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ROLE OF PLATELETS IN ASPIRIN-SENSITIVE BRONCHOCONSTRICTION IN THE GUINEA-PIG; INTERACTIONS WITH SALICYLIC ACID

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1 The bronchoconstriction caused in the guinea-pig by arachidonic acid (AA), bradykinin, adenosine diphosphate (ADP) and adenosine triphosphate (ATP) was correlated with effects on platelets. ATP and ADP produced a brief thrombocytopenia and AA a more prolonged one. Bradykinin had no effect on platelets.

2 Aspirin inhibited bronchoconstriction and thrombocytopenia produced by AA and part of the bronchoconstriction produced by ATP, but had no effect against ADP. Thrombocytopenia produced by ADP and ATP was not affected by aspirin or indomethacin.

3 Platelet depletion by antiserum prevented bronchoconstriction in response to ADP and to ATP, but not in response to bradykinin or to AA, showing that platelets are not involved in aspirin-sensitive bronchoconstriction. Infusions of ADP reduced bronchoconstriction and thrombocytopenia in response to ADP itself and to ATP, but not to AA. Bronchoconstriction by ADP or ATP involves an action on platelets. Only that due to ATP is partially dependent on the activity of prostaglandin synthetase.

4 ATP induced aggregation *in vitro* in guinea-pig platelet-rich plasma (PRP). Rabbit PRP responded only when ATP was first incubated with guinea-pig plasma. The aggregating compound formed was probably ADP, since it was destroyed by apyrase. Its formation was not inhibited by aspirin or indomethacin, indicating that aspirin inhibits ATP-induced bronchoconstriction by a different mechanism.

5 The aggregating effect of ATP on guinea-pig platelets was inhibited by concentrations of apyrase that block ADP-induced aggregation, and potentiated by lower concentrations of apyrase.

6 Adenosine 5'-tetraphosphate did not aggregate platelets *in vivo* or *in vitro*. *In vitro* aggregation occurred when apyrase was added, suggesting transformation into ADP. Adenosine 5'-tetraphosphate and apyrase inhibited aggregation due to ADP, but failed to affect that due to AA. This suggests that aggregation involving products of prostaglandin synthesis does not require ADP.

7 Salicylic acid did not interfere with bronchoconstriction or aggregation due to AA, but prevented inhibition by aspirin when the weight ratio, salicylic acid:aspirin was 4:1. Salicylic acid may be useful in studies of potential inhibitors of thromboxane A2 synthesis and of thromboxane A2-dependent processes *in vivo* and *in vitro*.

Introduction

Several chemically unrelated substances such as bradykinin, arachidonic acid (AA), slow reacting substances A and C, and adenosine triphosphate (ATP), induce bronchoconstriction in the guinea-pig, which is inhibited by non-steroidal anti-inflammatory drugs (NSAID), possibly because a common mediator of bronchoconstriction is released (Collier, 1969). The discovery that bradykinin, anaphylaxis (Piper & Vane, 1969), slow reacting substance C and the prostaglandin precursor AA (Vargaftig & Dao, 1971) release a substance initially called rabbit aorta contracting substance (RCS) whose generation was inhibited by NSAID, led to the proposal that RCS might be such a common mediator. Most of the activity bioassayed as RCS is accounted for by thromboxane

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A2, formed from prostaglandin endoperoxides by thromboxane synthetase (Hamberg, Svensson & Samuelsson, 1975). Since NSAID inhibit platelet aggregation (Vargaftig & Zirinis, 1973; Willis & Kuhn, 1973) and the accompanying generation of thromboxane A2, we suggested that platelets might be the common site of action of those agonists which produce *in vivo* NSAID-sensitive brochoconstriction as well as the common source of thromboxane A2 (Vargaftig, 1974). The role of platelets in aspirin-sensitive bronchoconstriction and certain aspects of salicylic acid-aspirin interaction are here investigated.

An account of some of this work was given to the British Pharmacological Society in September 1975 and March 1977.

