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Some effects of inhibiting endogenous prostaglandin formation on the responses of the cat spleen

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Summary

1. Cat isolated spleens release prostaglandins into the venous effluent in response to stimuli such as nerve stimulation, noradrenaline and angiotensin II.

2. The release of prostaglandins is abolished by pre-treatment of the spleen with indomethacin (0·3–5 μg/ml).

3. The capsular and vascular responses to the different stimuli are augmented after inhibition of prostaglandin release.

4. The prevention of prostaglandin release, as well as the augmentation of vascular and capsular responses, are reversible after indomethacin treatment is stopped.

5. The role of prostaglandins as modulators of the responses to several stimuli is discussed.

Introduction

Several workers have suggested that prostaglandins act as modulators of hormone action and of neurotransmission (Bergström, 1967 ; Horton, 1969 ; Hedqvist, 1970 ; Piper & Vane, 1971). The discovery that indomethacin and other anti-inflammatory acids block the synthesis of prostaglandins in various systems (Vane, 1971 ; Ferreira, Moncada & Vane, 1971 ; Smith & Willis, 1971) has provided a useful tool to assess some of these hypotheses. The present experiments were designed to test Hedqvist's proposal (Hedqvist, 1969a, b, 1970) that prostaglandin release from cat spleen is a negative feedback mechanism which attenuates the effects of nerve stimulation.

Methods

Thirteen cats weighing from 1·5–5 kg were anaesthetized with pentobarbitone sodium (30 mg/kg) intramuscularly. The abdomen was opened and the splenic pedicle carefully dissected to separate artery, vein and nerves. Heparin (1,000 I.U./kg) was injected intravenously and the splenic artery and vein were cannulated with polyethylene tubing. The spleen was then removed and perfused at 8–15 ml/min with Krebs bicarbonate solution at 37° C containing 3% dextran as previously described (Ferreira *et al.*, 1971).

A portion (5 ml/min) of the splenic outflow was used to superfuse a rat stomach strip (Vane, 1957) rat colon (Regoli & Vane, 1964) and chick rectum (Mann & West, 1950). These tissues were chosen because of their sensitivity to prostaglandins and specificity was further increased by infusing a mixture of antagonists

(Gilmore, Vane & Wyllie, 1968) which prevented any action of acetylcholine, 5-hydroxytryptamine, histamine or catecholamines. Contraction of the assay tissues were detected with auxotonic levels attached to 'Harvard' smooth muscle transducers and displayed on a multi-channel recorder (Watanabe). The overall amplification of the system was fourfold.

In all experiments indomethacin was infused continuously into the splenic outflow, so that the assay tissues were continuously exposed to a prostaglandin synthetase inhibitor throughout the experiment. Thus, any effects observed on the assay tissues were due to changes in prostaglandin output from the spleen. Indomethacin was applied to the spleen by changing the infusion into the splenic inflow.

In four experiments, samples of splenic outflow were collected for extraction and thin layer chromatography. Each sample (150 ml) was acidified to pH 3 with 1 N HCl and extracted twice with equal volumes of ethyl acetate. The two extracts

