Biochem. J. (1978) **174**, 921–929
Printed in Great Britain

Prostaglandin Receptors on Human Platelets

STRUCTURE–ACTIVITY RELATIONSHIPS OF STIMULATORY PROSTAGLANDINS

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(*Received* 23 *January* 1978)

1. Synthetic analogues of prostaglandins E_2 or $F_{2\alpha}$ (monocyclic bisenoic prostaglandins), like the endogenous prostaglandin endoperoxides (prostaglandins G_2 and H_2) from platelets, and like synthetic analogues of prostaglandin H_2 (bicyclic bisenoic prostaglandins), can induce aggregation of human platelets, although prostaglandins E_2 and $F_{2\alpha}$ themselves are inactive. 2. All the prostanoid compounds that induce platelet aggregation release 5-hydroxytryptamine from platelet dense bodies, but do not release β-*N*-acetylglucosaminidase from lysosomal granules. Arachidonic acid evokes a similar response. 3. All endoperoxide analogues tested (bicyclic compounds) were powerful platelet stimulants, and all active compounds (whether mono- or bi-cyclic) apparently acted via the same receptor as the endogenous prostaglandin endoperoxides. 4. The nature and stereospecificity of substituents at positions 11 and 15 (or 16) on prostaglandin E_2 are critical determinants for platelet-stimulating activity: deoxy substitution at position 11 plus methylation at position 15 (or 16) produces a potent stimulant, particularly if the groups around C-15 are in the *S* configuration. 5. The effects of these structural modifications are apparently due to, at least in part, a change in side-chain conformation.

The formation of platelet aggregates and the associated secretion of platelet constituents, reactions of great importance in haemostasis and thrombosis, are influenced by prostaglandins and related compounds formed by the platelets in response to stimuli and also when these compounds are added exogenously (Kloeze, 1967; Willis, 1974; Samuelsson *et al.*, 1976). The main precursor of the prostaglandins formed by most cell types (including platelets) is arachidonic acid, and its metabolic products have important and diverse effects on many cells, but with the notable exception of the corpus luteum (Powell *et al.*, 1975), the receptors that mediate these effects have not yet been well characterized. The platelet provides a valuable cellular model for studying such prostaglandin receptors. The prostaglandin endoperoxides G_2 and H_2 are potent platelet stimulants (Hamberg *et al.*, 1974; Willis *et al.*, 1974), as is their major metabolic product, thromboxane A_2 (Hamberg *et al.*, 1975; Needleman *et al.*, 1976). The study of these compounds is complicated by their lability in aqueous media, but analysis of their actions has been facilitated by the recent synthesis of stable analogues of prostaglandin H_2 (Corey *et al.*, 1975; Bundy, 1975) that are also powerful platelet stimulants (Corey *et al.*, 1977).

We have now investigated the structure–activity relationships of several such compounds and compared them with analogues of prostaglandin E_2, some of which also stimulate platelets (Fenichel *et al.*, 1975; Gordon & MacIntyre, 1976). Prostaglandin E_2 itself, which is a major prostaglandin formed by stimulated platelets (Smith *et al.*, 1973), does not induce either aggregation or secretion in human platelets (MacIntyre & Gordon, 1975). The present investigation has allowed us to define some major structural requirements for prostaglandins to stimulate platelets. In addition, we have found that platelet secretion induced by prostaglandins is qualitatively different from that induced by other stimuli such as thrombin and collagen.

Materials and Methods

Measurement of platelet aggregation

Human blood was obtained by antecubital venepuncture from volunteers who had allegedly ingested no drugs for at least 7 days: 1 vol. of trisodium citrate (0.13 M) was added to 9 vol. of blood, and platelet-rich plasma was prepared by centrifugation (1500*g*; 20°C; 1 min). Platelet aggregation was measured photo-

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metrically (Born, 1962) in 0.1 ml samples of platelet-rich plasma (Gordon & Drummond, 1974).

