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Mutual interaction of prostaglandin-like material and noradrenaline during periarterial nerve stimulation of rabbit intestine

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The present study was undertaken to examine if the hypothesis of negative feed-back loop between prostaglandins (PGs) and noradrenaline (NA) proposed by Hedqvist (1970b) holds for the rabbit ileum.

Methods

A segment of ileum (2–3 cm) from adult rabbits prepared according to Finkleman (1930), was set up in a 30 ml organ bath containing McEwen (1956) solution at 35·5 ± 1·0 °C continuously bubbled with oxygen. The longitudinal movements of the ileum were recorded by an isotonic frontal writing lever on a smoked drum (load 750 mg, magnification 10 times). The periarterial nerves were stimulated by bipolar platinum electrodes at supramaximal voltages and frequencies of 5, 10, 20 and 50 Hz with square wave pulses of 5 ms duration for 45 s. The cycle was repeated every 3 min.

The possible role of newly synthesized NA. The nerves were stimulated at supramaximal voltages; however, depending upon the frequency of stimulation, its duration was changed so that 300 shocks were always delivered. The cycle with each frequency was repeated every 3 min. After eliciting control responses the test preparations were exposed to indomethacin (10 μg ml^{-1}) or α-methyl-*p*-tyrosine (AMPT) or both for 30 min and responses were repeated in their presence. Unlike the previous experiments in which the inhibitory response was measured from the midpoint of effects, the inhibitory effect was calculated thus:

$$\frac{\text{area of the trace due to the inhibitory effect}}{\text{initial height of pendular movements.}}$$

Reserpine pretreatment. Preparations were exposed to reserpine (1 mg ml^{-1}) in-vitro for 30 min, the reserpine-containing solution being changed every 10 min. After 30 min, the preparation was washed with McEwen solution several times before the responses to nerve stimulation were recorded.

Test for the presence of PG-like activity in the bathing fluid. In experiments where the samples were assayed for PG-like activity, the period of incubation was kept constant at 90 s. The PG in the medium was extracted in ether (Samuelsson 1963) and bioassayed on the rat stomach strip (Vane 1957) set up in a 15 ml bath containing Krebs bicarbonate solution at 37 °C and bubbled with a mixture of 5% CO_2 and 95% O_2. Isotonic contractions were recorded (load 500 mg and magnification 15 times); 10 min cycle was used. A mixture of antagonists (sotalol 2, phentolamine 1, bromolysergic acid diethylamide 1, mepyramine 1 and atropine 1 μg ml^{-1}) was added to make the preparations specifically sensitive to detection and quantitation of PG-like material (Gilmore et al 1968). In addition, indomethacin (1 μg ml^{-1}) was added to increase sensitivity. Rat colon preparations set up according to Regoli & Vane (1964), served to monitor the presence of PGF-like substances in the bathing fluid.

Drugs used were: atropine sulphate and mepyramine maleate (BDH); 2 bromolysergic acid diethylamide (BOL-Sandoz); prostaglandins E_1, E_2 and F (Upjohn); phentolamine methanesulphonate, reserpine (CIBA); sotalol (Mead Jonson & Co) α-methyl-*p*-tyrosine and indomethacin (Merck, Sharp & Dohme).

Indomethacin was dissolved in 2% sodium carbonate solution and the pH was adjusted to 7·6 with HCl. Stock solutions of PGE_1, E_2 and F were prepared by dissolving 10 mg in 9 ml of a solution of 0·2% Na_2CO_3 and 1 ml of 95% ethanol.

Results

In almost all the preparations, responses to periarterial sympathetic nerve stimulations at frequencies ranging from 5 to 50 Hz resulted in frequency-related relaxation of the ileum (Fig. 1). Guanethidine (2 μg ml^{-1}), prevented the response of the ileum to periarterial nerve stimulation (n = 3).

The medium bathing the unstimulated ileum when tested on the rat isolated stomach strip contained 4·4 ± 0·27 ng, equivalents of PGE-like activity (n = 7). When the nerve was stimulated, there was a frequency-related decrease in the amount of PGE-like material in the bathing fluid (Fig. 1). However, when tested on the rat colon, though exogenously added, PGF (2–8 ng ml^{-1}) produced concentration-related contractions, the medium bathing the Finkleman preparation did not elicit any response, ruling out the presence of PGF.

