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A POSSIBLE NEGATIVE FEEDBACK CONTROL OF EXCITATORY TRANSMISSION VIA PROSTAGLANDINS IN CANINE SMALL INTESTINE

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1 Contractile responses of canine intestinal circular and longitudinal muscles to field stimulation (20 Hz, 1 ms, 30 V/cm, for 5 s) were inhibited by treatment with atropine (0.1 μg/ml), indicating that the response to field stimulation was mediated by acetylcholine (ACh).

2 Prostaglandins E_1 (PGE_1), PGE_2 and $PGF_{2\alpha}$ inhibited the response of circular but not longitudinal muscle to field stimulation, althouth $PGF_{2\alpha}$ was less effective than PGE_1 and E_2.

3 PGE_1 was much less active in inhibiting the response of circular muscle to ACh than to field stimulation, suggesting that prostaglandins might act predominantly at prejunctional sites to prevent the release of ACh.

4 Indomethacin (1 μg/ml) potentiated the response of circular muscle but not longitudinal muscle to field stimulation.

5 Release of PGE-like compounds from circular muscle only, was increased by field stimulation at 20 Hz (total of 1000 pulses) and ACh (10 μg/ml), but not by a lower frequency (2 Hz, total of 2400 pulses) which produced only a slight contraction. This finding may indicate that prostaglandins were released predominantly from the muscle.

6 Prostaglandins may exert a negative feedback mechanism of excitatory transmission in circular muscle but not in longitudinal muscle of canine small intestine.

Introduction

The existence of a negative feedback control mechanism of adrenergic transmission via prostaglandins was proposed by Hedqvist & Wennmalm (1971). Recently, a negative feedback control by prostaglandins of cholinergic transmission in rabbit heart (Junstad & Wennmalm, 1974) and in canine trachea (Nakanishi, Yoshida & Suzuki, 1976) was reported. However, Botting & Salzman (1974) observed that in the longitudinal intestinal muscle of the guinea-pig the response to field stimulation was not inhibited by prostagladins. E series prostaglandins relax most intestinal circular muscle but contract longitudinal muscle (Bennett, Eley & Scholes, 1968). Therefore, in the present experiments, longitudinal and circular muscle preparations were examined to see whether negative feedback control of cholinergic transmission by prostaglandins exists in this species.

Methods

Canine isolated intestine

Mongrel dogs of either sex, weighing 7 to 15 kg, were anaesthetized with sodium pentobarbitone (30 mg/kg, i.v.) and were bled from carotid arteries. A jejunal segment of about 2 cm length was removed. The segment was cut longitudinally and the lumen was opened. The muscular layers were stripped from the lamina muscularis mucosae, and were separated into longitudinal and circular muscles. Strips (approximately 1.5 × 0.2 cm) of longitudinal and circular muscles were suspended between two platinum rings for transmural stimulation in organ baths containing 20 ml of Tyrode solution at 37°C, aerated with a mixture of 95% O_2 and 5% CO_2. The composition of the solution was as follows (mM): NaCl 136.9, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 11.9

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and glucose 5.6. Tension development was recorded isometrically through an FD pick-up (Nihon Kohden, SB-1T, SB-1TH) and a carrier amplifier (Nihon Kohden, RP-3, RP-5). The resting tension was adjusted to 2.0 g at the beginning of the experiment and the tissue allowed to equilibrate for more than 1 h. The resting tension usually decreased to 0.5 to 1.0 g during the experiments. Field stimulation was applied with rectangular pulses of 1 ms duration, at 20 Hz and 30 V/cm with an electrical stimulator (Nihon Kohden, MSE-3R).

Drugs used were acetylcholine chloride (Daiichi Chemical Co. Ltd.), atropine sulphate (Wako Pure Chemical Co. Ltd.), indomethacin (Sumitomo Chemical Co. Ltd.), prostaglandins E_1, E_2 and $F_{2\alpha}$ (Ono Pharmaceutical Co. Ltd.). Indomethacin was dissolved in a weak basic solution in a concentration of 1 mg/ml by adding NaOH to pH 8.5, and was diluted with 0.9% w/v NaCl solution (saline). Prostaglandins were dissolved in 0.01 M phosphate buffer (pH 7.5) to make a concentration of 100 μg/ml, and were diluted with the same buffer (vehicle). Statistical significance was determined by Student's t test.