Measurement of platelet secretion

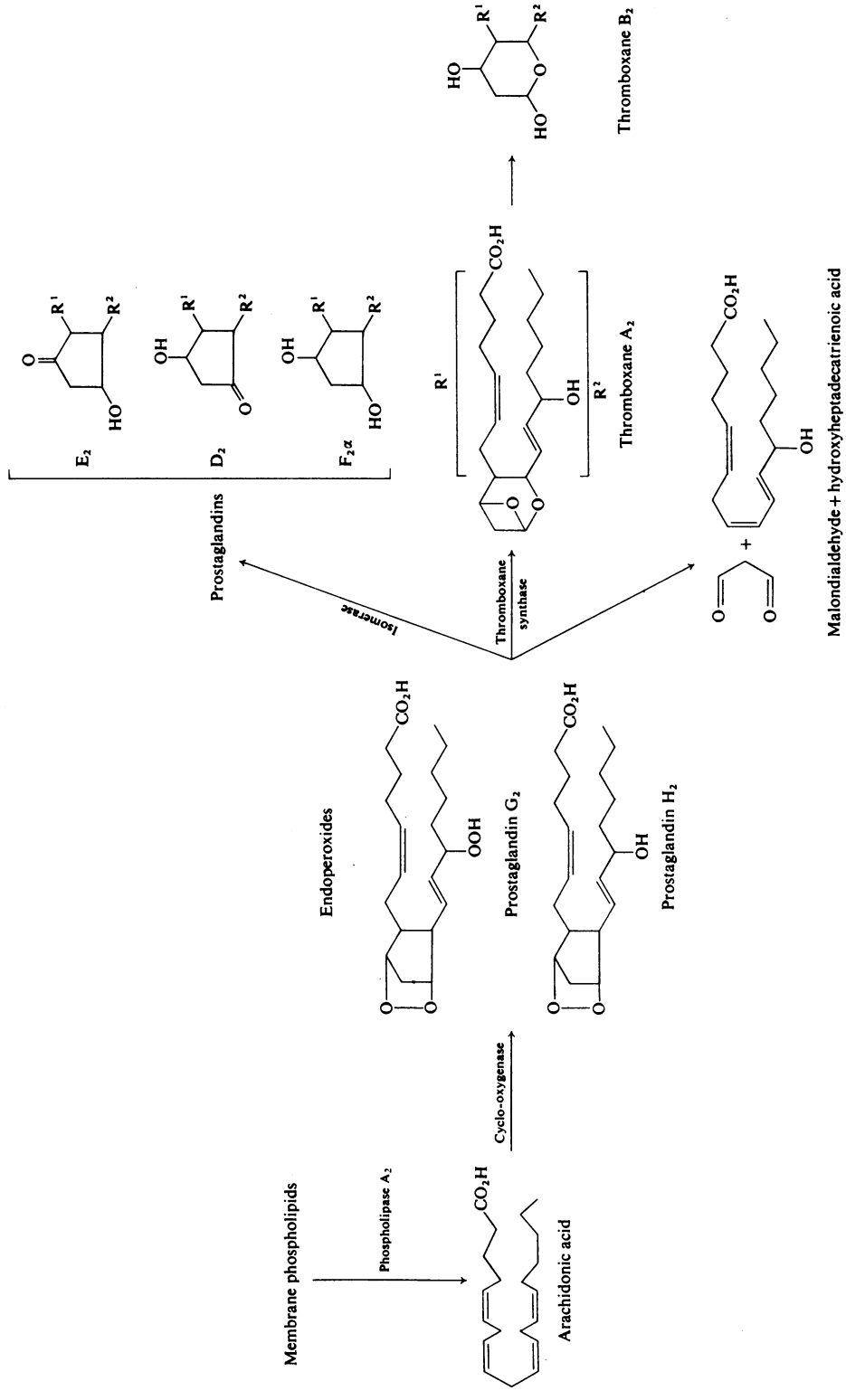
Platelet-rich plasma was preincubated at 37°C for 30 min with 1 μM-5-hydroxy[^{14}C]tryptamine (which is incorporated into platelet dense bodies) and 0.1 μM-[^{3}H]adenine (which is incorporated into the cytoplasmic pool of adenine nucleotides). After addition of an agonist to the stirred platelet samples, the reactions were terminated by the addition of 4 vol. of ice-cold 0.4% (w/v) EDTA in 154 mM-NaCl and immediate centrifugation (14700g; 20°C; 30s). Subsamples of cell-free supernatant for measurement of ^{3}H and ^{14}C release were transferred into scintillation vials containing 0.33% 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole in toluene plus 30% (v/v) ethoxyethanol. Platelet pellets were digested in 19 M-formic acid (37°C; 30 min) before transfer to scintillation vials. Radioactivity was measured in a Nuclear–Chicago mark 2 liquid-scintillation counter under conditions giving optimum discrimination between ^{3}H and ^{14}C. Increases in supernatant radioactivity were expressed as a percentage of the activity in a control unstimulated cell pellet. Replicate subsamples of cell-free supernatant were taken for fluorimetric measurement of β-*N*-acetylglucosaminidase (EC 3.2.1.30), a platelet lysosomal enzyme, essentially as described by Gordon (1975). Briefly, 100 μl volumes of cell-free supernatant of platelet pellets digested in 1% Triton X-100 (37°C; 30 min) were incubated at 37°C with 100 μl of 0.3 M-sodium citrate buffer, pH 4.9, and the reactions were initiated by adding 100 μl of 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (3 mM). After 60 min, the reactions were terminated by heating at 100°C for 2 min, and 700 μl of glass-distilled water was added to each sample. All samples were vortex-mixed, and 100 μl volumes of the diluted reaction mixture were added to 1.5 ml of 50 mM-glycine/NaOH buffer, pH 10.4. The supernatant was clarified by centrifugation (14700g; 20°C; 1 min), and the fluorescence of this solution was measured in a Farrand mark 1 spectrophotofluorimeter with activation and emission wavelengths 370 and 450 nm respectively (uncorrected instrument readings). Increases in supernatant fluorescence were expressed as a percentage of the fluorescence in pellets of control, unstimulated platelets.

