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INTERRELATION OF PROSTAGLANDIN ENDOPEROXIDE (PROSTAGLANDIN G_2) AND CYCLIC 3′,5′-ADENOSINE MONOPHOSPHATE IN HUMAN BLOOD PLATELETS

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Summary

The prostaglandin endoperoxide, prostaglandin G_2, in platelet-rich plasma may produce reversible platelet aggregation without secretion, irreversible aggregation with secretion of platelet constituents inhibited by indomethacin, or the latter effects despite indomethacin, depending on the concentration of the endoperoxide. Irreversible aggregation and platelet secretion induced by prostaglandin G_2 apparently result from the action of ADP, since these responses are inhibited by 2-*n*-amylthio-5′-AMP (an inhibitor of the actions of ADP on platelets) and they do not occur in heparinized platelet-rich plasma. Prostaglandin G_2 lowers the platelet level of cyclic 3′,5′-AMP. Its actions are inhibited by elevation of cyclic AMP levels by prostaglandin E_1 or dibutyryl cyclic AMP or adenosine. Like malondialdehyde production induced by thrombin, ADP, or arachidonic acid, prostaglandin G_2-induced malondialdehyde production is reduced by dibutyryl cyclic AMP and prostaglandin E_1. Platelet activation by prostaglandin G_2 is enhanced by the adenylate cyclase inhibitor, 9-(tetrahydro-2-furyl)-adenine.

The action of prostaglandin G_2 on platelets is more complex then previously reported.

Introduction

Secretion of the contents of intracellular platelet granules, termed the "release reaction", and platelet aggregation in response to many stimuli are inhibited by aspirin and other non-steroidal anti-inflammatory drugs. It is known that these drugs are potent antagonists of the enzyme cyclooxygenase, which in the pletelet converts arachidonic acid to the labile prostaglandin endoperoxides, prostaglandins G_2 and H_2 [1,2]. Addition of the endoperoxides to platelet suspensions is followed by the release of platelet constituents and by

aggregation [2]. For these reasons and because of much supporting collateral evidence, summarized in several recent reviews [3—5], it is now widely held that the biologically active endoperoxides and their product, thromboxane A_2, act as "second messengers" in the platelet and that their formation is common to the activation of platelets by a large number of different stimuli.

There is evidence that alterations in the platelet concentration of cyclic 3′, 5′-adenosine monophosphate (cyclic AMP) may also modulate platelet functions and that such effects may be related to prostaglandins and their precursors. Thus, prostaglandins E_1 and D_2, which inhibit platelet activities, elevate platelet cyclic AMP by stimulation of adenylate cyclase [6—8], whereas prostaglandin E_2, which at high concentrations has an action similar to prostaglandin E_1 and prostaglandin D_2, at low concentrations augments platelet function and has an opposite effect on cyclic AMP [9—11]. A decrease in platelet cyclic AMP has been observed in response to ADP, epinephrine, collagen, thrombin, and other agents that induce platelet aggregation [11—13]. This decrease is blocked by aspirin and indomethacin [14], which suggests that it results from the action of prostaglandins and related substances. Reduction of cyclic AMP in platelet-rich plasma has also been reported to accompany aggregation of platelets by arachidonic acid [15]. "LASS" or "labile aggregation stimulatory substance", a product of the incubation of lysed platelets with arachidonic acid, which is thought to consist of a mixture of endoperoxides and thromboxane A_2 [16], has been shown to reduce cyclic AMP in platelet-rich plasma [17]. The cyclic AMP changes follow the same time course as platelet aggregation.

Conversely, it is also apparent that alterations in platelet cyclic AMP levels may influence prostaglandins and related compounds. There is evidence [19, 20] that increased platelet cyclic AMP inhibits the production of prostaglandin endoperoxides and thromboxanes after stimulation of platelets by collagen, a result attributable to inhibition of platelet cyclooxygenase. Malmsten and coworkers [19] reported that cyclic AMP did not inhibit the actions of the endoperoxides themselves or other aspects of platelet behavior and suggested that reduction in cyclooxygenase activity might account for the inhibition of platelet activity by cyclic AMP.

We have reinvestigated this question and have found the actions of the endoperoxide prostaglandin G_2 to be more complicated than previously reported and to be substantially influenced by alterations in the platelet content of cyclic AMP.