In preparations exposed to reserpine in-vitro, the mean PG release into the bathing fluid of unstimulated ileum was 4·5 ± 0·14 ng (n = 4), which was not different from control release. After several washes,

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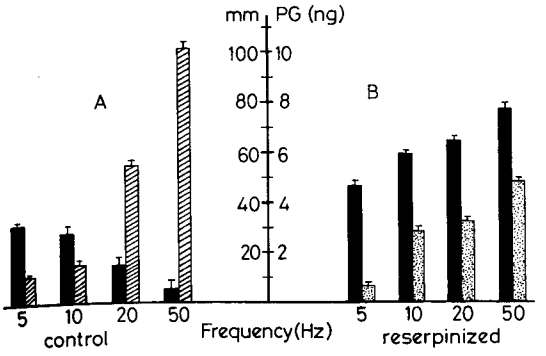

FIG. 1. Effect of mesenteric nerve stimulation on the movements and release into the medium of PG-like activity by Finkleman preparations. Panel A depicts responses of control preparations and panel B the responses of preparations reserpinized in-vitro. The solid histograms depict PG-like activity (ng/90 s), the hatched histograms represent relaxations (mm) and stippled histograms represent contractions (mm). Figures below each set of histograms depict frequency of stimulation. Means of 5 to 7 observations were used to construct each histogram; vertical bars represent s.e.m. The coefficient of correlation between relaxations and release of PG-like materials was $-0{\cdot}98$ ($P < 0{\cdot}05$) and that between the contractions and release of PG-like material was 0·99 ($P < 0{\cdot}001$).

responses to nerve stimulation were recorded. Instead of frequency-related relaxations there were frequency-related contractions associated with an increase in the release of PG-like material in the bathing fluid (102·2 ± 5·1%; 131·1 ± 2·2%; 142·2 ± 5·1% and 171·1 ± 5·5% at 5, 10, 20 and 50 Hz respectively; n = 4). Atropine (1–5), mepyramine (1) or BOL (1) did not modify the contractions. These concentrations of antagonists blocked responses to acetylcholine, histamine or 5-HT (1×10^{-6} M). The significant correlation coefficient between contraction and PG-release suggests a causal relationship between the contractions and PG release (Fig. 1).

Preparations treated with reserpine and higher frequencies were then exposed to indomethacin (10 μg ml^{-1}) for 60 min. The pendular movements were inhibited. Stimulation of superior mesenteric nerves (20 to 50 Hz) did not elicit any contractions (n = 6) and there was total block of the release of PGE-like material into the bathing medium.

The role of newly synthesized NA. Indomethacin (10 μg ml^{-1}) increased the inhibitory effect of nerve stimulation (Table 1). In the paired control preparations similarly stimulated in the absence of indomethacin, the responses to nerve stimulation at the beginning and end of the experiment were similar.

The pretreatment of Finkleman preparations with 100 μg ml^{-1} AMPT only, did not change the responses to stimulation but prevented the potentiating effect of indomethacin (Table 1).

Indomethacin (10 μg ml^{-1}) had no effect on the relaxant response to 1 μg ml^{-1} of exogenously added NA (control 8·0 ± 1·3; with indomethacin 8·0 ± 1·1; n = 4).

Effect of PGE_2 on responses to frequency stimulation. PGE_2 (0·2 μg ml^{-1}) had no effect on movements of the ileum but inhibited responses to frequency nerve stimulation (Table 2) without affecting those to 1 μg ml^{-1} NA (control: 8·0 ± 1·3, with PGE_2: 8·0 ± 1·1; n = 4).

Discussion

Isolated preparations of frog intestine (Vogt & Distelkotter 1967), bovine iris (Posner 1970) and rabbit jejunum (Ferreira et al 1972) release PGs into bathing fluid. These tissues are contracted by PGs. In all of them, the release of PGs probably represents fresh synthesis. Thus the evidence that the resting tone of some isolated smooth muscles may be maintained by the continuous generation of PGs has been accumulating. The present results support this. The lack of any significant effect of the bathing fluid on the rat isolated colon would seem to suggest release of PGs of the E series, since the rat stomach strip and colon can differentiate between PGs of the E and F series (Gryglewski & Vane 1972).