Materials

Aminophylline, arachidonic acid, ADP, bovine tendon collagen, EDTA, EGTA, 5-(4-biphenylyl)-2-(4-t-butylphenyl)-1-oxa-3,4-diazole and 4-methylumbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside were obtained from Sigma (London) Chemical Co., Kingston upon Thames, Surrey, U.K. Ionophore A23187 was a gift from Lilly Research Centre, Windlesham, Surrey, U.K. Aspirin was obtained from Addenbrooke's Hospital Pharmacy, Cambridge, as acetylsalicylic acid powder. Indomethacin was obtained from Merck, Sharpe and Dohme, Hoddesdon, Herts., U.K. 5-Hydroxy[*side-chain*-2-^{14}C]tryptamine creatinine sulphate (58 mCi/mmol) and [8-^{3}H]adenine (24 Ci/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Prostaglandins

For prostaglandin nomenclature see Nugteren *et al.* (1966), Cahn *et al.* (1956) and Table 1. For the major pathways of arachidonate metabolism in platelets see Scheme 1. The following compounds were kindly given by Dr J. E. Pike and Dr. G. L. Bundy, Upjohn Co., Kalamazoo, MI, U.S.A.: prostaglandins A_2, D_2, E_1, E_2, $F_{2\alpha}$; 15(*R*)-15-methylprostaglandin E_2 (U42842); 15(*S*)-15-methylprostaglandin E_2 methyl ester (U35960); 15(*R*)-16,16-dimethylprostaglandin E_2 (U37026); 15(*S*)-hydroxy-9α,11α-(epoxymethano)-prosta-5*cis*,13*trans*-dienoic acid (U44069); 15(*S*) - hydroxy - 11α,9α - (epoxy - methano)-prosta-5*cis*,13*trans*-dienoic acid (U46619); 15(*R*)-16,16-dimethylprostaglandin E_1 (U39598). 13,14-Dihydro-15(*S*)-16(*R*)-methylprostaglandin E_2 methyl ester (ONO 464) was kindly given by Dr. M. Tsuboshima, ONO Pharmaceutical Co., Osaka, Japan. 15(*RS*)-Hydroxy-9α,11α-(etheno)-prosta-5*cis*-13*trans*-dienoic acid (ICI 86841) was a gift from Dr. M. W. Senior, ICI Ltd., Macclesfield, Cheshire, U.K. 15(*S*) - Hydroxy - 9α,11α - azo - prosta - 5*cis*,-13*trans*-dienoic acid (azo-prostaglandin H_2) was synthesized by Dr. E. J. Corey, Harvard University, Boston, MA, U.S.A. All the above compounds were dissolved initially in ethanol at a concentration of 33 mM. They were subsequently diluted to 3.3 mM-stock solutions in 1.9 mM-Na_2CO_3, and further dilutions were made in 154 mM-NaCl. 11-Deoxy-15(*RS*)-15-methylprostaglandin E_2 (Wy 17186), 11-deoxy-15(*S*)-15-methylprostaglandin E_2 (Wy 40659), 11-deoxy-15(*S*)-15-ethynylprostaglandin E_2 (Wy 17256), 11-deoxy-15(*S*)-prostaglandin E_2 (Wy 18189), 11-deoxy-11α-methyl-15(*S*)-prostaglandin E_2 (Wy 16868), 11-deoxy-15(*S*)-16(*RS*)-methylprostaglandin E_2 (Wy 19110) and 11-deoxy-15(*R*)-16(*RS*)-methylprostaglandin E_2 (Wy 19068) were kindly supplied in 0.2 M-sodium phosphate buffer by Dr. R. L. Fenichel, Wyeth Laboratories, Radnor, PA, U.S.A. Subsequent dilutions were made in 154 mM-NaCl. Prostaglandin G_2 was a gift from Professor B. Samuelsson, Karolinska Institut, Stockholm, Sweden. Because of the lability of this compound in aqueous media, it was diluted initially in ice-cold dry acetone. Immediately before use, a subsample of the acetone



