RELEASE OF PROSTAGLANDINS FROM THE PASSIVELY DISTENDED WALL OF GUINEA PIG SMALL INTESTINE

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Abstract—The effects of the radial distension of the intestinal wall and the increased intraluminal pressure on the liberation of prostaglandins (PGs) were studied with the isolated guinea pig ileum. Both procedures were accompanied by an increased output of a prostaglandin-like substance and the release was proportional to the degree of distension or intraluminal pressure. Thin-layer chromatography coupled with bioassay suggested that this substance may be a mixture of PGF₂α, PGE₂ and PGE₁. The existence of the former two types of PGs were prominent. The distension-induced release of PG-like substance was assumed to be not mediated by nerve excitation since tetrodotoxin failed to inhibit the release. The increased output of the PG-like substance was not maintained after distension of the intestinal wall, despite continued application of stimulus, indicating that actual tissue deformation is an essential condition leading to increased PG output. It is concluded that distension of the intestinal wall to its circumferential direction is an appropriate stimulus for the release of PG-like substance from the small intestine. The present results favor the view that prostaglandins may participate in the peristaltic activity.

Prostaglandins (PGs) of E and F types have been found in the wall of the intestine of different species (1, 2) and the release of PGs is increased with transmural stimulation (3). In view of the potent pharmacological activity on smooth muscle contractions and on neural excitation, modulation of peristaltic activity by PGs has been extensively demonstrated (4–6). Distension of the intestinal wall is one of the most effective means of stimulation for the initiation of peristalsis. In guinea pig small intestine, many pharmacologically active substances are released during the distension of the intestinal wall and are considered to contribute to peristalsis (7, 8). However, little is known of the release of PGs during distension of the intestinal wall. It has been suggested that PGs can be liberated by simple distortion of muscle cells (9). If such is indeed the case in the intestine, it can be expected that a significant amount of PGs would be release during distension of the intestinal wall.

The present work was carried out to clarify the characteristics of the release of PGs during distension of the intestinal wall and to determine if PGs act as modulators in peristalsis.

MATERIALS AND METHODS

Male guinea pigs weighing 300 to 600 g were killed by a blow on the head and bled. Three to five segments of ileum about 6 cm long were excised excluding the most 10 cm distal portion and weighed after blotting both sides of the wall with filter paper. These segments were then incubated separately at 38°C in the organ-baths containing 10 to 20 ml Tyrode...
solution aerated with mixture of oxygen (95%) and carbon dioxide (5%) under any one of the procedures described below.

The procedures tested were— a) Radial distension: The segment was distended to its circumferential direction by inserting a glass rod of proper diameter, usually 7 mm, into intestinal lumen and then the segment was fixed on the rod by binding at both ends of the segment. Special care was taken to avoid any longitudinal distension. In these series of experiments, the samples for the estimation of PGs were collected by changing the bath fluid with fresh Tyrode solution every 10 min. During the first and second 10 min of collection period the intestinal segment was left undistended. At the beginning of the third 10 min collection period, the intestinal segment was distended and maintained in this state thereafter until the end of experiment. b) Distension by increasing intraluminal pressure: The intestinal segment was suspended as described by Trendelenburg (10) in a 20 ml organ-bath. Before obtaining a sample of bath fluid for estimation of PGs output, the intestine was left in the bath for 20 to 30 min. Thereafter, the bath fluid was changed with fresh Tyrode solution and incubation was further carried out for 30 min. During this period the intraluminal pressure was kept at zero mm H2O. After collecting the fluid, the bath was washed out quickly and fresh Tyrode solution and incubation was further carried out for 30 min. During this period the intraluminal pressure was kept at zero mm H2O. After collecting the fluid, the bath was washed out quickly and fresh Tyrode solution was added and preparation left in it for another 30 min. At the beginning of this period, intraluminal pressure was set at 20 or 40 mm H2O level. After 30 min the intraluminal pressure was again dropped to zero and all of the bath fluid was withdrawn. c) No distension: The segment was incubated without any treatment such as distension. Both ends of the segment were ligated to prevent any active substances possibly generated in the mucosa from leaking out into the surrounding medium during incubation. Samples were collected as in procedure (a).

All the samples collected were kept in an ice cold situation until biological estimation of the PGs.

