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INHIBITION BY NON-STEROID ANTI-INFLAMMATORY AGENTS OF RABBIT AORTA CONTRACTING ACTIVITY GENERATED IN BLOOD BY SLOW REACTING SUBSTANCE C

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1 A crude and a partially purified preparation of slow reacting substance C (SRS-C) as well as arachidonic acid decreased resistance to perfusion of the dog hind paw. This effect was suppressed by treatment with non-steroid anti-inflammatory drugs.

2 Injections of SRS-C or of arachidonic acid induced marked and reproducible contractions of strips of rabbit aorta and a rat stomach which were bathed in blood from an anaesthetized dog. The effect on the rabbit aorta is attributed to formation of a rabbit aorta contracting substance (RCS). The contractions were suppressed when the dog was treated with a non-steroid anti-inflammatory drug.

3 Incubation of blood or of platelet-rich plasma with SRS-C or arachidonic acid resulted in the formation of similar materials. This formation was suppressed by anti-inflammatory drugs.

4 SRS-C, linoleic, linolenic, and arachidonic acids are suitable substrates for soybean lipoxidase for the generation of RCS.

5 It is suggested that RCS and prostaglandin are formed within platelets, when SRS-C or arachidonic acid are injected into animals or added *in vitro*. Non-steroid anti-inflammatory drugs suppress these effects, possibly by inhibiting prostaglandin synthetase.

Introduction

Injections of slow reacting substance C (SRS-C) or of arachidonic acid to guinea-pig perfused lungs are followed by the release of a rabbit aorta contracting substance (RCS) (Vargaftig & Dao, 1971a). This release is suppressed by previous perfusion of the lungs with non-steroid anti-inflammatory drugs which have been shown to inhibit the release of RCS during *in vitro* anaphylaxis (Piper & Vane, 1969). The biosynthesis of prostaglandins is also inhibited by non-steroid anti-inflammatory agents (Vane, 1971; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971). It has been suggested that RCS is an intermediate in the biosynthesis of prostaglandin from arachidonic acid (Gryglewski & Vane, 1972; Vargaftig & Dao Hai, 1972). The hypotensive and bronchoconstrictor effects of SRS-C are also inhibited by non-steroid anti-inflammatory drugs (Vargaftig, Miranda & Lacoume, 1969). These effects of SRS-C were attributed to the release of an unidentified mediator in the circulation of the animals (Vargaftig & Dao Hai, 1972). The purpose of the present work was to investigate this suggestion. Contact of SRS-C with blood generates a substance that contracts rabbit aortic strip, rat stomach strip and rat isolated colon. Non-steroid anti-inflammatory agents blocked the generation of this factor. This substance shares a common property with lipid hydroperoxides in that it contracts rabbit aortic strip similarly to incubates of linoleic, linolenic and arachidonic acid with lipoxidase. A preliminary account of these results was given to the British Microcirculation Society (Ferreira & Vargaftig, 1973).

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Methods

Vascular resistance in the dog hind paw

In heparinized dogs (100 u/kg) anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.) the femoral flow was kept constant with a Watson Marlow pump supplying blood from a carotid artery. Perfusion pressure was measured by a Statham transducer placed between the output of the pump and the artery. Flow was initially adjusted to give a mean pressure similar to the systemic pressure. SRS-C and fatty acids were injected directly in the blood supplying the perfused paw, whereas the anti-inflammatory drugs were given by the jugular vein.

Biological assays

The following tissues were superfused as described by Vane (1964): spiral strips of rabbit aorta (Piper & Vane, 1969), rat stomach strips (Vane, 1957) and rat colon (Regoli & Vane, 1964). The samples and standards were injected into the tubing supplying Krebs solution (10 ml/min, 38° C) to the tissues. In some experiments an extra length of silicone tubing was added (incubating circuit) to extend by 2 min the time taken for the infused substances to reach the assay tissues. The Krebs solution contained a mixture of antagonists: atropine or hyoscine hydrochloride (0.1 μg/ml), phenoxybenzamine hydrochloride (0.1 μg/ml), propranolol hydrochloride (2 μg/ml), methysergide bimaleate (0.2 μg/ml) and mepyramine maleate (2 μg/ml) (Gilmore, Vane & Wyllie, 1968). Indomethacin (1 μg/ml) was also added to prevent generation of prostaglandins by the assay tissues. Contractions of the assay tissues were detected with auxotonic levers attached to Harvard smooth muscle transducers. The overall amplification of the system was four to eight fold.

Generation of rabbit aorta contracting substance in circulating blood

The blood bathed organ technique (Vane, 1964) was used with the same three assay tissues in order to test appearance of RCS activity in circulating blood. Anaesthetized dogs (pentobarbitone, 30 mg/kg i.v.) were heparinized (1000 u/kg) and blood was taken from a femoral artery and pumped at a rate of 10 ml/min over the assay tissues. Blood returned to the animal by gravity via the femoral vein. SRS-C and arachidonic acid were injected into the blood superfusing the tissues. The substances injected were in contact with the blood for about 5 s before reaching the assay organs. Anti-inflammatory drugs were injected intravenously.