Methods

Bronchoconstriction in guinea-pigs anaesthetized with pentobarbitone (30 mg/kg, i.p.) was recorded on a Beckman Dynograph by a slight modification of the Konzett-Rössler method (Vargaftig, Coignet, de Vos, Grijsen & Bonta, 1971). Percentage inhibition was calculated by comparison of the extent of bronchoconstriction before and after a potential inhibitor. Animals were ventilated with a Starling miniature pump at 72 strokes/minute. Interference by released bronchodilator catecholamines was prevented by injection of propranolol at the beginning of the experiments (3 mg/kg i.p. and 1 mg/kg i.v.). In some experiments, muscular relaxation was ensured with gallamine (5 mg/kg). The carotid arteries were used for measurement of the arterial pressure and for the collection of blood. All injections were given intravenously unless otherwise stated.

Platelet aggregation was studied in 0.2 ml of platelet-rich plasma (PRP) prepared from citrated arterial blood by standard procedures and adjusted to 300,000 platelets/μl; 0.2 ml of 0.9% NaCl solution (saline) was added (Born, 1962). Aggregating agents and potential inhibitors were added in volumes not exceeding 50 μl. Platelet counts were carried out in a Coulter Counter or in a chamber.

Bioassays of citrated blood or PRP, incubated with AA were carried out on isolated superfused (Vane, 1964) rat stomach strips, rabbit aortic strips and, in a few experiments, rabbit coeliac or mesenteric artery strips (Bunting, Moncada & Vane, 1976). The superfusing Krebs solution contained antagonists of catecholamines, 5-hydroxytryptamine, histamine and acetylcholine (Vane, 1964; Vargaftig, Tranier & Chignard, 1974). Indomethacin (1 mg/ml) was added to prevent generation of prostaglandins by the tissues.

Antiplatelet plasma (APP)

Both fore paws of two adult rabbits were injected with 0.5 ml of saline-washed guinea-pig platelets (Vargaftig *et al.*, 1974) which had been lysed by 5 cycles of freezing and thawing, concentrated five times in saline and suspended in 0.25 ml of Freunds complete adjuvant. Booster injections of platelet suspension without adjuvant were given twice subcutaneously. After three weeks, blood was collected from the ear artery into sodium citrate. The plasma was separated, heated for 30 min at 56°C and lyophilized. Activity of reconstituted APP was checked by adding 10–50 μl aliquots to citrated guinea-pig PRP, and observing the platelet lysis in the aggregometer.

Drugs

The following drugs were used: arachidonic acid (grade 1, 99% pure, Sigma), dissolved as described (Vargaftig *et al.*, 1974); lysine acetylsalicylate (Aspegic, Laboratoire Egic), for *in vivo* treatment, doses being calculated as acetylsalicylic acid: aspirin (acetylsalicylic acid) and salicylic acid (Prolabo); ATP (purest available, Sigma and Boehringer); apyrase (ATPase activity, 2.2 units/mg protein), bradykinin triacetate, adenosine 5'-tetraphosphate (ATPP) (97–99% pure, grade 1), adenosine-N'-oxide and ADP, (Sigma); Freund's complete adjuvant (Difco); indomethacin (Merck, Sharpe and Dohme); sodium pentobarbitone (Lathevet); (±)-propranolol (Avlocardyl, ICI); bradykinin potentiating peptide (BPP 9α, courtesy of Dr L. J. Green, Ribeirão Preto, Brazil); gallamine triethiodide (Flaxedil, Specia); water-insoluble compounds were solubilized in polyethyleneglycol 300 (Merck Schuchardt) which had no effect on platelets when used below 25 μl/ml.

Results

In vivo *effects*

Arachidonic acid (50–500 mg/kg) produced bronchoconstriction (Figure 1a). Thrombocytopenia was present within 10 s and 1 min after doses of 250 μg/kg and above; a residual effect was still present after 6 min (Figure 1a). Bronchoconstriction and thrombocytopenia were reproducible, and in some cases they were even slightly increased upon repeated injections given at 30 min intervals. The blood pressure effect of AA was less predictable, since hypotension was frequently preceded by a short-lasting hypertensive burst. When hypertension was pronounced after the first injection of AA, subsequent injections frequently produced less hypertension or even hypotension.

