The role of prostaglandins in cholinergic transmission in guinea-pig ileum

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The possible role of endogenous prostaglandin (PG) in cholinergic nerve-mediated responses of guinea-pig ileal longitudinal muscle has remained uncertain despite several studies using indomethacin to inhibit PG synthesis (see Bennett, Eley & Stockley, 1975 for discussion), although similar experiments with aspirin clearly suggest that endogenous PG contributes to these contractions (Hall, O'Neill & Sheehan, 1975).

The recent demonstration that exogenous acetylcholine (ACh) can inhibit the output of transmitter ACh by stimulating pre-synaptic muscarinic receptors (Fosbraey & Johnson, 1978) suggested a possible explanation for the varied results because some authors alternated doses of ACh with periods of electrical stimulation. The present experiments were designed to investigate the effect of indomethacin on neurogenic contractions before and after inhibition by exogenous ACh.

Segments of guinea-pig ileum were set up (Krebs' solution; 5% CO_2 in O_2; 37°C) for electrical field stimulation (supramaximal voltage; 0.5 ms; 0.2 Hz) and longitudinal muscle responses were recorded isotonically. Stimulation was interrupted for 1 min at about 7 min intervals while ACh (40–400 ng/ml for 30 s) was given to inhibit, submaximally, subsequent twitches.

Indomethacin (10 μg/ml; Figure 1) did not further reduce the twitches which were inhibited by ACh (88 ± 21% of control, $n = 6$) but the twitches gradually became more variable in size and their recovery between doses of ACh was reduced to 59 ± 5% of control ($n = 6$, $P < 0.01$) after 30–40 min. Responses of control preparations were unchanged at this time. In the presence of indomethacin, PGE_2 (1–4 ng/ml) caused rapid recovery of the twitches without contracting the muscle directly (Figure 1).

These results support the hypothesis that endogenous PG synthesis is necessary to maintain maximal cholinergic transmission at low frequencies. Furthermore, they suggest the possibility that activation of pre-synaptic muscarinic receptors reduces subsequent twitches by inhibiting PG synthesis. This supposition is also consistent with the extensive findings of Botting & Salzmann (1974).

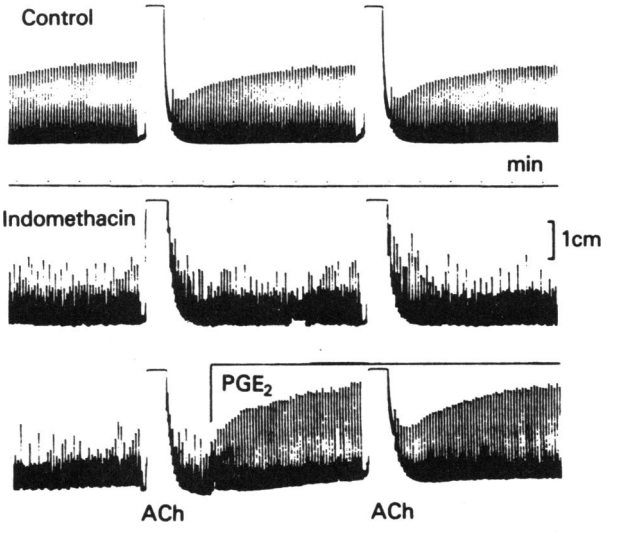

Figure 1 Contractions of guinea-pig ileum to electrical field stimulation (supramax. voltage; 0.5 ms; 0.2 Hz) and actylcholine (ACh; 400 ng/ml for 30 s). Top trace shows inhibition of twitches following ACh and their subsequent recovery. Middle trace commences 30 min after adding indomethacin (10 μg/ml); electrically-induced twitches are more variable in size and fail to recover to control height between ACh doses. Lower trace: PGE_2 (2 ng/ml) counteracts the effects of indomethacin.

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Prostaglandins and the response of rat isolated ileum and duodenum to bradykinin

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An involvement of prostaglandins in the contractile action of bradykinin upon the longitudinal muscle of rat isolated terminal ileum was reported by Crocker & Willavoys (1976). Preliminary data has been presented suggesting that prostaglandins may be involved in the contractile action of bradykinin during mucosal but not serosal perfusion of the rat ileum (Crocker, Walker & Wilson, 1978). We have investigated further this interrelationship of prostaglandins and bradykinin in the contraction of the ileum and extended the study to the involvement of prostaglandins in the relaxation of the duodenum to bradykinin.

The terminal ileum or proximal duodenum was removed from male Wistar rats (180–250 g), placed under a tension (1 g) and perfused through the Lumen at 3 ml/min with Krebs solution at 37°C, bubbled with 5% CO_2 in O_2. In experiments involving pretreatment, indomethacin (8 mg/kg) was injected subcutaneously 2 h prior to removal of the appropriate tissue. All contractions were recorded isometrically. Bradykinin (0.2 ml) was injected into the Krebs solution prior to perfusion of either the mucosal surface, or the serosal surface after eversion of the ileum or duodenum.

The longitudinal muscle contractions of the ileum, or relaxations of the duodenum, to bradykinin perfused over the serosal surface were unaffected by either indomethacin 2.8 μM ($n = 5$) or 28 μM ($n = 5$) added to the perfusate, or by pretreatment of the rats with indomethacin. However, during mucosal perfusion of the ileum the log-dose response curve of bradykinin on the longitudinal muscle was displaced to the right with a reduction in the maximal contraction of 54 ± 6% ($n = 5$) $P < 0.001$ and 80 ± 5% ($n = 5$) $P < 0.001$ by indomethacin 2.8 μM and 28 μM respectively when added to the perfusate. Pretreatment of rats with indomethacin also reduced the maximal contraction of the ileum to bradykinin during mucosal perfusion by 45 ± 6% ($n = 5$) $P < 0.001$. Similarly, during mucosal perfusion of the duodenum the maximal relaxation observed was reduced by 41.5 ± 6% ($n = 6$) $P < 0.001$ and 53.4 ± 5% ($n = 6$) $P < 0.001$ by indomethacin 2.8 μM and 28 μM respectively when added to the perfusate. Pretreatment of rats with indomethacin also reduced the maximal relaxation observed to bradykinin by 43.6 ± 16% ($n = 7$) $P < 0.01$.

These results provide further support for an involvement of prostaglandins in the response of the rat small intestine to bradykinin during mucosal perfusion but not during serosal perfusion. Thus it appears that the involvement of prostaglandins is not confined to the contractile action of bradykinin since it also occurred during relaxation of the duodenum to bradykinin.

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