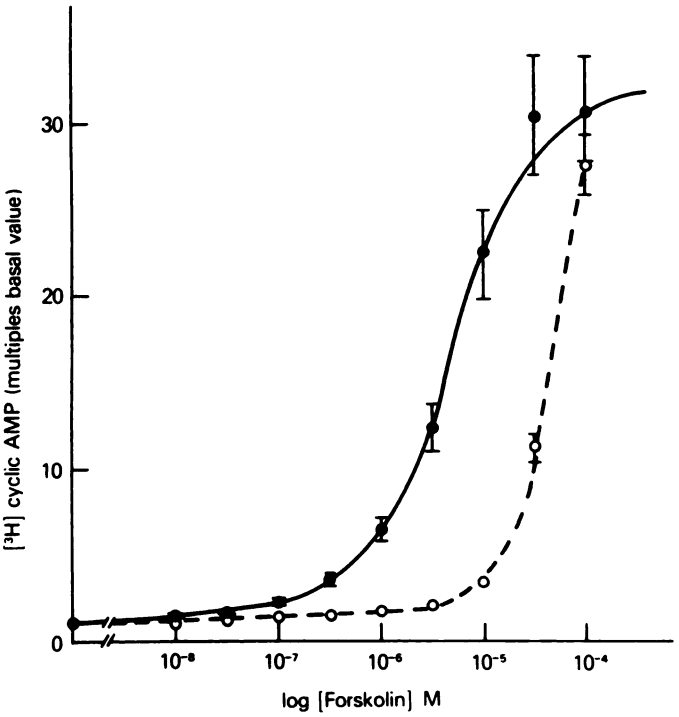

FIG. 5. *Forskolin-induced elevation of platelet cyclic AMP*
Prelabeled platelets were prepared and equilibrated as described under Materials and Methods. The incubation period was 5 min. ●——●, Stimulation of platelets in buffer. Each point represents the mean ± standard error of the mean of five experiments performed in triplicate. ○- - -○, Stimulation of platelets in plasma-buffer (1:2). Each point represents the mean ± standard error of the mean of three experiments performed in triplicate.

and for platelets in plasma-buffer, respectively. The ED_{50} values for forskolin as determined by double-reciprocal analysis of the data were 4.1×10^{-6} M and 1.9×10^{-5} M for platelets in buffer and for platelets in plasma-buffer, respectively.

Prostaglandins greatly augmented the accumulation of cyclic AMP elicited by forskolin. Thus, incubation of platelets with 300 nM PGI_2, PGD_2, or PGE_1 concurrently with forskolin produced a 5.2-fold increase in the maximal response for forskolin with PGD_2, a 6.6-fold increase with PGE_1, and a 12.7-fold increase with PGI_2 (in plasma) (Fig. 6). In addition, prostaglandins caused a left-shift in the dose-response curve for forskolin as summarized in Table 1.

Low concentrations of forskolin (0.8 μM) markedly augmented the response of platelet cyclic AMP-generating systems to PGI_2, PGE_1, and PGD_2. This concentration of forskolin, which produced only a 2- to 4-fold stimulation in basal cyclic AMP alone, produced a dramatic 10-fold increase in the response to saturating concentrations of prostaglandins (Fig. 7). In addition, as summarized in Table 2, forskolin caused a 2- to 3-fold increase in the apparent potency of these prostaglandins.

A number of compounds were incubated with platelets in the presence of 0, 0.8 μM, and 30 μM forskolin and the increases in platelet ^3H-labeled cyclic AMP were determined (Table 3). The response to saturating concentrations of PGE_1 and PGD_2 was augmented markedly in the presence of 0.8 μM forskolin. Forskolin at 30 μM caused a further augmentation of the prostaglandin response. In the case of PGE_2, $PGF_{1\alpha}$, and 6-keto-$PGF_{1\alpha}$, where these prostaglandins were not themselves present in a maximal stimulating concentration, incubation in the presence of 30 μM forskolin caused a 4- to 5-fold increase over the response in the presence of 0.8 μM forskolin. Forskolin at 30 μM also caused a further 5-fold increase in the response to 2-chloroadenosine over that in the presence of 0.8 μM forskolin. Forskolin did not reveal a stimulatory response to isoproterenol or norepinephrine. Indeed, norepinephrine caused approximately a 50% inhibition of the response to forskolin (Table 3). Inhibition of the forskolin response also occurred with 6-fluoronorepinephrine (data not shown), a relatively specific alpha$_2$-adrenergic agonist (14, 15).