Bronchoconstriction was induced by ATP (0.1–1.5 mg/kg) and by ADP (0.005–0.3 mg/kg) (Figures 1b, 1c and 2); even lower doses of ATP induced a brief fall in platelet counts (Figure 1b). The threshold doses of ADP were the same for both effects (Figure 1c).

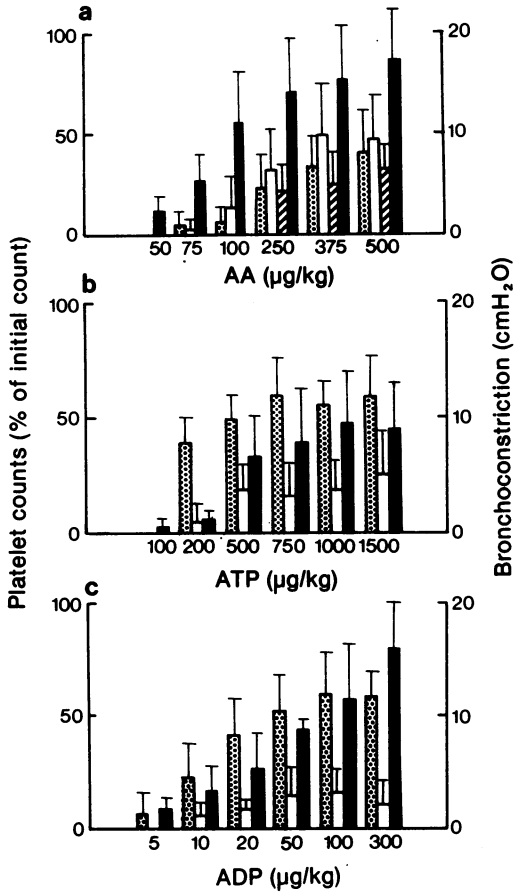

Figure 1 Dose-related thrombocytopenia and bronchoconstriction induced by arachidonic acid, ATP and ADP in guinea-pigs. (a) Arachidonic acid (AA), (b) ATP and (c) ADP were injected intravenously to guinea-pigs prepared for recording of bronchoconstriction and for platelet counts. Doses in μg/kg and times of blood sampling as indicated. Bronchoconstriction was measured at its height. Solid columns: bronchoconstriction; stippled column and open column: thrombocytopenia at 10 s and 1 min respectively; hatched columns: thrombocytopenia at 6 minutes.

Adenosine 5'-tetraphosphate (0.2–1 mg/kg) induced hypotension and bradycardia, in three animals, but no thrombocytopenia nor significant bronchoconstriction. Adenosine-N'-oxide (0.75 mg/kg) failed to induce bronchoconstriction or thrombocytopenia. Bradykinin (1–2 μg/kg) produced bronchoconstriction but no significant thrombocytopenia.

Aspirin (2–10 mg/kg) blocked the bronchoconstriction and thrombocytopenia induced by AA as well as the blood pressure effects (Table 1). When bronchoconstriction was only partially inhibited by the lowest dose of aspirin, thrombocytopenia was already inhibited completely. Aspirin caused only partial inhibition of the bronchoconstriction caused by ATP, whereas the thrombocytopenia and hypotension were unaffected (Table 1). Bronchoconstriction and thrombocytopenia due to ADP were not affected by aspirin up to 10 mg/kg or by 10 mg/kg of indomethacin, even when only 5 μg/kg of ADP was used.

In order to see whether the effects of AA were due to release of ADP from circulating platelets, and whether this might contribute to bronchoconstriction, guinea-pigs were infused with ADP at 1 mg kg^{-1} h^{-1} for one hour, and injected with ADP, ATP or AA after 30 minutes. In ten animals, the initial platelet counts were 470.200 ± 103.000 per μl, and 395.000 ± 78.000 per μl 30 min after starting the infusion ($P < 0.05$). The bronchoconstrictor effect of ATP was reduced in 5 animals by 55% ± 21% ($P < 0.001$), and of ADP in 7 animals by 84 ± 10% ($P < 0.001$). ADP (200 μg/kg), reduced platelet counts by 66.3 ± 15% within 10 s before the ADP infusion, and after the infusion by 33.6 ± 16.3% ($P < 0.01$). The equivalent values for ATP (500 μg/kg) were 64.2 ± 18% and 30 ± 19% ($P < 0.02$). In contrast, the bronchoconstriction and thrombocytopenia induced by AA (375 μg/kg), were not significantly affected by the infusion of ADP. In four animals, the thrombocytopenic effect was 45.5 ± 19% before ADP, and 40.5 ± 13% after ADP. Bronchoconstriction was inhibited by less than 8% and sometimes enhanced.