Generation of rabbit aorta contracting substance in blood and in blood fractions

Heparinized (50 u/ml) or citrated (see below) blood (20-40 ml) was collected from anaesthetized dogs (pentobarbitone, 30 mg/kg) in plastic tubes and kept at room temperature. Samples of blood (1-2 ml) were incubated at room temperature with equal volumes of the alcoholic extract of SRS-C or of the solutions of unsaturated fatty acids, to a final concentration of 25-100 μg/ml for arachidonic acid, and up to 800 μg/ml for linolenic and linoleic acids. Crude SRS-C (0.1-0.5 ml) was also mixed with 0.5 ml of blood. Non-steroid anti-inflammatory agents were added to blood (1-30 μg/ml) 1-2 min prior to the addition of fatty acids or SRS-C. For the preparation of blood fractions, 45 ml of venous blood was collected from a dog into 5 ml of sodium citrate (3.8% w/v). Platelet-rich plasma (PRP) was separated by centrifugation at 150 *g* at 20° C for 10 min in a Sorvall RC2-B centrifuge. The erythrocytes were separated from the white cells and resuspended in plasma or saline (0.9% w/v NaCl solution) at equal volumes. These blood fractions were incubated with SRS-C or arachidonic acid as described for blood (see above).

Generation of rabbit aorta contracting substance by lipoxidase

Two different incubation procedures were used:

(a) Soybean lipoxidase was infused into the incubating circuit to give a final concentration of 100 μg/ml, alone or together with one of the several unsaturated fatty acids or SRS-C.

(b) Soybean lipoxidase was incubated with the unsaturated fatty acids at 0° C and pH 9. The substrate (8 mg) was converted into the ammonium salt by addition of ammonium hydroxide (0.019 M, 2 ml). Lipoxidase (10 mg) was dissolved in 1 ml of a sodium borate buffer (0.1 M, pH 9). The reaction was started by addition of 2 ml of the enzyme solution to 2 ml of the substrate. After 15 to 30 min additional 2 ml aliquots of enzyme solution were added. After 1 h 1 N HCl was added to bring the pH to 7.5 and an equal volume of a solution containing twice the normal concentration of Krebs salts was added to the incubation mixture. Control solutions, one of them without the substrate, were prepared in the same way. With a similar procedure, complete conversion of the fatty acids to hydroperoxides occurs at the end of the incubation period (Hamberg & Samuelsson, 1967). The concentrations of hydroperoxide are expressed in terms of the initial concentrations of the fatty acids in the

incubation mixtures. Such mixtures were kept up to 14 days at -20° C, without apparent loss of activity. RCS activity was also obtained by incubating a 1 : 10 dilution in Krebs solution of an alcoholic extract of SRS-C (see below) with lipoxidase (100 μg/ml) for 3 h at 37° C.

Preparations of slow reacting substance C

The three different preparations of SRS-C were obtained as follows:

Crude slow reacting substance C. Crude SRS-C was prepared by incubating fresh egg yolk emulsions (40% v/v in saline) with 1-10 μg/ml of *Naja nigricollis* venom, at 37° C. One to four hours were allowed for the completion of the reaction between phospholipase A2 (phosphatide acylhydrolase, E.C. 3.1.1.4) contained in the venom and the egg yolk phospholipids. This reaction mixture was used after neutralization of the remaining venom by *Naja nigricollis* antiserum (1 ml to neutralize 500 mg of venom, as recommended by the supplier).

Alcoholic extract. Crude SRS-C was obtained from 100 ml of egg yolk emulsified with 125 ml of saline and mixed with 20 μg/ml of *Naja nigricollis* venom. After a 4 h incubation at 37° C the proteins were precipitated with 5 volumes of ethanol. The supernatant was filtered twice and then evaporated under reduced pressure. The residue was resuspended in 6 ml ethanol followed by 14 ml saline. Dilutions in Tyrode solution (10% v/v) were prepared at the beginning of the experiment.

Partially purified slow reacting substance C. Partially purified SRS-C was obtained by incubating egg yolk in ether with *Naja nigricollis* venom as described by Vogt (1957). The final product contains only the unsaturated fatty acids split from lecithin by phospholipase A2.

Drugs

The following drugs were used: linoleic and linolenic acids (BDH Chemicals Ltd.); arachidonic acid (Sigma London Chemical Ltd. London); soybean lipoxidase (Sigma); dihomo-γ-linolenic acid (gift from Upjohn U.S.A.); indomethacin (Merck, Sharp & Dohme); phenylbutazone (Midy); aspirin as a lysine salt (Aspegic, Laboratoire Egic, France); *Naja nigricollis* antiserum (Institut Pasteur, Paris); *Naja nigricollis* venom (Hans Hartge, Hamburg, Germany); prostaglandins E_2 and $F_{2\alpha}$ (Cambrian Chemicals); methysergide bimaleate (Sandoz); mepyramine bimaleate (Rhone Poulenc); atropine sulphate (Laboratoires Bruneau).

