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Prostaglandins, oxygen tension and smooth muscle tone

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Summary

1. By using indomethacin to inhibit their intramural synthesis, we have investigated the contribution of prostaglandins to the maintenance of (a) the intrinsic tone of isolated smooth muscle preparations and (b) contractions produced by drugs or high oxygen concentration.

2. When treated with indomethacin, the rat stomach strip and chick rectum preparation slowly relaxed, whether they were bathed in Krebs solution or blood. Although their sensitivity to added prostaglandin was somewhat enhanced, they became insensitive to changes in oxygen or glucose concentration. However, another smooth muscle preparation, the rat colon, was neither relaxed by indomethacin nor contracted by high oxygen concentration.

3. These results support the hypothesis that intramural generation of prostaglandin maintains the tone of some smooth muscle preparations.

4. Contractions of the guinea-pig isolated colon were induced by histamine. These contractions were normally well maintained but in Krebs solution lacking either oxygen or glucose, only the initial spike contraction remained. In the presence of indomethacin the histamine contraction was also poorly sustained, but maintenance was restored by a low concentration of prostaglandin E_2.

5. Thus, the effects on smooth muscle of oxygen or glucose lack may also be mediated by reduction in the synthesis or effects of an intramural prostaglandin. Extension of this hypothesis to intestinal and vascular smooth muscle *in vivo* is discussed.

Introduction

Some isolated smooth muscle preparations, such as the rat colon or guinea-pig ileum cannot be relaxed by catecholamines or spasmolytic drugs. Others, like the rat stomach strip, rabbit duodenum or jejunum, guinea-pig taenia caeci or ascending colon are relaxed by catecholamines, showing that in the 'normal' state in the isolated organ bath, the smooth muscle develops a continuous submaximal contraction. This resting tone is reduced when oxygen or glucose is withdrawn from the bathing fluid, or when the tissue is exposed to metabolic inhibitors such as 2:4-dinitrophenol (Gross & Clark, 1923; Garry, 1928; Prasad, 1935; Feldberg & Solandt, 1942; Born & Bülbring, 1955; Born, 1956; Blair & Clark, 1956). These effects have been attributed to an interruption of the normal supply of chemical energy to the muscle. However, Born (1956) found that anoxia or glucose lack reduced creatine phosphate but not ATP content in isolated taenia caeci of

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guinea-pigs and Northover (1971) found a rise in creatine phosphate and no change in ATP concentration in guinea-pig stomach muscle during anoxia.

Smith & Vane (1966) used rat stomach strip preparations for studying the effects of oxygen tension on smooth muscle. When superfused with blood or Krebs solution, the tone of the muscle was directly proportional to the pO_2. The contractor effect of high oxygen concentration was unaffected by hyoscine, phenoxybenzamine, hexamethonium, brom-lysergic acid diethylamide or mepyramine. The rat stomach strip is very sensitive to prostaglandins E_1, E_2 and $F_{2\alpha}$, the contractor activity of which is absent during anoxia (Coceani & Wolfe, 1966). Furthermore, enzymes which synthesize prostaglandins E_2 and $F_{2\alpha}$ have been isolated from homogenates of the rat stomach (Pace-Asciak, Morawska, Coceani & Wolfe, 1967) and synthesis of prostaglandins is dependent on molecular oxygen (Samuelsson, Granström & Hamberg, 1967; Nugteren, Beerthuis & Van Dorp, 1967).

We have used indomethacin, which strongly inhibits prostaglandin synthesis (Vane, 1971) in all systems so far tested (Aiken & Vane, 1971; Ferreira, Herman & Vane, 1972; Ferreira, Moncada & Vane, 1971; Smith & Lands, 1971; Smith & Willis, 1971), to determine whether local generation of prostaglandins in smooth muscle preparations can account for the effects induced by changes in oxygen concentration, or by glucose lack.

Methods

The isolated tissue preparations used were rat stomach strip (Vane, 1957), rat colon (Regoli & Vane, 1964), chick rectum (Mann & West, 1950), guinea-pig ascending colon and guinea-pig taenia caeci. Some tissues were superfused at 10 ml/min by the blood bathed organ technique (Vane, 1964), with arterial blood at 37° C from anaesthetized cats (chloralose, 80 mg/kg i.p.) or dogs (chloralose, 100 mg/kg i.v.). The animals were given heparin (1,000 i.u./kg) and ventilated with a positive pressure respiration pump ('Ideal', Palmer); the oxygen concentration of the arterial blood was increased by supplying the input of the respiration pump with oxygen instead of air. In some experiments, the tissues were superfused with Krebs solution, pre-gassed with oxygen containing 5% CO_2.