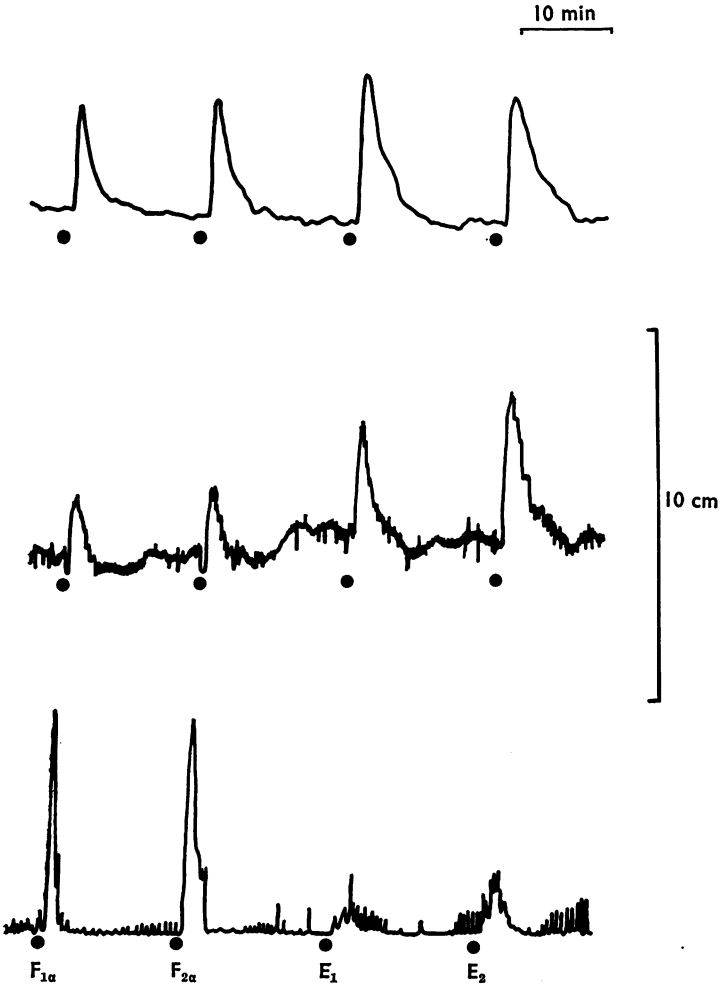

FIG. 1. Differential responses of a rat stomach strip, chick rectum and rat colon to prostaglandins $F_{1\alpha}$ (100 ng) $F_{2\alpha}$ (19 ng) E_1 (30 ng) and E_2 (14 ng). The F prostaglandins cannot be differentiated from each other, although they can be distinguished from prostaglandin E_1 and E_2, which each give an individual pattern of contractions. Time, 10 min ; vertical scale 10 cm.

were combined and evaporated to dryness under reduced pressure. The residues were taken up in 0·5 ml ethanol and an aliquot (75–100 μl) chromatographed in the AI system of Gréen & Samuelsson (1964) alongside markers of prostaglandin E_2 and $F_{2\alpha}$ (5 μg). Strips of the chromatograms corresponding to the markers were scraped off the plates, taken up in Krebs solution and bioassayed as prostaglandin E_2 or $F_{2\alpha}$ as previously described (Gilmore *et al.*, 1968). The rest of the plates were also divided into strips (1 or 2 cm) and assayed against prostaglandin E_2 as a standard.

Although the bioassay system was able to distinguish between prostaglandins E_1 and E_2 and the F prostaglandins by differential contractions of rat stomach strip, chick rectum and rat colon it was not possible to distinguish prostaglandin $F_{1\alpha}$ from $F_{2\alpha}$ (Fig. 1). Some samples were therefore chromatographed in the AII system (Gréen & Samuelsson, 1964) in order to identify the prostaglandins released. Areas of the developed AI plates corresponding to the E and F markers were scraped off the plates, shaken with 0·5 ml ethanol and centrifuged. Aliquots (25 μl) of the ethanol extract were then applied to the AII plates. Recovery of prostaglandin standards after sequential chromatography in both systems was 20–50%.

The spleen was supported on a plastic tray hanging from a strain gauge, the output from which was displayed on one channel of the recorder, thereby recording contractions of the spleen as decreases in weight. The mean splenic perfusion pressure was displayed on another channel of the pen recorder. The spleen was covered with gauze moistened with 0·9% w/v NaCl solution (saline) and kept warm by a lamp, the output of which was electronically controlled to maintain it at 37° C by a thermistor placed on the surface of the spleen. The splenic nerves were stimulated by trains of pulses lasting 20–120 s at a frequency of 1–10 Hz applied with platinum electrodes connected to a Bell stimulator.

Substances used were prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ (Upjohn) (−)-noradrenaline bitartrate (Sigma), angiotensin II (Hypertensin, Ciba) and indomethacin (Merck, Sharp & Dohme).

Results

Resting output

There was a basal output of prostaglandin-like material which increased as the experiment progressed. The initial basal output was estimated from the contractions produced when the assay tissues were superfused with splenic effluent instead of fresh Krebs solution. This varied from undetectable concentrations up to 4 ng/ml. Parallel to this increase in the basal output of prostaglandin-like material there was a decrease in the sensitivity of the assay tissues to calibrating doses of prostaglandins.

