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Acetylcholine release from the myenteric plexus of guinea-pig ileum by prostaglandin E_1

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Release of acetylcholine (ACh) by prostaglandin E_1 from the nerve terminals of the guinea-pig longitudinal muscle strip was studied in order to reveal the effect of PGE_1 on myenteric plexus activity. The ACh released was collected in the presence of physostigmine (2·1 μg ml^{-1}) and choline (0·1 μg ml^{-1}) at 38° C. Five to 100 ng ml^{-1} PGE_1 enhanced the release dose-dependently. The effect was maintained during the presence of PGE_1 in the organ bath, while rapid tachyphylaxis was observed with the ACh-releasing action of nicotine. Tetrodotoxin or morphine almost completely inhibited the effect of PGE_1 on ACh release. Hexamethonium, in a concentration which completely blocked the effect of nicotine, partially inhibited the effect of PGE_1. In the late phase of nicotine action, the tissue was still sensitive to PGE_1 despite the continued exposure to nicotine. These data suggest the presence in the myenteric plexus of PG receptors which can increase ACh release.

Prostaglandins (PGs) of several types have been found in many mammalian organs including the gastrointestinal tract. Recent studies from this laboratory have shown PGE release by circumferential distension of the guinea-pig intestinal wall to play a significant role in evoking peristalsis (Yagasaki et al 1980). PGE_1 or E_2 enhance the peristaltic activity of the longitudinal muscle in guinea-pig isolated ileum (Bennett et al 1968; Takai et al 1974; Sanger & Watt 1978). Two proposed sites of action of PGs in stimulating gastrointestinal movement are the myenteric nerves and the smooth muscle (Schulz & Cartwright 1976). In the myenteric plexus of guinea-pig ileum a facilitatory role has been attributed to PGE_1 and PGE_2. Since in the longitudinal muscle strip preparation of guinea-pig ileum all the acetylcholine derives from the myenteric plexus (Paton & Zar 1968), the mode of action of PGE_1 on the plexus was studied using this preparation.

METHODS

Male guinea-pigs, 300 to 600 g, were killed by a blow on the head and bled. A length of small intestine was isolated proximal to the last 10 cm, and a longitudinal strip was prepared as described by Paton & Zar (1968); a piece of the ileum (10–15 cm) was mounted on a glass rod of 7 mm diameter and the mesentery cut away. The longitudinal muscle layer was separated from the underlying circular muscle by stroking it away from its mesenteric attachment along the whole length. The strips, with adherent myenteric plexus, were weighed after blotting with filter paper. Three strips were then gathered together (total 200 to 300 mg), tied at each end with cotton thread, and set up in an organ bath containing 3 to 5 ml of Tyrode solution of composition (g $litre^{-1}$): NaCl, 8·0; KCl 0·2; $CaCl_2$ 0·2; $MgCl_2$ 0·1; NaH_2PO_4 0·05; $NaCHO_3$ 1·0 and glucose 1·0. The bath fluid was kept at 37 °C and was bubbled with 5% CO_2 in O_2. Physostigmine salicylate and choline chloride 2·1 μg ml^{-1} and 0·1 μg ml^{-1}, respectively, were added. The samples for the estimation of acetylcholine (ACh) were collected by replacing the bath fluid with fresh Tyrode solution at 37 °C every 2 or 5 min. Before the first samples were collected, the strips were equilibrated under resting conditions for 30 min. During this period the bath fluid was replaced every 5 min. All the collected samples were kept on ice until assayed (less than 3 h).

Assay of acetylcholine

The collected bath fluid was gently shaken with 0·1 volume of Amberlite XAD-2 for 10 min and centrifuged to remove prostaglandins (Keirse & Turnbull 1973) and any added nicotine. Aliquots were then assayed against ACh on the longitudinal muscle strip, 5 cm long, obtained from the most distal 8 cm of guinea-pig ileum and sensitized with 21 ng ml^{-1} eserine. To inhibit the release of endogenous ACh from the longitudinal muscle strip, 3·75 μg ml^{-1} morphine hydrochloride was added to the bath fluid. The assay bath contained 5 ml Tyrode solution at 37 °C bubbled with 5% CO_2 in O_2. When the intestinal preparations were exposed to drugs such as tetrodotoxin or hexamethonium, the bath fluids were compared with standard ACh solutions to

* Correspondence.

which both drugs were added to give the same final bath concentration. The active substance in the bath fluid was considered to be ACh since the contraction of muscle strip induced by bath fluid was prevented by atropine (0·1 μg ml^{-1}), and was lost after boiling the bath fluid for a few minutes in alkali.

