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The release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs

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Summary

1. Slices of rabbit spleen generate rabbit aorta contracting substance (RCS) and a prostaglandin of the E series when they are vibrated or stirred.

2. The release of both substances is increased by arachidonic acid and inhibited by aspirin-like drugs.

3. When the spleen effluent containing RCS and prostaglandin E is incubated for a further 3 min, the prostaglandin concentration increases and the RCS concentration declines.

4. RCS may therefore be an unstable intermediate in the biosynthesis of prostaglandins.

Introduction

Prostaglandins (PGs) are released from perfused dog spleen by catecholamines, sympathetic nerve stimulation, or by infusion of particles (Davis, Horton & Withrington, 1967, 1968 ; Ferreira & Vane, 1967 ; Gilmore, Vane & Wyllie, 1968, 1969). This release represents fresh synthesis, for more prostaglandin can be released by these stimuli than can be extracted from the unstimulated spleen (Davis *et al.*, 1968 ; Gilmore *et al.*, 1968). Blockade by anti-inflammatory drugs of prostaglandin production and release from the spleen (Ferreira, Moncada & Vane, 1971) provided additional evidence that aspirin-like drugs inhibit the prostaglandin synthetase system (Vane, 1971). These drugs also prevent the release from guinea-pig perfused or chopped lung of an unstable substance called rabbit aorta contracting substance or RCS (Piper & Vane, 1969). RCS is released from lungs by anaphylaxis, by mechanical agitation, by infusion of particles or by substances such as bradykinin (Piper & Vane, 1969 ; Palmer, Piper & Vane, 1970 ; Piper & Vane, 1971), ' slow reacting substance C ' or arachidonic acid (Vargaftig & Dao, 1971). Some connexion between the generation of prostaglandins and that of RCS is possible. We have now looked for RCS release in rabbit spleen, known to be capable of releasing prostaglandins (Willis, 1970a). Mechanical vibration of chopped spleen slices proved to be a suitable stimulus.

Methods

Dutch male rabbits were killed by a blow on the neck. The spleens were removed, cut with scissors into slices approximately 0·5–1·0 mm thick and washed

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several times with oxygenated Krebs bicarbonate solution. To improve fluid flow through them, 1 g of the spleen choppings was mixed with 0·5 g of thin polyethylene rings of 5 mm in diameter. This mixture was placed on a fine mesh diaphragm (cut from a nylon stocking) in the barrel of a 10 ml plastic syringe. Krebs solution at 37° C, pre-gassed with 5% carbon dioxide in oxygen and containing several antagonists (Gilmore *et al.*, 1968) was dripped through the chopped spleen at 5 ml per minute. Vibrations were applied to the chopped spleen (Fig. 1) by a plastic rod attached to a vibrator which consisted of a Ronson electric toothbrush handle. The rod was 10 cm long, 4 mm in diameter and had a polyethylene disc of 10 mm in diameter at the end. The frequency of vibration was 95 Hz and the amplitude of vibration at the end of the rod did not exceed 2 mm. Vibrations were generated intermittently for 30–60 s periods. In some experiments instead of applying vibrations, spleen choppings were gently stirred with a blunt plastic rod for 30 seconds.

The effluent from the chopped spleens superfused a series of 4–6 isolated bioassay tissues (Fig. 1) which, because of the antagonists, mepyramine (0·1 μg/ml) hyoscine (0·1 μg/ml), methysergide (0·2 μg/ml), phenoxybenzamine (0·1 μg/ml) and propranolol (2 μg/ml) in the Krebs solution, did not react to histamine, acetylcholine,