Effects of antiplatelet plasma (APP)

APP had to be administered very slowly to avoid bronchoconstriction, hypotension and death of the guinea-pigs. When 1 ml/kg was infused over a 1 h period to 30 animals all survived, and showed a platelet count of 13500 ± 9200, as compared to 454500 ± 85500 before infusion. Platelet depletion suppressed bronchoconstriction due to ADP and to ATP, but not the effects of bradykinin and of AA (Table 2; Figure 2). Since interaction of AA with platelets was so marked, it was possible that platelet lysis in blood by APP would not remove membrane-bound prostaglandin-synthesizing ability. Blood was thus collected from guinea-pigs before, treated with APP and then incubated with AA. Bioassays confirmed that APP abolished the ability to generate thromboxane A2 and prostaglandin-like activities.

Platelet aggregation in vitro

AA in final concentrations of 10–50 μM induced aggregation in guinea-pig PRP, which was inhibited by aspirin (50–100 μM, 5 min incubation), and by indomethacin (5–20 μM, 1 min incubation). Addition of apyrase 1 mg/ml before AA, or after aggregation was

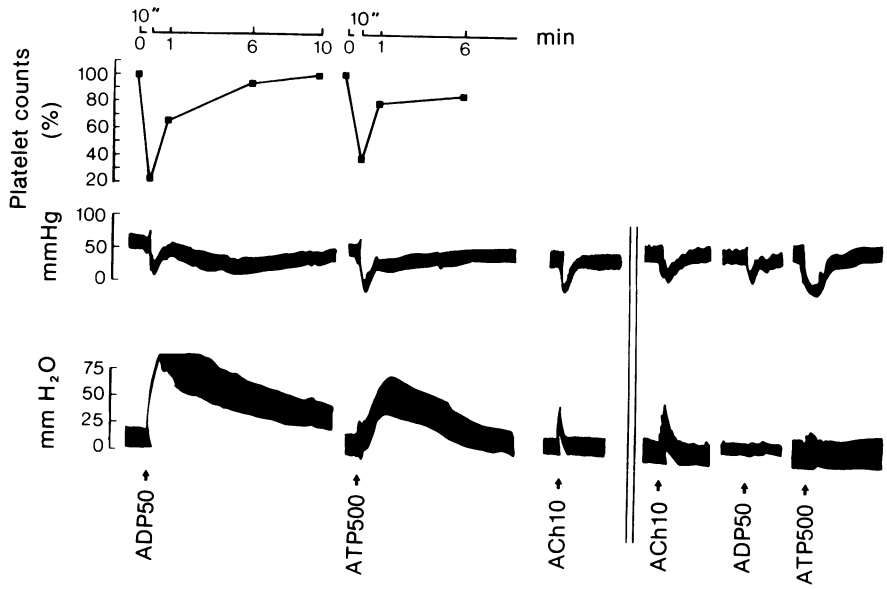

Figure 2 Suppression by antiplatelet plasma of bronchoconstriction due to ADP and to ATP. Platelet counts (% initial value), arterial pressure (mmHg) and bronchial resistance to inflation (mmH_2O) of a guinea-pig injected with ADP 50 μg/kg and ATP 500 μg/kg before (left panel) and after (right panel) anti-platelet treatment. Acetylcholine (ACh, 10 μg/kg) was given as a control bronchoconstrictor.