The reactions of other tissues were also studied in isolated organ baths of 15 ml capacity containing Krebs solution bubbled with 5% carbon dioxide in either oxygen or nitrogen. A pair of baths was used so that a treated tissue could be compared with an untreated one.

Changes in length of the isolated tissues were detected by Harvard smooth muscle transducers fixed with a pendulum lever (Paton, 1957); the output from the transducers was suitably amplified and displayed on a multi-channel pen recorder (Watanabe). The magnification for rat stomach strip was 4 times, for guinea-pig taenia caeci 8 times and for guinea-pig ascending colon and chick rectum 16 times. The Krebs solution was composed of the following in g/l. (mM): NaCl, 6·9 (118); KCl, 0·35 (4·7); $CaCl_26H_2O$, 0·55 (2·5); KH_2PO_4, 0·16 (1·2); $MgSO_47H_2O$, 0·29 (1·17); glucose, 1·0 (5·6); $NaHCO_3$, 2·1 (25·0).

The following drugs were used: heparin (Boots); histamine acid phosphate (British Drug Houses); indomethacin (Merck, Sharp & Dohme); niflumic acid (Squibb); phenylbutazone (Geigy); prostaglandin E_2 (PGE_2) (Upjohn); sodium meclofenamate (Parke, Davis & Co.). Indomethacin was diluted from a fresh solution made up by dissolving 10–25 mg in ethanol (0·2–0·5 ml) and diluting with

Krebs solution to give 1 mg/ml. Concentrations of histamine are expressed in terms of base.

Results

Superfusion with Krebs solution

Indomethacin (1–2 μg/ml) had no effect on the tone of the rat colon, but sometimes reduced the spontaneous activity. However, in the same concentration it always caused a gradual relaxation of the rat stomach strip and chick rectum. Even after exposure to indomethacin for one hour, relaxation was still incomplete. After stopping the indomethacin treatment, further relaxation occurred over the next two hours. During the course of these experiments, which were mainly done on rat stomach strips, the sensitivity of the preparations to prostaglandin E_2 increased. A similar increase was observed in untreated strips, making it difficult to assess whether indomethacin had any further effect on sensitivity. However, in isolated organ bath experiments (see later) indomethacin induced a clear augmentation of the effects of prostaglandin E_2.

Blood bathed organ experiments

When rat stomach strips or chick rectums are superfused with cat or dog blood in place of Krebs solution, they contract (Vane, 1964). Thereafter the tissues gradually relax until, after 30–90 min they stabilize at a higher tone than that exhibited in Krebs solution. Ventilation of the animal with oxygen instead of air then causes the rat stomach strip and chick rectum to contract further (Smith & Vane, 1966). Such an effect is shown in Fig. 1, in which the two tissues were