Prostaglandin determination

Prostaglandins released spontaneously or by field stimulation or ACh-administration into the nutrient solution were determined by radioimmunoassay. The whole Tyrode solution (10 ml), in which the tissue was incubated for 20 min, was collected four times with rest periods of 10 min in between. The prostaglandin extraction procedure was slightly modified from that of Zusman, Caldwell, Speroff & Behraman (1972). Total prostaglandins were extracted twice with 10 ml ethyl acetate from Tyrode solution which was acidified to pH 3.0 with 1 N HCl. Prostaglandins of the E and F series were separated from total prostaglandin fractions by chromatography on a column of 0.5 × 15 cm glass packed with 0.5 g silicic acid (Wako Gel C-100). The following solvent systems were used, I benzene, II benzene:ethyl acetate = 60:40; III benzene:ethyl acetate:methanol = 60:40:8; IV benzene:ethyl acetate:methanol = 60:40:20; V methanol and VI benzene:ethyl acetate:methanol = 60:40:2.5. After ethyl acetate was evaporated at 40°C, total prostaglandins were dissolved in 1 ml of solvent VI and applied to a column. Solvent I (1 ml) was added to a column and the eluate was discarded. The eluate from solvent II (5 ml), corresponding to prostaglandin A (PGA) and PGB, was also discarded in the present experiment. The eluates from solvents III (20 ml) and IV (8 ml), corresponding to PGE and PGF respectively were collected and evaporated. The evaporated PGE or PGF fraction was dissolved in 1 ml of 10 mM Tris-HCl buffer (pH 7.6) for radioimmunoassay. Anti-PGE_1 serum (350 times dilution) and anti-$PGF_{2\alpha}$ serum (1600 times dilution) were used as the specific binding proteins. The specific activities of [^{3}H]-PGE_1 and [^{3}H]-$PGF_{2\alpha}$ (Radio Chemical Centre) were 87 Ci/mmol and 9.2 Ci/mmol respectively. The assay mixture consisted of 0.1 ml of sample or standard, 0.1 ml of anti-serum and 0.1 ml of 0.1 μCi/ml [^{3}H]-PGE_1 or [^{3}H]-$PGF_{2\alpha}$ containing 0.5% bovine serum albumin (BSA) and 0.1 M Tris-HCl (pH 7.6). The mixture was incubated at 0 ~ 4°C for 24 h. After addition of 0.5 ml of dextran coated charcoal (1 g of Norit Extra, 150 mg of dextran and 1 g of BSA in 300 ml of water) to the mixture, the preparation was centrifuged for 2 min. [^{3}H]-PGE_1 or [^{3}H]-$PGF_{2\alpha}$ bound to anti-serum in the supernatant (0.5 ml) was counted with a liquid scintillation counter using toluene-based scintillator, the composition being as follows: 2,5-diphenyloxazole (PPO) 4.0 g and 1,4-bis(2-(5-phenyloxazolyl))-benzene(POPOP) 0.1 g in 1000 ml of toluene and 500 ml of triton X-100. The recoveries of PGE_1 (10 ng) and $PGF_{2\alpha}$ (10 ng) during the whole procedure including extraction and chromatography were 83.9 ± 3.91% (n = 18) and 43.7 ± 2.48% (n = 18), respectively.

Results

Effects of atropine on the response to field stimulation

The circular and longitudinal muscles of canine small intestine were contracted by field stimulation at 20 Hz for 5 s. Atropine (0.1 μg/ml) completely inhibited the response of the longitudinal muscle whereas the response of the circular muscle was only partially inhibited (Figure 1). A higher dose of atropine (1 μg/ml) did not abolish the response of circular muscle to field stimulation.