Table 1 Effect of aspirin on bronchoconstriction and thrombocytopenia induced by ATP, ADP and arachidonic acid

	ATP (0.75 mg/kg)			*ADP* (0.1 mg/kg)			*Arachidonic acid* (0.5 mg/kg)		
Aspirin (mg/kg)	n	*Broncho-constriction*[1]	*Thrombo-cytopenia*[2]	n	*Broncho-constriction*[1]	*Thrombo-cytopenia*[2]	n	*Broncho-constriction*[1]	*Thrombo-cytopenia*[2]
0	27	100	56.5 ± 12	59	100	59.3 ± 19	26	100	37.8 ± 15
2	10	67.1 ± 39	52.3 ± 14	8	77.5 ± 31	62.7 ± 14	5	11.3 ± 8	0
5		—	—	—	—	—	5	0	0
20	10	29.5 ± 24	58 ± 12	8	95.5 ± 10	62.9 ± 13	5	0	0

n = number of animals; [1]bronchoconstriction induced by ATP, ADP or arachidonic acid before injection of aspirin was adjusted to 100%; [2] % thrombocytopenia 10 s after ATP and ADP, and 1 min after arachidonic acid.

Table 2 Effect of antiplatelet plasma on bronchoconstriction induced by intravenous arachidonic acid, Bradykinin, *ADP* and *ATP*

Agonists	n	*Dose* (μg/kg)	% *bronchoconstriction* ± *s.d. after antiplatelet treatment*[1]	P
Arachidonic acid	5	375	97.8 ± 2.5	NS
	6	500	91.2 ± 2.5	NS
Bradykinin	6	1	97.0 ± 6	NS
ADP	10	100	11.2 ± 15	<0.001
ATP	5	750	15.6 ± 13	<0.001

n = number of animals; [1] as % of bronchoconstriction before antiplatelet treatment.

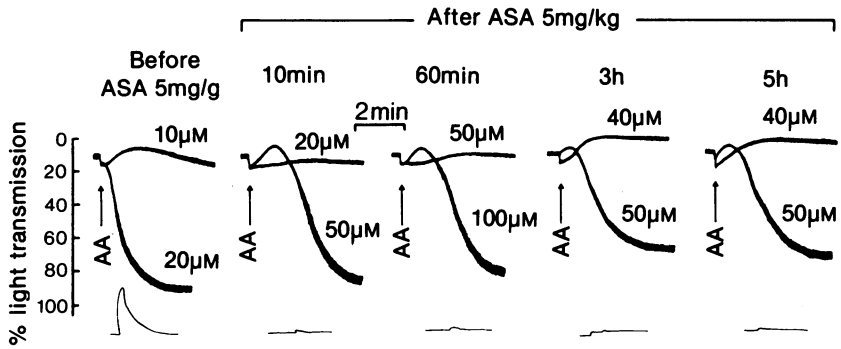

Figure 3 Effect of aspirin on aggregation of guinea-pig platelets and on generation of thromboxane A2 activity. Superimposed tracings of aggregation of guinea-pig platelets prepared from blood collected before (extreme left panel) and at indicated intervals after aspirin (ASA, 5 mg/kg i.v.). The threshold dose of arachidonic acid (AA) for aggregation was 20 μM before aspirin and 50–100 μM at various intervals afterwards. Lower tracing: contractions of rabbit aorta induced by PRP incubated with AA 0.1 mM for 2 minutes. Time: 2 minutes.

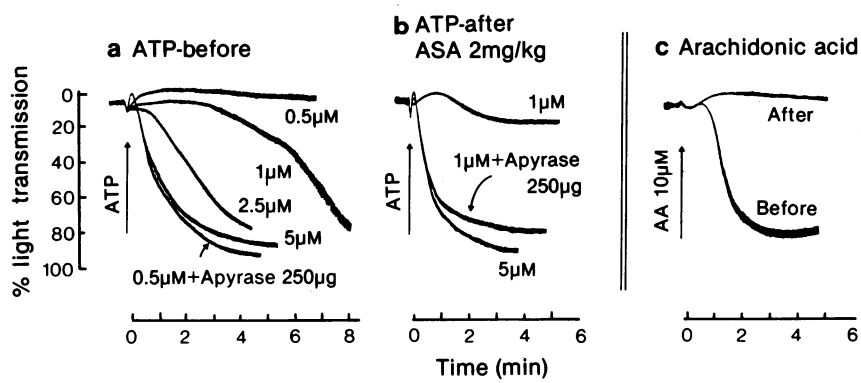

Figure 4 Effect of aspirin on aggregation of guinea-pig platelets by ATP and by arachidonic acid. Aggregation of guinea-pig platelets by 0.5, 1.0, 2.5 and 5 μM ATP (final concentrations). PRP was prepared from blood collected before (a) and after (b) aspirin (ASA) administration (2 mg/kg). Whereas 1 μM ATP aggregated platelets collected before aspirin, 5 μM ATP was required afterwards. In both instances, 250 μg/ml of apyrase allowed sub-threshold amounts of ATP to trigger aggregation. Aggregation by arachidonic acid (c) was inhibited after aspirin 2 mg/kg.

