J. Pharm. Pharmac., 1977, **29**, 222–228 *Received October* 18, 1976

Carrageenan and thrombin trigger prostaglandin synthetase-independent aggregation of rabbit platelets: inhibition by phospholipase A_2 inhibitors*

BERNARDO B. VARGAFTIG

Centre de Recherche Merrell International, 16, rue d'Ankara, 67084-Strasbourg Cedex, France

Acetylsalicylic acid, salicylic acid and indomethacin were equally effective in inhibiting aggregation of plasma-free rabbit platelets induced by carrageenan and by thrombin. In contrast, only acetylsalicylic acid and indomethacin suppressed the accompanying formation of prostaglandin-like activities. Blockade of aggregation required the presence of the inhibitors in the platelet preparation, whereas blockade of prostaglandin synthetase remained even when the inhibitors were washed out. Prostaglandin synthetase-derived products appear not to be involved in the development of aggregation by carrageenan or by thrombin. Such aggregation was inhibited by two phospholipase A_2 inhibitors, bromophenacyl bromide and mepacrine. It is suggested that carrageenan and thrombin-induced aggregation are mediated by non-prostaglandin, phospholipase A_2-derived products.

Aggregation of washed human platelets induced by high concentrations of thrombin is accompanied by generation of prostaglandins (PGs). The latter is inhibited by non-steroidal anti-inflammatory drugs (NSAID), whereas neither aggregation nor release of platelet enzymes are suppressed (Smith & Willis, 1971). This implies that formation of PGs accompanies thrombin-induced aggregation but does not cause it. Aggregation by the PG precursor arachidonic acid is also suppressed by NSAID (Ingerman, Smith & others, 1973; Vargaftig & Zirinis, 1973; Willis & Kuhn, 1973). The latter agents inhibit the formation of an unstable 'rabbit aorta contracting substance' (RCS) in arachidonic acid-platelet incubates. It has been suggested that RCS, now identified as a mixture of PG endoperoxides that preceded natural PGs during their biosynthesis and of the non-prostanoid derivative thromboxane A_2 (Nugteren & Hazelhof, 1973; Hamberg, Svensson & Samuelsson, 1974), is responsible for aggregation by arachidonic acid (Vargaftig & Zirinis, 1973; Willis & Kuhn, 1973), by collagen (Vargaftig & Zirinis, 1973) or by thrombin (O'Brien, 1968). This 'single pathway' theory for aggregation due to different agonists that share some responsiveness to NSAID can be criticized on the basis that only threshold concentrations of collagen are completely inhibited by aspirin while inhibition declines for higher amounts (O'Brien, 1968; Gordon & MacIntyre, 1974). In contrast, inhibition by aspirin or by indomethacin of aggregation due to arachidonic acid is unsurmountable. The correlation between aggregation and PG formation, by comparison of aggregation of plasma-free rabbit platelets by thrombin and by the pro-inflammatory polysaccharide carrageenan in the presence of potential antagonists, has been examined. The role of the PG synthetase pathway for aggregation by carrageenan and by thrombin was investigated using NSAID to inhibit generation of PGs, and phospholipase A_2 inhibitors to suppress availability of unsaturated fatty acids, including arachidonic acid.

* Part of this work was presented at the joint meeting of the British and French Pharmacological Societies, Oxford, September, 1976.

METHODS AND MATERIALS

Aggregation of rabbit platelets in citrated platelet-rich plasma or of washed platelets resuspended in Tyrode solution (Tyrode-platelets) (Vargaftig, Tranier & Chignard, 1974) was recorded turbidimetrically (Born, 1962). Technical details were previously described (Vargaftig, Tranier & Chignard, 1975). Platelet incubates were bioassayed on the following superfused tissues: a rabbit aorta strip to detect RCS (Piper & Vane, 1969), a rat stomach strip to detect PG-like activity (Piper & Vane, 1969) and a rabbit coeliac artery. The last tissue has been claimed to respond specifically to thromboxane A_2 (Bunting, Moncada & Vane, 1976). Assays were at 37°, since at room temperature carrageenan had no aggregating activity, nor triggered release of pharmacologically active substances, in contrast to effectiveness of ADP and of arachidonic acid. Potential inhibitors of aggregation or of release of substances were added to 0·4 ml platelet suspensions, in a volume of 10 μl, 1 min before adding the aggregating

agent, unless otherwise stated. Drugs were dissolved either in 0·9% (w/v) NaCl or in polyethyleneglycol, in which case controls were run with the solvent alone.