Effects of prostaglandins E_1, E_2 and $F_{2\alpha}$ on the response to field stimulation

PGE_1, PGE_2 or $PGF_{2\alpha}$ (10 ng/ml) alone had almost no effect on the longitudinal and circular muscles. Sometimes, slight rhythmic contractions of both muscles were observed after the addition of PGE_1, PGE_2 or $PGF_{2\alpha}$ (0.1 μg/ml). None of these prostaglandins (10 ng and 0.1 μg/ml) had any effect on the response of the longitudinal muscle to field stimulation. All three prostaglandsins caused a dose-dependent inhibition of the response of circular muscle although $PGF_{2\alpha}$ was less potent than PGE_1 or E_2 (Figures 2 and 3).

Effects of prostaglandins E_1, E_2 and $F_{2\alpha}$ on the response to acetylcholine

ACh (10 μg/ml) produced contractions in both circu-

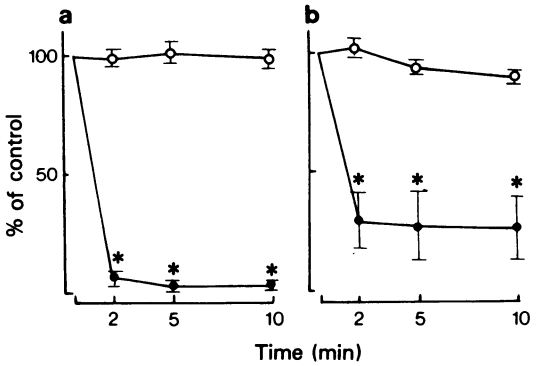

Figure 1 The effect of atropine (0.1 μg/ml) on the responses to field stimulation (20 Hz, for 5 s) in longitudinal (a) and circular (b) muscles of canine small intestine. Mean values are given; vertical bars show s.e. mean. (○): Non-treated; (●): atropine (0.1 μg/ml)-treated. Significance of the difference from non-treated: * $P < 0.05$. Number of experiments = 6.

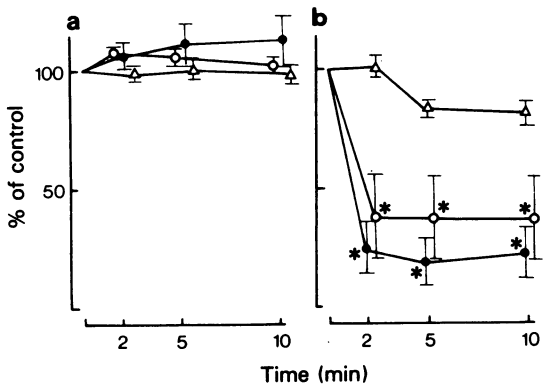

Figure 2 The effect of prostaglandin E_1(PGE_1) on the responses to field stimulation (20 Hz, for 5 s) in longitudinal (a) and circular (b) muscles of canine small intestine. Mean values are given; vertical bars show s.e. mean. (△): Vehicle; (○): PGE_1 10 ng/ml; (●): PGE_1 0.1 μg/ml. Significance of the difference from vehicle: * $P < 0.05$. Number of experiments = 6.

lar and longitudinal muscle of similar amplitude to those produced by field stimulation at 20 Hz for 5 s (Figure 4). None of the prostaglandins (0.1 μg/ml) had a significant effect on the dose-response regression of contractions of longitudinal muscle to ACh (10 ng to 10 μg/ml). The prostaglandins had little effect on circular muscle, PGE_1 causing a significant reduction of the response to only one concentration of ACh (0.1 μg/ml) (Figure 5).

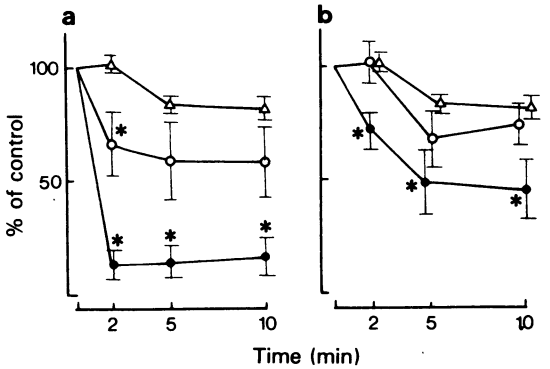

Figure 3 The effects of prostaglandin E_2(PGE_2) (a) and $F_{2\alpha}$ ($PGF_{2\alpha}$) (b) on the responses of circular muscle to field stimulation (20 Hz, for 5 s). Mean values are given; vertical bars show s.e. mean. (△): Vehicle; (○): PGE_2 or $PGF_{2\alpha}$ 10 ng/ml; (●): PGE_2 or $PGF_{2\alpha}$ 0.1 μg/ml. Significance of the difference from vehicle: * $P < 0.05$. Number of experiments = 6.