Methods and Materials

Platelet-rich plasma was prepared from whole blood anticoagulated with trisodium citrate or heparin by previously described techniques [7]. Blood donors denied the ingestion of any medicines within the week before venipuncture. Platelet aggregation [21] and the release of ^{14}C- or ^{3}H-labelled 5-hydroxytryptamine (serotonin) [22] were measured by published methods, employing a Chronolog aggregometer. Assay of cyclic 3′,5′-adenosine monophosphate was by a modification [23] of Gilman's method [24]. The validity of the technique and its sensitivity were established in a prior report [23].

Malondialdehyde was measured by a modification of the method of Macfarlane et al. [25], detailed in Table II.

Purified endoperoxide prostaglandin G_2 was furnished through the kindness of Dr. Bengt Samuelsson, Karolinska Institute, Stockholm, SQ22536 (9-(tetrahydro-2-furyl)-adenine), through the kindness of Dr. D.N. Harris, Squibb Inst. for Medical Research, Princeton, N.J., 2-*n*-amylthio-5′-adenosine monophosphate through the generosity of Dr. D.E. McIntyre, Department of Pathology, University of Cambridge, England, and stable prostaglandins through the kindness of Dr. J.L. Pike, Upjohn Co., Kalamazoo, Mich.

Other reagents employed were enzyme grade and were obtained from commercial sources.

Results

Addition to citrated platelet-rich plasma of prostaglandin G_2 was followed by platelet shape change and aggregation (Fig. 1). The effective dose varied somewhat in platelet-rich plasma samples from different blood donors, but a characteristic pattern of response to a range of doses was observed. At relatively low concentrations of the endoperoxide (usually less than 0.3 μM) the pattern was characteristic of "primary" or reversible aggregation, resembling that seen with 5′-adenosine diphosphate (ADP) at concentrations insufficient to induce the platelet release reaction. There was no release of [^{14}C]serotonin at these concentrations of prostaglandin G_2. Often at higher prostaglandin G_2

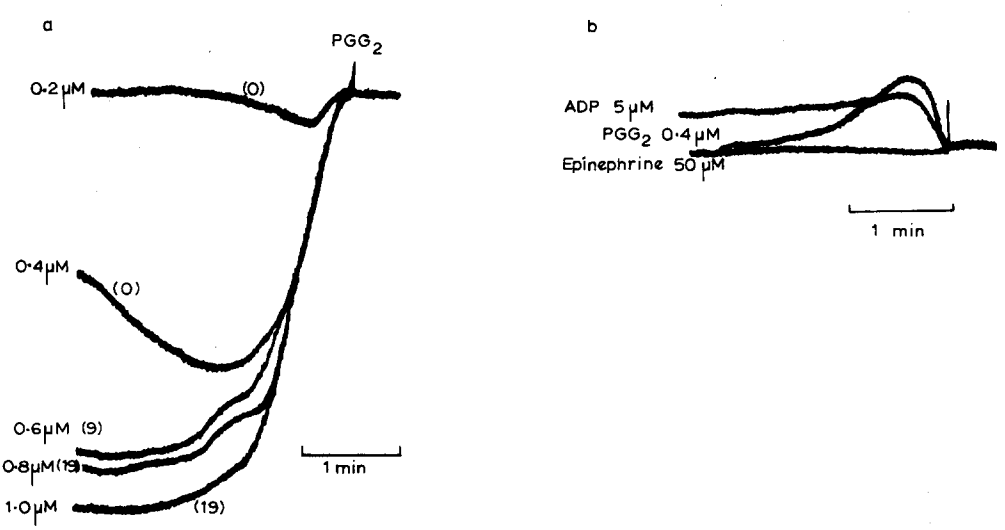

Fig. 1. Platelet aggregation in citrated platelet-rich plasma in response to different concentrations of prostaglandin G_2 (a). Numbers in parentheses in this and subsequent tracings of aggregation curves often refer to release of labelled 5-hydroxytryptamine, expressed as percent of radioactivity (^{3}H or ^{14}C) previously taken up by the platelets. Time of addition of prostaglandin G_2 is indicated by the injection artifact, which obscures the effect on light transmittance of shape change. In Fig. 1b, addition of 7 mM EDTA to block aggregation and expansion of the optical scale [52] allow demonstration of the initial excursion of light transmittance due to shape change. The effects of ADP and epinephrine are shown for comparison.

concentrations, and consistently above 0.6 μM, the pattern resembled that termed "second phase" or irreversible aggregation and was accompanied by release of [^{14}C]serotonin. At concentrations of prostaglandin G_2 below 0.7 μM, the second wave of aggregation and serotonin release could be inhibited with indomethacin (Figs. 2 and 3). The inhibition increased with increasing concentrations of indomethacin but never resulted in inhibition of "primary" aggregation, regardless of how great the concentration of indomethacin (up to 10^{-4} M). Increasing the concentration of prostaglandin G_2 reduced the effect of indomethacin, and beyond 1.0 μM prostaglandin G_2, the inhibitory effect of indomethacin was entirely overcome.