Drugs were as follows: carrageenan (Viscarin, Marine Colloids); acetylsalicylic acid (Rhône Poulenc); salicylic acid (Prolabo); indomethacin (Merck, Sharpe & Dohme); apyrase, 5-hydroxytryptamine, arachidonic acid (dissolved as described by Vargaftig & others, 1974), *p*-tosyl-1-arginine methyl ester; *N*-ethylmaleimide and phenylmethylsulfonylfluoride (Sigma); bovine thrombin (Roche); ethylenediaminetetracetic acid, disodium salt (EDTA) and ethyleneglycol bis (amino-2 ethylether) tetracetic acid (EGTA) (Prolabo); adenosine (Fluka); prostaglandin E_1 (PGE_1) (Ono); heparin (Choay); reserpine (Ciba); mepacrine (Specia); bromophenacyl bromide (Koch-Light); polyethyleneglycol 300 (Merck Schuchardt); 6*N*-2′-*O*-dibutyryl adenosine-3′5′ cyclic monophosphate (dibutyryl AMP) (Boehringer Mannheim).

RESULTS

Effects of carrageenan on platelets

The lowest concentration of carrageenan that induced a 25% aggregation of platelet-rich plasma in 5 min or less (threshold concentration, determined according to intensity of aggregation) was 100 μg ml^{-1} or below in 65 samples out of 97, and above in the remaining (up to 1·25 mg ml^{-1} in 9). In 11 samples the threshold was 12·5 μg ml^{-1} and provided a scattered mean (with s.d.) of 83 (160) μg ml^{-1} for the threshold. In contrast, when washed platelets were used the threshold was reduced to 14 (9·6) μg ml^{-1}. The threshold for thrombin was of 0·005 (0·01 U ml^{-1}) and the tracings obtained resembled those due to carrageenan. Both were inhibited by EGTA and by EDTA (2–5 mM), by PGE_1 (0·5–2 μM), by adenosine (0·5–1 mM), by the ADP scavenger apyrase (1–4 mg ml^{-1}), and by dibutyryl AMP (0·5–1 mM). These antagonists reversed aggregation when added after its completion. Heparin (20 U ml^{-1}) and tosyl-1-arginine methyl ester (0·5–1 mM) inhibited aggregation due to thrombin, but failed to interfere when induced by carrageenan.

The three isolated tissues were contracted by incubates of platelet-rich plasma with carrageenan (250–500 μg ml^{-1}) (Fig. 1). The smooth muscle-contracting activity was present 2–3 min after addition of carrageenan, and best seen at 4 min, remaining stable for at least 30 min. Despite the presence of standard anti-5HT agents in the super-

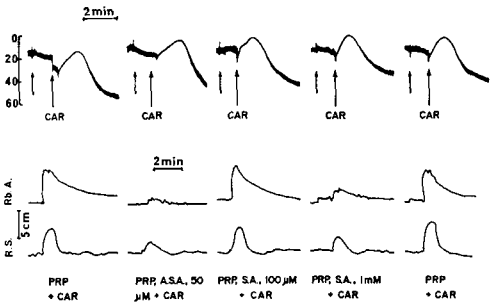

FIG. 1. Interference of aspirin and of salicylic acid with the effects of carrageenan on rabbit platelets. Platelet-rich plasma was stirred at 1100 rev min^{-1} with a metal bar, placed in the bottom of an aggregometre cuvette, and aggregation recorded after addition of 200 μg of carrageenan (CAR) (at the arrow) (upper panel). The indicated concentrations of aspirin or of salicylic acid were added to platelet-rich plasma 1 min before carrageenan, in 10 μl of polyethyleneglycol. This solvent was added before carrageenan in control samples. 4 min later, the incubates were bioassayed (lower panel) on two superfused isolated tissues, Rb.A.—rabbit aorta strip, R.S.—rat stomach strip. 1 mM of salicylic acid was required to inhibit generation of smooth muscle-contracting activity only partly, whereas 0·05 mM of aspirin was sufficient to suppress most activity. Vertical scale for aggregation indicates % light transmission. Horizontal scale—Time (min); vertical scale—cm of pen deflection.