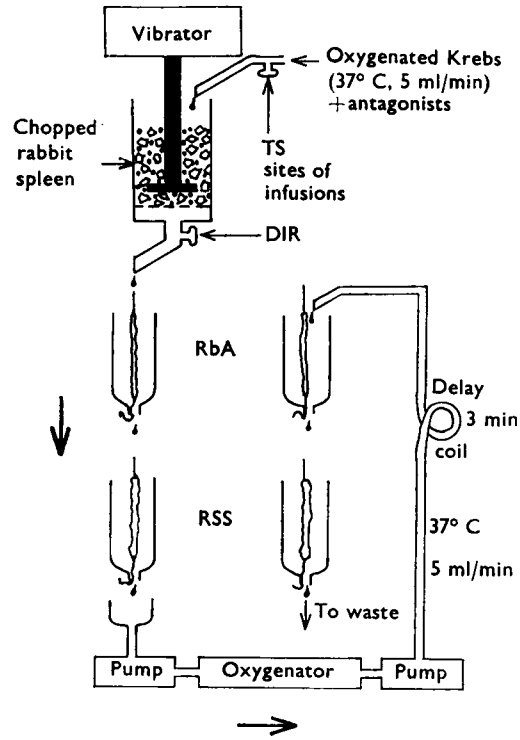

FIG. 1. Diagram of apparatus for the vibration and superfusion of the chopped spleen. The rabbit spleen slices were mixed with polyethylene rings and placed on the mesh diaphragm in the barrel of a 10 ml syringe contacting the plastic rod of the vibrator (95 Hz). A roller pump dripped Krebs solution containing combined antagonists at 37° C at 5 ml/min through the chopped spleen. The effluent from the syringe superfused the first bank of the assay tissues, which consisted of rabbit aortic strip (RbA), rat stomach strip (RSS) and sometimes rat colon. The fluid was collected in a reservoir, pumped through an oxygenator and then through a delay coil (3 min) before superfusing the second bank of tissues. The vibrator was switched on for periods of 30–60 s. Drugs were infused directly over tissues (DIR) or through the spleen (TS) as shown.

5-hydroxytryptamine or catecholamines. They were therefore relatively specific detectors of prostaglandins and RCS (Piper & Vane, 1969). The following tissues were used: rat stomach strip (Vane, 1957); rat colon (Regoli & Vane, 1964) and spirally cut strips of thoracic aorta of the rabbit. Rat stomach strip and rat colon differentiated between prostaglandins of the E and F series (Gilmore *et al.*, 1968); the rabbit aorta was insensitive to most vaso-active substances except RCS and angiotensin II (Piper & Vane, 1969).

The assay tissues were arranged in two banks with 2–3 tissues in each as described by Gilmore *et al.* (1968). However, after the effluent of the chopped spleen had passed over the first bank of tissues (Fig. 1), it was re-oxygenated by mixing with a stream of 5% carbon dioxide in oxygen in a 10 ml plastic syringe barrel filled with stainless steel wool and then pumped through a coil of silicone tubing kept at 37° C so that it reached the second bank of assay tissues after a delay of 3 minutes. Thus the assay tissues in the second bank detected any change in the activity of the released substances induced by 3 min incubation in the Krebs solution. The movements of the assay tissues were detected by auxotonic (Paton, 1957) levers attached to Harvard smooth muscle transducers. The transducer output was suitably amplified for display on a multichannel pen recorder (Beckman-Offner or Watanabe). The degree of amplification was adjusted so that the rat stomach strip and rat colon in each bank gave similar recorded responses to control infusions of PGE_2. The amplification for the two strips of rabbit aorta was usually equal. Since both strips were cut from the same piece of aorta, it can be assumed that their sensitivity to RCS was similar. Even so, in some experiments, the amplification of the second strip was increased 2–4 times.

In some experiments the assay tissues were superfused with fresh Krebs solution (containing antagonists) and the effluent from the chopped spleen was run to waste. After 1–2 h spleen effluent was superfused over the assay tissues; in this way the basal output of active substances from the chopped spleen could be estimated. Drugs were infused directly (DIR) to the assay tissues for calibration of their reactions or through the chopped spleen (TS) as shown in Fig. 1.

Further information about the type of prostaglandin released from the spleen was obtained by extraction and chromatography. The effluent (10 ml) was collected immediately after vibration or stirring of the spleen choppings, then acidified to pH 3 with 1 N HCl. Half this volume was extracted three times with ethyl acetate and the combined extracts were evaporated to dryness; the residue was taken up in 0·1 ml of ethanol and chromatographed in the AI system (Gréen & Samuelsson, 1964) on thin layers of silica gel F_{254} (prepared in 0·25 mm thickness on glass plates; Merck Darmstadt) (Willis, 1970b) together with markers of 5 μg of PGE_2 or $PGF_{2\alpha}$. Strips (2 cm) of chromatograms corresponding to the marker spots were separated, scraped into test tubes, shaken with 1 ml of Krebs solution and centrifuged. The supernatants were bioassayed on rat stomach strips and rat colons in cascade using PGE_2 and $PGF_{2\alpha}$ as standards. The other half of the acidified effluent was brought to pH 7·4 and the concentration of prostaglandins was determined on rat stomach strips in terms of PGE_2.

The content of prostaglandins in spleen slices was estimated after they had been superfused with Krebs solution for 1 hour. They were transferred into 4 ml saline, acidified with 1 N HCl to pH 3 and the mixture heated in a boiling water bath for one minute. It was then homogenized in a Potter Elvehjem homogenizer and

centrifuged at 800 g. The supernatant was adjusted to pH 7·4 and assayed for prostaglandin content in terms of PGE_2 on rat stomach strips.