The purine derivative 2-*n*-amylthio-5'-AMP inhibits the action of ADP on platelets [26], and there is evidence that its activity, like that of ATP [27], is highly specific. It blocks both primary and second phase aggregation and release induced by ADP and the release reaction and irreversible aggregation induced by collagen or epinephrine but has no effect on primary aggregation induced by epinephrine, serotonin, or vasopressin. Unlike adenosine and 5'-AMP [15], the action of 2-*n*-amylthio-5'-AMP is not blocked by the adenylate cyclase inhibitor SQ22536 (McIntyre, D.E. Gordon, J.L. and Salzman, E.W., unpublished) (see below). The effects of 2-*n*-amylthio-5'-AMP on prostaglandin G_2-induced platelet clumping and secretion are shown in Fig. 4. There is marked inhibition of platelet secretion and the second phase of platelet aggregation, but the primary phase of aggregation is much less affected.

Mustard et al. [28] have reported that the release reaction induced by ADP in citrated platelet-rich plasma is an artifact of the low ionized calcium concentration and have observed that ADP causes platelet aggregation without secretion in heparinized platelet-rich plasma or in a suspension of washed platelets in an artificial medium with physiological calcium concentration. When one adds prostaglandin G_2 to heparinized platelet-rich plasma (Fig. 5), only primary

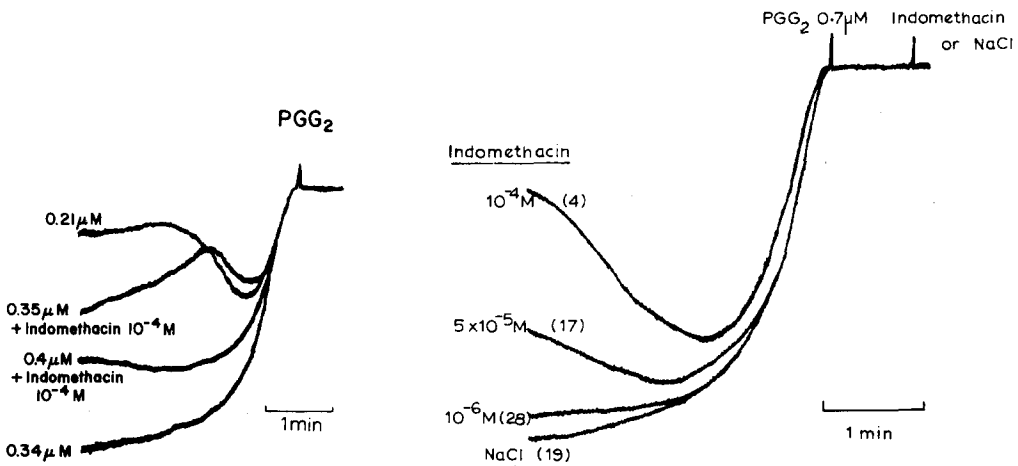

Fig. 2. Effect of indomethacin on platelet aggregation induced by prostaglandin G_2. For description, see text.

Fig. 3. Effect of indomethacin in different concentrations (or saline control) on platelet aggregation and [^{14}C] serotonin release induced by prostaglandin G_2.