fusing Krebs solution (Piper & Vane, 1969), this suggested involvement of 5-HT, particularly since each sample was calculated to contain 1–2 μg of 5-HT (1–1·5 $\times$ 10^8 platelets) (Maupin, 1967), and since 5-HT contracted the tissues. The coeliac artery was particularly resistant to anti-5-HT treatment. Interference of 5-HT was avoided by using platelet-rich plasma and Tyrode-platelets prepared from reserpinized rabbits (3 mg kg^{-1}, s.c., 20 h beforehand, Baumgartner, 1969), which aggregated to similar amounts of carrageenan or of thrombin as did control platelets. The aorta and coeliac artery-contracting activities obtained in these incubates were labile and decayed to nil within 4–12 min at room temperature. In contrast, the rat stomach-contracting activity decayed by only 50% during the same interval, indicating presence of labile PG endoperoxides and thromboxanes (vessel contracting) and of resulting natural PGs (stomach contracting, stable). Aggregation was not required for the generation of smooth-muscle contracting activity, since omission of the metal bar used to stir the plasma-rich plasma—and thus lack of the platelet-to-platelet interaction required for aggregation—increased the yield of smooth muscle-contracting activity compared with stirred samples.

Table 1. *Inhibition by non-steroidal anti-inflammatory drugs and by potential antagonists of phospholipase A_2 of release of smooth muscle-contracting activities in incubates of platelet-rich plasma and carrageenan*.*

Inhibitor mM		% inhibition of contractions (s.d.) of Coeliac artery		Aorta		Stomach	
Indomethacin							
0·00001	(4)	22	(23)	23	(15)	12	(8)
0·0001	(4)	52	(4)	45	(10)	23	(10)
0·001	(4)	92	(6)	97	(7)	77	(16)
Salicylic acid							
0·1	(3)	4	(4)	20	(19)	4	(6)
0·5	(5)	29	(15)	37	(20)	15	(11)
1	(5)	70	(16)	81	(20)	38	(14)
Aspirin							
0·01	(5)	17	(14)	21	(13)	14	(8)
0·05	(3)	63	(35)	66	(31)	34	(14)
0·1	(5)	93	(10)	97	(7)	76	(18)
Mepacrine							
0·005	(3)	26	(14)	27	(10)	15	(7)
0·01	(3)	57	(22)	62	(21)	43	(11)
0·02	(3)	95	(7)	—		85	(13)
Bromophenacyl bromide							
0·005	(4)	+87	(52)	+99	(63)	+41	(29)
0·01	(4)	+65	(69)	+91	(61)	+21	(25)
0·1	(3)	34	(29)	62	(31)	48	(20)
0·2	(3)	92	(7)	100		94	(10)

* Platelet-rich plasma (0·2 ml) collected from a reserpinized rabbit was mixed with NaCl 0·9% (w/v) (0·2 ml), and incubated for 4 min with 25–100 μg of carrageenan, in presence of drug solvents or of the indicated amounts of potential inhibitors. Stirring was omitted. Number of separate assays is shown in parentheses.