Effect of indomethacin on the response to field stimulation

Indomethacin (1 μg/ml), which inhibits prostaglandin synthesis (Vane, 1971), had no effect on the response of longitudinal muscle to field stimulation but potentiated the response of circular muscle (Figure 6).

Release of prostaglandins during stimulation

If a negative feedback mechanism via prostaglandins exists in the intestinal muscle, prostaglandins must be released by nerve stimulation. The release of prostaglandins of the E and F series was determined during field stimulation (2 and 20 Hz) or administration of ACh (10 μg/ml)(Figure 7). When field stimulation at 2 Hz for 20 min (total of 2400 pulses) was applied to both longitudinal and circular muscles, a slight increase in muscle tension was observed. Field stimulation at 20 Hz for 5 s every 2 min for 20 min (total of 1000 pulses), resulted in a marked increase in tension with every stimulation. When ACh (10 μg/ml) was administered for 20 min, both longitudinal and circular muscles contracted as strongly as when field stimulation of 20 Hz was applied. The release of PGE-like material from longitudinal muscle was not increased by stimulation at 2 Hz, 20 Hz or by ACh administration. However, there was a significant increase in prostaglandin release from circular muscle during field stimulation at 20 Hz and during ACh administration. Field stimulation at 2 Hz did not increase prostaglandin release from circular muscle, although the total number of pulses at 2 Hz was larger than at 20 Hz. The release of PGF-like material

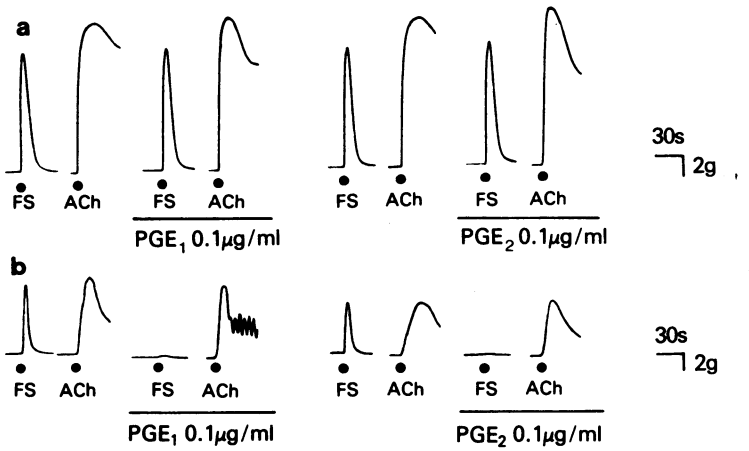

Figure 4 Comparison of the effect of prostaglandin E_1 (PGE_1) and prostaglandin E_2 (PGE_2) on the response to field stimulation (FS: 20 Hz, for 5 s) and the response acetylcholine (ACh) (10 μg/ml) in longitudinal (a) and circular (b) muscles of canine small intestine.

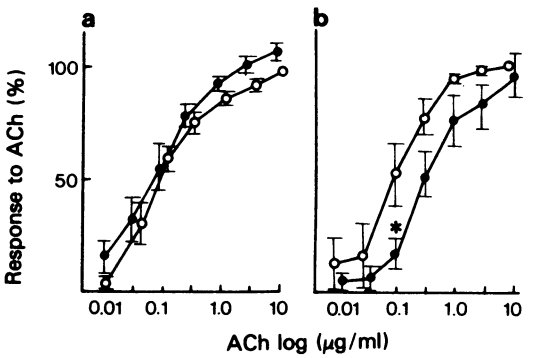

Figure 5 The effect of prostaglandin E_1 (PGE_1) on the contractile response to acetylcholine (ACh) in longitudinal (a) and circular (b) muscles of canine small intestine. Mean values are given; vertical bars show s.e. mean. (○): Vehicle; (●): PGE_1 0.1 μg/ml treated 5 min before ACh. Data calculated as percentage responses to ACh (10 μg/ml). Significance of the difference from vehicle: * $P < 0.05$. Number of experiments = 6.