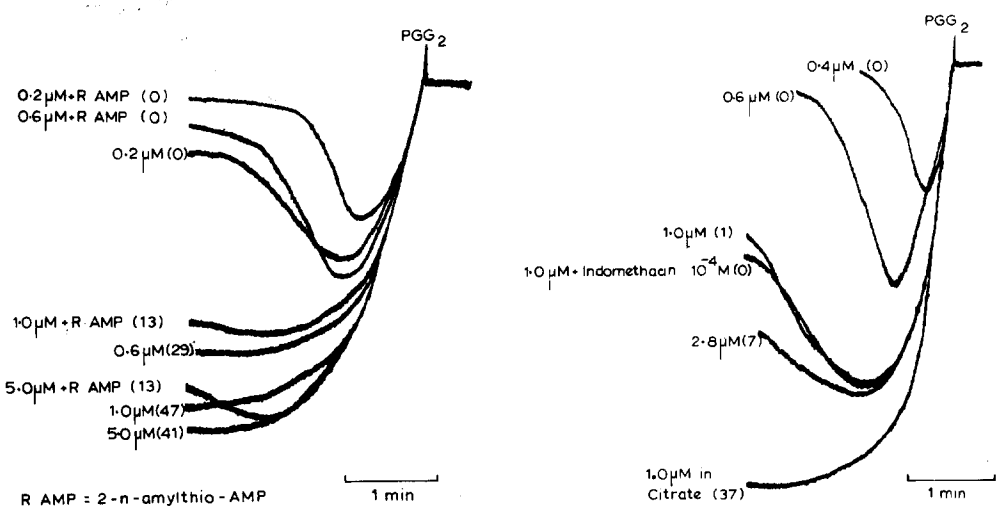

Fig. 4. Effect of 2-*n*-amylthio-5′-AMP on platelet aggregation and secretion of [^{3}H]serotonin induced by various concentrations of prostaglandin G_2.

Fig. 5. Platelet aggregation without release of [^{3}H]serotonin induced by prostaglandin G_2 at different concentrations in platelet-rich plasma anticoagulated with 5 I.U. heparin/ml. The response in citrated platelet-rich plasma is shown for comparison. 10^{-4} M indomethacin has no effect on prostaglandin G_2-induced aggregation in heparinized platelet-rich plasma.

aggregation is seen. There is no release of [^{3}H]serotonin at prostaglandin G_2 concentrations (e.g. 1.0 μM) that in citrated platelet-rich plasma induce substantial release and only minimal release at concentrations (e.g. 2.8 μM) which in citrated platelet-rich plasma are supramaximal. Indomethacin has no effect on aggregation of platelets induced by prostaglandin G_2 in heparinized platelet-rich plasma. These experiments and those described above with 2-*n*-amylthio-5′-AMP suggest that the release of serotonin and the second or irreversible phase of aggregation are indirect effects of prostaglandin G_2 and result from the action of ADP, but the primary phase of platelet aggregation induced by prostaglandin G_2 appears to be a more direct effect.

Addition of prostaglandin G_2 to a platelet suspension was regularly followed by a rapid decrease in the content of cyclic AMP (Table I). In platelet-rich plasma, prostaglandin G_2 produced a variable effect on cyclic AMP. In nine experiments in platelet-rich plasma, prostaglandin G_2 reduced cyclic AMP 6—18%, but in three experiments no fall in cyclic AMP was detected. The reduction in cyclic AMP was not inhibited by indomethacin.

Platelet aggregation and serotonin release by prostaglandin G_2 were inhibited by agents that increase platelet cyclic AMP. Increasing concentrations of such inhibitors first blocked the second wave of aggregation and serotonin release, and at higher concentrations they inhibited primary aggregation as well. Inhibition of platelet aggregation by N^6-2′-1-dibutyryl cyclic AMP is shown in Fig. 6. Fig. 7 illustrates the effects of prostaglandin E_1. In Figs. 8a and 8b, inhibition of platelet aggregation and serotonin release by adenosine and 5′-AMP are shown. Like prostaglandin E_1 [7], adenosine has been reported to stimulate platelet adenylate cyclase and raise the platelet content of cyclic

TABLE I

EFFECT OF PROSTAGLANDIN G_2 ON CYCLIC AMP (pmol/ml platelet suspension)

Citrated platelet-rich plasma was filtered through Sepharose 2B by the method of Tangen et al. [29] as modified by Lindon et al. [30]. To the resultant platelet suspension in Tyrode's buffer was added prostaglandin G_2 in various concentrations, and after incubation at 37°C for 1 min, 1/10 volume of cold 90% trichloroacetic acid was added, and cyclic AMP was measured [23]. Results are mean ± S.D. of quadruplicate samples.