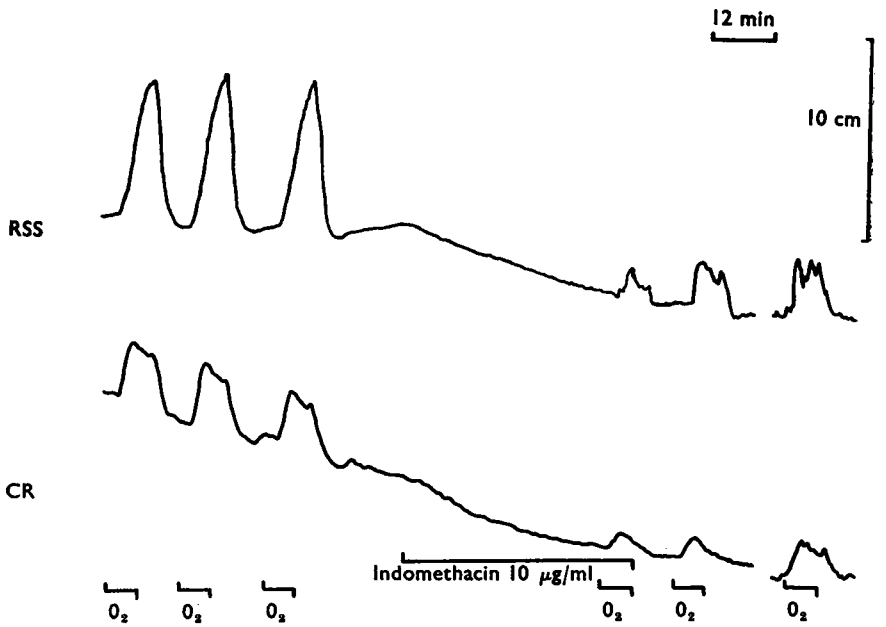

FIG. 1. The tracings are from a rat stomach strip (RSS) and chick rectum (CR) superfused at 10 ml/min with arterial blood from a dog (10·8 kg). The first three contractions of each tissue were induced by ventilating the dog with pure oxygen (O_2) for 6 min instead of air. Indomethacin was then infused into the bathing blood to give a concentration of 10 μg/ml. This caused relaxation of both tissues and thereafter the contraction induced by high oxygen tension was almost abolished. The last contraction shows that there was little recovery 2 h later. Time, 12 min; vertical scale 10 cm pen movement, which represents a muscle shortening of 2·5 cm, or about 25%.

superfused with blood from a dog. After the initial contraction due to exposure to blood the tone of the rat stomach strip had stabilized but that of the chick rectum was still gradually declining. Both tissues contracted each time the dog was ventilated with oxygen for 6 minutes. Indomethacin was then infused into the extracorporeal circulation to give a concentration in the blood of 10 μg/ml. This gradually reduced the resting tone of the rat stomach strip and increased the rate of relaxation of the chick rectum. At the end of the infusion (total dose of 4·4 mg or 0·41 mg/kg), the contractions of the tissues induced by ventilating the dog with oxygen were almost abolished. There was only slight recovery of the effect 3 hours later.

Similar effects were obtained in 10 experiments in which rat stomach strips (and sometimes chick rectums) were superfused with blood from cats. In all of these, an infusion of indomethacin (10 μg/ml) into the bathing blood caused a reduction in the resting tone of the preparations. This was always accompanied by a reduction or abolition of the contraction induced by ventilating the cat with oxygen. Injection of indomethacin (2 mg/kg) intravenously gave similar effects.

The cats received proportionally larger doses of indomethacin than did the dogs, making it even more difficult to demonstrate recovery. Reversibility of the effect of indomethacin was demonstrated, however, by using a second cat, as in Fig. 2. The first three responses (upper trace) show the contractor effects produced by respiring the cat with oxygen. Indomethacin (10 μg/ml) was then infused into the blood bathing the rat stomach strip and this caused a gradual relaxation in resting tone. At the end of the infusion (total dose of indomethacin to cat, 5 mg or 2·5 mg/kg), the effects of increased oxygen concentration were almost abolished. The rat stomach strip was then superfused with blood from a second cat which had not received indomethacin (lower trace). Initially, the response to increased