Scheme 1. *Arachidonic acid metabolism in human platelets*

stock solution was diluted with ice-cold 154mM-NaCl. All stock solutions of prostaglandins were stored at −20°C under N_2.

Results

Platelet aggregation induced by prostaglandins

At concentrations from 0.03 to 300 μM, all prostaglandins that induced platelet aggregation elicited the same pattern of response: a modest, reversible clumping of platelets ('primary aggregation') at the lowest effective concentrations, and a more intense irreversible aggregation ('secondary aggregation') at higher concentrations. Over a narrow range of intermediate concentrations, primary and secondary responses were kinetically distinct, resulting in biphasic aggregation patterns, whereas at high concentrations the primary and secondary aggregation responses merged (Fig. 1). The minimum active concentration of each prostaglandin was determined (that is, the lowest concentration that elicited a detectable primary aggregation response) and the results of these studies are shown in Table 1. The same rank order of potency was obtained if the minimum concentration of each compound required to induce secondary aggregation was determined. The absolute potency of these stimulants varied up to 5-fold in different subjects, but the rank order was the same in all subjects.

Not all the monocyclic compounds tested (nos. 1–13) induced platelet aggregation. Prostaglandins E_2, $F_{2\alpha}$ and A_2 (nos. 1–3) were inactive even at 300 μM, but 11-deoxy substitution on prostaglandin E_2 (nos. 4, 5) resulted in modest stimulatory activity. Methylation of prostaglandin E_2 at position 15 (nos. 6, 7) conferred activity only if the groups were in the *S* configuration. Dimethylation at position 16 (no. 8) produced a more potent stimulant. The combination of a deoxy substituent on position 11 and a methyl or ethynyl substituent on position 15 or 16 (nos. 9–13) further enhanced aggregating activity. In these 11-deoxy 15- or 16-substituted prostaglandins, the orientation of the substituents at C-15 markedly influenced activity: compounds with the C-15 groups in the *S* configuration were more potent stimulants than was the corresponding 15(*R*) enantiomer (compare nos. 12 and 13) or the mixture of C-15 epimers (compare nos. 10 and 11). The bicyclic prostaglandins tested (nos. 14–18) were all effective platelet stimulants, showing roughly comparable activity whether the linkage between C-9 and -11 contained an azo, epidioxy or epoxymethano substitutent (nos. 14–17). The compound with an etheno linkage (no. 18) was less active. These results indicate that a bimolecular 9α,11α linkage confers platelet-aggregating activity on 15(*S*)-hydroxyprostadienoic acid, that the composition of this linkage is relatively unimportant, and that the potency of the most active of such compounds is comparable with that of monocyclic compounds nos. 11 and 13.