	Prostaglandin G_2 (μm)						
	0	0.05	0.1	0.2	0.4	0.6	0.8
Control	24.2 ± 0.63	19.6 ± 1.7	9.2 ± 0.4	9.7 ± 0.12	9.7 ± 0.27	9.6 ± 0.75	9.7 ± 0.42
Indomethacin (10^{-4} M)	19.1 ± 2.0	14.0 ± 2.0	14.5 ± 1.1	—	—	13.0 ± 0.34	9.7 ± 0.10

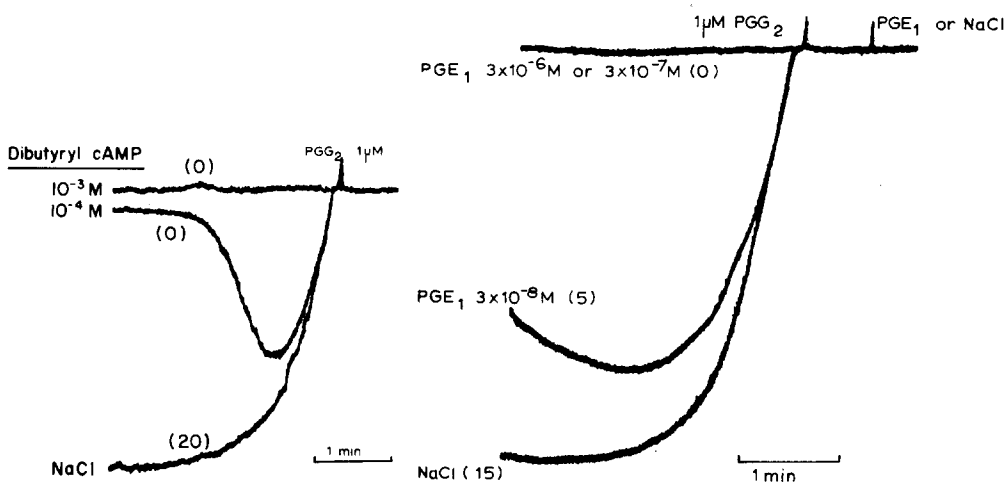

Fig. 6. Effect of dibutyryl cyclic AMP (10 min preincubation, 37°C) on platelet aggregation and [^{14}C] serotonin release induced by prostaglandin G_2.

Fig. 7. Effect of prostaglandin E_1 on platelet aggregation and [^{14}C] serotonin release induced by prostaglandin G_2.

AMP [31]. That its inhibitory effect on platelet aggregation and release results from this property of adenosine is indicated by the ability of the compound SQ22536 (9-(tetrahydro-2-furyl)-adenine), an inhibitor of adenylate cyclase [32], to block the inhibitory effects of adenosine (Fig. 8a). There is evidence that 5'-AMP is converted in plasma to adenosine and that inhibition of platelet activity by 5'-AMP is due to this transformation [33]. The effects of 5'-AMP and prostaglandin E_1 were also blocked by SQ22536.

Malmsten and associates [19] have reported that an increase in platelet cyclic AMP leads to inhibition of thromboxane production by platelets stimulated by collagen. An analogous effect is implied by the observation that malondialdehyde production induced by thrombin, ADP, or arachidonic acid is reduced by prostaglandin E_1 and dibutyryl cyclic AMP, which elevate platelet cyclic AMP levels [7]. In Table II malondialdehyde production is shown following addition of prostaglandin G_2 to platelet-rich plasma. Dibutyryl cyclic AMP and prostaglandin E_1 decreased malondialdehyde production in response to prostaglandin G_2.

There is evidence (Salzman, E.W., McIntyre, D.E., Gordon, J.L., Levine, L. and Smith, M., unpublished) that platelet aggregation and secretion are enhanced by the adenylate cyclase inhibitor 9-(tetrahydro-2-furyl)-adenine (SQ22536) when the initiating stimulus is an agonist whose action on platelets is accompanied by a fall in platelet cyclic AMP, such as ADP, collagen, thrombin, or epinephrine, but not when the agent is one that is not associated with such changes in cyclic AMP concentration (e.g. vasopressin, 5-hydroxytryptamine, or the calcium ionophore Lilly A23187 [17]). Fig. 9 illustrates the effect of SQ22536 on platelet activation by prostaglandin G_2. The effects of prostaglandin G_2 were augmented by prior addition of the adenylate cyclase