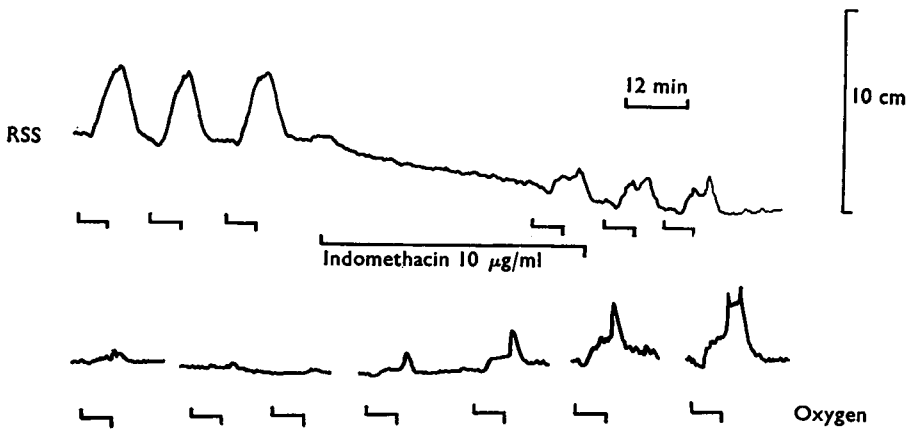

FIG. 2. The tracing is from a rat stomach strip (RSS) superfused at 10 ml/min with blood from a cat (2·0 kg). The first three contractions (upper trace) were induced by ventilating the cat with oxygen for 6 min instead of air. Indomethacin (10 μg/ml) in the bathing blood caused relaxation of the RSS after which the response to high oxygen tension was substantially reduced. The RSS was then superfused with blood from a cat untreated with indomethacin. The lower tracing shows the return of the effects of high oxygen tension 30, 45, 55, 75, 90, 100 and 120 min after starting the superfusion of the RSS with blood from the untreated cat. Time 12 min; vertical scale 10 cm, which represents a muscle shortening of 2·5 cm, or about 25%.

oxygen was still abolished but there was then a slow increase in the size of contraction induced by high oxygen so that four hours later the contraction was similar to that at the beginning of the experiment. However, this did not necessarily mean that the full effect had returned, for without indomethacin treatment, contractions of the rat stomach strip induced either by high oxygen or by prostaglandin E_2 (5–20 ng/ml) gradually increased throughout the experiment, so that after 6 h, half to one-fifth of the concentration of prostaglandin E_2 used initially was needed to produce a similar contraction. The effects of prostaglandin E_2 were not reduced by indomethacin, but because of the gradual increase in sensitivity to prostaglandin E_2 it was difficult to estimate whether indomethacin further increased the sensitivity of the strips to prostaglandin E_2.

In one experiment, the mixture of Krebs solution and ethanol used to dissolve the indomethacin was infused in an equivalent amount into the blood bathing the rat stomach strips. This had no effect on the basal tone, on the oxygen response, or on the contraction of the rat stomach induced by prostaglandin E_2.

Other anti-inflammatory acids which inhibit prostaglandin synthesis (Vane, 1971 ; Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972) produced a similar effect to indomethacin. These included niflumic acid (10 μg/ml, one experiment), phenylbutazone (20–40 μg/ml, four experiments) and meclofenamate (20 μg/ml, one experiment).

Isolated organ bath experiments

Rat stomach strips were bathed in Krebs solution at 38° C in a pair of isolated organ baths. When the organ baths were bubbled with a nitrogen/CO_2 mixture, the rat stomach strips relaxed. Such an experiment is shown in Fig. 3. The first contraction of each rat stomach strip was induced by prostaglandin E_2 (3 ng/ml) and the relaxations were due to bubbling the bath with 95% nitrogen/CO_2. One strip (lower tracing) was then treated with indomethacin (2 μg/ml) which caused a gradual reduction in the tone. Prostaglandin E_2 (3 ng/ml) was now more effec-

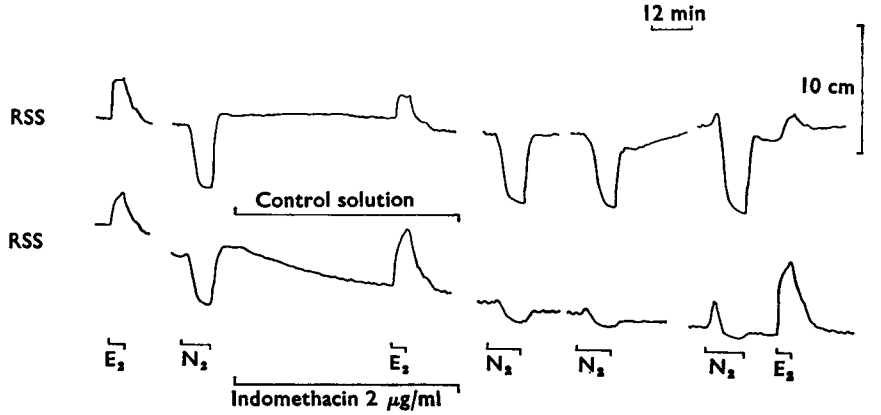

FIG. 3. Two rat stomach strips (RSS) were bathed in Krebs solution in isolated organ baths. Prostaglandin E_2 (3 ng/ml) caused similar contractions of both. Bubbling the baths with a 95% N_2, 5% CO_2 mixture caused relaxations. Indomethacin (2 μg/ml) was then added to one bath (lower tracing) and the vehicle to the other. The indomethacin-treated strip gradually relaxed and became more sensitive to prostaglandin E_2. At the same time, the relaxation induced by nitrogen was almost abolished. The last three nitrogen effects were obtained at 60, 100 and 200 min after indomethacin treatment. Time 12 min ; vertical scale 10 cm which represents a muscle shortening of 2·5 cm or about 25%.