Results

Vasodilator effects

Injections of arachidonic acid and SRS-C evoked a drop in the vascular resistance of the dog paw. This drop amounted to 33% ± 8 (mean ± s.d.) and to 26% ± 9 (mean ± s.d.) of the basal resistance, for 500 μg arachidonic acid and 0.5 ml crude SRS-C respectively (see methods section). Similar effects were only obtained with 2.5 mg of dihomo-γ-linolenic acid. Non-steroid anti-inflammatory drugs prevented the effects of arachidonic acid and of SRS-C and partially those of dihomo-γ-linolenic acid but not those of acetylcholine or prostaglandin E_2. Figure 1 illustrates the abolition of the effects of SRS-C and of arachidonic acid by 0.5 mg/kg indomethacin. Table 1 shows that the inhibitory effects of indomethacin and of phenylbutazone are dose-dependent. Low doses of the antagonists were more effective against SRS-C than against arachidonic acid. Methysergide (0.5 mg/kg), mepyramine (5 mg/kg) and atropine (0.3 mg/kg) did not affect vasodilation due to SRS-C or to arachidonic acid.

Generation of rabbit aorta contracting substance in circulating blood

Agonists were initially tested on the isolated organs superfused with Krebs solution before the circulating blood was connected. Crude SRS-C, up to 0.5 ml, had no effect upon the resting tone of the isolated organs, whereas arachidonic acid (50-100 μg) contracted the rabbit aorta, and the rat stomach strip, but not the rat colon. Prostaglandin $F_{2\alpha}$ in a few preparations at the high doses used (0.2 μg-0.5 μg) induced small contractions of the rabbit aorta preparation. When the tissues were superfused with blood, the resting tone increased and responsiveness of the rat stomach to prostaglandins decreased, but the rat colon responded as under Krebs solution. Arachidonic acid (5-20 μg), crude SRS-C (0.2-0.5 ml) and partially purified SRS-C (2-5 mg) when injected into the blood bathing the tissues, consistently induced marked contractions of the rabbit aorta, rat stomach and colon. Intravenous injections of phenylbutazone (1-5 mg/kg, 6 experiments), indomethacin (0.2-1 mg/kg, 4 experiments) and aspirin (5 mg/kg, 2 experiments) prevented the effects of SRS-C and of arachidonic acid, but did not interfere with those of prostaglandins (Figure 2).

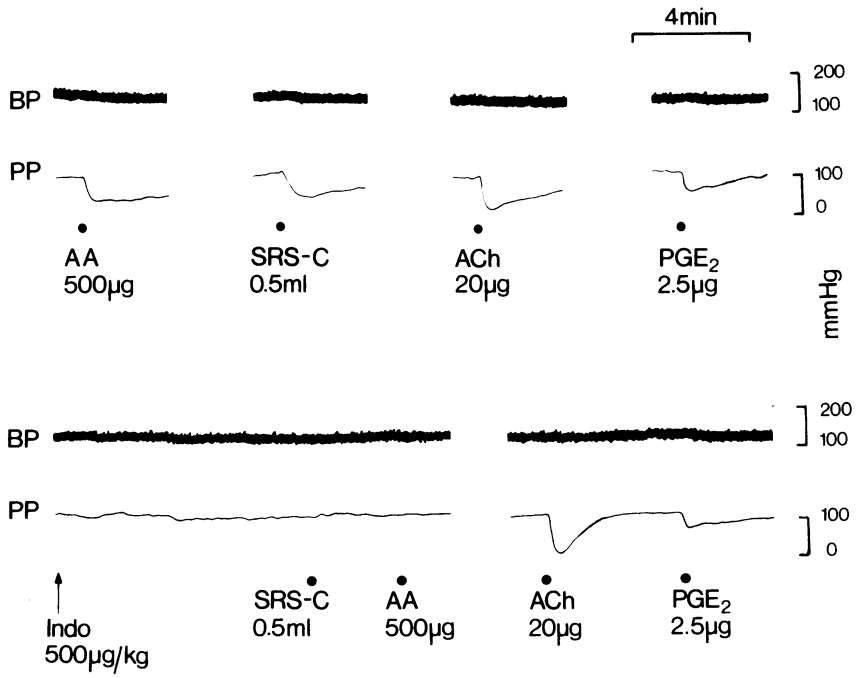

Fig. 1 Inhibition by indomethacin (Indo) of the vasodilator effects of slow reacting substance C (SRS-C) and of arachidonic acid (male dog, 12 kg). Arachidonic acid (AA) and SRS-C, acetylcholine (ACh) and prostaglandin E_2 (PGE_2) were injected into the blood perfusing the paw. The intravenous injection of indomethacin suppressed the effects of SRS-C and of arachidonic acid without interfering with those of acetylcholine and prostaglandin E_2. BP, arterial blood pressure in mmHg; PP, perfusion pressure of the dog's paw.