Interference by anti-inflammatory drugs

Aspirin, indomethacin and salicylic acid delayed aggregation by carrageenan in platelet-rich plasma and inhibited it when threshold concentrations of the aggregating agent were used. Inhibitory concentrations of aspirin and of salicylic acid were similar, which contrasts with their widely different effectiveness against PG synthetase (Vane, 1971; Vargaftig & Zirinis, 1973). In contrast, rabbit aorta contracting and PG-like activities were suppressed by 0·01–0·1 mM aspirin, and by 0·005–0·01 mM indomethacin, even though these concentrations failed to inhibit aggregation. Salicylic acid reduced the generation of activities only when added at 1 mM (Fig. 1). Inhibition by NSAID was also seen when stirring was omitted, effective concentrations falling to 1 μM for indomethacin and to 0·01–0·05 mM for aspirin (Table 1).

Table 2. *Inhibition by non-steroidal anti-inflammatory drugs of aggregation of washed rabbit platelets induced by carrageenan.*

Antagonist concn (mM)	n*	% Inhibition (s.d.)† in Tyrode-platelets	
Indomethacin			
1	4	84	(3)
0·5	10	72	(22)
0·1	9	48	(26)
0·01	4	8	(13)
Aspirin			
5	2	100	
1	7	79	(17)
0·5	5	71	(29)
0·1	5	23	(31)
Salicylic acid			
5	3	100	
1	5	92	(16)
0·5	4	50	(16)

* n = number of separate assays.

† Inhibition of speed of aggregation as compared to control assays with drug solvent and carrageenan. Speed was calculated for the first 30 s of linear aggregation.

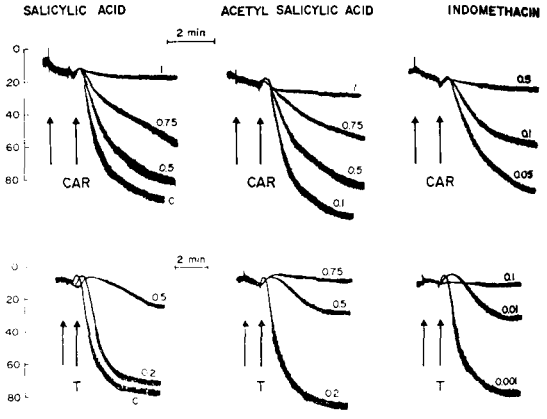

FIG. 2. Inhibition by salicylic acid, by acetylsalicylic acid and by indomethacin of aggregation of Tyrode-suspended platelets by carrageenan and by thrombin. Washed rabbit platelets suspended in Tyrode solution were challenged with 5 μg ml^{-1} of carrageenan (CAR, upper panel), or with thrombin (T, 0·012 U ml^{-1}), lower panel), in the aggregometer. Potential inhibitors dissolved in polyethyleneglycol were added 1 min before the aggregating agent, at final mM concentrations indicated on the figure. A control aggregation test, (C) in the presence of 10 μl of polyethyleneglycol, was run every 2–3 cycles but is only shown twice for simplicity. Salicylic acid and aspirin were equiactive against aggregation by carrageenan and by thrombin, indomethacin was more active, although below its anti-PG synthetase potency. Scales as in Fig 1.