Secretion of granule constituents from platelets

The pattern of secretion associated with aggregation induced by prostaglandins is unlike that induced by other stimuli such as collagen or ionophore A23187, but similar to that induced by ADP. Primary aggregation induced by prostaglandins was not associated with secretion of either 5-hydroxy[^{14}C]-tryptamine or β-*N*-acetylglucosaminidase, but secondary aggregation was accompanied by secretion of

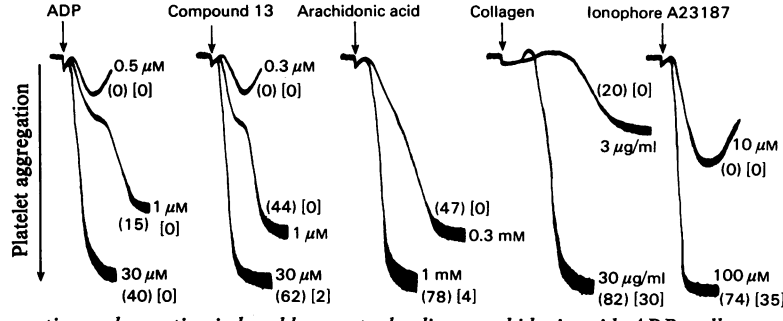
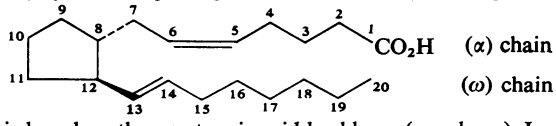


Fig. 1. *Platelet aggregation and secretion induced by prostaglandins, arachidonic acid, ADP, collagen and ionophore A23187*
Platelet-rich plasma was incubated for 2min at 37°C before the addition of agonist at the concentrations indicated. Platelet aggregation and release of [8-^{3}H]adenine, 5-hydroxy[^{14}C]tryptamine and β-*N*-acetylglucosaminidase were measured as described in the Materials and Methods section. Compound 13 [11-deoxy-15(*S*)-16(*RS*)methyl-prostaglandin E_2] was used as a representative prostaglandin stimulus. Similar results were obtained with other prostaglandins. Values in parentheses are percentage release of 5-hydroxy[^{14}C]tryptamine; those in square brackets are percentage release of β-*N*-acetylglucosaminidase. In all cases [8-^{3}H]adenine release was less than 10%. Results are mean values of duplicate determinations.

Table 1. *Activity of bis-enoic prostaglandins as inducers of human platelet aggregation*



Prostaglandin nomenclature is based on the prostanoic acid backbone (see above). In all cases the compounds have a *cis*-double bond between C-5 and -6, and a *trans*-double bond between C-13 and -14. Prostaglandin A_2 has an additional double bond between C-10 and -11. Results shown are mean values from two or three experiments on different plasma samples from the same donor, in which the lowest concentration of agonist that elicited a primary aggregation response was measured. The rank order of potency of these compounds was the same in all donors, but there was substantial inter-subject variability, and the absolute potency varied up to fivefold in different donors.

No.	Compound	Substituents 9	11	15	16	Minimal active concentration (μM)
1	Prostaglandin E_2	=O	OH	H, OH	H	>300
2	Prostaglandin $F_{2\alpha}$	OH	OH	H, OH	H	>300
3	Prostaglandin A_2	=O	H	H, OH	H	>300
4	11-Deoxyprostaglandin E_2	=O	H	H, OH	H	5
5	11-Deoxy-11α-methylprostaglandin E_2	=O	CH_3	H, OH	H	10
6	15(*R*)-15-methylprostaglandin E_2	=O	OH	OH, CH_3	H	>300
7	15(*S*)-15-methylprostaglandin E_2 methyl ester	=O	OH	CH_3, OH	H	10
8	15(*R*)-16,16-dimethylprostaglandin E_2	=O	OH	OH, H	CH_3, CH_3	1
9	11-Deoxy-15(*S*)-15-ethynyl-prostaglandin E_2	=O	H	C≡CH, OH	H	1
10	11-Deoxy-15(*RS*)-15-methyl-prostaglandin E_2	=O	H	OH, CH_3 / CH_3, OH	H	3
11	11-Deoxy-15(*S*)-15-methyl-prostaglandin E_2	=O	H	CH_3, OH	H	0.1
12	11-Deoxy-15(*R*)-16(*RS*)-methyl-prostaglandin E_2	=O	H	OH, H	H, CH_3 / CH_3, H	1
13	11-Deoxy-15(*S*)-16(*RS*)-methyl-prostaglandin E_2	=O	H	H, OH	H, CH_3 / CH_3, H	0.1
14	15(*S*)-Hydroperoxy-9α,11α-epidioxy-prostadienoic acid (prostaglandin G_2)	–O———O–		H, OOH	H	0.2
15	15(*S*)-Hydroxy-9α,11α-epoxy-methanoprostadienoic acid	–O———CH_2–		H, OH	H	0.2
16	15(*S*)-Hydroxy-11α,9α-epoxy-methanoprostadienoic acid	–CH_2———O–		H, OH	H	0.1
17	15(*S*)-Hydroxy-9α,11α-azoprostadienoic acid	–N═══N–		H, OH	H	0.1
18	15(*RS*)-Hydroxy-9α,11α-ethenoprostadienoic acid	–C═══C–		H, OH / OH, H	H	3