tive on the indomethacin-treated strip but applicaton of nitrogen had a much reduced effect. Two hours later the relaxant effect of nitrogen was even smaller whereas the effect of prostaglandin E_2 was further augmented. After 4 to 5 h, application of nitrogen gave an increasing initial contraction of the rat stomach strip exposed to indomethacin. However, there was also a tendency for a contractor response to show in the untreated rat stomach strip at this stage of the experiment. In five other experiments indomethacin (1–2 μg/ml) caused a relaxation of the rat stomach strips and a gradual abolition of the response induced by bubbling with nitrogen. In five of the six experiments, the contractions induced by prostaglandin E_2 were increased by indomethacin and in the sixth, they were unaffected. The contractor effects of prostaglandin E_2 were always either abolished or substantially reduced when tested during application of nitrogen.

Similar effects were seen on a chick rectum preparation, but increase in the oxygen tension of the fluid bathing the rat colon did not cause contraction, either when it was bathed in blood or in Krebs solution.

Effects of oxygen or glucose lack on contractions induced by histamine and prostaglandin E_2

West, Hadden & Farrah (1951) using isolated intestine from rabbits and Born (1956) using taenia caeci from guinea-pigs showed that during anoxia, the longitudinal smooth muscles still contracted in response to chemical stimulants but did not sustain the contraction, as they did with oxygen present. We have confirmed these observations on the guinea-pig taenia caeci. However, in most experiments, we used the guinea-pig ascending colon preparation, which we chose because it showed a maintained contraction to histamine in normal conditions but a reduction in basal tone coupled with non-maintenance of the histamine response when the tissue was made anoxic. Figure 4 shows an experiment in which bubbling the bath with nitrogen caused a relaxation of the guinea-pig colon, which was then much less sensitive to prostaglandin E_2. With nitrogen instead of oxygen, the

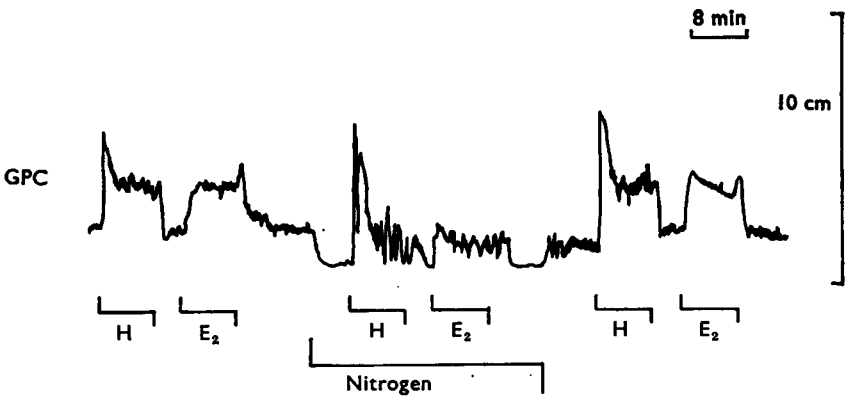

FIG. 4. The tracing is from a guinea-pig colon bathed in Krebs solution in an isolated organ bath. Both histamine (50 ng/ml) and prostaglandin E_2 (5 ng/ml) caused a contraction which was well maintained. When the bath was bubbled with 95% N_2, 5% CO_2 the colon relaxed. The histamine response showed only the initial contraction and the contraction due to prostaglandin E_2 was severely attenuated. The effects were reversed when the bath was bubbled with 95% O_2, 5% CO_2. Time 8 min; vertical scale 10 cm pen movement (magnification 16:1).

contraction induced by histamine was not maintained. Deprivation of glucose (Fig. 5) had a similar effect. In both instances, the reduced prostaglandin effect, unlike that of histamine, showed a plateau response with no initial spike.