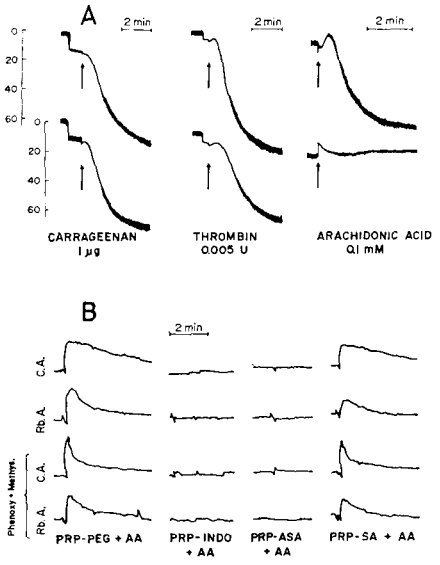

FIG. 3. Failure of pre-incubation of rabbit platelets with aspirin, followed by resuspension in drug-free plasma, to prevent aggregation by carrageenan, as compared to effectiveness against arachidonic acid. Platelet-rich plasma was incubated for 10 min at 20° with 1 mM (final concentration) of aspirin or salicylic acid, and then washed thoroughly (Vargaftig & others, 1974), and resuspended either in Tyrode solution or in drug-free autologous citrated plasma. Panel A shows the aggregation tracings. In the first two columns, the upper tracing was obtained after addition of carrageenan or of thrombin to control (untreated) Tyrode platelets, whereas the lower tracing was obtained after similar additions to aspirin-treated platelets. Only a minor increase in the delay required for aggregation by carrageenan and by thrombin was observed. In contrast, aggregation by arachidonic acid (last column, on the right) of aspirin-treated platelets resuspended in drug-free plasma was inhibited (lower tracing), as compared to washed platelets incubated only with the drug solvent and resuspended in plasma. Aspirin irreversibly blocked PG synthetase, which did not influence thrombin or carrageenan-induced aggregation noticeably. This was confirmed in the experiment shown in panel B, where similar incubates of platelets, treated for 10 min with indomethacin (INDO), aspirin (ASA) or salicylic acid (SA), were resuspended in drug-free plasma, and incubated with arachidonic acid (AA) for two min, before being bioassayed on two parallel batches of a coeliac artery (C.A.) and of a rabbit aorta (Rb.A.) strip each. In this experiment, the Krebs solution superfusing one batch of tissues, did not contain phenoxybenzamine nor methysergide, and 5-HT contracted the tissues at 10–20 ng (not shown). To Krebs solution superfusing the other pair of isolated tissues, phenoxybenzamine and methysergide were infused, at amounts calculated to reconstitute their usual concentration (Vane, 1964). Platelet-rich plasma was prepared from a reserpinized rabbit, reducing even more the possibility of 5-HT interference. This was confirmed by comparing the responses of both pairs of tissues. Pre-treatment of platelets with indomethacin and with aspirin suppressed the generation of vessel contracting substances (RCS activity, mixture of prostaglandin endoperoxides and of thromboxane A_2), whereas salicylic acid failed to do so, although, as seen in Figs 2 and 3, these antagonists are equiactive against carrageenan-induced aggregation. Scales for aggregation as in Fig. 1.

Neither aggregation by carrageenan nor its inhibition was due to interaction with plasma factors, since Tyrode-platelets aggregated to carrageenan and to thrombin. This aggregation was inhibited by equimolar concentrations of aspirin, indomethacin or salicylic acid (Table 2 and Fig. 2). Further evidence that blockade of aggregation was unrelated to the ability to inhibit PG synthetase was provided by experiments in which aspirin was incubated for 5–10 min with platelet-rich plasma or with Tyrode-platelets and then challenged with carrageenan or with thrombin (the latter only in Tyrode-platelets). The concentration of aspirin used (0·1–0·5 mM) inhibited carrageenan or thrombin-induced aggregation partially when added for one min, but effectiveness was not increased by prolonging incubation up to 10 min. This contrasts with arachidonic acid-induced aggregation, which is highly time-dependent, a partial inhibition after 1 min incubation being converted to full inhibition within 5–10 min.

In another series of experiments platelet-rich plasma was incubated with 1 mM aspirin or salicylic acid, or 0·5 mM indomethacin. Platelets were washed and half were resuspended in drug-free plasma, the other half in Tyrode solution. No aggregation or release of RCS or of PG-like activities were obtained in aspirin or indomethacin pre-treated platelets resuspended in plasma, upon challenge with arachidonic acid, whereas platelets incubated with salicylic acid reacted to arachidonic acid as did controls. In contrast, aggregation by threshold amounts of carrageenan or of thrombin was only slightly retarded or unaffected by treatment with any of the drugs (Fig. 3).