Results

Content of prostaglandins in unstimulated spleen slices

In three experiments the concentration of prostaglandins in the spleen slices (in terms of PGE_2) was 360, 520 and 600 ng/g.

Basal output of prostaglandins from spleen slices

In four experiments, the basal output of prostaglandins from the spleen slices was estimated by superfusing the assay tissues in turn with fresh Krebs solution or with effluent from the spleen choppings. The output (as PGE_2) was 4, 6, 10 and 15 (ng/ml)/minute. This output slowly decreased with time. When the antagonists were added to the Krebs solution after it had superfused the spleen choppings, the basal output of PG-like material was in the same range. The spleen effluent did not contract the rabbit aorta or the rat colon showing that neither $PGF_{2\alpha}$ nor RCS could be detected. The basal output of prostaglandins was abolished by sodium meclofenamate (0·2 μg/ml) or indomethacin (0·5 μg/ml) added to the superfusion fluid passing through the spleen slices (Fig. 2).

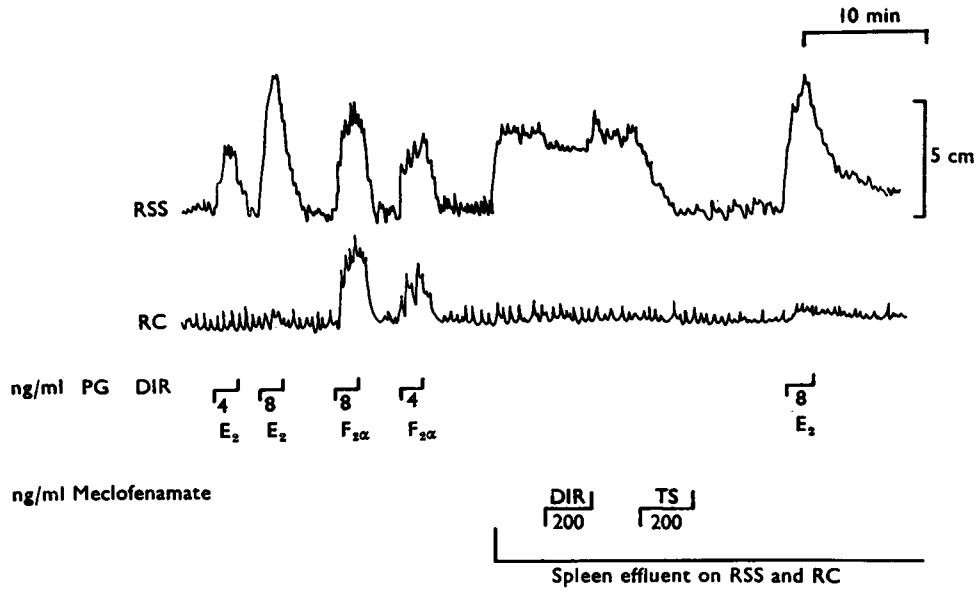

FIG. 2. Rat stomach strip (RSS) and rat colon (RC) were superfused at 5 ml/min with Krebs solution containing combined antagonists, heated to 37° C. Where indicated the Krebs solution was changed for the effluent from chopped spleen (Fig. 1). The contraction of rat stomach strip but not of rat colon denoted the basal output of PGE_2 from the spleen of about 6 (ng/ml)/minute. Sodium meclofenamate (0·2 μg/ml) only slightly affected the tone of the rat stomach strip when infused directly over the assay tissues, but when infused through the spleen choppings (TS) it completely abolished the output of PGE_2. The rabbit aortic strip (tracing not shown) did not change in tone. Time scale 10 min; vertical scale 5 cm.

Stirring

Gentle stirring (7 experiments) of the spleen slices for 30 s released substances into the perfusate which contracted rat stomach strips and rabbit aortas. The contraction of the rat stomach strip in the first bank of tissues corresponded to PGE_2 at a concentration of 107 ± 23 ng/ml (range 50–200 ng/ml), while the stomach strip contraction in the second bank of tissues corresponded to PGE_2 at a concentration of 148 ± 17 ng/ml (range 100–200 ng/ml). With these high concentrations, the dose-response curve of the rat stomach strip to PGE_2 was shallow, making precise assay difficult (Fig. 3).