completed (approx. 1 min later), failed to prevent or to reverse the aggregation. Addition of bradykinin (up to 20 μg/ml) in the presence of up to 250 μg/ml of BPP 9α had no effect on platelets. Thromboxane A2 and prostaglandin-like activities were found in incubates of PRP with AA, but not of PRP with bradykinin.

When ADP (0.05–1 μM)-aggregated guinea-pig platelets were tested, the typical double wave was inhibited by indomethacin (0.05 μM) and by aspirin (25–50 μM). Aggregation by AA, and the double wave due to ADP did not occur in PRP from aspirin-treated animals (see below) (Figure 3). Apyrase (1 mg/ml or more) prevented or reversed the aggregation by ADP. The speed, but not the extent of the aggregation induced by ATP 1–5 μM was dose-dependent (Figure 4). Aggregation by the low concentrations of ATP was accelerated by apyrase 0.01–1 mg/ml, whereas 2–4 mg/ml failed to potentiate sub-threshold amounts of ATP and inhibited higher amounts. Next, the possibility was investigated that ATP might act indirectly, after transformation into ADP. Rabbit PRP was used because rabbit platelets are not aggregated by ATP. Guinea-pig plasma was incubated with ATP and 10–20 μl aliquots mixed with rabbit PRP. Guinea-pig plasma transformed ATP into a substance that aggregated rabbit platelets. This generation was the same when plasma was collected

after aspirin treatment (20 mg/kg), or when either aspirin or indomethacin (1 mM) was added 5 min before ATP. The active substance was destroyed by apyrase. When ADP was incubated with guinea-pig plasma for different times and then assayed on rabbit platelets at final concentrations of 0.5–5 μM, 100% of 0.5 μM ADP and less than 50% of 5 μM ADP were destroyed in 30 min (Figure 5).

Adenosine 5'-tetraphosphate (ATTP), which had no platelet-aggregating effect up to 1 mM, became active in concentrations as low as 0.01 mM when added together with apyrase 100 μg/ml or more. Aggregation was also triggered when apyrase was added up to 5 min after ATTP. Aggregation by ADP was inhibited by 86% by 0.1 mM ATTP and by 58% by 0.05 mM; 0.01 mM ATTP was inactive. Aggregation by AA was not inhibited by 0.1 mM ATTP, and reduced by 37% by 1 mM.

Action of aspirin and of salicylic acid on the effects of arachidonic acid

Five animals were injected with aspirin (5 mg/kg i.v.) and blood was collected after 10 min, 1, 3 and 5 hours. PRP was prepared and incubated with AA and in two animals, with ATP as well. Generation of thromboxane A2 and of prostaglandin-like activities as well as aggregation, were inhibited. In two out of the five animals, increments in the added amounts of AA reversed inhibition (Figure 3) but in the other three, 20–50 fold increments failed to induce aggregation. Aggregation by ATP was also blocked but inhibition was surmounted by larger amounts of ATP. Inhibition was abolished when apyrase (100–500 μg/ml) was added together with ATP.

Salicylic acid (up to 200 mg/kg) had no effect on the bronchoconstriction or aggregation caused by AA, but prevented the inhibition by aspirin. This required a salicylic acid:aspirin weight ratio of 4. The following doses of salicylic acid (in mg/kg) were injected intravenously: 5, to three animals, 10 to four animals, 20 to four animals, 50 to three animals, and 200 to two animals. Thirty min later all 16 animals were injected with 5 mg/kg of aspirin. PRP was prepared from blood collected before and after salicylic acid, and 10 min after aspirin. The threshold concentration of AA for aggregation was 35 ± 15 μM both before and after administration of salicylic acid. Aggregation by up to 0.5 mM AA was inhibited in