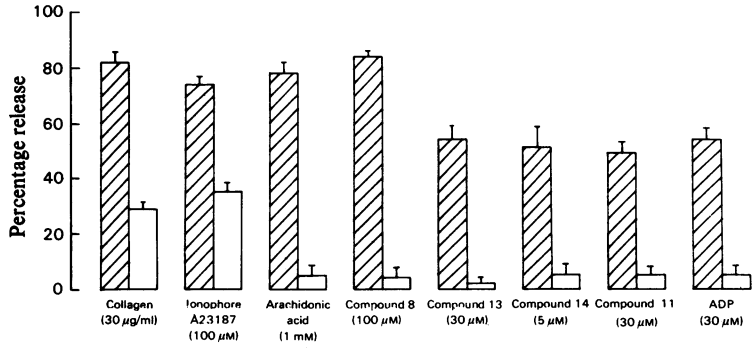


Fig. 2. *Secretion of platelet constituents induced by prostaglandins, arachidonic acid, ADP, collagen and ionophore A23187* Release of 5-hydroxy[^{14}C]tryptamine (▨) and β-*N*-acetylglucosaminidase (□) were measured in platelet-rich plasma samples incubated for 2 min at 37°C before addition of agonist at the concentration indicated (see the Materials and Methods section). The concentrations of agonists chosen were those that induced maximal, irreversible responses (secondary aggregation). In all cases [8-^{3}H]adenine release was less than 10%. Results are mean values ± S.E.M. of six determinations.

up to 80% of the 5-hydroxy[^{14}C]tryptamine in platelets, without significant release of the lysosomal enzyme or of the cytoplasmic marker, adenine. Arachidonic acid also induced platelet aggregation with release of 5-hydroxy[^{14}C]tryptamine (but not the lysosomal enzyme) from platelets. This pattern of secretion was found to accompany secondary aggregation induced by ADP (Mills *et al.*, 1968). In contrast, aggregation induced by collagen (30 μg/ml) or by ionophore A23187 (100 μM) is associated with 20–40% release of lysosomal enzymes as well as 70–80% release of 5-hydroxy[^{14}C]tryptamine; again, less than 10% of [8-^{3}H]adenine was released. Patterns of aggregation and secretion induced by these different stimuli in representative experiments are shown in Fig. 1, and detailed measurements of secretion in replicate experiments are given in Fig. 2.

Inhibition of prostaglandin-induced platelet aggregation

Primary aggregation induced by prostaglandins was unaffected by aspirin and indomethacin, which inhibit the cyclo-oxygenase enzyme responsible for forming prostaglandins G_2 and H_2, and hence abolish aggregation induced by arachidonic acid (Roth & Majerus, 1975). Secondary aggregation induced by high concentrations of prostaglandins was also unaffected, but the cyclo-oxygenase inhibitors had a modest effect against the narrow range of prostaglandin concentrations that induced biphasic aggregation (i.e. when secondary aggregation was only just detectable) (Fig. 3). These results are analogous to those reported with the native endoperoxide prostaglandin G_2 (Salzman, 1977). Thus secondary platelet aggregation and secretion induced by native endoperoxides or prostaglandin analogues do not depend on initiation of the platelet prostaglandin-synthesis pathway, although there is apparently some positive feedback through this pathway via activation of the cyclo-oxygenase enzyme. This observation is consistent with the results obtained in other tissues (Lands *et al.*, 1976).

Agents that chelate bivalent cations (EDTA, EGTA) and agents that elevate the cellular concentration of cyclic AMP, either by stimulating adenylate cyclase (prostaglandin E_1, prostaglandin D_2) or by inhibiting cyclic AMP phosphodiesterase (aminophylline), inhibit prostaglandin-induced platelet aggregation (Fig. 3). This indicates that the platelet response to stimulatory prostaglandins is subject to the same cellular control mechanisms as for other stimuli such as ADP, collagen and thrombin (Mills & Macfarlane, 1976).