Interference of the phospholipase A_2 inhibitor bromophenacyl bromide, and of other reagents with generation of activities and with platelet aggregation

If PG synthetase has a role in carrageenan or thrombin-induced aggregation, one might expect blockers of phospholipase A_2 to be effective inhibitors, since this enzyme provides free arachidonic acid for conversion to PGs, after it has been hydrolysed from phospholipids (Schoene & Iacono, 1975). One of such potential inhibitors, mepacrine (Vargaftig & Dao, 1972; Flower & Blackwell, 1976)

nhibited aggregation by carrageenan when used at 0·01–0·05 mM, but since it displays direct anti-aggregating effects as well (anti-ADP), it is debatable whether its activity is specific or not. Bromophenacyl bromide, an 'active site' inhibitor of phospholipase A_2 (Volwerk, Pieterson & De Haas, 1974) suppressed aggregation by carrageenan or by thrombin of Tyrode-platelets, when added at 5–10 μM one min before either of them (Table 3 and

Table 3. *Inhibition by bromophenacyl bromide of aggregation of washed rabbit platelets induced by carrageenan and by thrombin.*

Final concn of bromophenacyl bromide (μM)	% Inhibition of velocity (s.d.) of aggregation due to Carrageenan		n	Thrombin		n
1	19·6	(20)	5	6		2
2·5	38	(5·3)	3		—	
5	69	(24)	4	31·5	(26)	4
7·5	100		2	81·6	(9·5)	3
10	100		2	90·6	(9)	3

n = Number of separate assays.

Fig. 4). Aggregation of platelet-rich plasma by arachidonic acid or by ADP was inhibited by 50–100 μM of bromophenacyl bromide. Those concentrations had no suppressive activity on PG synthetase as seen in the bioassay with arachidonic acid. Generation of PG-like activities in carrageenan-platelet-rich plasma incubates showed a biphasic pattern, whether stirring took place or not: at 5–10 μM bromophenyl bromide potentiated the yield of smooth muscle-contracting substances, and above 50 μM generation was inhibited (Table 1). Mepacrine did not potentiate generation of pharmacological activities.

Addition of EDTA (5 mM), of *N*-ethylmaleimide (0·1 mM) and of phenylmethylsulphonylfluoride (2 mM) to platelet-rich plasma before carrageenan, in the absence of stirring and thus of notable aggregation, resulted in suppression of the formation of PG-like and RCS activities.

DISCUSSION

Activation of phospholipase A_2 or of an equivalent enzyme is a necessary step for providing PG synthetase with its substrate for PG formation. Our findings are consistent with phospholipase A_2-activation by carrageenan and by thrombin, with consequent release of RCS and PG-like activities. Nevertheless, since inhibition by NSAID of formation of these substances was not paralleled by

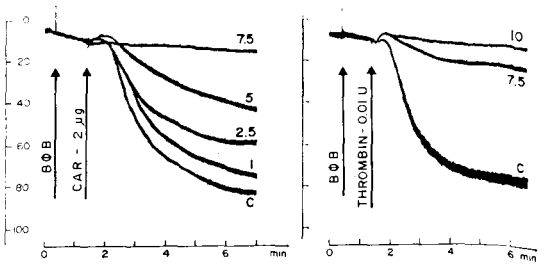

FIG. 4. Inhibition by bromophenacyl bromide of aggregation of Tyrode-suspended platelets by carrageenan and by thrombin. Washed Tyrode-suspended platelets were aggregated by the indicated concentrations of carrageenan or of thrombin, in presence of bromophenacyl bromide (BϕB) at the indicated final μM concentrations. Tracings are superimposed, and show the ability of BϕB to inhibit aggregation in a concentration-related fashion. Scales as in Fig. 1.

inhibition of aggregation, whereas that due to arachidonic acid was fully suppressed, it seems that bromophenacyl bromide and mepacrine do not act only by preventing PG formation. In fact, it is logical to assume that activation of phospholipase A_2, besides providing substrates for PG synthetase, release other fatty acids, that induce direct or ADP-dependent aggregation (Fig. 5), possibly after peroxidation (Okuma, Steiner & Baldini, 1971). The role of PG synthetase for aggregation of rabbit platelets by thrombin or by carrageenan thus appears as minor, and the possibility that phospholipase A_2-derived products participate in aggregation is highlighted. The mechanisms responsible for inhibition by NSAID of aggregation due to arachidonic acid, on one side, and to thrombin or to carrageenan, on the other, are different, particularly since salicylic acid was as active as aspirin against the two latter, although it is ineffective against arachidonic acid (Vargaftig & Zirinis, 1973). Finally, the time-course of inhibition of carrageenan-induced aggregation by aspirin was also different from that against arachidonic acid. The mechanism of inhibition by NSAID of carrageenan-induced aggregation was not solved, and may involve interference with Ca^{2+}, as found for inhibition of histamine release from mast cells (Whittle, 1976).