DISCUSSION

Forskolin, a diterpene from a genus of plants used in Aurvedic medicine for heart disease, spasmodic pain, painful micturation, and convulsions (16), has proven to be a potent inhibitor of human platelet aggregation caused by a wide variety of endogenous compounds (Fig. 1). The greater potency of forskolin against arachidonic acid-induced aggregation as compared with aggregation induced by ADP and other agents, a pattern which is also seen with PGD_2, is most likely a reflection of effects at different stages of platelet aggregation in this system. In citrated platelet-rich plasma, aggregation induced by ADP consists of two stages. The first of these involves a reversible aggregation with no granule release or prostaglandin synthesis. The second stage is irreversible and appears to require prostaglandin synthesis for the release of the contents of the dense granules (17). It has been shown that prostaglandin synthesis is very sensitive to levels of platelet cyclic AMP (18). It is not surprising, therefore, that aggregation induced by arachidonic acid, which requires conversion to the prostaglandin endoperoxides for its effects (19), should be inhibited more easily than ADP-induced aggregation by forskolin and PGD_2, both of which are compounds known to increase cyclic AMP levels in this system (Figs. 6 and 7).

The onset of the effects of forskolin on platelet aggregation is nearly as rapid as that of the effects of PGI_2 (Fig. 2). Again, the immediate 50% inhibition of ADP-induced aggregation seen with both PGI_2 and forskolin is probably due to inhibition of the second, arachidonic acid-dependent, stage of aggregation. The time course for the onset of inhibition agrees well with the time course for increases in platelet cyclic AMP levels (Figs. 2 and 4). The reversibility of the effect of forskolin on platelet cyclic AMP levels (Fig. 4) agrees well with the reversibility of its effects in other systems (3).

Forskolin causes a 30- to 40-fold elevation of cyclic AMP in human platelets (Fig. 5). The dose-response curve for forskolin is sigmoidal, indicative of a single site of action. The markedly lower potency of forskolin in the presence of plasma-buffer as compared with buffer alone (Fig. 5) is most likely due to the binding of forskolin to plasma proteins but could also reflect the presence of an