Chemicals

Prostaglandin E_1 (Ono Pharmaceutical Co. Osaka, Japan), acetylcholine chloride (Sigma), choline chloride, hexamethonium chloride (Wako Chemical Co. Osaka, Japan), tetrodotoxin (Sankyo Co. Tokyo, Japan), morphine hydrochloride (Takeda Pharmaceutical Co. Osaka, Japan), physostigmine salicylate (eserine, Merck), Amberlite XAD-2 (Rohm & Hass, U.S.A.). All other chemicals were of analytical grade. Potassium-rich Tyrode solution was made by increasing KCl concentration up to 40 mM and reducing NaCl by the appropriate amount. The results were analysed using Student's *t*-test for paired and unpaired data. Where probability values are not given 'significant' means $P > 0.05$.

RESULTS

Release of ACh by prostaglandin

The preparations of guinea-pig ileum longitudinal strip at rest released ACh spontaneously. The amount was fairly constant for at least 60 min, but varied with different preparations. PGE_1 in concentration of 1·0 to 100 ng ml^{-1} dose-dependently increased the ACh release (Fig. 1). At the highest PGE_1 concentration the ACh release increased from 92·7 ± 7·0 to 182·2 ± 16·1 ng g tissue^{-1} min^{-1} (mean ± s.e. of 6 experiments, $P < 0.001$). The increased ACh release was sustained during the presence of PGE_1, and after withdrawal of PGE_1 the ACh release gradually returned to initial resting levels in about 10 min, as shown by measurements every 2 min following exposure to PGE_1 100 ng ml^{-1} for 10 min (Fig. 2).

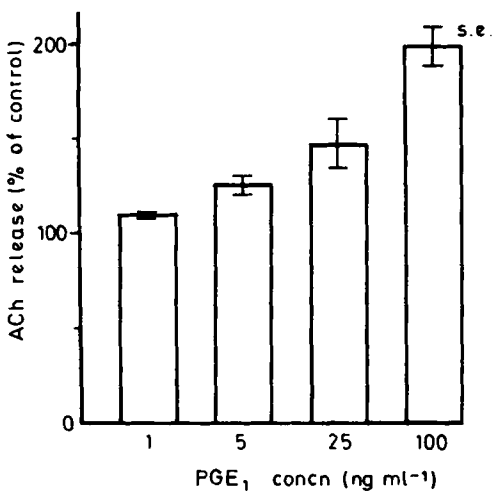

FIG. 1. Dose-dependent release of acetylcholine by PGE_1 from the longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum. The amount of acetylcholine released spontaneously from each preparation before application of PGE_1 was used as the control value. Vertical bars represent s.e. (three to six experiments).

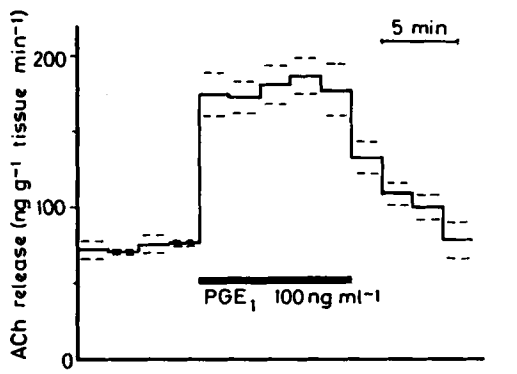

FIG. 2. Release of acetylcholine by PGE_1 from longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum. Mean of three experiments with identical treatment schedules. Dashed lines represent s.e. Time mark, 5 min.

Action of some drugs which depress myenteric plexus activity

Nicotine releases ACh by stimulating nicotinic receptors on cell bodies in the myenteric plexus (Vizi 1973). As shown in Fig. 3, nicotine 2 μg ml^{-1}

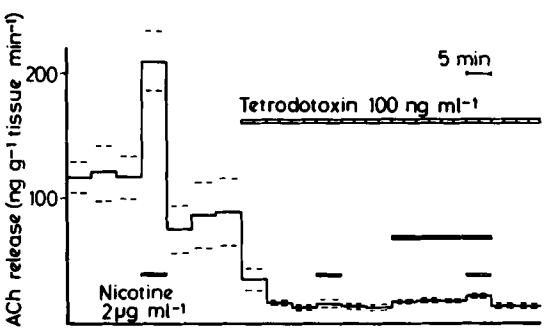

FIG. 3. Release of acetylcholine by nicotine or PGE_1 from the longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum, and its reduction by tetrodotoxin. Mean of six similar experiments. Dashed lines represent s.e. Tetrodotoxin (100 ng ml^{-1}), nicotine (2 μg ml^{-1}) and PGE_1 (100 ng ml^{-1}) were present as indicated. Time mark, 5 min.