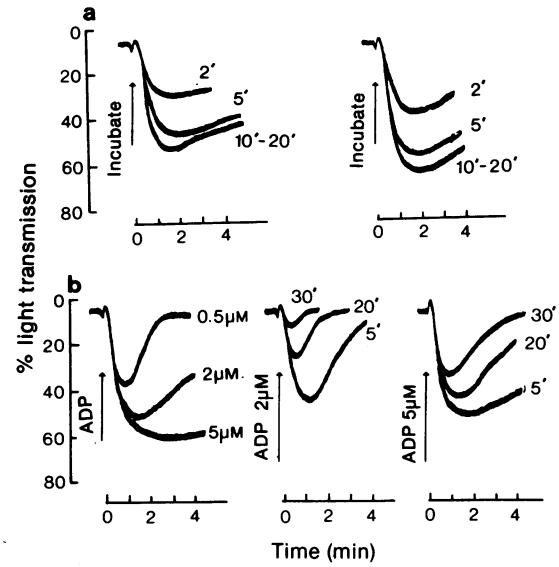

Figure 5 Conversion of ATP into a substance aggregating rabbit platelets. (a) Aggregation of rabbit platelets induced by incubates of guinea-pig plasma with ATP, tested at the times indicated. Indomethacin (1 mM) did not modify generation of aggregating activity (right hand tracings). Final concentration of ATP equivalents in rabbit platelet-rich plasma (PRP), was 5 μM. (b) Effects of incubates of guinea-pig plasma and ADP, added at the indicated concentrations to rabbit PRP immediately (left panel) and after different intervals (middle and right hand tracings).

Table 3 Interference of salicylic acid with aspirin-induced inhibition of bronchoconstriction and thrombocytopenia due to arachidonic acid (AA)

Group	Injections of arachidonic acid	Thrombocytopenia (% ± s.d.) 10 s	1 min	Bronchoconstriction (mmH_2O ± s.d.)
I	Before	38 ± 15	32 ± 9	18.7 ± 2
	After	0	0	0
II	Before	46 ± 20	43 ± 17	20.4 ± 6
	After	44 ± 16	26 ± 11	21.4 ± 4.6

Groups of five guinea-pigs were injected with AA (0.5 mg/kg) before and after aspirin (5 mg/kg) in group I, or with salicylic acid (200 mg/kg), followed after 30 min by aspirin (5 mg/kg), in group II.

PRP from the animals treated with the 1:1 drug ratio; the 2:1 ratio inhibited in two animals aggregation by 0.5 mM AA but did not counteract 0.1 mM AA in two other animals. When the ratio was 4:1, all samples aggregated, but the required concentration of AA was 125 ± μM, i.e., significantly above the threshold before drug administration ($P < 0.02$). Finally, when the drug ratio was 10:1 or 40:1, the thresholds for aggregation by AA were the same, demonstrating that salicylic acid fully antagonized the inhibition by aspirin.

A similar salicylic acid–aspirin antagonism was observed *in vivo*, since both bronchoconstriction and thrombocytopenia induced by AA were unaffected by aspirin if the latter was preceded by salicylic acid (Table. 3).

Discussion

AA induced thrombocytopenia which seemed to depend on cyclo-oxygenase, since it was inhibited by low doses of aspirin or indomethacin. However, platelet-depleted animals. Finally, bronchoconstric- by AA. Bradykinin had no effect on platelets *in vivo* or *in vitro*, and produced bronchoconstriction in platelet-depleted animals. Finally, bronchoconstriction by ADP and by ATP was accompanied by a marked short-lasting thrombocytopenia, and was completely suppressed by platelet depletion. Accordingly, a continuous infusion of ADP prevented bronchoconstriction by ADP and by ATP, but not that due to AA or to bradykinin.

