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Substances that Increase the Cyclic AMP Content Prevent Platelet Aggregation and the Concurrent Release of Pharmacologically Active Substances Evoked by Arachidonic Acid

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Abstract

Arachidonic acid-induced platelet aggregation was inhibited by prostaglandins E_1 and $F_{2\alpha}$ (PGE_1 and $PGF_{2\alpha}$), papaverine and dibutyryl cyclic AMP. Prostaglandin E_2 displayed a biphasic effect, as concentrations below 2 μM potentiated aggregation, whereas concentrations above it were inhibitory. Isoproterenol (up to 10 mM) failed to block aggregation but inhibition was uncovered in presence of adrenergic α-blocking agents. Isoproterenol potentiated aggregation due to sub-threshold amounts of arachidonic acid, and this effect, but not that due to PGE_2, was suppressed by the α-blocking agents. Isoproterenol and PGE_2 appear thus to enhance arachidonic acid-induced platelet aggregation after interacting with different receptor sites. The yield of rabbit aorta contracting activity formed during AA-induced aggregation was markedly reduced by PGE_1, dibutyryl cyclic AMP and high concentrations of PGE_2, and was increased by low concentrations of the latter. PG-like activity was not significantly reduced when aggregation and generation of rabbit aorta contracting activity were inhibited by bibutyryl cyclic AMP. It is hypothesized that interaction of human platelets and arachidonic acid results in formation of different pharmacologically active materials, possibly bearing similar lipoperoxide structures. Generation of one portion of these materials is controlled by the adenyl cyclase-cyclic AMP system, whereas another portion, that comprises the natural PG, is cyclic AMP-independent. Prostaglandins formed during platelet aggregation have a regulatory role and modulate the platelet response, rather than constitute a trigger stimulus for aggregation.

Introduction

Release of histamine and of slow reacting substance A from different cells by antigens or chemical agents is modulated by the cyclic AMP system (for a review, see [1]). Prostaglandin E_1 (PGE_1) markedly increases production of cyclic AMP by adenyl cyclase, and inhibits platelet aggregation [2], including that due to the PG precursor arachidonic acid (AA) [3]. This observation lead us now to study the interference with AA-induced aggregation and with the accompanying release of potential inflammatory mediators, of various drugs known to affect the cyclic AMP system. Incubation of platelets with AA triggers the biosynthesis of PGs [4] and of what has been named rabbit aorta contracting substance (RCS), initially hypothesized to be a PG precursor [5, 6], as its generation was inhibited by those anti-inflammatory drugs (AID) that suppress PG formation [7–9]. Evidence has been provided that rabbit aorta contracting activity is not accounted for solely by the cyclic endoperoxide that preceeds PG's during their biosynthesis [9–11], as initially thought [5, 6], but includes as well other products, possibly formed during peroxidation of AA. Generation of these materials is reduced by catalase, pointing to a role for H_2O_2 or for a related organic peroxide for its production [12].

We now bring evidence that rabbit aorta contracting activity in incubates of platelet rich plasma (PRP) with AA is reduced by manoeuvres that increase cyclic AMP content or activity, whereas platelet aggregation is abolished. The cyclic AMP system modulates not only histamine or SRS-A release from leukocytes and mast cells, but influences as well the generation of potential inflammatory materials distinct from PG from platelets challenged with AA.

Materials and methods

Human blood provided by the Centre de Transfusion (Strasbourg) or collected from volunteers members of the laboratory was anticoagulated with $^1/_{10}$th volume of 4% sodium citrate and centrifuged at 200 g for 8 min-

utes. The upper plasma layer was collected and used as PRP. Aggregation was studied in a Bryston aggregometer with samples of 0.2 ml of PRP and 0.2 ml of apyrogenic 0.9% NaCl solution, thermostated at 37 °C and stirred at 1100 rpm [11]. The method was essentially that of BORN [13]. Release of pharmacologically active substances was studied by injecting incubates of PRP with AA into a stream of Krebs solution superfusing a rabbit aorta and a rat stomach strip. Appropriate inhibitors were added to Krebs solution [3, 14] to prevent interference by histamine or serotonin, possibly released from platelets. The drugs and sources were: arachidonic acid (AA) and isoproterenol hydrochloride, Sigma; prostaglandins E_1, E_2 and $F_{2\alpha}$, Cambrian Chemicals; papaverine hydrochloride, Bruneau; 6 N-2′-0-dibutyryl adenosine-3′,5′ cyclic monophospate (dbc AMP, dibutyryl cyclic AMP) and 8-bromo-cyclic 3′,5′-guanosine monophosphate (8-bromo-cyclic GMP), Boehringer Mannheim; phentolamine (Regitine), Ciba and dihydroergotamine tartrate (DHE), Sandoz. Prostaglandins and AA were initially dissolved to 5 mg/ml in 96° ethanol, further dilutions being performed with a 20 mg % solution of Na_2Co_3. Two concentrations of AA were used; the lower was chosen to allow potentiation of aggregation, and was the highest amount of AA that did not induce aggregation or induced it by less than 10%; it usually amounted to 0.1 m*M*. The higher concentration of AA was used to study inhibitors, and was the smallest concentration that induced a complete and spontaneously irreversible aggregation; it usually amounted to 0.5 m*M*.

Results

Twenty-nine (55.7%) out of 52 samples of PRP prepared from blood provided by the blood bank aggregated in response to AA, whereas 27 (93%) out of 29 samples collected from the volunteers responded positively. This difference was highly significant ($\chi^2 = 12.2$, $p < 0.001$). Whether the comparatively higher

Table 1
Inhibition of arachidonic acid-induced platelet aggregation by prostaglandins, papaverine and two nucleotides.

Inhibitor[1])	μM[2])	n[3])	% Inhibition ± S.E.M.[4])
PGE_1 (1 min)	0.05	10	97.80 ± 0.85**
	0.02	7	80.25 ± 11.65**
	0.01	6	52.54 ± 15.60**
	0.005	11	23.75 ± 6.90*
	0.002	5	15.60 ± 9.35
$PGF_{2\alpha}$ (1 min)	10	5	86.60 ± 12.20**
	5	7	39.65 ± 5.95**
	1	6	21.95 ± 7.10*
	0.5	6	12.60 ± 4.70*
	0.1	5	4.45 ± 2.60
PGE_2 (1 min)	10	5	87.45 ± 25.85**
	5	5	40.25 ± 9.25**
	2.5	4	5.95 ± 2.45
Papaverine (2 min)	10	6	90.40 ± 4.05**
	5	8	46.05 ± 13.55**
	1	6	5.60 ± 3.70
Dibutyryl cyclic AMP (5 min)	1000	5	95.80 ± 1.40**
	700	3	92.25 ± 2.00**
	500	4	79.45 ± 4.70**
	300	3	61.00 ± 8.25**
	200	3	26.50 ± 15.05
8 bromo cyclic GMP (2 min)	500	3	92.50 ± 2.15**
	100	3	58.40 ± 18.50*
	50	3	26.25 ± 14.10*
	10	4	3.20 ± 2.10

[1]) Duration of incubation with inhibitor in parenthesis. [2]) Concentration. [3]) Number of assays. [4]) % inhibition was calculated from the extent of aggregation, expressed in percent of light transmission as compared to that of platelet poor plasma adjusted to 100%. Velocity, which was affected similarly, is not given to simplify the table.
* = $p < 0.05$; ** = $p < 0.01$.