Table 1 Inhibition by phenylbutazone and indomethacin of the drop in vascular resistance induced in the dog hind paw by arachidonic acid (AA) and slow reacting substance C (SRS-C)

Drug	*mg/kg*	*% Drop in vascular resistance of perfused dog hind paw induced by:*					
		AA			*SRS-C*		
		n	*Control*	*After treatment*	n	*Control*	*After treatment*
Phenylbutazone	0.25	1	30	29	3	33 (26-28)	3 (0-10)
	0.50	3	31* (22-44)	9 (0-17)	4	24 (23-44)	0 (0)
	1	4	33 (27-42)	0 (0)	2	26 (26)	0 (0)
Indomethacin	0.25	2	42.5 (35-50)	22 (11-31)	4	33 (22-40)	15 (0-26)
	0.5	6	34 (22-40)	15.5 (0-31)	6	25 (13-44)	0 (0)

* Range in brackets.

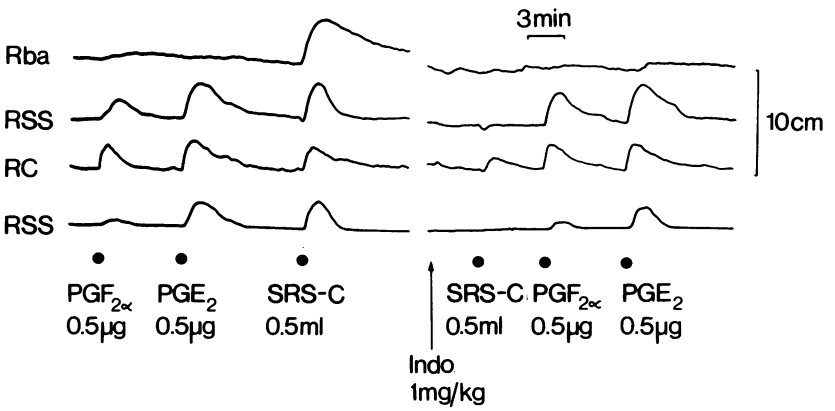

Fig. 2 Interference of indomethacin (Indo) with the generation of rabbit aorta contracting substance in the blood of a dog by slow reacting substance C (SRS-C). Assay tissues were superfused with 10 ml/min of blood from the femoral artery: substances injected remained in contact with blood for about 5 s before reaching the tissues. Crude SRS-C induced contractions of all preparations. Indomethacin (1 mg/kg, intravenously) prevented the effects of SRS-C but did not modify the effects of prostaglandins $F_{2\alpha}$ and E_2. Vertical scale: 10 cm of pen displacement. RbA, rabbit aorta spiral strip; RSS, rat stomach strip; RC, rat colon.

In vitro *generation of rabbit aorta contracting substance from blood*

Despite the use of doses of SRS-C and of arachidonic acid that did not contract the assay tissues superfused by Krebs solution, there was the possibility that when blood superfused the isolated organs the threshold for contraction could change, due to increased oxygen tension in blood. To exclude the possibility that generation of substances was occurring in the isolated tissues, samples of blood from donor dogs were mixed with SRS-C or with arachidonic acid at room temperature for 1-2 min, and then assayed on isolated organs superfused with Krebs solution containing the described mixture of antagonists, as well as indomethacin. Blood alone (up to 0.5 ml) had no effects, but after incubation with either crude SRS-C alcoholic extract or partially purified SRS-C, the blood induced contractions similar to those obtained in circulating blood. It was noticeable that doses of the incubation mixtures giving equal contractions of the rat stomach strips did not contract the rabbit aorta equally and that the mixture with SRS-C usually was less active on the rabbit aorta (Figure 3). This indicates that the materials generated from arachidonic acid and from SRS-C on incubation with blood are not identical. Prolonged incubation of SRS-C (8 experiments) or arachidonic acid (3 experiments) with blood (up to 60 min) was followed by a marked loss of activity (more than 50%) upon rabbit aortic strips. However, on the rat stomach

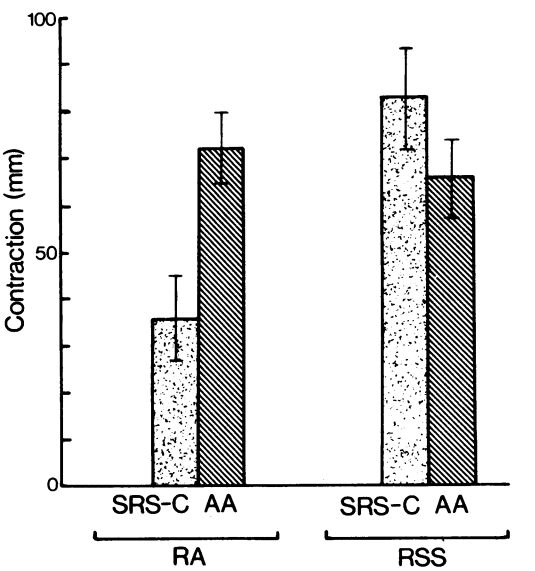

Fig. 3 Generation of contractor activity by incubating blood with slow reacting substance C (SRS-C) or arachidonic acid. Blood (0.5 ml) was incubated with the alcoholic extract of SRS-C (0.5 ml) or with arachidonic acid (AA) (final concentration, 25 μg/ml). Aliquots of 0.5 ml of the incubation mixture were tested on the assay tissues. RA, rabbit aortic strips; RSS, rat stomach strips. The columns show the results of five experiments and the bars are the standard error of the mean.