Stimulated output

Stimulation of the splenic nerves, repeated every 10–20 min, induced a rise in the perfusion pressure (vasoconstriction) and a decrease in the spleen weight (splenic contraction). The first three or four responses tended to increase but subsequent responses were thereafter reproducible. There was an associated output of prostaglandin-like material which gradually increased as the experiment progressed. The output occurring with each of the first few stimulations was (in terms of

prostaglandin E_2) generally less than 1 ng/ml, and reached concentrations of 10 to 30 ng/ml after two or three hours of perfusion.

The greater increases in output were obtained in the experiments where the basal output also substantially increased. As the prostaglandin output associated with nerve stimulation became larger, so the change in perfusion pressure tended to become biphasic (9 out of 13 experiments) so that the pressor response was followed by a prolonged fall in perfusion pressure below resting levels. The maximum of the after-vasodilatation response occurred when the prostaglandin output was at a peak. In 4 out of 13 experiments there was a secondary rise in the perfusion pressure after stopping nerve stimulation. In 4 experiments instead of nerve stimulation noradrenaline (0·5–2 μg) or angiotensin II (0·5 μg) were injected in 0·1 ml saline. Noradrenaline produced a similar effect to nerve stimulation on perfusion pressure, spleen weight and release of prostaglandin, whereas angiotensin produced a relatively greater effect on the vascular than on the capsular muscle; amounts of prostaglandin released were comparable to those with the other two stimuli.

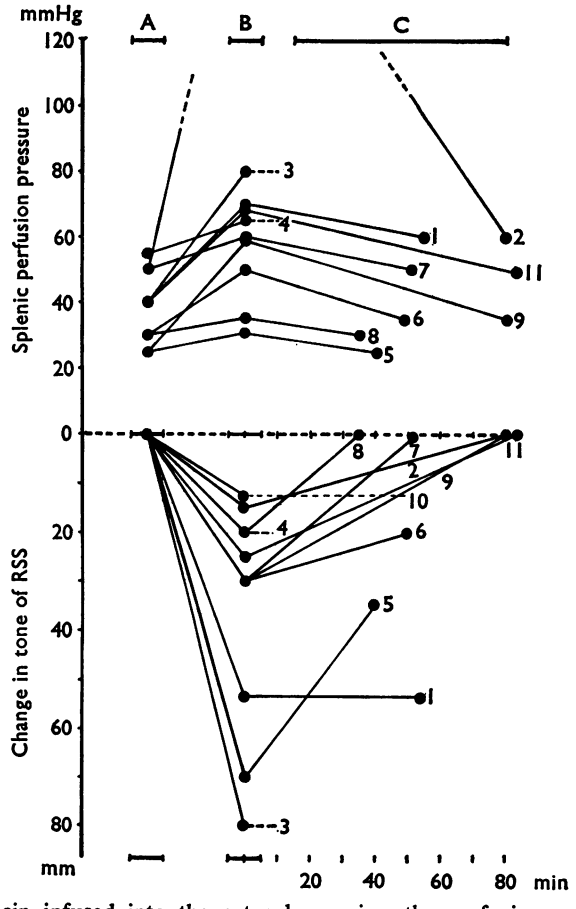

FIG. 2. Indomethacin infused into the cat spleen raises the perfusion pressure and relaxes the assay tissues. A, B, C show the values for splenic perfusion (mmHg) and change in tone of rat stomach strip (RSS) (mm) before, during and after indomethacin infusion. Indomethacin concentrations were 0·3 μg/ml in experiment 1; 1 μg/ml in experiments 2, 7, 8, 9; 1·5 μg/ml in experiments 10, 11 and 5 μg/ml in experiments 3, 4, 5, 6. The scale on the abscissae shows the time at which the measurements were made.

Identification of prostaglandins

Prostaglandins of the E and F group were measured by bioassay after chromatography in the AI system. There was approximately 10 times as much E present as F. Further chromatography in the AII system combined with bioassay showed that prostaglandin E_2 was the major component (85–95%), with traces of $F_{2\alpha}$ (5–15%); no E_1 or $F_{1\alpha}$ could be detected.

Effects of indomethacin

When indomethacin (0·3–5 μg/ml) was infused into the spleen 30–60 min from the start of the perfusion, at a time when neither basal nor stimulated release of prostaglandins was observed, it had no effect on the resting spleen weight or basal perfusion pressure and there was no relaxation of the assay tissues. It was not possible to determine the effects of indomethacin on stimulation of the spleen at this stage of the experiment, for the responses to stimulation were not reproducible.