PGE_2 slightly contracted some rabbit aortic strip preparations; however, during stirring, a substance appeared in the effluent which strongly contracted the aortic strip (Fig. 3). This substance disappeared after passage through the delay circuit

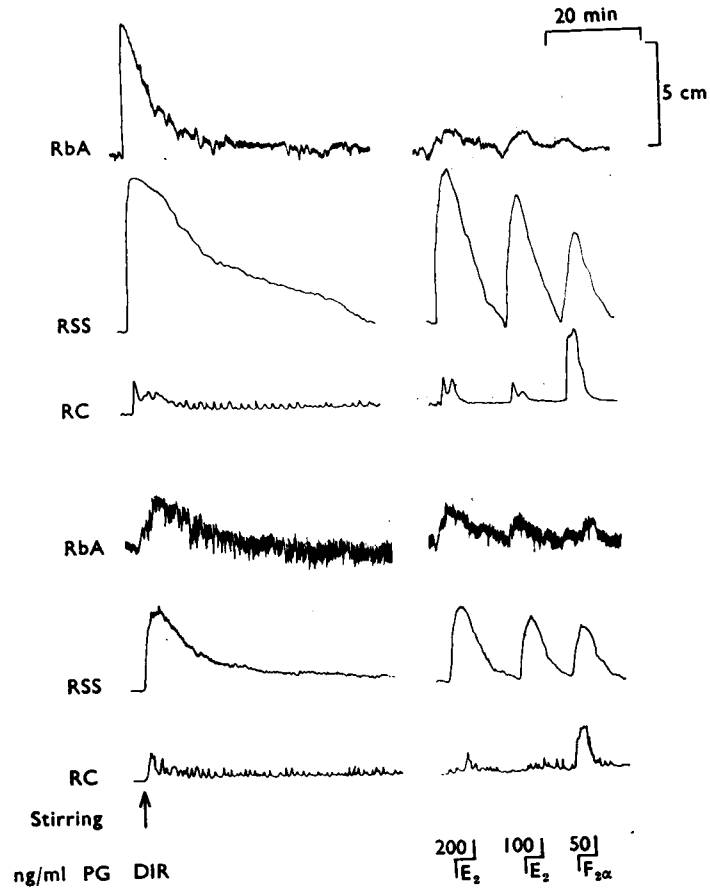

FIG. 3. The assay tissues were rabbit aortic strip (RbA), rat stomach strip (RSS) and rat colon (RC), all superfused with the effluent from the rabbit spleen slices in two banks as shown in Fig. 1. The upper three tracings are of the first bank and the lower three tracings of the second (delayed) bank of tissues. The first contraction of the assay tissues was induced by stirring the spleen slices (for 30 s). The next three contractions were induced by infusions of PGE_2 and $PGF_{2\alpha}$ directly over tissues (DIR). The contraction of the rat stomach strip in the first bank induced by stirring was matched by PGE_2 at a concentration 200 ng/ml, whereas the contraction of the rat stomach strip in the second bank was greater. The relative potencies of PGE_2 and $PGF_{2\alpha}$ on rat stomach strip and colon indicated that little or no $PGF_{2\alpha}$ had been released by stirring. The rabbit aortic strip in the first bank of tissues contracted strongly ,whereas that in the second bank showed only a weak contraction, mostly attributable to PGE_2. Time scale 20 min; vertical scale 5 cm.

so that the second aortic strip hardly contracted. Figure 3 shows that substances released from the stirred slices scarcely contracted the rat colon; thus little or no $PGF_{2\alpha}$ had been released.

Vibration

Vibration for 15–30 s resulted in a similar but smaller release of prostaglandins and a substance contracting the rabbit aortic strip. The mean concentration of prostaglandins detected by the first bank of tissues (14 experiments) was 23 ± 2 ng/ml expressed in terms of PGE_2, while at the second bank, the concentration was 35 ± 4 ng/ml. This increase in prostaglandin concentration was statistically significant ($t=2{\cdot}15$, $f=26$, $P=0{\cdot}01$). Again, the substance contracting the aortic strip was always detected by the first bank of tissues but little or none was present after passage through the delay coil (Fig. 4). The results were unaffected by adding the antagonists to the spleen superfusate, rather than passing them through the spleen slices.