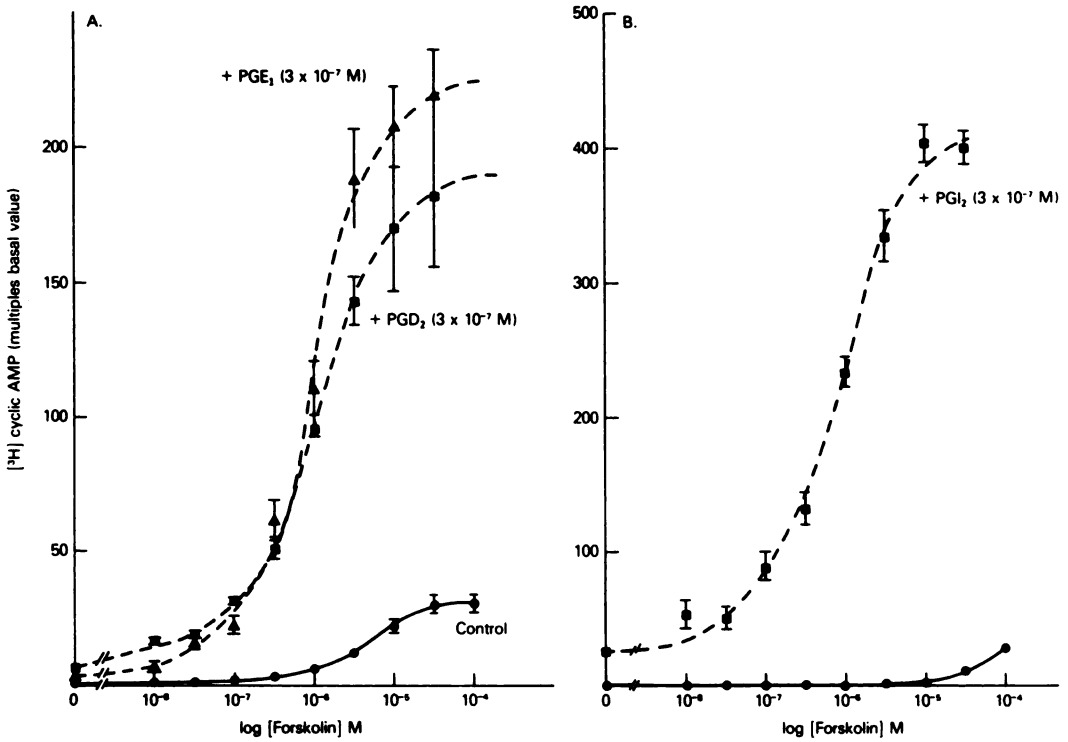

FIG. 6. *Effect of prostaglandins on forskolin-induced elevation of platelet cyclic AMP*

A. Platelets suspended in buffer. Experiments were performed as described under Materials and Methods. The stimulation period was 5 min for both forskolin and forskolin plus PG. ●——●, Forskolin ($N = 5$); ■- - -■, forskolin plus 300 nM PGD_2 ($N = 5$); ▲- - -▲, forskolin plus 300 nM PGE_1 ($N = 4$). Each point represents the mean ± standard error of the mean of N experiments performed in triplicate.

B. Platelets suspended in plasma-buffer (1:2). ●——●, Forskolin; ■- - -■, forskolin plus 300 nM PGI_2. Each point represents the mean ± standard error of the mean of three experiments performed in triplicate.

endogenous inhibitor of forskolin action. In other systems, all of which were assayed in buffer-media, the potency of forskolin for activation of cyclic AMP generation has been in the 5–10 μM (ED_{50} values) range (2–5), comparable to its potency with platelets in the absence of plasma.

TABLE 1

Effects of prostaglandins on stimulation of platelet cyclic AMP-generating systems for forskolin

Experiments were performed as described under Materials and Methods. Each number represents the mean ± standard error of the mean of N experiments performed in triplicate. ED_{50} and E_{max} values were obtained from the intercepts of double-reciprocal analysis of individual dose-response curves summarized in Fig. 6. Statistical differences from forskolin controls were determined by a two-tailed Student's t-test analysis.

Suspension medium and stimulus	^{3}H-labeled cyclic AMP		
	Forskolin ED_{50} (μM)	E_{max} (multiples of basal values)	N
Buffer			
Forskolin	4.4 ± 0.96	34.2 ± 3.4	5
Forskokin + 300 nM PGD_2	0.85 ± 0.09[a]	178 ± 12.40[a]	3
Forskolin + 300 nM PGE_1	1.13 ± 0.06[a]	226 ± 20.2[a]	4
Plasma-buffer			
Forskolin	19.05 ± 2.59	33.10 ± 1.60	3
Forskolin + 300 nM PGI_2	0.90 ± 0.19[a]	423 ± 9.7[a]	

[a] $p < 0.001$ (difference from appropriate control).

The most remarkable aspect of the interaction of forskolin with platelets is the augmentation of both the forskolin response and the prostaglandin response when these two types of compounds are combined (Tables 1, 2, and 3). This is manifest both with respect to inhibition of platelet aggregation (*vide infra*) and with respect to activation of cyclic AMP systems, i.e., adenylate cyclase. PGI_2, PGE_1, and PGD_2, at concentrations approximately equivalent to the K_D values for these compounds for activation of cyclic AMP-generation, caused not only a 4- to 5-fold increase in the maximal response to forskolin but also caused a significant shift in the ED_{50} for forskolin (Table 1). In other words, it appears that a receptor-mediated activation of adenylate cyclase enhances the ability of forskolin to affect the system. Conversely, low concentrations of forskolin (0.8 μM) caused a 10-fold increase in the maximal response to each of the prostaglandins (Fig. 7) and a statistically significant, 3-fold decrease in the ED_{50} for these prostaglandins (Table 2). These effects may be the result of an enhanced receptor-adenylate cyclase coupling induced by forskolin. Augmentation of hormonal activation by forskolin has also been seen in brain slices, where a low concentration of forskolin augments the accumulation of cyclic AMP elicited by histamine, isoproterenol, norepinephrine, adenosine, PGE_2, and vasoactive intestinal peptide (3, 5). In some cases, the main effect of forskolin was to increase the maximal response to the hormone while in other