Inhibition of carrageenan and thrombin-mediated aggregation by Ca^{2+} chelating agents, by *N*-ethylmaleimide, adenosine, phenylmethylsulphonylfluoride, dibutyryl cAMP and PGE_1, indicates a true aggregation, and not just particle agglutination (Zucker, 1972, 1974). Calcium is needed for aggregation by most agents (Mustard & Packham, 1970), is not required for PG synthetase activity, but is

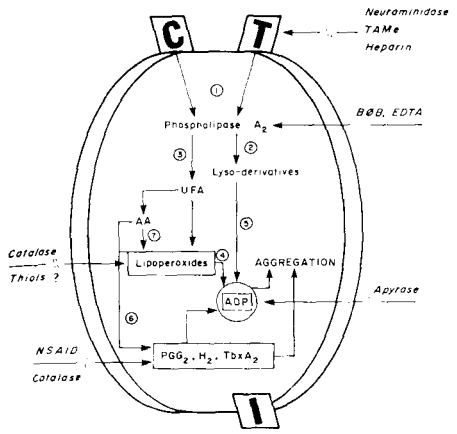

FIG. 5. Hypothesized mechanism for aggregation by carrageenan and by thrombin of washed rabbit platelets. Receptors for thrombin and for carrageenan on the platelet surface are indicated by T and C, respectively. Addition of either triggers activation of phospholipase A_2 (pathway 1, in circle) which can be inhibited by bromophenacyl bromide (BϕB, inhibition being indicated by interrupted arrows). Phospholipase A_2 releases unsaturated fatty acids (UFA), among which are arachidonic acid (AA), and lyso-derivatives (pathways 2 and 3). Lyso-derivatives and lipoperoxides formed from UFA trigger release of ADP from dense granules leading to aggregation (pathways 4 and 5). Non-steroidal anti-inflammatory drugs interfere at least at two sites: on pathway 6, which is PG synthetase-dependent, but also elsewhere, since they exhibit inhibitory properties unexplained by inhibition of PG synthetase. Box labelled I represents inhibitory mechanisms, which exert control over some pathways (ADP antagonism, cyclic AMP effects, lipoperoxide scavenging by endogenous catalase and/or glutathion peroxidase etc.). Neuraminidase and thrombin inhibitors (heparin, tosyl arginine methyl ester) prevent the effects of thrombin. In contrast, neuraminidase potentiates the effects of carrageenan (Vargaftig, to be published).

essential for phospholipase A_2 (Hanahan, 1971). Effectiveness of EDTA and of EGTA in suppressing generation of pharmacological activities in carrageenan-platelet-rich plasma incubates even though aggregation was avoided by lack of stirring, suggests that carrageenan triggers the release of active substances by acting at least one step before precursors are set free for further conversion by PG synthetase and similar enzyme systems.

It is unclear whether or not the labile vessel-contracting substances which were bioassayed actually cause, or only accompany aggregation. The fact that the yield of these substances was increased in carrageenan-platelet-rich plasma incubates when stirring was omitted, and thus aggregation avoided, may indicate a dissociation between aggregation and generation of mediators, or it may simply result from the fact that aggregation is absent by lack of platelet-to-platelet contact when no stirring is ensured. This suggests that during aggregation the activity formed by carrageenan-stimulated platelets is partially neutralized. A plausible hypothesis is that platelet catalase and/or glutathione peroxidase interfere at this stage as a mechanism ensuring peroxide scavenging (Okuma & others, 1971), particularly since catalase, which has been shown to inhibit aggregation by arachidonic acid (Vargaftig & others, 1975) was also active against aggregation and release of mediators by carrageenan and by thrombin (to be published; see Fig. 5).