The first period of vibration usually produced the greatest amount of prostaglandins and RCS. Thereafter vibrations generated reproducible amounts of both substances for 3 trials, provided that the period of vibration was not longer than

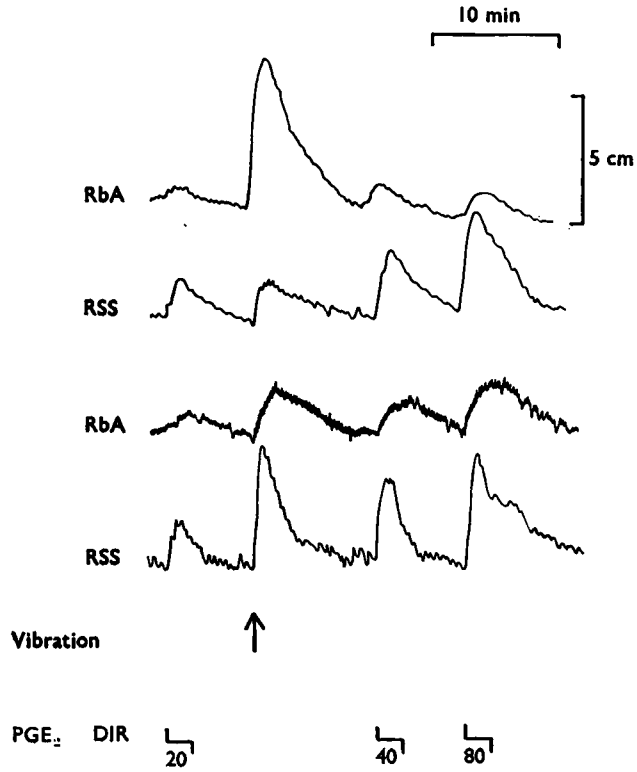

FIG. 4. The assay tissues were rabbit aortic strip (RbA) and rat stomach strip (RSS), arranged in two banks and superfused with the rabbit spleen effluent as shown in Fig. 1. The upper two tracings are of the first bank of tissues and the lower two tracings of the second (delayed) bank. Vibration for 30 s (marked by the arrow) resulted in the appearance of a substance contracting the rabbit aortic strip in the first bank and of prostaglandins, which contracted the rat stomach strip. After the delay coil there was little RCS left but the amount of prostaglandins increased from about 20 ng in the first bank to about 80 ng in the second bank (assayed as PGE_2). Time scale 10 min; vertical scale 5 cm.

30 s and the intervals between vibrations were not shorter than 20 minutes. If the period of vibrations was increased or the intervals between vibrations shortened a decreased release of substances contracting the rabbit aortic strip and rat stomach strip resulted. When this occurred, a continuous infusion of arachidonic acid (1 μg/ml) through the chopped spleen restored the generation of active materials in response to vibration (Fig. 5), but did not cause generation without vibration.

The decrease in RCS concentration and increase in prostaglandin concentration, as the effluent from the incubated spleen passed through the 3 min incubation coil, was unaffected by infusions of indomethacin (1 μg/ml) directly into the spleen effluent. Reversal of the sequence of superfusion of the banks of tissues also reversed the relative heights of the contractions of the aortic strip, showing that the smaller contraction of the second aortic strip was not due to differing sensitivities of the tissues.

Chromatographic separation

Extracts obtained from the perfusate during vibration or stirring of spleen slices were chromatographed in the AI system. Out of 50 ng of prostaglandin-like activity in the effluent of vibrated spleen slices 49 ng were detected in the zone