However, after 2–3 h, when basal output of prostaglandins had increased, although indomethacin produced no change in the spleen weight, the resting perfusion pressure always increased. At the same time, there was a decrease in the

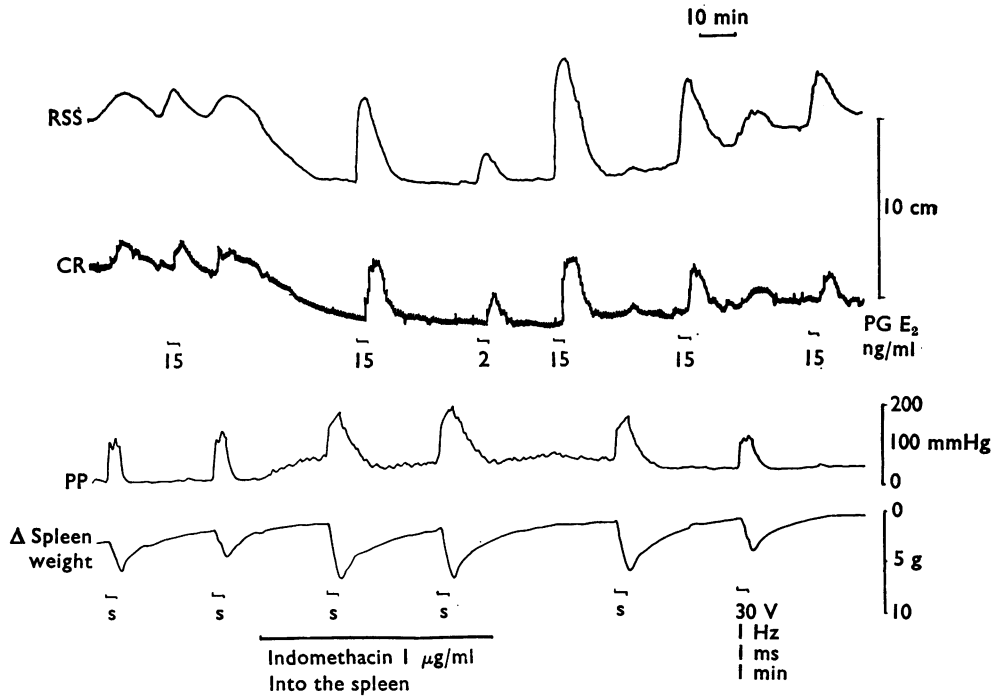

FIG. 3. Inhibition of prostaglandin synthesis increases the effects of splenic nerve stimulation. Indomethacin (1 μg/ml) was infused into the splenic outflow except when applied to the spleen. The upper two tracings show the contractions of rat stomach strip (RSS) and chick rectum (CR) to prostaglandin infusion or release from spleen following nerve stimulation (s). The lower two tracings show the splenic perfusion pressure (PP) (mmHg) and the change in splenic weight (g). The infusion of indomethacin (1 μg/ml) into the spleen causes (a) relaxation of the assay tissues together with an increase in sensitivity to exogenous prostaglandin E_2 (PGE_2) (b) increased perfusion pressure and augmentation of the effects of nerve stimulation (c) abolition of prostaglandin output induced by nerve stimulation (experiment 9). Time 10 min; vertical scales 10 cm, 200 mmHg and 10 g.

resting output of prostaglandins as shown by the relaxation of the assay tissues (Fig. 2). When the relaxation of the assay tissues was complete the previous basal output was again estimated. This averaged 4·2 ng/ml (range 1–11 ng/ml). The relaxation of the assay tissues could be reversed by infusions of prostaglandin E_2 (1–11 ng/ml) into the spleen. The sensitivity of the assay tissues to prostaglandins was increased when the splenic output of prostaglandins had been abolished by indomethacin.

During indomethacin treatment of the spleen the effects of nerve stimulation, noradrenaline or angiotensin were augmented (Figs. 3 and 4). The peak of the effects was increased in only six out of thirteen experiments for the perfusion pressure and in eight out of thirteen for the splenic weight. However, in all the experiments the duration of the effects was substantially increased and when present, the after-vasodilatation of the vessels was abolished. In the four experiments in which a secondary rise of the perfusion pressure after stopping nerve stimulation was observed it was also augmented by indomethacin.

