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The effect of indomethacin on contractions of guinea-pig stomach following cessation of vagal stimulation in the presence of hyoscine or pempidine

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Following muscarinic receptor blockade, transmural electrical stimulation relaxes guinea-pig taenia caecum by exciting non-cholinergic, non-adrenergic nerves. This relaxation is followed on cessation of stimulation by an after-contraction (Bennett 1966). The prostaglandin (PG) biosynthesis inhibitor indomethacin was shown by Burnstock et al (1975) to inhibit this after-contraction, implicating a role for PG, without affecting the relaxation.

Stimulation, during muscarinic receptor blockade, of the non-cholinergic, non-adrenergic vagal fibres innervating guinea-pig stomach, produced a gastric relaxation without an after contraction (Paton & Vane 1963). Recently Downing & Morris (1979) have shown that in the presence of ganglion blocking drugs, vagal stimulation produced a relaxation of the guinea-pig stomach followed by a rapid return to the baseline or an after contraction. This contraction was potentiated by physostigmine and prevented by hyoscine, implicating a role for acetylcholine (ACh).

We now report studies with indomethacin on responses of the guinea-pig stomach to vagal stimulation in the presence of hyoscine or pempidine.

Methods

Whole stomachs with vagi attached were removed from albino guinea-pigs of either sex (200–500 g). Any stomach contents were carefully washed out with McEwen's (1956) solution. An incision was made in the fundus and a glass cannula of 0·5 cm internal diameter was inserted and tied in. The pyloric sphincter and oesophagus were then ligated.

The preparation was lowered into a 100 ml organ bath containing McEwen's solution and set up in the manner of Paton & Vane (1963) for intraluminal pressure recording using a Devices low pressure transducer (UP1) and pen recorder. At the start of each experiment the baseline pressure was adjusted to 0·2 kPa. The vagi were stimulated supramaximally using bipolar platinum ring electrodes (pulse width 0·2 ms; 20–30 V; 30 Hz) for 20 s every 5 min.

All drugs were added directly to the bath. Contact times were: Indomethacin 1 h; sodium nitroprusside 5 min; papaverine 10 min. Drugs used were: Hyoscine hydrobromide (BDH); indomethacin (Sigma); papaverine (base) (BDH); pempidine tartrate (M & B); $PGE_2 \times PGF_{2\alpha}$ (pure subst) (Upjohn); sodium nitroprusside (BDH).

Results

When vagal stimulation was stopped in the presence of hyoscine (0·46 μM) intraluminal pressure rose abruptly towards control levels followed by a slower rise of approximately 2 min before pre-stimulation pressure (baseline) is reached (Figs 1A; 2A; 3A; 4A). In the presence of pempidine (15·0 μM), using the stimulus parameters given above relaxation was followed by an after contraction (Figs 1D; 3D; 4D).

Indomethacin (2·8 to 28 μM) lowered the tone of the preparation and reduced the after contractions seen in the presence of pempidine (n = 9) and the abrupt post-stimulation contraction seen in the presence of hyoscine (n = 9) (Fig. 1). As expected, there was a concomitant reduction in the size of neurogenic relaxations. The greater the fall in tone, the greater the change in form of the neurogenic responses. In the presence of indomethacin, PGE_2 (5·7 nM) caused an immediate restoration of tone and of neurogenic responses to their control form. Fig. 2 shows the effect of PGE_2 on neurogenic relaxations and tone seen in the presence of hyoscine and indomethacin. Similar observations were made when pempidine was used in place of hyoscine. $PGF_{2\alpha}$ (4·2 μM) used in place of PGE_2 had no demonstrable effect on tone or form of the responses.

To be certain that the reductions in the after contractions were not a consequence of the fall in tone, the effects of

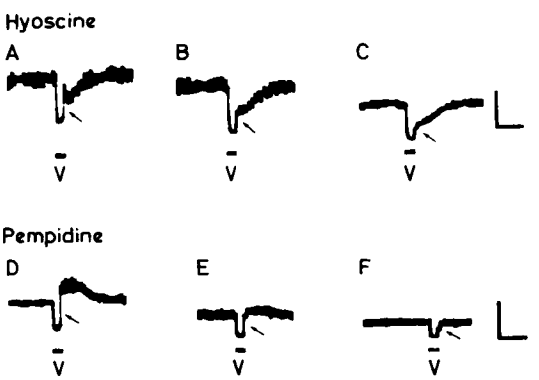

FIG. 1. Effect of indomethacin on responses of isolated stomach to supramaximal vagal stimulation (V) in the presence of hyoscine (0·46 μM; A, B, C) or pempidine (15·0 μM; D, E, F); (A) and (D) control responses; (B) and (E) 60 min after the addition of indomethacin (14·0 and 5·6 μM respectively). (C) and (F) 60 min after the addition of 28·0 μM indomethacin. Separate preparations were used for hyoscine and pempidine. Arrows indicate the post-stimulation after contractions referred to in the text. Vertical bar = 0·5 kPa, horizontal bar = 1 min.