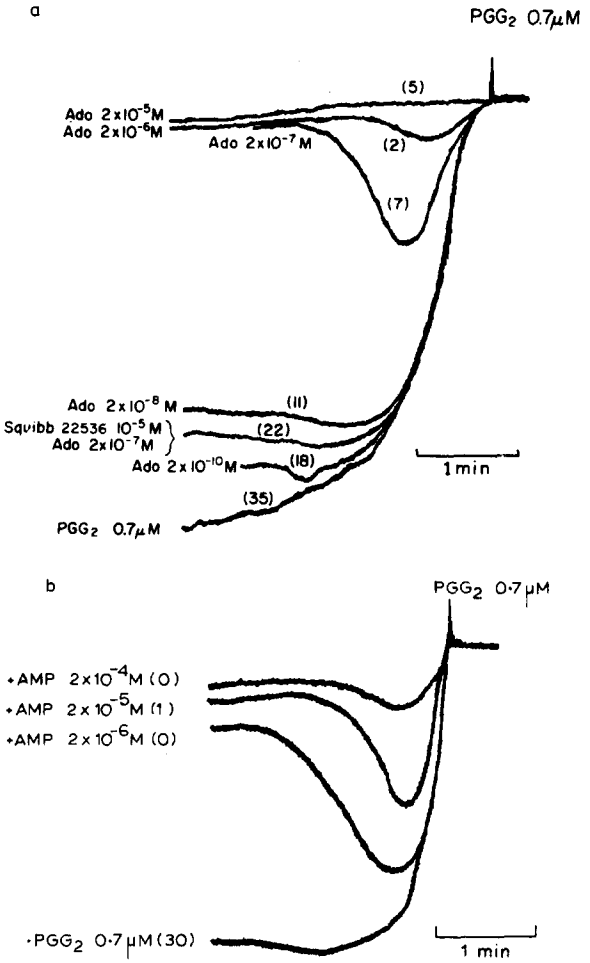

Fig. 8. Effect of adenosine (a) and 5′-AMP (b) on platelet aggregation and release of [^{14}C] serotonin induced by prostaglandin G_2. In the presence of SQ22436, the inhibitory action of adenosine is reduced. Fig. 8a is reprinted from ref. 15, by permission.

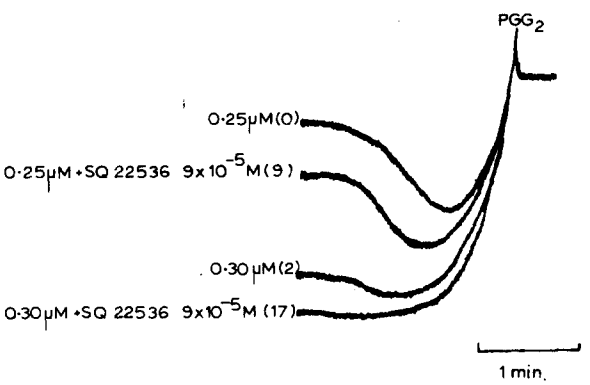

Fig. 9. Effects of SQ22536 added to citrated platelet-rich plasma 1 min before the addition of 0.25 μM prostaglandin G_2. There is enhancement of platelet aggregation and of the release of [^{14}C] serotonin.

TABLE II

MALONDIALDEHYDE PRODUCTION WITH PROSTAGLANDIN G_2; EFFECT OF INCREASED CYCLIC 3',5'-AMP

Citrated platelet-rich plasma or platelet-poor plasma (2 ml) was incubated for 10 min at 37°C with dibutyryl 3',5'-AMP or prostaglandin E_1 or a like volume of 0.145 M NaCl (control) before addition of prostaglandin G_2. After 3 min the reaction was stopped by addition of 1.5 ml of 40% cold trichloroacetic acid containing 1 M HCl and 0.1 M $NaAsO_2$. After centrifugation the supernatant was mixed with 0.6 ml 0.1 M sodium thiobarbiturate and heated to 70°C for 30 min. After standing overnight in the cold, samples were concentrated on a DEAE-cellulose column, eluted with 6 M KOH, and read at 548, 518, and 578 nm against a KOH blank. The mean of the readings at 518 and 578 nm was subtracted from the 548 nm reading and compared with a standard curve prepared from malondialdehyde tetramethylacetal (modified from method of Macfarlene et al. [25].) Reagent blanks were obtained by constructing standard curves in platelet-poor plasma, platelet-poor plasma with 10^{-6} M prostaglandin E_1 and platelet-poor plasma with 10^{-3} M dibutyryl cyclic AMP. Analysis of linear regressions by least squares yielded values that were not significantly different in the three instances ($y = my + b$, for y = absorbance units and x = nmol malondialdehyde; platelet-poor plasma: $m = 0.189$, $b = 0.0124$; platelet-poor plasma/prostaglandin E_1: $m = 0.201$, $b = 0.0165$; platelet-poor plasma/libatoral cyclic AMP: $m = 0.235$, $b = 0.0276$). Results shown are typical of those in four replicate experiments.