Identification of prostaglandins: Two to five samples of bath fluid were combined, acidified to pH 3 immediately with hydrochloric acid and extracted three times with an equal volume of peroxide-free ether. The combined ether phase was evaporated to dryness at a room temperature below 30°C under a stream of nitrogen gas. The dried material was dissolved in 0.5 ml ethanol and then subjected to thin-layer chromatography on silica gel G containing three per cent silver nitratated. The A II solvent system of Green and Samuelsson (11) was used as a developing solvent. Authentic PGE1, E2 and F20 were treated similarly, chromatographed concurrently and made visible by spraying the plate with 10 per cent molibdeic acid followed by heating. Prior to visualization, consecutive centimeter bands of the plate to which the fluid extract was applied and then developed, were extracted with 5 ml of 50 per cent chloroform in methanol. One gram of NaCl was added to this extract to precipitate the silver nitrate therein. After separation from solid matter, the solvent was again evaporated to dryness as described above and the residue was dissolved in 1 ml Tyrode solution for biological assay.

Assay of prostaglandins: PGs in a sample of bath fluid were assayed on a rat fundus strip suspended in oxygenated Tyrode solution at 37°C. To increase the specificity of the
preparation for PGs, hyoscine, phenoxybenzamine, pyrilamine (0.1 µg/ml each), propranolol 2 µg/ml and methysergide 10 ng/ml were added to the Tyrode solution. The contractions of the muscle strip to the bath fluid were compared with standard doses of PGE\textsubscript{1}. The amount of released PGs was expressed a ng PGE\textsubscript{1} equivalent/g tissue/min, although the bath fluid contained PGE\textsubscript{1}, E\textsubscript{2} and F\textsubscript{2a}, as shown in results. When the intestinal segment was exposed to tetrodotoxin, the bath fluid was compared with standard PG solutions to which the drug concerned was added to give the same final concentration in the assay bath. A stock solution of PGE\textsubscript{1} was prepared by dissolving 10 µg PGE\textsubscript{1} in 1 ml ethanol and kept at -18°C until use. The standard solution was made by diluting the stock solution with an appropriate volume of Tyrode solution, immediately before assay.

Chemicals used were prostaglandin E\textsubscript{1}, E\textsubscript{2}, F\textsubscript{2a} (Ono Pharmaceutical Company, Osaka), phenoxybenzamine hydrochloride (Tokyo Kasei, Tokyo), propranolol hydrochloride (Sumitomo Chemical Co., Takarazuka), pyrilamine maleate (K & K Laboratories, New York), methysergide maleate (Sandoz Pharmaceuticals, Hanover). All other chemicals were of analytical grade. The data were analyzed using Student's t-test for paired and unpaired data.

RESULTS

Identification of a prostaglandin-like substance released from intestinal segment: Samples of bath fluid from both unstimulated and radially distended segments contracted rat fundus strips. The responses were resistant to hyoscine, pyrilamine (0.1 µg/ml each) and 10 ng/ml methysergide. The activity of the bath fluid was not altered after incubating the fluid with 10^{-4} g/ml chymotrypsin for 30 min at pH 8.0. Furthermore, such contractile activity of the bath fluid was not observed when the experiments were carried out in the presence of 10^{-5} g/ml indomethacin (indomethacin was added to the bath fluid at the beginning of incubation). From these series of experiments the active substances in the collected bath fluid were considered to be prostaglandin-like substances. Figure 1 shows the results of identification of active substances in the bath fluid from circumferentially distended intestinal segments subjected to thin-layer chromatography. The bioassay on rat fundus strip which was specified to PG (see METHOD) revealed that large activities resided in the zone which corresponded to the authentic PGF\textsubscript{2a} and PGE\textsubscript{2}. The activity resided also in the zone which corresponded to the authentic PGE\textsubscript{1}, but it was relatively small. With undistended gut segment, much the same tendency was seen.

Radial distension and prostaglandin release: The undistended gut segment released spontaneously fairly constant amounts of PGs over one hr. The release of PGs during six runs of sequential 10 min incubation periods ranged from 22.8±2.0 (mean±S.E.) to 28.0±4.5 ng/g tissue/10 min. A radial distension of the intestinal wall increased the release to about nine times the resting release. The release, however, gradually decreased even though the distension of the intestinal wall was maintained (Fig. 2). In the other series of experiments, the intestinal segments were distended to different degrees by inserting glass rods of various diameters. When a glass rod of 5 mm, 7 mm or 8 mm in diameter was used
the release was $108.2 \pm 25.0$, $239.8 \pm 50.1$ and $337.1 \pm 68.0$ ng/g tissue/10 min, respectively. Tetrodotoxin ($5 \times 10^{-7}$ g/ml), added to the bath fluid 5 min before the collection period, affected neither the release of PG at rest nor during radial distension of the intestinal wall (Fig. 3).