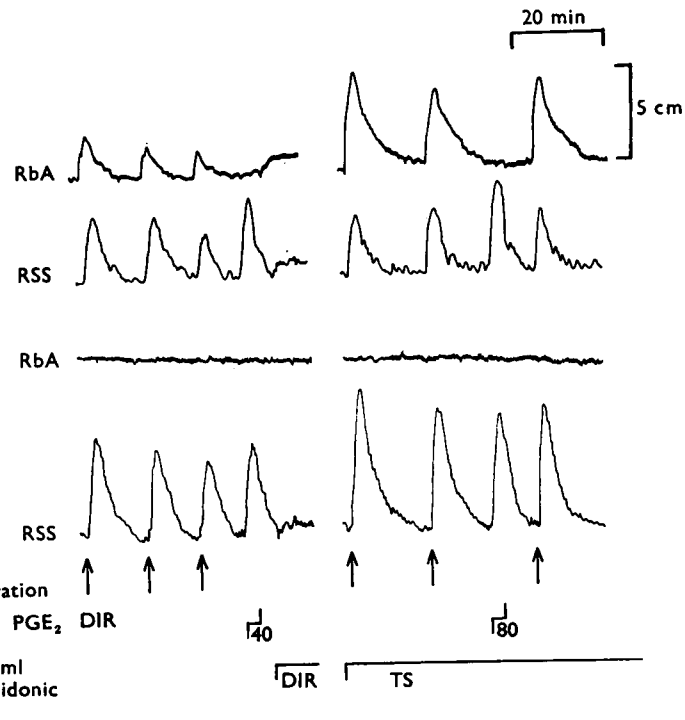

FIG. 5. Tracings as in Fig. 4. Four consecutive vibrations of 60 s duration at 15 min intervals (three of which are shown in the picture) resulted in a gradual decrease in the production of RCS and prostaglandins. The production was restored by arachidonic acid (1 μg/ml) infusion through the chopped spleen (TS). Arachidonic acid caused only a slight contraction of the assay tissues when infused directly over them (DIR), but made the rat stomach strip less sensitive to PGE_2. Note the disappearance of RCS and the increase in concentration of prostaglandins in the fluid reaching the second bank of tissues after each vibration. The increase in prostaglandin concentration in the delayed bank is even greater during treatment with arachidonic acid. Time scale 20 min ; vertical scale 5 cm.

corresponding to a PGE_2 marker. Of 133 ng in the effluent obtained from the stirred spleen slices, 125 ng were detected in the PGE_2 zone. No activity was found in zones corresponding to the $PGF_{2\alpha}$ marker, so that the content of $PGF_{2\alpha}$ was less than the threshold sensitivity of the bioassay (5 ng).

Anti-inflammatory drugs

The release of substances that contracted the rat stomach strip and rabbit aortic strip induced by stirring or vibrating spleen slices was reduced by anti-inflammatory drugs. Infusion of arachidonic acid (1 μg/ml) through the chopped spleen did not affect this inhibition by anti-inflammatory drugs.

Aspirin (40 μg/ml; 3 experiments) reduced the output of prostaglandins by 45, 50 and 75% and the output of RCS by 90% in all three experiments. Oxyphenbutazone (10 μg/ml: 3 experiments) reduced the output of prostaglandins by 10, 25 and 40% and the output of RCS by 50, 50 and 75%. Indomethacin (1 μg/ml; 5 experiments) reduced the output of prostaglandins by 50, 60, 75, 90 and 90% and the output of RCS by 90, 80, 90, 100 and 100% (Fig. 6). Meclofenamic acid (0·5 μg/ml; 5 experiments) reduced the output of prostaglandins by 50, 60, 60, 75 and 100% and the output of RCS by 70, 70, 89, 90 and 100%.

These values were calculated from the reactions of the first bank of tissues. Reduction in prostaglandin-like activity was calculated in terms of PGE_2. Reduction in RCS-like activity was calculated from the change in height of contraction of the rabbit aorta (even though it assumed a linear dose-response relationship), since no standard of comparison was available.

Discussion

Guinea-pig lungs release RCS and prostaglandins E_2 and $F_{2\alpha}$ when provoked by several stimuli including anaphylaxis, injection of bradykinin (Piper & Vane, 1969) and gentle massage or stirring of chopped lungs (Palmer *et al.*, 1970). We have

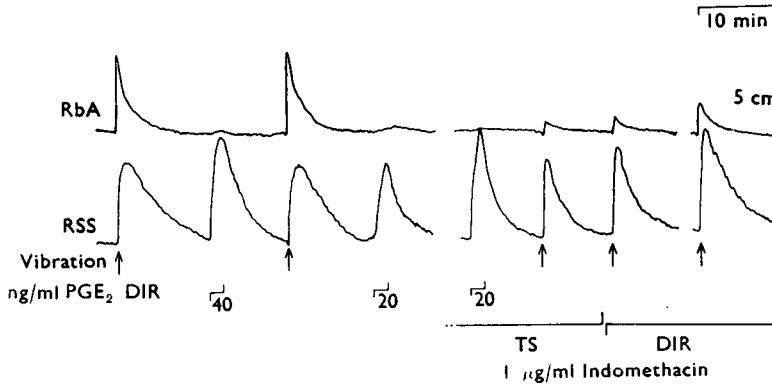

FIG. 6. Rabbit aortic strip (RbA) and rat stomach strip (RSS) were arranged in one bank and superfused with the effluent from the chopped spleen. Vibration for 30 s was applied at arrows. This resulted in a fairly reproducible generation of RCS and prostaglandins. Infusion of indomethacin (1 μg/ml) through the spleen (TS) almost abolished release of RCS due to vibration while release of prostaglandins was partially reduced. After indomethacin had been infused through the spleen for 45 min, it was infused only over the assay tissues (DIR). The last vibration was applied 30 min after this change. A partial recovery of RCS output and a more complete recovery of prostaglandins output from the spleen was observed. Note the sensitization of the rat stomach strip to PGE_2 due to the presence of indomethacin in the superfusing fluid. Time scale 10 min; vertical scale 5 cm.

now shown that RCS and prostaglandins are released from rabbit chopped spleen when it is stirred or vibrated. Both from the results of parallel assay and chromatographic separation, the prostaglandin released was of the E series. Willis (1970a) has found the effluent from superfused contracting strips of rabbit spleen to contain an E-type prostaglandin.