Discussion

The results of the present study indicate that stimulation of platelets by native endoperoxides (prostaglandins G_2 and H_2) can be duplicated not only by endoperoxide analogues with different 9α,11α linkages, but also by monocyclic analogues of prostaglandin E_2. All the analogues induced similar aggregation patterns: at low concentrations, primary aggregation with no secretion; and secondary aggregation accompanied by 5-hydroxytryptamine secretion at higher concentrations. These results support our hypothesis that all bisenoic prostaglandins (whether mono- or bi-cyclic) that stimulate platelets act at a single receptor site (MacIntyre & Gordon, 1977). The pattern of platelet secretory responses induced by the prostaglandin analogues is similar to

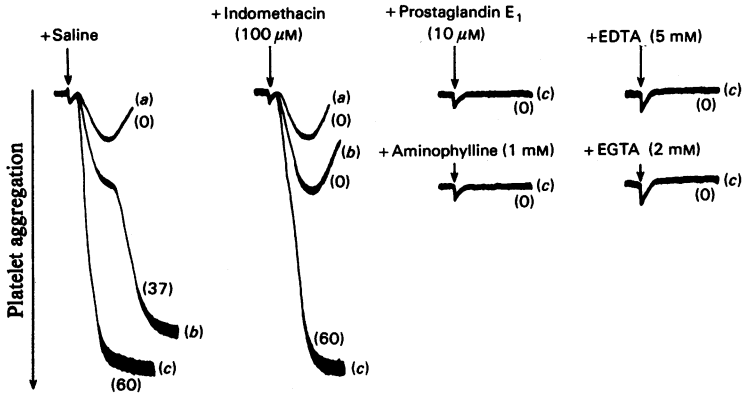


Fig. 3. *Inhibition of prostaglandin-induced platelet aggregation*

Platelet-rich plasma was preincubated for 5min at 37°C with indomethacin (100μM) and aminophylline (1mM), and for 2min at 37°C with prostaglandin E_1 (10μM), EDTA (5mM) and EGTA (2mM). Control samples received 154mM-NaCl in place of drug. Platelet aggregation was initiated by the addition of compound 8 [15(*R*)-16,16-dimethylprostaglandin E_2]: 2μM (*a*), 5μM (*b*) or 30μM (*c*). Similar results were obtained with other prostaglandins. Values in parentheses indicate percentage release of 5-hydroxy[^{14}C]tryptamine. Results are mean values of duplicate determinations.

that induced by ADP, in that no lysosomal enzyme secretion (β-*N*-acetylglucosaminidase) accompanied the 5-hydroxytryptamine release. Our preliminary findings with two analogues of prostaglandin E_2 (MacIntyre *et al.*, 1977) have now been confirmed with several other analogues and with prostaglandin G_2, and the new data support our earlier conclusion that although prostaglandins may play a role in lysosomal enzyme secretion from platelets, they alone are not sufficient to initiate the process. Consequently the lysosomal enzyme secretion from platelets induced by stimuli such as collagen and ionophore A23187 must involve other intracellular control systems apart from prostaglandin biosynthesis.

For structure–activity studies, concentrations of prostaglandins that induced only primary aggregation were used, thus ensuring that there was no activation of endogenous platelet prostaglandin synthesis.

All of the bicyclic compounds investigated (nos. 14–18) are active platelet stimulants. The nature of the linkage between C-9 and -11 appears to be relatively unimportant, since compounds bearing epidioxy (no. 14), 9α,11α-epoxymethano (no. 15), 11α,9α-epoxymethano (no. 16) and 9α,11α-azo (no. 17) linkages are virtually equipotent. However, the compound with a 9α,11α-etheno linkage (no. 18) was less active. It is possible that any bicyclo(2.2.1) system can confer platelet-aggregating activity on bisenoic prostaglandins, and indeed evidence from Portoghese *et al.* (1977) indicates that such activity is not restricted to bicyclo(2.2.1) systems; they found that prostaglandin $F_{2\alpha}$ acetal [a bicyclo(3.2.1) system] was also a powerful platelet stimulant.