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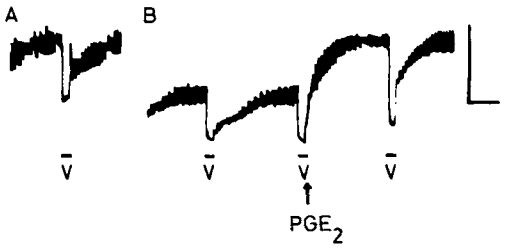

FIG. 2. Responses of the isolated stomach to supramaximal vagal stimulation (V) in the presence of hyoscine (0·46 μM) (A) control responses; (B) responses following 60 min contact with indomethacin (28·0 μM). Note restoration of tone and form of response following the addition of PGE_2 (5·7 μM) vertical bar = 0·5 kPa, horizontal bar = 1 min.

indomethacin were compared with those of two smooth muscle relaxants; sodium nitroprusside and papaverine. Both drugs were added separately in cumulative doses to give a final bath concentration which lowered the tone of the preparations by a similar degree to that produced by 28 μM indomethacin. The maximum fall in tone produced by indomethacin was 0·46 ± 0·07 kPa (mean ± s.e. mean; n = 18). In individual preparations, between 2 and 8 μM sodium nitroprusside was required, the mean fall in tone was 0·44 ± 0·08 kPa (n = 13). With papaverine (14 to 43 μM) the mean fall in tone was 0·44 ± 0·12 kPa (n = 9). These falls in tone were not significantly different from those produced by indomethacin ($P >> 0.05$).

Neither sodium nitroprusside (Fig. 3) nor papaverine (Fig. 4) caused changes in form of the after contractions similar to those seen with indomethacin, although some

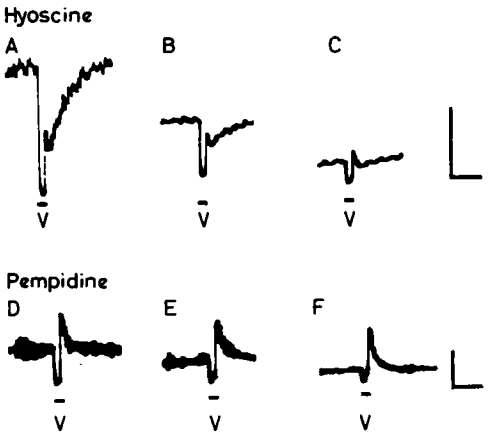

FIG. 3. Effect of sodium nitroprusside on responses of isolated stomachs to supramaximal vagal stimulation (V) in the presence of hyoscine (0·46 μM; A, B, C) or pempidine (15·0 μM; D, E, F). (A) and (D) control responses; (B) and (E) 5 min after the addition of sodium nitroprusside (0·2 and 0·1 μM respectively). (C) and (F) 5 min after the addition of 4·0 and 2·0 μM sodium nitroprusside respectively. Separate preparations were used for hyoscine and pempidine. Vertical bars = 0·5 kPa, horizontal bars = 1 min.

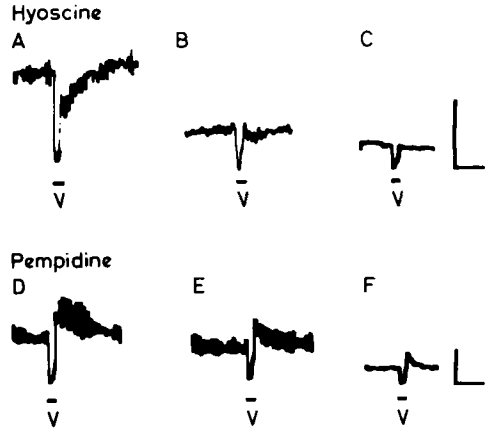

FIG. 4. Effect of papaverine on responses of isolated stomachs to supramaximal vagal stimulation (V) in the presence of hyoscine (0·4 μM; A, B, C) or pempidine (15·0 μM; D, E, F). (A) and (D) control responses; (B) and (E) 10 min after the addition of papaverine (10·7 and 2·7 μM respectively); (C) and (F) 10 min after the addition of 42·6 and 14·1 μM papaverine respectively. Separate preparations were used for hyoscine and pempidine. Vertical bars = 0·5 kPa, horizontal bars = 1 min.

reduction was seen with papaverine. As expected lowering the tone resulted in smaller relaxations.

PGE_2 (5·7 μM) did not restore the tone-lowering effect of sodium nitroprusside or papaverine by more than 50%. With sodium nitroprusside PGE_2 raised the tone by 0·21 ± 0·03 kPa (n = 13) and with papaverine by 0·19 ± 0·02 kPa (n = 9).