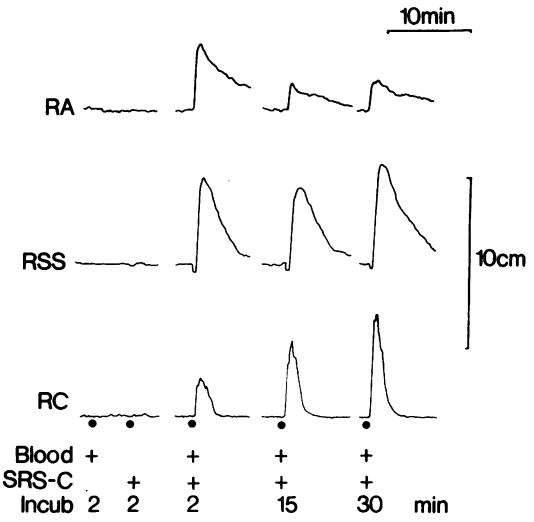

Fig. 4 Time course of generation of rabbit aorta contracting substance in blood by slow reacting substance C (SRS-C). Blood (2 ml) was incubated with 2 ml of the ethanol extract of SRS-C and 0.5 ml aliquots tested 2, 15 and 30 min after incubation (Incub) at room temperature. Neither blood nor SRS-C incubated with an equal volume of saline for 2 min contracted the rabbit aortic strips (RA), rat stomach strips (RSS) or the rat colon (RC).

strip or rat colon there was little change or, as shown in Fig. 4, and increase of activity occurred. Incubation of prostaglandin E_2 in blood for up to 30 min did not modify its activity on the rat stomach strip. Previous incubation of blood for 1 min with indomethacin or phenylbutazone, before adding SRS-C, resulted in a concentration-related inhibition of the generation of RCS. Phenylbutazone at 1, 3 and 9 μg/ml inhibited 26.5 66 and 79% respectively, whereas indomethacin at similar concentrations inhibited 31, 77 and 87% respectively of the contractions elicited by incubation mixtures of blood and arachidonic acid.

Release of rabbit aorta contracting substance from blood fractions

Incubation of SRS-C with PRP generated more rabbit aorta contracting activity than did incubation with blood, whereas the effects on the rat stomach strip were of the same order of magnitude for PRP and blood (Figure 5). Incubation of SRS-C with erythrocytes suspended in saline or in autologous plasma produced less RCS than incubation with blood or PRP. However, the contractor effect on the rat stomach strip was the same whether blood, PRP or erythrocytes were incubated with SRS-C. Incubation of SRS-C with plasma generated little or no contractor activity. Blood (0.5 ml) and blood haemolyzed with distilled water evoked small organ responses. Incubation of PRP with indomethacin greatly reduced the generation of contractor activity on the rabbit aorta and rat stomach strip. Indomethacin also inhibited the generation by blood, although less effectively for rat stomach strip than for rabbit aorta. Little or no effect of indomethacin was seen on the contractor activity generated by erythrocyte suspensions. Phenylbutazone inhibited release of contractor materials from blood and PRP (Table 2).

Generation of rabbit aorta contracting substance from unsaturated fatty acid and slow reacting substance C

The RCS activity of the fatty acid hydroperoxides was estimated by infusing the fatty acid or SRS-C together with lipoxidase into the stream of Krebs solution that superfused the assay tissues. An extra length of tubing was added between the injection point and the assay tissues to allow the enzyme and substrate to mix for 2 minutes. When linoleic acid (5-20 μg/ml; 2 experiments), linolenic acid

Table 2 Inhibition by phenylbutazone of the release of pharmacologically active substances from dog plasma rich in platelets (PRP) by slow reacting substance C (SRS-C)

Phenylbutazone concentration (μg/ml)	*% Inhibition of rabbit aorta contraction*					
	SRS-C and blood			*SRS-C and PRP*		
	No. of experiments	*Mean*	*Range*	*No. of experiments*	*Mean*	*Range*
1	2	26.5	23-30	5	40	23-66
3	2	66	62-70	2	46.5	30-63
9	3	79	68-87	5	83	50-100