increased ACh release from 118 ± 19·2 to 209 ± 24·0 ng g tissue^{-1} min^{-1}. Tetrodotoxin reduced the resting ACh release by about 85% and almost completely inhibited the release by nicotine or PGE_1 (Fig. 3). The release of ACh under the influence of tetrodotoxin alone and with PGE_1 was 15·5 ± 2·6 and 19·5 ± 1·6 ng g tissue^{-1} min^{-1}, respectively (6 experiments; $0{\cdot}1 > P > 0{\cdot}05$). Hexamethonium 100 μg ml^{-1} reduced the resting release of ACh by about 50% and completely prevented the release of ACh by nicotine, and it also partly prevented the response to PGE_1. The ACh release with PGE_1 100 ng ml^{-1} alone and in the presence of hexamethonium was 88·3 ± 11·4 and 49·8 ± 10 ng g tissue^{-1} min^{-1} (6 and 5 experiments; $0{\cdot}05 > P > 0{\cdot}01$) (Fig. 4).

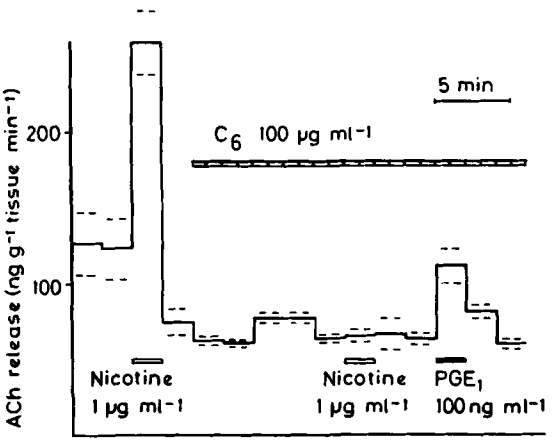

FIG. 4. Effect of hexamethonium on the release of acetylcholine induced by nicotine or PGE_1 from the longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum. Mean of five similar experiments. Dashed lines represent s.e. Hexamethonium (100 μg ml^{-1}), nicotine (1 μg ml^{-1}) and PGE_1 (100 ng ml^{-1}) were present as indicated. Time mark, 5 min.

Paton & Perry (1953) showed that nicotine blocks ganglion transmission initially by depolarizing the ganglion cell; in the continued presence of nicotine, selective block to nicotinic stimulants persisted even after repolarization. We therefore tested the effect of PGE_1 on ACh release during selective block to nicotine. PGE_1 applied 18 min after 10 μg ml^{-1} nicotine increased ACh release from 107·8 ± 7·6 to 168·2 ± 6·9 ng g^{-1} tissue min^{-1} despite the continued exposure to nicotine. In these experiments, output of ACh in response to PGE_1 before nicotine (control period) was enhanced to a similar extent (97·8 ± 9·8 to 179·6 ± 11·2 ng g tissue^{-1} min^{-1}) (Fig. 5).

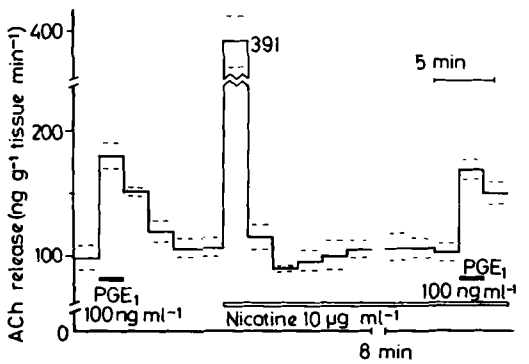

FIG. 5. Release of acetylcholine by PGE_1 from the longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum during continuous exposure to nicotine. Mean of five similar experiments. Dashed lines represent s.e. Time mark, 5 min. Note the release of acetylcholine by PGE_1 (100 ng ml^{-1}) in the presence of nicotine. During the 8 min break acetylcholine release was not measured, but the tissue was still exposed to nicotine.

Morphine 10 μg ml^{-1} reduced resting ACh release by 81 ± 2·7% (8 experiments) and inhibited the release by PGE_1, whereas that by 40 mM potassium was not significantly affected ($P > 0{\cdot}8$) (Fig. 6).