Consideration of the structural requirements for monocyclic prostaglandins to stimulate platelets shows that the orientation of the groups at position 15 and the nature of the substituents on positions 11, 15 and 16 are important determinants of reactivity. Prostaglandins E_2, $F_{2\alpha}$ and A_2 (nos. 1–3) are inactive as platelet stimulants; the minimal structural modification to produce an active platelet stimulant is either 11-deoxygenation (no. 4) or alkylation at position 15 or 16 (nos. 5 and 7). A combination of 11-deoxygenation and 15- or 16-alkylation enhances activity (nos. 11 and 13). Compounds in which the groups at position 15 are in the natural or (*S*) configuration are more potent agonists than the corresponding 15(*R*) enantiomer (compare nos. 6 and 7; nos. 12 and 13) or the mixture of C-15 epimers (compare nos. 10 and 11). However, a 15(*S*) configuration is not an absolute requirement for aggregating activity, as compound no. 12, with a 15(*R*) configuration, is still active. The compounds bearing 11-deoxy-15(*S*)-15-methyl or 16(*RS*)-methyl substituents (nos. 11 and 13) are as active as the native endoperoxides or their bicyclic analogues (nos. 14–17). It would be interesting to test a 15- or 16-methylated bicyclic prostaglandin analogue, and to compare the potency of 16(*R*)- and 16(*S*)-methylated compounds.

From structure–activity relationships and receptor-affinity data it was suggested that, as well as the constituents at positions 9 and 11 of the cyclopentane ring, close alignment of the α- and ω-alkyl

side chains was important for the interaction of prostaglandins E and F with receptors on smooth muscle (Rabinowitz *et al.*, 1971; Andersen & Ramwell, 1974). This 'hairpin conformation hypothesis' implied a multi-point contact of a prostaglandin molecule with its receptor, regulated in part by the configuration of the side chains. This concept was supported by physicochemical evidence of the existence of such 'hairpin' conformations in the crystalline state and in solution (Leovey & Andersen, 1975; Andersen *et al.*, 1976). Structural models show that in prostaglandin endoperoxides the α- and ω-chains project slightly upwards from the plane of the cyclopentane ring, whereas in prostaglandin E_2 they project parallel to the cyclopentane ring (Andersen *et al.*, 1976). The results of the present study are consistent with the concept that there are multiple sites of contact between the receptor that initiates platelet aggregation and a stimulatory prostaglandin molecule; in particular, with the substituents at C-11, C-15 and C-16. How deoxygenation at position 11 and/or alkylation at position 15 or 16 confers stimulating activity on the molecule is not yet established, but it presumably reflects facilitated binding to the stimulatory receptor. This could be partly due to a change in the physicochemical characteristics (e.g. electron density, lipophilicity, molecular size) at these positions, but it is likely that altering the orientation of the side chains is even more important. Two pieces of evidence support this concept. First, geminal substitution at position 16 produces a bending of the ω chain (Andersen *et al.*, 1976), so that the side-chain configuration more closely resembles that in the endoperoxides, and 16,16-dimethylprostaglandin E_2 (compound no. 8) is a powerful platelet-aggregating agent. Secondly, removal of the double bond at position 5,6 or 13,14 (which distorts the hairpin conformation of bisenoic prostaglandins) abolishes stimulatory activity: for example, 15(*R*)-16,16-dimethylprostaglandin E_1 (lacking the 5,6 double bond) and 13,14-dihydro-15(*S*)-16(*R*)-methylprostaglandin E_2 methyl ester (lacking the 13,14 double bond) do not induce platelet aggregation (D. E. MacIntyre, unpublished work). Taken together, these results suggest that orientation of the side chains is of paramount importance in determining the activity of prostaglandins.

Previous studies from our laboratory using sodium *p*-benzyl-4-[2-(4-chlorobenzyl)-1oxo-3-phenylpropyl]phenyl phosphonate (N 0164), a competitive inhibitor of bisenoic prostaglandins, indicated that all stimulatory prostaglandins act on the same platelet receptor (MacIntyre, 1977; MacIntyre & Gordon, 1977); in other words, appropriate modification of the prostaglandin E_2 molecule could alter its activity to that of the prostaglandin endoperoxides. Studies with isolated smooth-muscle strips established that such prostaglandin E_2 analogues also had effects characteristic of prostaglandin endoperoxides on these preparations (MacIntyre *et al.*, 1978), which suggests that further studies of this type could help to characterize the receptor sites by which prostaglandins stimulate many different cells.

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