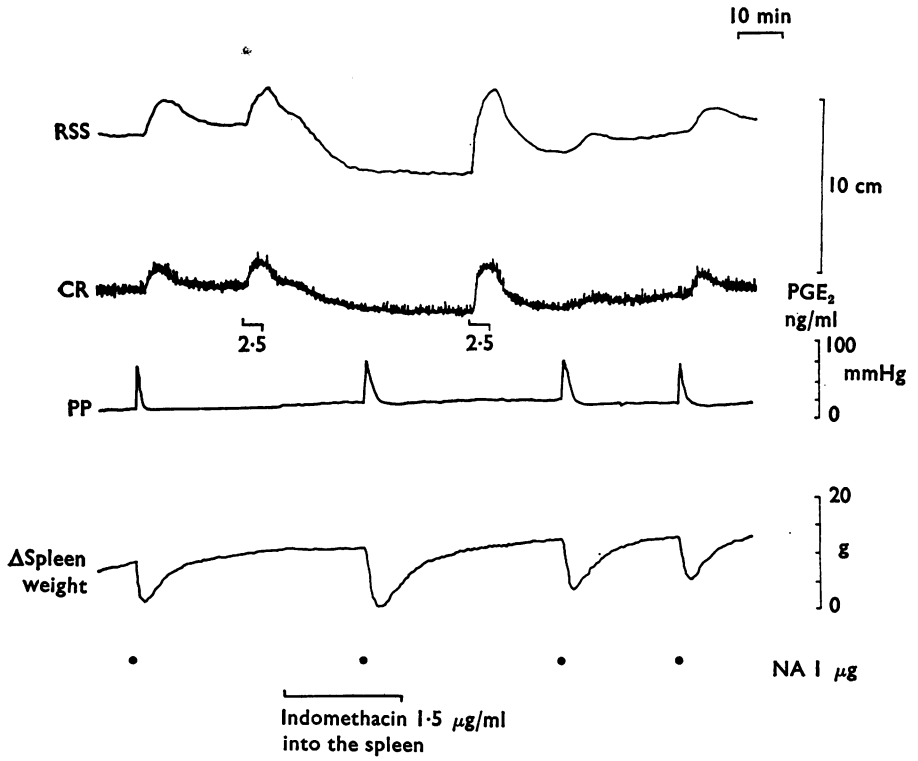

FIG. 4. Inhibition of prostaglandin synthesis increases the effects of noradrenaline injections. Indomethacin (1·5 μg/ml) was infused into the splenic outflow except when applied to the spleen. The upper two tracings show the contractions of a rat stomach strip (RSS) and a chick rectum (CR) to prostaglandin (PG) infusions and release of prostaglandin by noradrenaline injections. The lower two tracings show the splenic perfusion pressure (PP) (mmHg) and change in spleen weight (g). The infusion of indomethacin (1·5 μg/ml) into the spleen caused (a) relaxation of the assay tissues together with an increase in the sensitivity to calibrating infusions of prostaglandin E_2 (b) increased perfusion pressure and augmentation of the effects of noradrenaline injections (NA) (c) abolition of prostaglandin output induced by noradrenaline injections (experiment 12). Time 10 min; vertical scales 10 cm, 100 mmHg and 20 g.

An estimate of the overall changes was obtained by measuring the areas under the curves of the responses (Fig. 5). In all experiments (except no. 8 for the effects on weight) both the rise in perfusion pressure and the fall in spleen weight induced by nerve stimulation were augmented by indomethacin. At the same time, the release of prostaglandins induced by nerve stimulation was abolished. In 11 experiments the perfusion was continued for long enough (30–80 min) after the indomethacin was withdrawn from the spleen, for the augmentation of the effects of stimulation to be reversed. There was also a recovery of prostaglandin output.

Infusions of prostaglandin E_2 (1·5–5 ng/ml) into the spleen before indomethacin produced a small decrease in the resting perfusion pressure, no change in resting splenic weight and a small diminution in the effects of nerve stimulation. After indomethacin, infusions of the same concentrations of prostaglandin E_2 caused a greater decrease in the resting perfusion pressure and a reduction of the effects of nerve stimulation towards pre-treatment levels (Fig. 6).