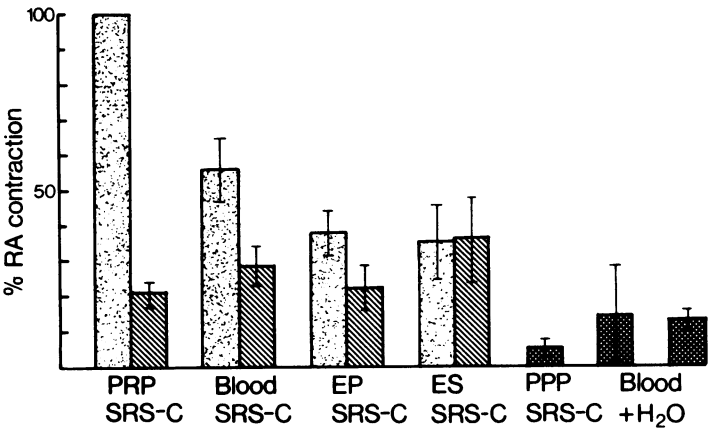

Fig. 5 Generation of rabbit aorta contracting substance from blood and fractions by slow reacting substance C (SRS-C). PRP, plasma rich in platelets; ES, erythrocytes resuspended in saline; PPP, plasma poor in platelets; EP, erythrocytes resuspended in plasma. Blood or fractions (0.5 ml) were incubated with 0.5 ml of the ethanol extract of SRS-C or distilled water for 2 min at 37° C. For each group: control, stippled column; indomethacin (added to the mixture to give the final concentration of 5 μg/ml), diagonally hatched column. The results are the means of four experiments in which the height of the concentrations of the rabbit aorta induced by 0.5 ml of the mixture PRP and SRS-C was taken as 100%.

(5-20 μg/ml; 2 experiments) and arachidonic acid (5-20 μg/ml; 5 experiments) dihomo-γ-linolenic acid (10-20 μg/ml; 2 experiments) or alcoholic extract of SRS-C (diluted to 1/50; 9 experiments) were infused together with soybean lipoxidase (100 μg/ml) there was a generation of an activity which strongly contracted rabbit aorta, rat stomach and rat colon preparations (Figure 6). With the exception of arachidonic acid, the fatty acids and SRS-C infused alone in the same final concentration produced little or no effect on the assay tissues. Although arachidonic acid when infused alone over the tissues may cause them to contract, a greater contraction was always caused when the acid was infused in combination with lipoxidase.

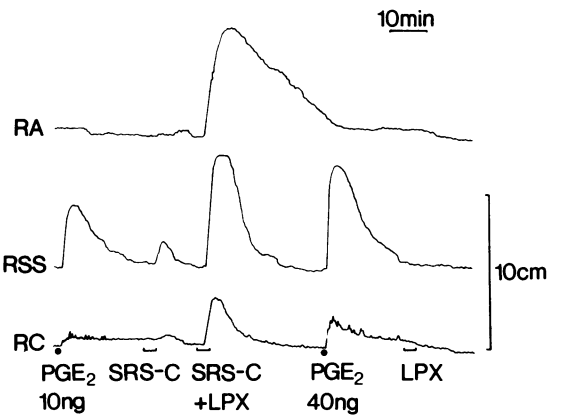

Fig. 6 Generation of a rabbit aorta contracting activity from slow reacting substance C (SRS-C) by soybean lipoxidase. The alcoholic extract of SRS-C (0.2 ml/min) and the soybean lipoxidase (LPX, 1 mg/min) were infused into a circuit which allowed the reagents to mix for 2 min before reaching the assay tissues. The rabbit aorta (RA), rat stomach strip (RSS) and rat colon (RC) were superfused in cascade with Krebs at a rate of 10 ml/minute.

Discussion

We have shown in this paper that SRS-C and arachidonic acid cause a fall in the vascular resistance of dog paws. This effect was blocked by non-steroid anti-inflammatory agents as was the systemic hypotension induced in dogs by an intravenous injection of SRS-C (Vargaftig & Dao, 1971b). The decrease in peripheral vascular resistance could be either a direct effect of arachidonic acid and of SRS-C on the vessels or an indirect effect due to the release of active materials from tissues or blood. The latter alternative was supported by the findings that (a) SRS-C or arachidonic acid when injected into a stream of Krebs solution superfusing isolated preparations had little or no contractor activity on the rabbit aorta, rat stomach strip and rat colon, whereas on blood perfused isolated tissues they

had strong contractor activity; (b) strong contractor activity could be generated by incubating SRS-C or arachidonic acid with blood and with PRP; (c) non-steroid anti-inflammatory agents blocked the generation of this activity either when given intravenously to dogs or added to blood *in vitro*.