The graded diminution of the release of PG-like material into the bathing fluid after periarterial nerve stimulation at different frequencies, demonstrated in the present study, supports the results that adrenergically innervated tissues, such as canine and feline spleen (Gilmore et al 1968), rabbit heart and ear (Wennmalm 1971) and guinea-pig and rat vas deferens (Hedqvist & Van Euler 1972) release PG-like material. Although surgical trauma may cause an increase in basal PG release, the present results cannot be considered as artifactual since there was frequency-related decrease in PGE-like material in the bathing fluid. In acutely reserpinized preparations in-vitro, nerve stimulation produced an increase in the PG content of the bathing fluid and also of the motor response of the preparation, both being frequency-dependent.

In the rabbit jejunum, indomethacin abolishes both the resting tone and the release of PGs in the bathing

Table 1. Responses* of Finkleman preparations to sympathetic nerve stimulation in the presence of indomethacin, AMPT and combinations of indomethacin and AMPT.

	Mean response ± s.e.m.			
Frequency (Hz)	Control	in the presence of indomethacin (10 μg ml^{-1})	in the presence of AMPT (100 μg ml^{-1})	in the presence of AMPT (100 μg ml^{-1}) and indomethacin
5	6·0 ± 0·05	14·0 ± 2·00**	5·0 ± 0·66	5·0 ± 1·90
10	7·0 ± 0·44	17·0 ± 1·90**	6·0 ± 0·49	6·0 ± 2·22
20	7·0 ± 0·72	17·0 ± 2·29**	6·0 ± 0·75	6·0 ± 0·00
50	8·0 ± 0·49	17·0 ± 2·27**	7·0 ± 1·11	6·0 ± 2·77

* Response = $\frac{\text{Area of the trace due to the inhibitory effect}}{\text{initial height of pendular movements.}}$

** $P < 0{\cdot}05$ compared with control.

Table 2. Responses* of Finkleman preparations to sympathetic nerve stimulation in the presence of PGE_2.

Frequency (Hz)	Mean response ± s.e.m. Control	in the presence of PGE_2 (2 μg ml^{-1})
5	7·0 ± 0·66	6·0 ± 0·50
10	8·0 ± 0·42	6·0 ± 0·39**
20	8·0 ± 0·12	5·0 ± 0·70**
50	9·0 ± 0·89	4·0 ± 0·80**

* Response = $\frac{\text{Area of the trace due to inhibitory effect}}{\text{initial height of pendular movements.}}$

** $P < 0·05$ compared with control.

fluid (Ferreira et al 1972). In the present study indomethacin completely inhibited normal pendular movements. The motor response of the reserpinized preparation to nerve stimulation was also blocked. The view is therefore strengthened that in reserpinized preparations contraction due to nerve stimulation may involve the release of PGs. Indomethacin blocks the release of PG and simultaneously enhances the release of NA by nerve stimulation, in rabbit heart (Chanh et al 1972), guinea-pig vas deferens (Stjarne 1973), rabbit kidney (Frame & Hedqvist 1975) and mesenteric arteries from cat and man (Hedqvist 1974). We observed that indomethacin caused potentiation of responses to the periarterial nerve stimulation that could be ascribed to facilitation of the release of NA, since indomethacin has no effect on monoamine oxidase, catechol-*O*-methyl transferase, or on NA uptake in different tissues (Hedqvist 1977) but it could also be due to block of synthesis of prostaglandins involved in contraction. Similarly the postsynaptic action of indomethacin was excluded by its lack of interaction with exogenous NA. PGE_2 caused inhibition of the responses to nerve stimulation but had no effect on those to exogenous NA. Newly synthesized NA plays an important role in transmitter release due to nerve stimulation. AMPT is an effective inhibitor of NA synthesis (Udenfriend et al 1965; Kupferman et al 1970) and abolished the indomethacin-induced potentiation of nerve stimulation. These results indicate therefore, that potentiation by indomethacin of response to nerve stimulation, may be due to increased release of newly synthesized NA.

The results are consistent with the hypothesis that locally formed PGE_2 is able to restrict the release of NA by a negative feedback loop, and hence acts as significant modulator of adrenergic transmission (Hedqvist 1970a,b). The PGs and NA released by sympathetic nerve impulses could be viewed as physiological antagonists. If the release of PGs is inhibited, the release of NA is augmented and vice versa. The PGs may be concerned with the maintenance of basal tone of intestine; release of NA tending to overcome increased tone produced by PGs, and the two may regulate the release of each other mutually.

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