Thromboxane A2 is released from isolated lungs injected with bradykinin (Piper & Vane, 1969) or with AA (Vargaftig & Dao, 1971). These lungs are probably free from platelets since thrombin fails to induce bronchoconstriction or to release thromboxane A2 (unpublished observations). Thus bradykinin triggers the release of, and AA is transformed into, bronchoconstrictor prostaglandin endoperoxides and thromboxane A2 (Piper & Vane, 1969; Vargaftig & Dao, 1971; Hamberg & Samuelsson, 1974), in lung tissue itself. This release may account for the platelet-independent effect *in vivo*. In contrast, ATP and ADP fail to release thromboxane A2 from isolated lungs (Vargaftig & Dao, 1971), and required platelets for *in vivo* bronchoconstriction.

The secondary wave of aggregation of guinea-pig platelets by ADP is accompanied by a release reaction. Both are inhibited by NSAID, whereas the primary aggregating effect of ADP is unaffected. Bronchoconstriction and thrombocytopenia by ADP occurred although aspirin completely inhibited the ability of platelets to form thromboxane A2; bronchoconstriction (but not thrombocytopenia) due to ATP was partly inhibited. This might suggest that aspirin interferes with conversion of ATP into ADP, which would also explain the inhibition of the effects of ATP and the resistance of those of ADP. However, although ATP was indeed transformed into an aggregating compound, probably ADP, by guinea-pig plasma, neither aspirin nor indomethacin interfered with this conversion. It is conceivable that ADP formed from ATP *in vivo*, is responsible for bronchoconstriction involving a platelet-dependent and prostaglandin-independent mechanism. Mechanical obstruction by microthrombi induces bronchoconstriction in the guinea-pig (Daly, 1974), and may thus explain the aspirin-resistant but platelet-dependent bronchoconstriction by ADP.

Apyrase transforms ATP into ADP, and less potently ADP into AMP (Molnar & Lorand, 1961). This explains why apyrase (500 μg/ml) inhibited aggregation by ATP and by ADP, whereas lower concentrations promoted aggregation by otherwise non-aggregating concentrations of ATP. Moreover, apyrase 20–50 μg/ml restored the inhibited aggregation in PRP prepared from aspirin-treated guinea-pigs, probably by forming an overwhelming amount of ADP within seconds. Similar results have been obtained with rat platelets (Ts'ao, 1976). ATTP failed to induce aggregation of guinea-pig platelets or bronchoconstriction, and was thus not transformed into an aggregating substance unless apyrase was added. Like other adenosine derivatives, ATTP induced hypotension and bradycardia. Inhibition of ADP-induced aggregation by ATTP is probably competitive, as recorded also in other species (Harrison & Brossmer, 1976).

In contrast to rabbit platelets, aggregation of guinea-pig platelets by AA was unaffected by amounts of apyrase or of ATTP which inhibited ADP-induced aggregation. This is in agreement with the concept that platelet aggregation by thromboxane A2 is not mediated by ADP, although the possibility that intracellular rather than extracellular release of ADP determines whether platelets will or will not aggregate remains open (Vargaftig, 1977b).

Salicylic acid failed to inhibit the effects of AA mediated by cyclo-oxygenase and prevented the inhibitory action of aspirin on platelet aggregation and bronchoconstriction *in vivo* and *in vitro*. Salicylic acid also prevents inhibition by aspirin of AA-induced hypotension in rabbits (Vargaftig & Lefort, 1977; Vargaftig, 1977a), inhibits the acetylation of human albumin by aspirin (Pinkard, Hawkins, & Farr, 1970) and may interfere with the binding of aspirin to a component of the platelet membrane, thus preventing its acetylation (Rosenberg, Gimber-Phillips, Groblewski, Davison, Phillips, Goralnick & Cahill, 1971; Roth, Stanford, & Majerus, 1975). Interference between salicylic acid and aspirin, or between various NSAID has been described (Van Arman, Nuss & Ris-

ley, 1973; Ezer, Palosi, Hajos & Szporny, 1976), but this is the first time a cyclo-oxygenase site of action has been shown, both *in vivo* and *in vitro*. Bronchoconstriction by AA in the guinea-pig can be used to confirm *in vivo* conclusions on anti-inflammatory agents drawn from *in vitro* work. Studies with other inhibitors of thromboxane A2 generation and/or activity should clarify how specific is the antagonism by salicylic acid of aspirin-induced inhibition, and consequently whether the *in vivo* guinea-pig model can be used to discriminate between sites of action of drugs.

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