Discussion

Indomethacin inhibits PG biosynthesis (Vane 1971). In the experiments reported here, it appears that PG generation is important for the maintenance of tone by the isolated stomach. Similar conclusions have been made for PGs in other gastrointestinal muscle (Eckenfels & Vane 1972; Ferreira et al 1972). PG generation also appears to be important for the abrupt post stimulation contraction seen in the presence of hyoscine. This observation is thus in accord with that of Burnstock et al (1975) for the after contraction seen in the guinea-pig taenia-caecum.

The present results also suggest that PG generation is involved in the after contraction seen in the guinea-pig stomach in the presence of pempidine. We have previously implicated a role for ACh in this rebound contraction (Downing & Morris 1979). The present results are not at variance with this conclusion since the after contraction seen in the presence of pempidine could involve both the release of PGs and ACh. A decrease in PG generation may result in a reduction in ACh release as suggested to occur in the guinea-pig ileum by Ehrenpreis et al (1973) and Bennett et al (1975).

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Effects of autonomic drugs on the electrical activity of intestinal circular muscle

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Although a considerable amount of information is available on the intrinsic nervous control (Crema et al 1970; Hirst & McKirdy 1974) of the circular layer of intestinal smooth muscle, relatively little is known about the mechanisms subserving its excitation (Holzer et al 1980). Circular muscle is usually quiescent; fast electrical activity is absent or very rarely detectable in the resting state but can be easily recorded during peristalsis segmentation (Frigo et al 1972) or by the intestinal stimulant cerulein (Lecchini & Gonella 1973). Spike activity is also produced in circular muscle by tetrodotoxin, which may unmask tonically-inhibited myogenic activity (Tonini et al 1974; Bortoff & Muller 1975). Our aim has been to investigate the effect of hexamethonium, noradrenaline (NA) and isoprenaline on evoked and spontaneous electrical activity of circular muscle.

Rabbits of either sex (900–1800 g) were used. Segments (5–6 cm long) of terminal ileum were removed and mounted horizontally in a organ bath 100 ml containing Tyrode's solution aerated with 5% CO_2 in O_2, at 36 °C. The oral end of the segment was tied over a glass tube connected to a Mariotte bottle containing Tyrode's solution and the lumen was perfused continuously at a flow rate of 2 ml min^{-1}. The aboral end was connected to an isotonic force transducer under a tension of 1–2 g to measure smooth muscle contractions. The intraluminal pressure was measured by means of a pressure transducer as described by Gonella (1971).

Extracellular electrical activity was measured with glass electrodes, tip diameter 0·10–0·20 mm, filled with Tyrode's solution and placed on the serosal surface of the preparations. The electrode arrangement was of the floating type to permit flexibility of movement with that of the bowel. Signals were led via chlorided silver wires to an AC-preamplifier (time constant 0·2 s). The electromyograms were recorded on an inkwriting polygraph. Drugs used were: hexamethonium chloride, (−)-noradrenaline bitartrate, (−)-isoprenaline bitartrate, cerulein (Farmitalia) and tetrodotoxin.

The electrical activity of the small intestine of the rabbit consists of rhythmic fluctuations of resting membrane (slow waves) and of rapid action potentials (spikes) which appear during slow wave depolarization. Spikes are accompanied by contraction of muscular layers and it has been found that the spikes synchronous with the contraction of circular fibres always occur after spike activity of the longitudinal fibres (Gonella 1971).

In all preparations (25 experiments) the slow waves were accompanied by small spikes (2–4 mV) similar to those obtained from isolated longitudinal muscle by Small & Weston (1971) and associated with longitudinal contractions. The frequency of the waves was 10–14 cycles min^{-1}. As shown in Fig. 1a, faster action potentials (more than 8 mV), related to the circular activity, occurred in bursts during segmentation or peristalsis. The mean (±s.e.) frequency of burst was 6·8 ± 0·4 min^{-1} and lasted 2 ± 0·1 s. When propagated during peristalsis, each burst travelled aborally at a velocity of 2 ± 0·08 cm s^{-1}. Hexamethonium (at a final concentration of $5{\cdot}6 \times 10^{-5}$ M, 10 experiments) abolished both mechanical and fast electrical activity of circular layer. It has been found that NA is capable of inhibiting both the release of acetylcholine from Auerbach's plexus in ileal muscle (Paton & Vizi 1969; Kazic 1971) and the peristaltic reflex in isolated colon (Frigo et al 1974), these actions being mediated via α-adrenoceptors on presynaptic nerve terminals (Vizi 1979). As shown in Fig. 1b, NA (3×10^{-8} M, 10 experiments) abolished both mechanical and fast electrical activity of the circular muscle, the latter after a latency of 1 min. The inhibitory effect of isoprenaline has been considered in terms of a direct action on the smooth muscle of intestinal tract (Vizi 1979). In our preparations isoprenaline (3×10^{-6} M, 10 experiments) abolished both mechanical and electrical activity of the circular coat.

The cholecystokinin-related polypeptide caerulein increases peristaltic activity probably by acting on presynaptic enteric nervous structures (Frigo et al 1971). In 12

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