*Intraluminal pressure and prostaglandin release:* The intraluminal pressure of the intestine was raised from zero to 20 or 40 mm water for 30 min. In such a case, the regular peristaltic activity was observed for a few minutes, diminished gradually, and finally the intestine became inflated and there were occasional circular muscle contractions. Increase in the intraluminal pressure also produced an increase in the release of PG from the intestinal segment. A linear relationship was obtained when the output of PG was plotted against the intraluminal pressure (Fig. 4).

**DISCUSSION**

The present experiments show that distension of intestinal wall to its circumferential muscle direction is an appropriate stimulus for release of a PG-like substance from the isolated guinea pig ileum. The main types of released PGs were tentatively identified as
PGE₂ and PGF₂α. On thin layer chromatography using the A II solvent system of Green and Samuelsson, PGF₁α and some of PGF₂α metabolites such as 13,14-dihydro-oxo PGF₂α, 15-oxo PGF₂α are indistinguishable from PGE₂ since they have very close Rf values (11, 12). These metabolites and PGF₁α are, however, only weakly active on the rat stomach strip (13, 14). Therefore, it is likely that the fraction tentatively expressed as PGE₂ is composed mainly of PGE₂. PGD₂ is not distinguishable from PGF₂α with respect to the Rf value and biological potency to rat fundus strips (12, 15). Thus, the activity residing in the zone which corresponded to the zone PGF₂α was probably a PGF₂α-like substance. Although it is unlikely that unstable PGG₂, PGH₂, PGI₂ or thromboxane A₂ would be present in the collected bath fluid, since these compounds are metabolized very rapidly in the tissue (16), the contribution of other substances, such as 6-keto-PGF₁α or thromboxane B₂, to the biological activity is not known. In the present study, samples were assayed on rat fundus strips and the amount was expressed in terms of PGE₁, which is about equipotent with PGE₂ (12) but about five times more potent than PGF₂α and PGD₂ (14, 15). The total amount of PGs in the collected bath fluid is therefore probably underestimated, but correction cannot be made as the relative amount and the potencies of all the types of PGs are unknown.

In the gastrointestinal tract, considerable amounts of PGs are released from the mucosa.
during incubation (12, 14). Thus, it may be that the increased output of PG-like substance seen in the present experiments was derived from the mucosal PG. However, this is unlikely since both ends of the intestinal segment were ligated and PGs hardly diffuse from the mucosal to the serosal side (4). PGs detected in the bathing fluid, therefore, are assumed to originate from the muscle of the intestinal wall. The presence of two types of PGs, PGE_2 and PGF_2α, in guinea pig longitudinal muscle preparation has been reported by Ambache et al. (1).

Concerning the release of PGs from the tissue, Botting (17) suggested that the prostaglandin-like activity from isolated guinea pig ileum during field stimulation was caused by the release of noradrenaline from the intramural sympathetic nerves. Involvement of nervous activity in the release of PGs has also been reported in the case of other organs such as heart (18), stomach (14) and vas deferens (19). Ramwell et al. (20) also suggested the involvement of neural activity in the release of PG from phrenic nerve-diaphragm preparations. In the present experiment, however, the release of PG-like substances during distension was not inhibited by tetrodotoxin. Thus, at least under the present conditions, increases of PG output do not appear to be mediated by nerve excitation. Previous reports have also noted that PG release can be evoked by many different forms of stimulation of smooth muscle, other than nerve stimulation (21, 22).
FIG. 4. Effect of increased intraluminal pressure on prostaglandin release from the isolated guinea pig ileum. Initially the intraluminal pressure was kept at 0 mm H₂O. After collecting the bath fluid, the bath was washed out quickly and filled with fresh Tyrode and another 30 min was allowed to elapse. At the beginning of this period, the intraluminal pressure was set at 20 or 40 mm H₂O. Each point represents the mean of 5 experiments ± S.E.

In the present experiments, the increased output of PG-like substances was not maintained after distension of the intestinal wall, despite continued application of stimulus, indicating that actual cell deformation was the essential condition which led to the augmented output of PGs. This finding parallels that seen in the case of other tissues (23) where increased output of PG from the spleen, as induced by stimulation of splenic nerves or by the application of adrenaline declined almost to the basal level after completion of contraction of the tissue, despite continued application of stimulus. The decline of PG release during sustained stimulus may reflect the generally accepted idea that PGs are stored to a limited extent in tissues.

The augmented release of a PG-like substance during radial distension favors the view that these substances may participate in peristaltic activity (4–6) for the following reasons. a) Both types of PGs, PGE₂ and PGF₂α, have relatively high stimulating activity on the guinea pig ileum (24, 25), b) PGE₂ increased the output of acetylcholine from the intestine, and this compound presumably plays an important role in initiating peristalsis (26) and c) radial distension is an effective physiological stimulus for peristalsis.

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