In conclusion, we found that carrageenan aggregates rabbit platelets, in presence or in absence of plasma, and that aggregation in the latter case resembles aggregation by thrombin (Fig. 5). Both are mostly PG-synthetase independent, and blocked by a phospholipase A_2 inhibitor. The pattern of inhibition of carrageenan-induced platelet aggregation strongly suggests participation of labile PG-related and unrelated lipoperoxides, thus providing new sites for interference with aggregation and its consequences.

Acknowledgements

The competent technical assistance of Mrs M. L. Part, J. Kintz, C. Berg and of Miss M. Haegeli, is gratefully acknowledged.

REFERENCES

BAUMGARTNER, H. R. (1969). *J. Physiol., Lond.*, **201**, 409–423.

BORN, G. V. R. (1962). *Ibid.*, **162**, 67P.

BUNTING, S., MONCADA, S. & VANE, J. R. (1976). *Br. J. Pharmac.*, **57**, 462P–463P.

FLOWER, R. J. & BLACKWELL, G. J. (1976). *Biochem. Pharmac.*, **25**, 285–291.

GORDON, J. L. & MACINTYRE, D. E. (1974). *Br. J. Pharmac.*, **50**, 469P.

HAMBERG, M., SVENSSON, J. & SAMUELSSON, B. (1974). *Proc. natn. Acad. Sci. U.S.A.*, **71**, 3824–3828.

HANAHAN, D. J. (1971). *The Enzymes*, pp. 71–85, New York: Academic Press.

INGERMAN, C., SMITH, J. B., KOCSIS, J. J. & SILVER, M. J. (1973). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **32**(I), 219 abs.

MAUPIN, B. (1967). *Biol. Med.*, **65**, 143–191.

MUSTARD, J. F. & PACKHAM, M. A. (1970). *Pharmac. Rev.*, **22**, 97–187.
NUGTEREN, D. H. & HAZELHOF, E. (1973). *Biochim. biophys. Acta*, **326**, 448–461.
O'BRIEN, J. R. (1968). *Lancet*, 1968i, 779.
OKUMA, M., STEINER, M. & BALDINI, M. (1971). *J. Lab. clin. Med.*, **77**, 728–742.
PIPER, P. J. & VANE, J. R. (1969). *Nature*, **223**, 29–35.
SCHOENE, N. W. & IACONO, J. M. (1975). *Fedn Proc. Fedn Am. Socs exp. Biol.*, **34**, 257 abs.
SMITH, J. B. & WILLIS, A. L. (1971). *Nature New Biol.*, **231**, 235–237.
VANE, J. R. (1964). *Br. J. Pharmac. Chemother.*, **23**, 360–373.
VANE, J. R. (1971). *Nature New Biol.*, **231**, 232–235.
VARGAFTIG, B. B. & DAO, N. (1972). *J. Pharm. Pharmac.*, **24**, 159–161.
VARGAFTIG, B. B. & ZIRINIS, P. (1973). *Nature New Biol.*, **244**, 114–116.
VARGAFTIG, B. B., TRANIER, Y. & CHIGNARD, M. (1974). *Prostaglandins*, **8**, 133–156.
VARGAFTIG, B. B., TRANIER, Y. & CHIGNARD, M. (1975). *Eur. J. Pharmac.*, **33**, 19–29.
VOLWERK, J. J., PIETERSON, W. A. & DE HAAS, G. H. (1974). *Biochemistry, N.Y.*, **13**, 1446–1454.
WHITTLE, B. J. R. (1976). *Br. J. Pharmac.*, **58**, 446P.
WILLIS, A. L. & KUHN, D. C. (1973). *Prostaglandins*, **4**, 127–130.
ZUCKER, M. B. (1972). *Thromb. Diath. haemorrh.*, **28**, 393–407.
ZUCKER, M. B. (1974). *The Inflammatory Process*, pp. 511–543. New York: Academic Press.