As in guinea-pig lungs and in whole spleen preparations, we consider for the following reasons that synthesis accompanies release of prostaglandins from the rabbit chopped spleen by vibration or stirring. First, the amounts of prostaglandins that were released during a single experiment far exceeded the amount of prostaglandins that could be extracted from homogenized tissue. Second, an infusion of the precursor, arachidonic acid through spleen slices restored their ability to produce prostaglandins and third, the release of prostaglandins from the vibrated or stirred spleen choppings was inhibited by anti-inflammatory drugs in the same order of potency as that observed when these drugs inhibited the biosynthesis of prostaglandins from arachidonate in guinea-pig lung homogenates (Vane, 1971) or in dog spleen homogenates (Cedro, Flower, Gryglewski & Vane, unpublished).

Our experiments throw little light on the mechanism by which mechanical stimulation of chopped lung or spleen tissue leads to prostaglandin synthesis and release. One possibility would be by activation of phospholipase, thus producing the precursor arachidonic acid from phospholipids. However, this seems unlikely as the only mechanism, for infusion of arachidonic acid through the spleen slices did not promote the release of prostaglandins until vibrations were applied. The fact that prolonged stimulation was needed to induce some exhaustion of prostaglandin synthesis suggests that rabbit spleen, like rat stomach (Pace-Asciak, Morawska, Coceani & Woolfe, 1968) possesses a rich endogenous source of the precursor. Another possibility would be that mechanical agitation leads to actual release of a prostaglandin synthetase (together with precursor) into the superfusion fluid. Certainly, the use of two banks of assay tissues separated by an incubation coil showed that there was a continued generation of prostaglandins in the effluent from the vibrated or stirred chopped spleen. The assessment of this continued generation during stirring was difficult because the concentrations of prostaglandins were too high for accurate direct bioassay. The milder procedure of vibration caused a smaller release of prostaglandins and with this, it was more easily shown that there was a continued generation of prostaglandins in the incubation coil during spleen vibration. However, this was unlikely to be due to enzymic synthesis of prostaglandins by a synthetase released into the effluent, for infusions of indomethacin directly into the spleen effluent did not prevent the continued generation. The remaining possibility, therefore, is that mechanical agitation in some way activates the prostaglandin synthetase within the cell.

At the time that the concentration of prostaglandin in the effluent from vibrated spleen slices was increasing, the concentration of RCS was declining. This RCS was similar in characteristics to that released from guinea-pig lung in that it was unstable, losing activity in 2–3 min, and its release was favoured by arachidonic acid and inhibited by anti-inflammatory drugs (Piper & Vane, 1969 ; Vargaftig & Dao, 1971).

Thus, both in guinea-pig lungs and in rabbit spleen, the biosynthesis and release of prostaglandins is accompanied by the release of RCS. These results, coupled

with the observations that prostaglandin activity in the incubation coil increases as the RCS activity declines, and that a RCS is generated from arachidonate by a crude cell-free prostaglandin synthetase extracted from dog spleen (Gryglewski & Vane, 1971), suggest that RCS is an unstable intermediate in the biosynthesis of prostaglandins. The cyclic endoperoxide, postulated by Samuelsson, Granström & Hamburg (1967) as an intermediate in biosynthesis of prostaglandins from arachidonic acid, is thought to be an unstable substance (Nugteren, Beerthuis & Van Dorp, 1967). Pace-Asciak & Wolfe (1971) and Pace-Asciak (1971) have described the formation from arachidonic acid of new prostanoic acid derivatives by prostaglandin synthesizing systems. However, these are stable and have little biological activity.

The prostaglandin precursors, arachidonic acid and bis-homo-γ-linolenic acid have several pharmacological activities (Horton & Main, 1966) although in general they are much weaker than the prostaglandins. Dakhil & Vogt (1962) showed that some of the smooth muscle stimulating activity of unsaturated long-chain fatty acids was due to the formation of peroxides. RCS has strong pharmacological activity in that it contracts rabbit aorta (Piper & Vane, 1969) and many other arterial muscle strips from several species (Piper, unpublished). Thus, if RCS is one of the peroxide intermediates in the formation of prostaglandins, it is possible that liberation of arachidonic acid, followed by generation of prostaglandins, will lead to a spectrum of pharmacological actions, some of which may be due to transient intermediates and some to the prostaglandins themselves. Since the production of RCS is inhibited by anti-inflammatory acids (Piper & Vane, 1969), as is the synthesis of prostaglandins (Vane, 1971) some of the intermediates may also contribute to the genesis of pain, inflammation and pyresis.