Control (pmol)		Dibutyryl cyclic 3', 5'-AMP			Prostaglandin E_1		
		10^{-5} M	10^{-4} M	10^{-3} M	10^{-8} M	10^{-7} M	10^{-6} M
Platelet-rich plasma *	12	—	—	—	—	—	—
Prostaglandin G_2 ($0.7 \cdot 10^{-6}$ M)	162	171	87	12	87	72	27
Prostaglandin G_2 ($1 \cdot 10^{-6}$ M	219	114	122	108	191	94	53

* 410 000 platelets/mm^3.

inhibitor. As with the other agents mentioned above (Salzman, E.W., McIntyre, D.E., Gordon, J.L., Levine, L. and Smith, M., unpublished) the effect of SQ22536 was manifest only in a narrow range of concentrations of prostaglandin G_2 at which there was transition from primary aggregation without serotonin release to a second phase response with release. At prostaglandin G_2 concentrations above or below this range, SQ22536 had no demonstrable effect.

Discussion

In many respects, aggregation of platelets by prostaglandin G_2 resembles the response of platelets to ADP, which at low concentrations causes shape change and reversible aggregation without secretion of platelet constituents and at higher concentrations leads to the release reaction and irreversible platelet aggregation. The second phase of aggregation and serotonin release at low concentration of prostaglandin G_2 can be inhibited with indomethacin, but high concentrations of prostaglandin G_2 escape the indomethacin inhibitory effect. The release reaction induced by ADP was been attributed to stimulation of prostaglandin endoperoxide synthesis [5], which is blocked by indomethacin. One might expect that addition of prostaglandin G_2 to platelet-rich plasma would bypass the indomethacin-vulnerable cyclooxygenase step, but the susceptibility of prostaglandin G_2 to inhibition by indomethacin may be explained by an autocatalytic effect of exogenous prostaglandin G_2 on cyclo-

oxygenation of endogenous arachidonic acid [34], which would be subject to inhibition by indomethacin. A high concentration of exogenous prostaglandin G_2 in platelet-rich plasma would presumably offset the effects of blockade of this indomethacin-vulnerable step. The explanation is less likely to be indomethacin's weaker inhibitory effect on thromboxane synthetase [35], which would reduce the conversion of prostaglandin endoperoxides to the more potent thromboxane A_2, since Moncada and associates [35] found thromboxane synthetase activity of equine platelet microsomes to be 50% inhibited by $3 \cdot 10^{-4}$ M indomethacin and unaffected by the compound at $5 \cdot 10^{-5}$ M, whereas platelet aggregation induced by prostaglandin G_2 appears substantially more sensitive (Fig. 3).

A decrease in the basal level of cyclic AMP has been observed with many agents that aggregate platelets, including ADP, thrombin, collagen, epinephrine, arachidonic acid, and physical stimuli such as adsorption on kaolin particles or centrifugation [11,23]. The fall in cyclic AMP is inhibited by aspirin and indomethacin [14]. These stimuli lead also to platelet prostaglandin synthesis [36, 37,23] and therefore presumably, although not directly measured in each case, to production of prostaglandin endoperoxides. Reduction of platelet cyclic AMP by prostaglandin G_2 might explain the reduction in cyclic AMP by the above stimuli. That the effect is likely to have physiologic significance is indicated by the ability of the adenylate cyclase inhibitor SQ22536 to enhance platelet activity.

Miller and Gorman [38] did not observe a fall in basal cyclic AMP upon addition of prostaglandin G_2 to platelet-rich plasma, but they found that prostaglandin G_2 inhibited the rise in cyclic AMP produced by prostaglandin E_1. They examined only a single concentration of the endoperoxide, 2.8 μM, which is substantially more than is necessary for a maximal effect and a concentration at which other effects such as conversion to prostaglandin D_2 might have been predominant [39]. Since prostaglandin D_2 is a stimulant to adenylate cyclase [40], its formation in platelet-rich plasma [39] could account for the inconstant effects of exogenous prostaglandin G_2 on cyclic AMP in platelet-rich plasma compared to the response in a gel filtered platelet suspension.

How prostaglandin G_2 reduces platelet cyclic AMP is not certain. At low concentrations, at which it enhances platelet activation [9,41], prostaglandin E_2, a product of prostaglandin G_2, can decrease platelet cyclic AMP [10,11]. It has been shown [7] that a labile prostaglandin intermediate termed "LASS" or "labile aggregation stimulatory substance" [16], presumably a mixture of endoperoxides and thromboxane A_2, decrease platelet cyclic AMP.