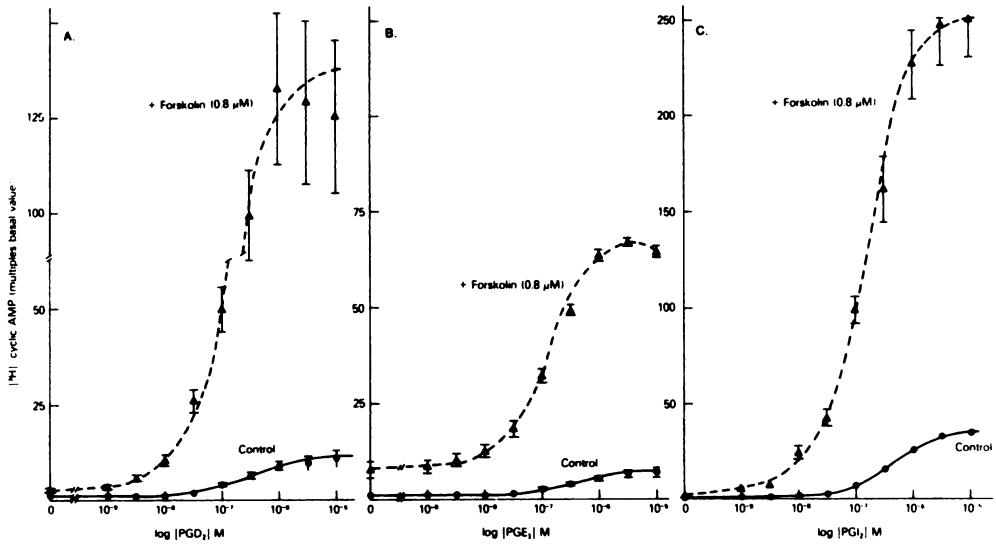

FIG. 7. *Effect of forskolin on prostaglandin-induced elevation of platelet cyclic AMP*
Experiments were performed as described under Materials and Methods.

A. Platelets in buffer stimulated for 5 min by either PGD_2 alone (●——●) or by PGD_2 in the presence of 0.8 μM forskolin (▲- - -▲). Each point represents the mean ± standard error of the mean of four experiments performed in triplicate.

B. Platelets in buffer stimulated for 5 min by either PGE_1 alone (●——●) or by PGE_1 in the presence of 0.8 μM forskolin (▲- - -▲). Each point represents the mean ± standard error of the mean of three experiments performed in triplicate.

C. Platelets in plasma-buffer (1:2) stimulated for 5 min by either PGI_2 alone (●——●) or by PGI_2 in the presence of 0.8 μM forskolin (▲- - -▲). Each point represents the mean ± standard error of the mean of three experiments performed in triplicate.

cases the main effect was to increase the apparent potency. The enhancement of receptor-mediated responses with forskolin in the platelet system appears to be much larger than that seen in brain slices. The augmentation in platelets appears to require the presence of stimulatory receptors coupled to adenylate cyclase; i.e., forskolin enhances responses to prostaglandins and 2-chloroadenosine, which have stimulatory receptors linked to cyclase, but does not reveal or potentiate responses to isoproterenol and norepinephrine (Table 3). There are no *beta*-adrenergic receptors on human platelets, and *alpha*-adrenergic receptors on platelets are inhibitory to adenylate cyclase (20). Norepinephrine did cause an inhibition of the response of cyclic AMP-generating systems to both 0.8 μM and 30 μM forskolin. This inhibition was also seen with a selective *alpha*$_2$-adrenergic agonist (14, 15), namely 6-fluoronorepinephrine (data not shown). Thus, although forskolin directly activates adenylate cyclase and enhances hormone receptor-mediated activation of the enzyme, it does not eliminate hormone receptor-mediated inhibition of the enzyme.