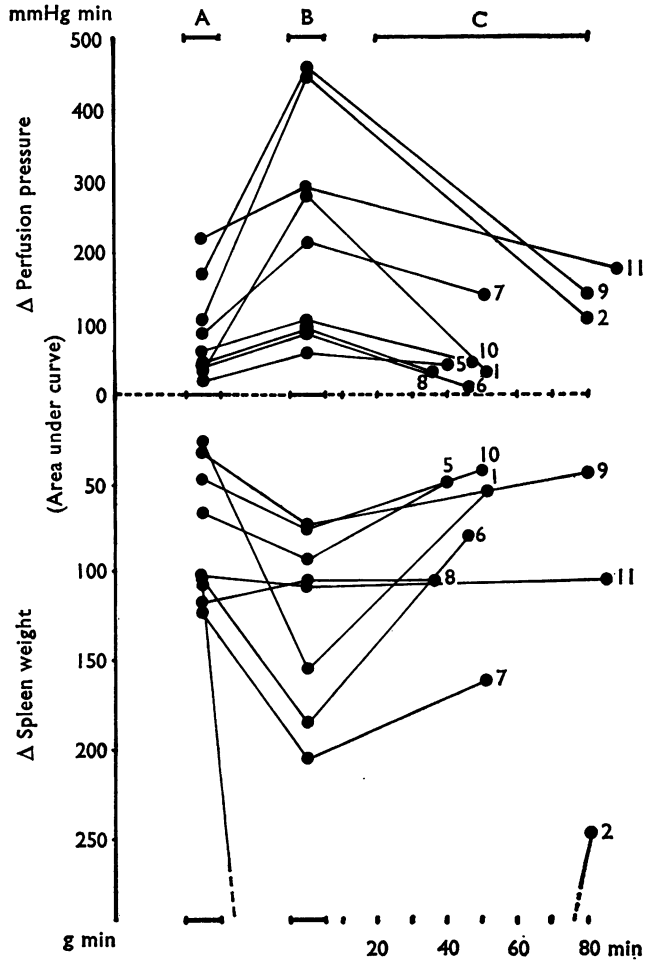

FIG. 5. Augmentation of the effects of splenic nerve stimulation by indomethacin. A, B, C show the change in perfusion pressure and spleen weight due to nerve stimulation before, during and after infusion of indomethacin into the spleen (for final concentrations see Fig. 2). The values are expressed as the integral (mmHg×min for perfusion pressure and g×min for spleen weight). The scale on the abscissae shows the time (in min) at which the measurements were made after the end of indomethacin infusion.

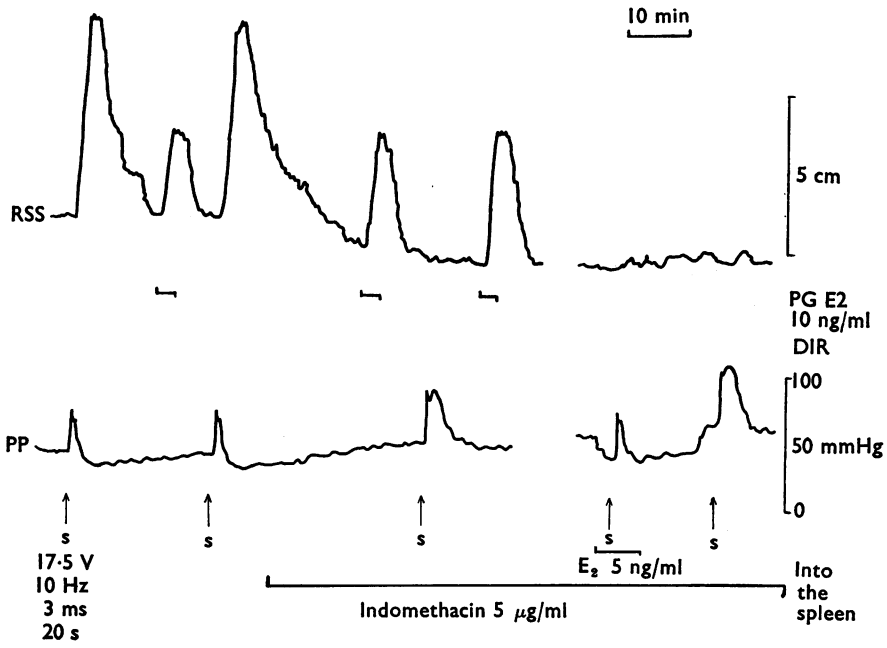

FIG. 6. Presence of prostaglandin reduces the effects of nerve stimulation. The upper tracing shows the contractions of rat stomach strip (RSS) to prostaglandin infusion or release after nerve stimulation (s). Indomethacin (5 μg/ml) infused into the spleen caused (a) relaxation of the RSS together with an increase of sensitivity to exogenous prostaglandin E_2 and (b) augmentation of the rise in perfusion pressure (PP) of the spleen due to nerve stimulation. An infusion of prostaglandin E_2 (PGE_2) (5 ng/ml) into the spleen during indomethacin infusion caused vasodilatation and reduced the rise in perfusion pressure due to nerve stimulation (experiment 4). Time 10 min ; vertical scales 5 cm and 100 mmHg.