Effects of indomethacin on contractions induced by histamine and prostaglandin E_2

A pair of tissues was used so that an untreated tissue could be compared with one exposed to indomethacin. Figure 6 shows part of an experiment in which

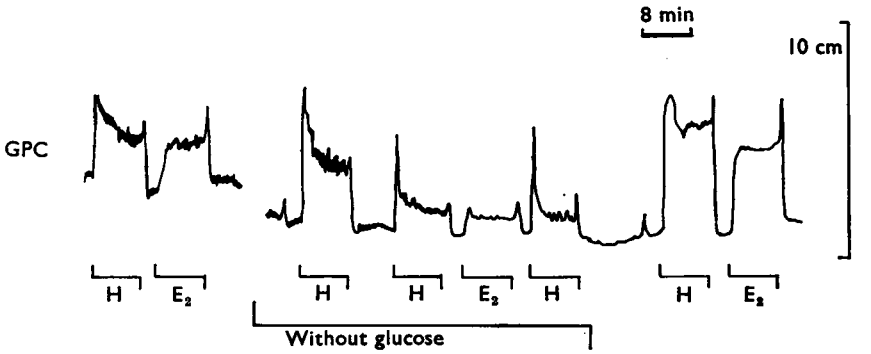

FIG. 5. The tracing is from a guinea-pig colon bathed in Krebs solution in an isolated organ bath. Histamine (50 ng/ml) and prostaglandin E_2 (5 ng/ml) caused contractions. When the tissue was bathed in Krebs solution without glucose, it gradually relaxed and 30 min later the contraction due to histamine was poorly sustained and the prostaglandin E_2 response was almost abolished. These effects were reversed when glucose was added to the Krebs solution. Time 8 min; vertical scale 10 cm pen movement (magnification 16:1).

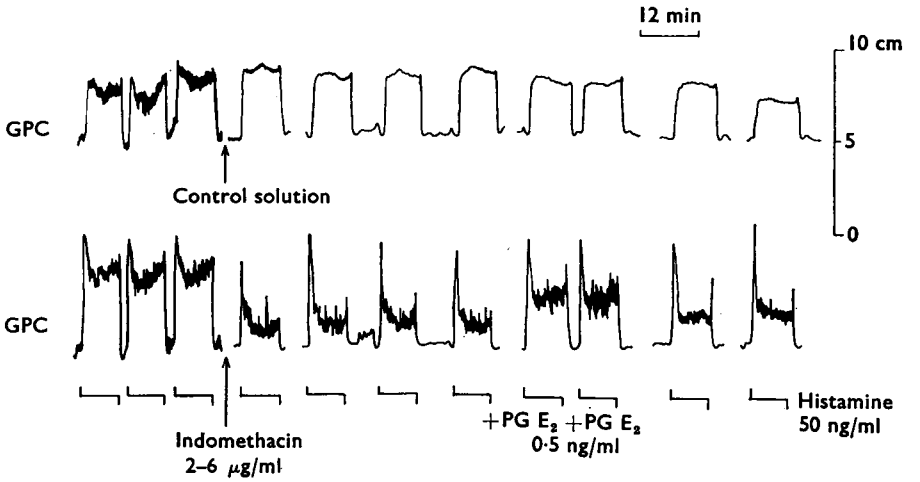

FIG. 6. Two guinea-pig colons were bathed in Krebs solution in isolated organ baths. Histamine (50 ng/ml) was added at regular intervals; it induced well-maintained contractions. Indomethacin was added to one tissue (lower trace) to give a concentration of 2 μg/ml for 40 min and 6 μg/ml for 30 minutes. This changed the histamine response so that it was no longer well maintained. The next two responses were obtained 20 and 60 min after stopping the indomethacin treatment. A further 120 min later, the response was poorly sustained but addition of a small concentration of prostaglandin E_2 (0·5 ng/ml) increased the maintenance of the histamine contraction. Time 12 min; vertical scale 10 cm pen movement (magnification 16:1).

three control contractions each lasting for 8 min were induced by histamine (50 ng/ml). One colon (lower tracing) was then treated with indomethacin for 30 minutes. The other tissue was treated with the solution used to dissolve the indomethacin. Exposure to indomethacin slightly reduced the peak response induced by histamine and substantially reduced the maintained response. Contractions due to prostaglandin E_2 were, if anything, potentiated. After the indomethacin was washed out the initial histamine contraction returned to pre-treatment levels but it was not sustained, giving a response very similar in shape to that seen during anoxia or glucose lack. The addition of a small concentration of prostaglandin E_2 (0·5 ng/ml) at the same time as the histamine tended to restore the maintenance of the contraction induced by histamine. This concentration of prostaglandin E_2 by itself did not cause contraction of the colon. Similar effects were seen in 10 other experiments.