The active material generated by incubation of SRS-C or arachidonic acid with blood shares some features with the rabbit aorta contracting substance (RCS) released from guinea-pig lungs during anaphylaxis (Piper & Vane, 1969). It contracted the rabbit aorta, the rat stomach strip and rat colon preparations treated with a mixture of antagonists and its generation was blocked by non-steroidal anti-inflammatory agents. It has been suggested that RCS is the cyclic endoperoxide intermediate in the biosynthesis of prostaglandins (Gryglewski & Vane, 1972; Vargaftig & Dao Hai, 1972). In the present paper we strengthen further the hypothesis that RCS is related to lipid hydroperoxides, by showing that rabbit aorta is very sensitive not only to arachidonic acid peroxide (Gryglewski & Vane, 1972) but also to those generated from linoleic, linolenic, dihomo-γ-linolenic acids and SRS-C soybean lipoxidase. Lipid hydroperoxides, besides contracting isolated preparations, will cause pain and cutaneous vasodilation in man (Ferreira, 1972). This last effect could explain the fall of peripheral resistance observed in the dog paw. When polyunsaturated long chain fatty acids are oxidized by soybean lipoxidase, a hydroperoxy group is introduced into the ω 6 position (Hamberg & Samuelsson, 1967). Our results demonstrate that this is sufficient to elicit contraction of the isolated organs. In the case of the prostaglandin synthetase, oxidation of ω 6 is preceded by formation of ω 8 peroxy-derivatives, which by themselves might be active upon rabbit aorta strips. At this step there is still a straight chain carbon skeleton as there is in the peroxides generated by lipoxidase. The next step in the synthesis of prostaglandin is the formation of a cyclic endoperoxide, having a hydroperoxy group in the ω 6 position. This derivative, though no longer a straight chain, may well possess activity of its own on the isolated preparations. In fact, Hamberg & Samuelsson (1973) isolated the cyclic endoperoxide and showed that it can contract the rabbit aorta. This cyclic endoperoxide, however, is unstable, being converted rapidly and spontaneously into prostaglandins. The rabbit aorta contracting material we now describe was relatively stable, which is compatible with its being a mixture of aliphatic and cyclic hydroperoxides, possibly formed by the prostaglandin synthetase enzymes (Lands, Le Tellier, Rome & Vanderhoek, 1973).

Neither plasma nor red cells seem to contribute significantly to the generation of RCS-like activity, when SRS-C or arachidonic acid were incubated with dog blood. Although a small generation could be detected with red cells resuspended in plasma, most of the activity was generated in platelet-rich plasma. Our experiments do not exclude the possible contribution of contaminant leucocytes which are known to synthesize prostaglandin (Higgs & Youlten, 1972).

Platelets are nevertheless involved. Non-steroid anti-inflammatory agents inhibit thrombin-induced platelet aggregation (Packham & Mustard, 1969) and prostaglandin formation (Smith & Willis, 1971). Recently Vargaftig & Zirinis (1973) found that arachidonic acid cause platelet aggregation with the parallel formation of rabbit aorta contracting substance and prostaglandins; these effects are dose-dependent and are inhibited by low concentrations of non-steroid anti-inflammatory agents. Peroxidation of lipids was suggested to be, at least in part, the mode of action by which thrombin, latex and similar aggregating agents induce the 'release reaction' (Okuma, Steiner & Baldini, 1971). The rabbit aorta contracting substances, like other lipid peroxides, may damage cell membranes (Lewis & Wills, 1962; Desai & Tappel, 1963) thus facilitating platelet aggregation. Inhibition of the intracellular generation of peroxides may then explain the inhibitory effects of non-steroid anti-inflammatory drugs against platelet aggregation induced by some agents.

Our experiments did not exclude the possibility that the vasodilatory effect of SRS-C and of arachidonic acid are at least in part due to the generation of RCS by the tissue components. This possibility must also be taken into account, since SRS-C and arachidonic acid are known to generate RCS from guinea-pig lungs perfused with Krebs solution (Vargaftig & Dao, 1971a). SRS-C is a mixture of unsaturated fatty acids released from phospholipids by phospholipase A2 contained in snake venom (Vogt, 1957). It is possible that the hypotensive effect, as well as the generation of RCS from blood by SRS-C, is due to its content of unsaturated fatty acids, especially arachidonic acid. Against this correlation of the action of SRS-C with its content of arachidonic acid is the fact that the material generated in blood showed different ratios of activity on the rabbit aorta and on the rat stomach strips.

Haemolysis caused by linoleic or linolenic acid or osmotic shock did not generate RCS-like material from blood. Thus a direct cytolytic effect of arachidonic acid or of SRS-C cannot explain the generation of RCS from blood or from PRP. It may well be that both act in these circumstances

as substrates for an oxidizing enzyme system sensitive to non-steroidal anti-inflammatory agents, like the prostaglandin synthetases. This is supported by our demonstration that SRS-C can be a substrate for soybean lipoxidase. The failure of linoleic and linolenic acids to be oxidized by platelets can also be explained by their known inhibitory action upon the prostaglandin synthetase system (Lands *et al.*, 1973). However, whether SRS-C or arachidonic acid are acting as releasing factors or being converted to active materials cannot be concluded from the present experiments.