Discussion

When the cat spleen contracts, there is a release into the venous effluent of a mixture of prostaglandins which we have identified as predominantly E_2 with a little $F_{2\alpha}$. In this respect, therefore, the spleen of the cat behaves similarly to that of the dog (Davies, Horton & Withrington, 1968 ; Ferreira & Vane, 1967 ; Gilmore, *et al.*, 1968). Results with dog spleen suggested that prostaglandins were released from contracting trabecular muscle (Ferreira & Vane, 1967 ; Gilmore *et al.*, 1968). Our finding that angiotensin provoked an equivalent release of prostaglandins to that caused by noradrenaline, even though the splenic contraction was much less, suggests that other cells can also participate in prostaglandin release. Gilmore, Vane & Wyllie (1969) came to a similar conclusion, for they found that an infusion of particles into the dog spleen caused prostaglandin release without splenic contraction.

From his results on cat spleen, Hedqvist (1969a) suggested that prostaglandin E_2 released during splenic contraction had a homeostatic function, reducing both the amount of noradrenaline released from sympathetic neurones and its effects on the smooth muscle. Our results show that when release of prostaglandin from the cat spleen is abolished by indomethacin, there is an augmentation of, not only the effects of nerve stimulation, but also those of noradrenaline and angiotensin injections. There was also augmentation of the effects of adrenaline infusions in dog spleen when indomethacin prevented prostaglandin production (Ferreira, *et al.*,

1971). These results, therefore, support Hedqvist's proposal and furthermore show that antagonism of the effects of noradrenaline on smooth muscle is at least as important a homeostatic mechanism as is the reduction in noradrenaline release. This conclusion is reinforced by the results of Holmes, Horton & Main (1963) and Weiner & Kaley (1969) who showed in various preparations that prostaglandin injections or topical application diminished the effects of nerve stimulation, noradrenaline or angiotensin. Since prostaglandin release may also have a regulatory function on the sympathetic innervation of the heart (Wennmalm & Hedqvist, 1970 ; Hedqvist, Stjärne & Wennmalm, 1970 ; Wennmalm & Stjärne, 1971) and the vas deferens (Swedin, 1971 ; Hedqvist & von Euler, 1972), Hedqvist's hypothesis may have a much wider application than to the sympathetic innervation of the spleen.

In our present results, abolition of prostaglandin release augmented more the duration than the peak of the effects of the various stimuli. This is consistent with the fact that the maximum release of prostaglandins occurred after the end of each stimulation and was co-incident with the vascular after-vasodilatation which was sometimes present. Thus, the released prostaglandins act more by limiting the residual actions of the agonists (or the release of the transmitter) after the stimulus has been withdrawn. Holmes *et al.* (1963) also found that prostaglandin E_1 reduced the duration, but not the force of contraction of the cat nictitating membrane elicited by nerve stimulation. These results support the hypothesis that prostaglandin release attenuates the effects of nerve stimulation by a feed-back loop, but suggest that such a loop has a time constant measured in minutes rather than seconds. They also fit in with the more general proposal (Piper & Vane, 1971 ; Collier, 1971) that prostaglandin production and release is a local mechanism by which cells can adapt themselves to many different types of stimuli, including those of a pathological, chemical or physiological nature.

As the spleen perfusion proceeded, there was a gradual increase in both the continuous basal release and the stimulated release of prostaglandins. The concentration of prostaglandin measured in the venous effluent can be regarded as an overflow, which represents a balance between prostaglandin production and inactivation by the tissue. Thus, the increases in overflow observed with time may have been due to increased formation or to decreased inactivation of prostaglandin. Our experiments do not distinguish between these possibilities, but they do show that the activity of the released prostaglandin on the vasculature of the spleen increases with time. For instance, indomethacin infusion at an early stage of the experiment did not increase the perfusion pressure, as it did later on ; furthermore, as the stimulated prostaglandin output increased, so the vascular after-vasodilatation was seen.