After treatment with indomethacin was stopped, there was only slight recovery of the maintenance of the histamine response over several hours.

Discussion

Evidence is accumulating that the resting tone of some isolated smooth muscles is maintained by the continuous generation of a prostaglandin. Isolated preparations of frog intestine (Vogt & Distelkötter, 1967), bovine iris (Posner, 1970) and rabbit jejunum (Ferreira *et al.*, 1972) release prostaglandin into the bathing fluid. These tissues are all contracted by prostaglandins; in all of them, the release of prostaglandins probably represents fresh synthesis, and in the two in which it has been measured (iris and jejunum) the release is related to the basal tone of the tissue. In the rabbit jejunum, indomethacin abolishes both the resting tone and the release of prostaglandin into the bath fluid (Ferreira *et al.*, 1972) and in several isolated tissues, prostaglandin antagonists reduce the resting tone (Bennett & Posner, 1971).

Our present results add further support to this concept. For instance, it is already established that prostaglandins can be synthesized (Pace-Asciak *et al.*, 1967) and released (Coceani & Wolfe, 1966; Bennett, Friedmann & Vane, 1967) by preparations of rat stomach. We have now shown that indomethacin, a potent inhibitor of prostaglandin biosynthesis gradually reduces the basal tone of the rat stomach strip. A concentration of 1–2 μg/ml was sufficient to produce this effect in Krebs solution but when blood was used as the bathing fluid, the indomethacin concentration was increased to 10 μg/ml to allow for 90% binding to plasma proteins (Hucker, Zacchei, Cox, Brodie & Cantwell, 1966). The fact that other non-steroid anti-inflammatory substances, in concentrations proportional to their activity against prostaglandin synthesis also reduce the basal tone of the rat stomach strip, makes it more likely that this is a specific effect on prostaglandin synthesis rather than some unrelated and unspecific action of indomethacin. The results with the rat colon also reinforce this conclusion, for it was neither relaxed by indomethacin or oxygen lack nor contracted in blood by increased oxygen tension. Presumably in this isolated tissue and in others which lack tone, there is little or no prostaglandin synthesis, or the tissue is insensitive to the amounts (or the type) synthesized.

The evidence, then, supports the concept (Bennett & Posner, 1971; Ferreira, *et al.*, 1972) that the tone of some isolated smooth muscles is maintained by an

intramural generation of prostaglandin(s). It follows from this conclusion that some procedures which abolish smooth muscle tone, such as anoxia or glucose lack, may do so by interfering with either the intramural generation of, or with the reactions of the tissue to, prostaglandin(s). Anoxia would be expected to reduce both prostaglandin synthesis, which depends upon molecular oxygen, (Samuelsson *et al.*, 1967; Nugteren *et al*, 1967) and the contractions of the tissue produced by prostaglandin, as shown by Coceani & Wolfe (1966) and our present results. Glucose lack also prevents the contractor activity of prostaglandin E_2; thus, if the tone is maintained by the action of locally generated prostaglandin, glucose lack would be expected to reduce the tone.

The contraction of the rat stomach strip induced by an increase in oxygen tension in the bathing blood could reflect an increased synthesis of prostaglandin within the tissue, because more oxygen is available. This interpretation, which suggests that here, provision of oxygen is the rate-limiting step in prostaglandin synthesis, is supported by the fact that indomethacin abolishes the contractions of the rat stomach strip and chick rectum which are induced by raising the oxygen tension of the bathing blood, but does not antagonize the action of prostaglandin E_2. The results with the rat stomach strips bathed in Krebs solution allow a similar conclusion, for when the tone of the strip had been abolished by indomethacin, it no longer contracted when the bath was bubbled with oxygen rather than nitrogen, but became responsive once more to PGE_2. In this context, prostaglandin synthetase becomes the 'oxygen receptor' discussed by Smith & Vane (1966) as responsible for the contractions of smooth muscle induced by high oxygen tension.