Gerrard et al. [42] have indicated that thromboxane A_2 can function as an ionophore for calcium. Such an action might raise local cytoplasmic ionized calcium levels and reduce cyclic AMP concentration by inhibition of adenylate cyclase [43]. It is also possible that prostaglandin G_2 or thromboxane A_2 has a direct effect on platelet adenylate cyclase. Gorman et al. [44] have described inhibition of activity of this enzyme in the fat cell by prostaglandin endoperoxides.

It seems unlikely that the reduction in platelet cyclic AMP by prostaglandin G_2 results from released ADP. ADP's effect on platelet cyclic AMP is blocked by aspirin, which implies that ADP's own action is mediated through the ara-

chidonate-cyclooxygenase system. Furthermore, attempts to demonstrate a direct effect of ADP on platelet adenylate cyclase or phosphodiesterase have been unsuccessful [7].

The release of ADP does not appear to be essential for platelet aggregation by prostaglandin G_2. Kinlough-Rathbone et al. [45] have shown that arachidonic acid aggregates platelets degranulated and therefore depleted of stored ADP by prior exposure to thrombin. Plasma ADP was not measured directly in our experiments, but we did not find serotonin release at prostaglandin G_2 concentrations which gave rise to "primary" or reversible aggregation, and the secretion of serotonin and of adenine nucleotides from storage granules are thought to occur simultaneously during the release reaction [46]. Primary aggregation due to prostaglandin G_2 was not inhibited by 2-*n*-amylthio-5'-AMP, an ADP antagonist. On the otherhand, it appears that the "second" or irreversible phase of platelet aggregation induced by prostaglandin G_2 can be attributed to the action of ADP. Both irreversible aggregation and serotonin release are inhibited by the ADP antagonist 2-*n*-amylthio-5'-AMP and are not observed in heparinized platelet-rich plasma, in which ADP does not cause release of platelet constituents. The source of the ADP that initially sets off the release reaction and thus leads indirectly to second phase aggregation in response to released ADP is not clear, but since there is no accompanying secretion of serotonin, it presumably derives from a site other than the storage granules, e.g. breakdown of membrane-bound ATP.

How changes in platelet cyclic AMP levels affect platelet function is not established. Stimulation of platelets by the ionophore A23187 is inhibited by increased cyclic AMP [17], which is consistent with the suggestion that the nucleotide operates to reduce platelet cytoplasmic calcium levels [18]. Such a mechanism is supported by the recent observation of Käzer-Glanzmann et al. [47] that platelet membrane vesicles can concentrate calcium in the presence of cyclic AMP, ATP, and protein kinase. This hypothesis would also be compatible with Berridge's [48] proposal of a bidirectional system in which the effects of increased ionized calcium levels are offset by the results of increased platelet levels of cyclic AMP.

Malmsten et al. [19] reported inhibition of platelet production of thromboxanes by dibutyryl cyclic AMP, prostaglandin E_1, and other substances that increase platelet cyclic AMP and suggested a direct inhibitory effect of cyclic AMP on the enzyme cyclooxygenase. The reported inhibition of malondialdehyde production following addition of thrombin, ADP, or arachidonic acid to platelet-rich plasma containing dibutyryl cyclic AMP or prostaglandin E_1 [15] would be consistent, and it would also account for the autocatalytic effect of prostaglandin G_2 on its own production [34]. By altering local calcium concentration, cyclic AMP levels could also regulate the activity of phospholipase A_2, the calcium-sensitive enzyme thought to make arachidonic acid available to prostaglandin synthetic pathways by hydrolysis of platelet membrane phospholipids [49,50].

Additional loci of action are necessary to account for other effect of cyclic AMP. Inhibition of platelet aggregation and the release reaction induced by prostaglandin G_2 (or by LASS [16]) requires that cyclic AMP inhibit some later step in platelet activation which must also account for the ability of dibutyryl

cyclic AMP and prostaglandin E_1 to inhibit the production of malondialdehyde induced by prostaglandin G_2.

In addition, yet another action is implied by the effects of dibutyryl cyclic AMP and prostaglandin E_1 on platelet function. At high concentrations these substances block not only the release reaction and platelet aggregation but also primary aggregation [7] and adhesion of platelet to artificial surfaces [51]. Neither primary aggregation by ADP nor adhesion of platelet to foreign surfaces is inhibited by indomethacin or aspirin [51]. These observations suggest an effect of cyclic AMP levels on fundamental aspects of platelet adhesivity.

Acknowledgements

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