An augmentation of prostaglandin responses by forskolin also occurs with respect to platelet aggregation (Fig. 3), as would be expected if forskolin affects aggregation through its action on cyclic AMP generation. Stimulation of cyclic AMP production by forskolin appears to be equivalent to stimulation by PGE_1 with respect to the resulting inhibition of ADP-induced platelet aggregation, since a given increase over basal cyclic AMP levels, whether elicited by PGE_1 or forskolin, causes

TABLE 2

Effect of forskolin on stimulation of platelet cyclic AMP-generating systems by prostaglandins

Experiments were performed as described under Materials and Methods (see legend to Fig. 7). Each value represents the mean ± standard error of the mean of *N* experiments (in parentheses) performed in triplicate. ED_{50} values were obtained by double-reciprocal analysis of individual dose-response curves from each experiment. Statistically significant differences were determined by the use of a two-tailed Student's *t*-test.

Stimulus	Prostaglandin (ED_{50}, μM)		Significance
	Control	+ 0.8 μM Forskolin	
PGD_2	3.97 ± 0.69 (4)	1.16 ± 0.38 (3)	$p < 0.002$
PGE_1	3.23 ± 0.94 (3)	1.21 ± 0.31 (3)	$p < 0.001$
PGI_2	5.51 ± 0.14 (3)	1.76 ± 0.13 (3)	$p < 0.001$

TABLE 3

Effect of forskolin on receptor-mediated responses of platelet cyclic AMP-generating systems

Experiments were performed as described under Materials and Methods using platelets suspended in buffer. Either buffer or forskolin was added concurrently with each stimulant. Each number represents the mean of three experiments performed in triplicate.

Stimulant	Multiple basal cyclic AMP		
	Control	+ 0.8 μM Forskolin	+ 30 μM Forskolin
Basal	1	2.00	33.36
10 μM PGE_1	11.88	175	349
10 μM PGE_2	3.73	78.38	278
10 μM PGD_2	9.17	142	307
10 μM $PGF_{1\alpha}$	2.31	21.36	68.12
10 μM 6-Keto-$PGH_{1\alpha}$	2.26	30.05	154
30 μM Isoproterenol	1.59	2.54	34.65
100 μM Norepinephrine	1.47	1.26	16.84
300 μM 2-Chloroadenosine	2.72	22.34	111

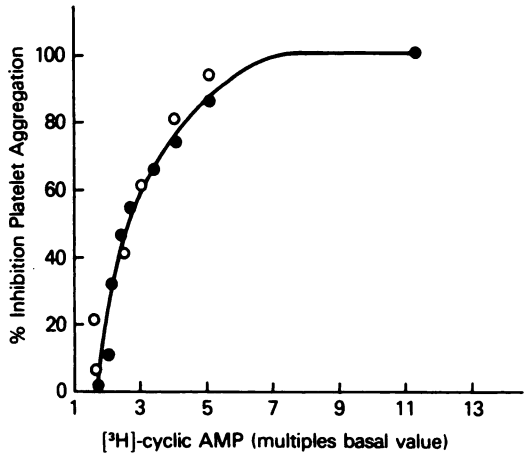

FIG. 8. *Correlation between inhibition of platelet aggregation and stimulation of ^{3}H-labeled cyclic AMP production*

The concentration of forskolin (●) or PGE_1 (○) required to produce each level of inhibition of ADP-induced platelet aggregation were obtained from Fig. 1A (forskolin) and Fig. 3 (PGE_1). The amount of ^{3}H-labeled cyclic AMP induced by these concentrations were then obtained from Fig. 5 (forskolin in plasma) and Fig. 7B (PGE_1). The appropriate values were then plotted against one another.

the same degree of inhibition of platelet aggregation (Fig. 8).

In summary, forskolin is a potent activator of cyclic AMP-generating systems in human platelets. Effects on cyclic AMP levels appear to be the mechanism whereby forskolin inhibits platelet aggregation. Forskolin, because of its ability to activate adenylate cyclase selectively, provides a powerful tool for the investigation of the role of cyclic AMP in physiological functions, such as platelet aggregation. In addition, by potentiating receptor-mediated activation of adenylate cyclase, forskolin may provide a tool for selectively manipulating specific cyclase systems in intact organisms. A case in point would be the selective action of forskolin or forskolin derivatives in combination with a low concentration of a stable PGI_2 analogue as a combination antithrombotic, vasodilator, and cardiotonic agent. Forskolin has been recognized as a potentially useful cardiotonic and hypotensive agent (21; see also ref. 16).

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