In conclusion, our results suggest that the vasodilator effects of SRS-C and of arachidonic acid are at least partly due to the generation by platelets of RCS-like activity and that some of this activity could be due to the formation of fatty acid hydroperoxides. The enzymic system involved in the generation of the RCS-like activity, like that of prostaglandin synthesis, is inhibited by non-steroidal anti-inflammatory drugs.

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References

DESAI, I.D. & TAPPEL, A.I. (1963). Damage to proteins by peroxidized lipids. *J. Lipid Research*, **4**, 204-207.

FERREIRA, S.H. (1972). Prostaglandins, aspirin-like drugs and analgesia. *Nature, New Biol.*, **240**, 200-203.

FERREIRA, S.H., MONCADA, S. & VANE, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature, New Biol.*, **231**, 237-239.

FERREIRA, S.H. & VARGAFTIG, B.B. (1973). Generation of rabbit aorta contracting substance in blood by slow reacting substance C and its blockade by non-steroid anti-inflammatory drugs. *Biorheology*, **10**, 288-289.

GILMORE, N., VANE, J.R. & WYLLIE, H.J. (1968). Prostaglandins released by the spleen. *Nature, Lond.*, **218**, 1135-1140.

GRYGLEWSKI, R. & VANE, J.R. (1972). The generation from arachidonic acid of rabbit aorta contracting substance (RCS) by a microsomal enzyme preparation which also generates prostaglandins. *Br. J. Pharmac.*, **46**, 449-457.

HAMBERG, M. & SAMUELSSON, B. (1967). On the specificity of the oxygenation of unsaturated fatty acids catalyzed by soybean lipoxydase. *J. Biol. Chem.*, **242**, 5329-5335.

HAMBERG, M. & SAMUELSSON, B. (1973). Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proc. Nat. Acad. Sci.*, **70**, 899-903.

HIGGS, G.A. & YOULTEN, L.J.F. (1972). Prostaglandin production by rabbit peritoneal polymorphonuclear leukocytes *in vitro*. *Br. J. Pharmac.*, **44**, 330P.

LANDS, W.E.M., LE TELLIER, P.R., ROME, L.H. & VANDERHOEK, J.Y. (1973). Inhibition of prostaglandin biosynthesis. In: *Advances in the Biosciences*, 9, ed Bergström, S. & Barnhard, S. Braunschweig: Pergamon Press.

LEWIS, S.E. & WILLS, E.D. (1962). The destruction of –SH groups of proteins and amino acids by peroxides of unsaturated fatty acids. *Biochem. Pharmac.*, **1**, 901-912.

OKUMA, M., STEINER, M. & BALDINI, M.G. (1971). Studies on lipid peroxides in platelets: II. Effect on aggregating agents and platelet antibody. *J. Lab. Clin. Med.*, **77**, 728-742.

PACKHAM, M.M. & MUSTARD, J.F. (1969). The effect of Pyrazole on thrombin-induced platelet aggregation. *Proc. Soc. exp. Biol. Med.*, **130**, 72-75.

PIPER, P.J. & VANE, J.R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature, Lond.*, **223**, 20-35.

REGOLI, D. & VANE, J.R. (1964). A sensitive method for the assay of angiotensin. *Br. J. Pharmac. Chemother.*, **23**, 351-359.

SMITH, J.B. & WILLIS, A.L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. *Nature, New Biol.*, **231**, 235-237.

VANE, J.R. (1957). A sensitive method for the assay of 5-hydroxytryptamine. *Br. J. Pharmac. Chemother.*, **12**, 344-349.

VANE, J.R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *Br. J. Pharmac. Chemother.*, **23**, 360-373.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. *Nature, New Biol.*, **231**, 232-235.

VARGAFTIG, B.B. & DAO, N. (1971a). Release of vasoactive substances from guinea-pig lungs by slow reacting substance C and arachidonic acid. *Pharmacology*, **6**, 99-108.

VARGAFTIG, B.B. & DAO, N. (1971b). Mode d'action et antagonisme de la substance a contraction differée C liberée par la phosphalipase A a patir du jaune d'oeuf. *J. Pharmac., Paris*, **2**, 287-304.

VARGAFTIG, B.B. & DAO HAI, N. (1972). Paradoxical inhibition of the effects of bradykin by some sulfhydric reagents. *Experientia*, **28**, 59-62.

VARGAFTIG, B.B., MIRANDA, E.P. & LACOUME, B. (1969). Inhibition by non-steroidal anti-inflammatory agents of *in vivo* effects of slow reacting substance. *Nature, Lond.*, **222**, 883-885.

VARGAFTIG, B.B. & ZIRINIS, P. (1973). Arachidonic acid induced platelet aggregation accompanied by release of potential inflammatory mediators distinct from PGE_2 and $PGF_{2\alpha}$. *Nature, New Biol.*, **244**, 114-116.

VOGT, M. (1957). Pharmacologically active substances formed in egg yolk by cobra venom. *J. Physiol. Lond.*, **135**, 131-147.

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