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REFERENCES

DAKHIL, T. & VOGT, W. (1962). Hydroperoxyde als Träger der darmerregenden wirkung hochungesättigter Fettsäuren. *Arch. exp. Path. Pharmak.*, **243**, 174–186.

DAVIS, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1967). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *J. Physiol.*, **188**, 38–39*P*.

DAVIS, B. N., HORTON, E. W. & WITHRINGTON, P. G. (1968). The occurrence of prostaglandin E_2 in splenic venous blood of the dog following splenic nerve stimulation. *Br. J. Pharmac.*, **32**, 127–135.

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. & VANE, J. R. (1967). Prostaglandins: their disappearance from and release into the circulation. *Nature*, **216**, 868–873.

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

GILMORE, N., VANE, J. R. & WYLLIE, J. H. (1969). Prostaglandin release by the spleen in response to an infusion of particles. In: *Prostaglandins, Peptides and Amines*, ed. Mantegazza, P. & Horton, E. W., pp. 21–29. London: Academic Press.

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors: XIX. Thin layer chromatography of prostaglandins. *J. Lipid Res.*, **5**, 117–120.

GRYGLEWSKI, R. & VANE, J. R. (1971). Rabbit-aorta contracting substance (RCS) may be a prostaglandin precursor. *Br. J. Pharmac.*, **43**, 420–421*P*.

HORTON, E. W. & MAIN, I. H. M. (1966). The relationship between the chemical structure of prostaglandins and their biological activity. *Mem. Soc. Endocr.*, **14**, 29–36.

NUGTEREN, D. H., BEERTHUIS, R. K. & VAN DORP, D. A. (1967). Biosynthesis of prostaglandins. In: *Proceedings of* IInd *Nobel Symposium*, ed. Bergström, S. & Samuelsson, B., pp. 45–50. Interscience Publishers.

PACE-ASCIAK, C. (1971). Polyhydroxy cyclic ethers formed from tritiated arachidonic acid by acetone powders of sheep seminal vesicles. *Biochemistry*, **10**, 3664.

PACE-ASCIAK, C., MORAWSKA, K., COCEANI, F. & WOLFE, L. S. (1968). The biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ in homogenates of the rat stomach. In: *Prostaglandin Symposium of the Worcester Foundation for Experimental Biology*, ed. Ramwell, P. W. & Shaw, J. E., pp. 371–378. New York: Interscience.

PACE-ASCIAK, C. & WOLFE, L. S. (1971). A novel prostaglandin derivative formed from arachidonic acid by rat stomach homogenates. *Biochemistry*, **10**, 3657–3664.

PALMER, M. A., PIPER, P. J. & VANE, J. R. (1970). The release of rabbit aorta contracting substance (RCS) from chopped lung and its antagonism by anti-inflammatory drugs. *Br. J. Pharmac.*, **40**, 581*P*.

PATON, W. D. M. (1957). A pendulum auxotonic lever. *J. Physiol., Lond.*, **137**, 36*P*.

PIPER, P. J. & VANE, J. R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature*, **223**, 29–35.

PIPER, P. J. & VANE, J. R. (1971). The release of prostaglandins from lung and other tissues. *Ann. N.Y. Acad. Sci.*, **180**, 363–385.

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

SAMUELSSON, B., GRANSTRÖM, E. & HAMBURG, M. (1967). On the mechanism of biosynthesis of prostaglandins. In: *Proceedings of* II*nd Nobel Symposium*, ed. Bergström, S. & Samuelsson, B., pp. 31–44. Interscience Publishers.

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

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. (1971). Release of vaso-active substances from guinea-pig lungs by slow reacting substance C and arachidonic acid: its blockade by non-steroid anti-inflammatory agents. *Pharmacology*, **6**, 99–108.

WILLIS, A. L. (1970a). A method for studying release of prostaglandins from superfused strips of isolated spleen. *Br. J. Pharmac.*, **40**, 470*P*.

WILLIS, A. L. (1970b). Simplified thin-layer chromatography of prostaglandins in biological extracts. *Br. J. Pharmac.*, **40**, 583*P*.

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