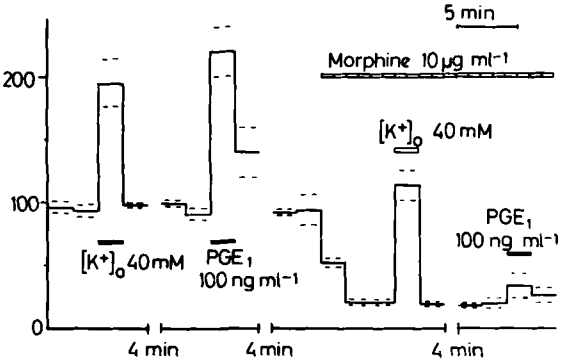

FIG. 6. Inhibitory action of morphine on acetylcholine release induced by PGE_1 from the longitudinal muscle strip-myenteric plexus preparation of guinea-pig ileum. Morphine (10 μg ml^{-1}) and PGE_1 (100 ng ml^{-1}) were present as indicated. The concentration of potassium in the Tyrode solution was increased to 40 mM as indicated. Mean of six similar experiments. Dashed lines represent s.e. Time mark, 5 min. During the 4 min break acetylcholine release was not measured, but the bath fluid was renewed every 2 min with Tyrode solution alone or containing morphine.

DISCUSSION

PGs have been suggested as modulators of the release or effectiveness of ACh in guinea-pig ileum (Ehrenpreis et al 1976; Schultz & Cartwright 1976). Our results clearly demonstrate that PGE_1 can release ACh from Auerbach's plexus in the longitudinal muscle strip preparation of guinea-pig ileum. This agrees with the findings that tetrodotoxin, hyoscine or atropine reduced the contraction of guinea-pig ileum caused by PGE_1 (Bennett et al 1968, 1975; Akanuma 1970).

Direct action of PGE_1 on prejunctional nerve endings seems unlikely since tetrodotoxin almost completely prevented the ACh release by PGE_1; tetrodotoxin does not inhibit ACh release excited by direct depolarization of nerve terminals (Katz & Miledi 1967; Paton et al 1971; Vizi 1973). Since it is generally accepted that tetrodotoxin inhibits nerve conduction (Narahashi 1974) the site of action is presumably more central than the axon, namely the cell bodies in the myenteric plexus.

Hexamethonium, in a concentration which prevented the ACh release by nicotine, only reduced that by PGE_1. Furthermore, PGE_1 was still effective when a selective block to nicotinic stimulants occurred during the continuous presence of nicotine (Fig. 5). The action of nicotine on parasympathetic ganglion cell is tachyphylactic. According to Vizi (1973), the output of ACh induced by nicotine from the myenteric plexus-longitudinal muscle preparation of guinea-pig ileum declined with time to a lower level which continued during the presence of nicotine. In contrast, we observed no tachyphylaxis with PGE_1. All these results suggest that PGE_1 primarily acts on a non-nicotinic site of ganglion cells.

The release of ACh was reduced to about half by hexamethonium, and more extensively by tetrodotoxin. This implies that the nerves of the plexus discharge spontaneously, so releasing ACh. The difference between hexamethonium and tetrodotoxin in the degree of inhibition of spontaneous release of ACh may mean that, in addition to nicotinic receptors, activation of other receptors such as those for 5-HT are involved in the spontaneous release of ACh. Actually, 5-HT is released from nerve terminals in Auerbach's plexus of guinea-pig small intestine (Wood & Mayer 1979) and can activate myenteric ganglion cells which release ACh (Brownlee & Johnson 1965). PGE_1 might similarly increase the excitability of ganglion cells and increase ACh release. This possibility is supported by the finding that inhibitors of PG synthesis block ACh release from myenteric the plexus induced by nicotine or 5-HT; the block was reversed by PGE_2 (Funaki & Yagasaki 1977).

PGE_1 or PGE_2 inhibit noradrenaline release from symphathetic nerves (Euler & Hedqvist 1969; Stjaerne 1973; Hedqvist 1974; Knoll et al 1975). Some authors (Botting 1977; Kadlec et al 1978) therefore suggested that PGEs may increase ACh release from the myenteric plexus by reducing the adrenergic inhibition on the myenteric plexus. However, this seems unlikely in our experiments, since the spontaneous output of ACh is little affected by the sympathetic nerve activity (Paton & Vizi 1969).

Our findings that PGE_1 augmented the release of ACh from guinea-pig myenteric plexus is in contrast to the report that PGE_2 produced no detectable changes in the electrically stimulated release of ACh (Hedqvist et al 1980). The difference in the type of PGE does not explain the discrepancy, since both substances induced contractions of guinea-pig ileum that were antagonized by tetrodotoxin and hyoscine (Bennett et al 1968). An alternative explanation is that PGEs may not affect ACh release induced by electrical nerve stimulation. This is supported by the observations that indomethacin, a potent inhibitor of PG synthesis, significantly reduced nicotine but not transmural stimulation-induced contractions of guinea-pig ileum (Sokunbi 1979).

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