An increase in production of prostaglandins with time may be an expression of a gradual deterioration of the isolated perfused spleen, perhaps due to slight oedema formation. Such oedema formation would explain the increased resistance to perfusion which became evident after indomethacin had abolished the prostaglandin release and it is known that tissue trauma of various kinds leads to prostaglandin formation (Piper & Vane, 1971). This reasoning implies that at least part of the continuous basal release of prostaglandin is a function of tissue damage, rather than of a local mechanism for physiological regulation of vascular tone. In the isolated perfused spleen of the dog, we did not clearly see a basal release of

prostaglandins (Ferreira, *et al.*, 1971) such as we find here in similar experiments on cat spleen. From their experiment in anaesthetized dogs, Aiken & Vane (1971) concluded there was a continuous prostaglandin release in the kidney which contributed to local regulation of vascular tone. They could not, however, show a similar basal release in hindlegs. Thus, the role of prostaglandin formation in local regulation of vascular tone may vary, not only from organ to organ, but also from species to species.

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REFERENCES

AIKEN, J. & VANE, J. R. (1971). Blockade of angiotensin-induced prostaglandin release from dog kidney by indomethacin. *The Pharmacologist*, **13**, 293.

BERGSTRÖM, S. (1967). Prostaglandins: members of a new hormonal system, *Science, N.Y.*, **157**, 382–391.

COLLIER, H. O. J. (1971). Prostaglandins and aspirin. *Nature (London)*, **232**, 17–19.

DAVIES, 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. Chemother.*, **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 (London)*, **216**, 868–873.

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

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

GRÉEN, K. & SAMUELSSON, B. (1964). Prostaglandins and related factors. XIX; thin layer chromatography. *J. Lipid Research*, **5**, 117–120.

HEDQVIST, P. (1969a). Modulating effect of prostaglandin E_2 on noradrenaline release from isolated cat spleen. *Acta physiol. scand.*, **75**, 511–512.

HEDQVIST, P. (1969b). Antagonism between prostaglandin E_2 and phenoxybenzamine on noradrenaline release from cat spleen. *Acta physiol. scand.*, **76**, 383–384.

HEDQVIST, P. (1970). Control by prostaglandin E_2 of sympathetic neuro-transmission in the spleen. *Life Sci.*, **9**, 269–278.

HEDQVIST, P., STJÄRNE, L. & WENNMALM, Å. (1970). Inhibition by prostaglandin E_2 of sympathetic neurotransmission in the rabbit heart. *Acta physiol. scand.*, **79**, 139–141.

HEDQVIST, P. & VON EULER, U. S. (1972). Prostaglandins control neuromuscular transmission in guinea-pig vas deferens. *Nature New Biol.*, **236**, 113–115.

HOLMES, S. W., HORTON, E. W. & MAIN, I. H. M. (1963). The effect of prostaglandin E_1 on the responses of smooth muscle to catecholamines, angiotensin and vasopressin. *Br. J. Pharmac. Chemother.*, **21**, 538–543.

HORTON, E. W. (1969). Hypothesis on physiological role of prostaglandins. *Physiol. Rev.*, **49**, 122–169.

MANN, M. & WEST, G. B. (1950). The nature of hepatic and splenic sympathin. *Br. J. Pharmac.*, **5**, 173–177.

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

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.

SWEDIN, G. (1971). Endogenous inhibition of the mechanical response of the isolated rat and guinea-pig vas deferens to pre- and post-ganglionic nerve stimulation. *Acta physiol. scand.*, **83**, 473–485.

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

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

WEINER, R. & KALEY, G. (1969). Influence of prostaglandin E_1 on the terminal vascular bed. *Am. J. Physiol.*, **217**, 563–566.

WENNMALM, Å. & HEDQVIST, P. (1970). Prostaglandin E_1 as an inhibitor of the sympathetic neuroeffector system in the rabbit heart. *Life Sci.*, **9**, 931–937.

WENNMALM, Å. & STJÄRNE, L. (1971). Inhibition of the release of adrenergic transmitter by a fatty acid in the perfusate from sympathetically stimulated rabbit heart. *Life Sci.*, **10**, 471–479.

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