Anoxia or glucose lack, like dinitrophenol, also prevent the maintenance of histamine-induced smooth muscle contraction, leaving only the initial spike. To find whether these effects might also be attributed to a lack of prostaglandin activity, caused either by an interference with the generation of prostaglandin(s) or by a loss of sensitivity to them, we investigated the effects of indomethacin on the contractions of the guinea-pig colon induced by histamine. There was a striking parallelism between the effects of this prostaglandin synthetase inhibitor and those of oxygen or glucose lack. In each instance the initial contractor effect of histamine was relatively unaffected but the contraction was not maintained. Whereas the effects of anoxia and glucose lack were readily reversible and could be ascribed to a loss in sensitivity to prostaglandin, those of indomethacin were long lasting. Furthermore, in the indomethacin-treated tissue, addition of prostaglandin E_2 in a concentration too low to cause a contraction by itself, tended to restore the maintenance of the histamine response. Thus, the maintenance of the contractor effect of a drug such as histamine (but not that of prostaglandin E_2) on isolated smooth muscle, may depend on a local and continuous basal release of prostaglandin(s).

This conclusion has important implications in the interpretation of the phenomenon of 'fade' (Paton, 1961), another facet of the non-maintenance of a contractor response. Changes in 'fade' may reflect changes in prostaglandin synthesis. Tissues which demonstrate 'fade' most easily may be those which are insensitive to prostaglandin, or generate little or no intramural prostaglandin(s) and therefore have little or no intrinsic tone in the isolated organ bath.

Indomethacin has been reported to uncouple oxidative phosphorylation

(Whitehouse, 1964) and dinitrophenol, a potent uncoupling agent, also produces similar effects on smooth muscle (Born & Bülbring, 1955) to those seen with anoxia, glucose lack or indomethacin. It seems unlikely, however, that our results with indomethacin are due to an effect on oxidative phosphorylation. First, the concentration (90 μg/ml) of indomethacin needed to produce uncoupling in rat liver mitochondria (Whitehouse, 1964) was far in excess of that used here. Second, using isolated stomach muscle from guinea-pigs, Northover (1971) has shown that indomethacin in concentrations as high as 200 μg/ml caused no significant depletion of ATP or creatine phosphate, and this was at a time when contractions of muscle were strongly inhibited.

Our results have other implications. When rat stomach strips are relaxed by indomethacin, they become more sensitive to prostaglandin E_2. This suggests that indomethacin treatment may be advantageous in some tissues used for bioassay of prostaglandins.

Another implication is that local prostaglandin generation may have a physiological function *in vivo* in maintaining intestinal tone. However, various forms of gentle trauma induce prostaglandin synthesis (Piper & Vane, 1971 ; Ferreira *et al.*, 1972), so it is also possible that the prostaglandin production in isolated smooth muscle is associated with, or exaggerated by, the trauma of removing the strip of intestine and bathing it in an artificial medium.

It is also interesting to speculate on the possibility that our conclusions with respect to intestinal muscle can be extended to vascular smooth muscle where prostaglandins generally cause relaxation. Griesemer & Coret (1959, 1960), Coret & Hughes (1964) and Hughes & Coret (1968) found that strips of rabbit aorta showed a biphasic response, starting with relaxation, when oxygen was introduced to the hypoxic muscle. Detar & Bohr (1968a, 1968b) found that the contractor response of strips of rabbit aorta to adrenaline was decreased in the absence of oxygen. Furchgott (1966), in a review of the metabolic factors that influence contractility of vascular smooth muscle, concluded ' that it is highly probable that locally produced tissue metabolites contribute to the regulation of peripheral blood flow '. The vasoconstriction produced by indomethacin in dog kidneys *in vivo* (Aiken & Vane, 1971) and in isolated cat spleen perfused with Krebs solution (Ferreira & Moncada, 1971) support the possibility that a prostaglandin fulfils such a role in some vascular beds. The local oxygen tension may contribute to the control of the rate of prostaglandin generation. This might be particularly relevant to the closure of the ductus arteriosus at birth, for Kovalcik (1963) showed this muscle to be strongly contracted by increased oxygen concentration.

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