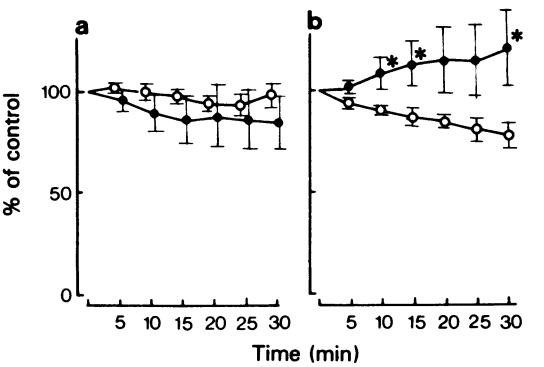

Figure 6 The effect of indomethacin on the response to field stimulation (20 Hz, for 5 s) in longitudinal (a) and circular (b) muscles of canine small intestine. Mean values are given; vertical bars show s.e. mean. (○): Control (equal volume of saline); (●): indomethacin 1 μg/ml. Significance of difference from control: * $P < 0.05$. Number of experiments = 6.

was not increased by stimulation of either longitudinal or circular muscle.

Discussion

The contractile responses of longitudinal and circular muscles to field stimulation were inhibited by treatment with atropine (0.1 μg/ml). However, the inhibitory effect of atropine on the response of circular muscle to field stimulation was weaker than that of longitudinal muscle. The finding cannot exclude the existence of non-cholinergic excitatory nerves in the circular muscle. PGE_1 and PGE_2 inhibited the response of circular but not longitudinal muscle to field stimulation. $PGF_{2\alpha}$ had a weak inhibitory effect on the response of circular muscle to field stimulation. There is a clear difference between the effect of PGE compounds on the response of circular muscle and that of longitudinal muscle to field stimulation. In the circular muscle, PGE compounds have an inhibitory effect on the response to field stimulation, as they

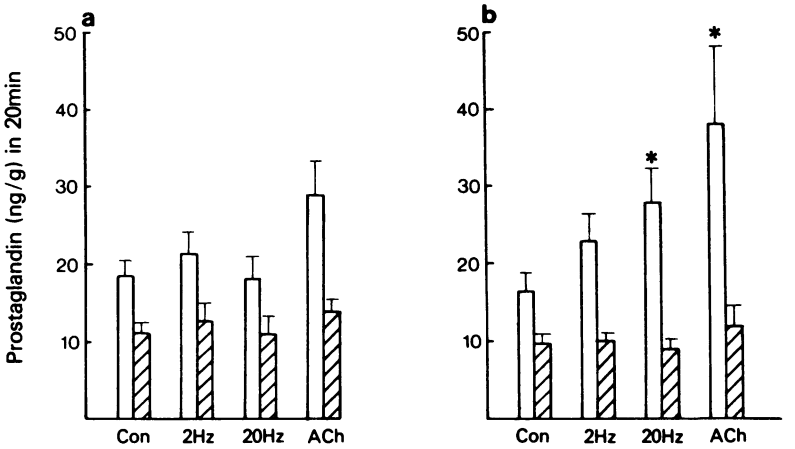

Figure 7 The release of prostaglandin E series (open columns) and F series (hatched columns) by field stimulation and acetylcholine (ACh) for 20 min. Control (Con): spontaneous release; 2 Hz: release by field stimulation at 2 Hz; 20 Hz: release by field stimulation at 20 Hz for 5 s every 2 min; ACh: release by administration of ACh (10 μg/ml). Vertical bars show s.e. mean. Significance of the difference from control: $*P < 0.05$. Number of experiments = 6.

have on various adrenergically stimulated preparations (Hedqvist & Wennmalm, 1971; Stjärne, 1972; Frame & Hedqvist, 1975; Hedqvist, 1976). PGE_1 inhibited the ACh (0.1 μg/ml)-induced contraction of circular muscle, indicating that PGE_1 acted on the postsynaptic membrane as Bennett *et al.* (1968) observed. The response to ACh (10 μg/ml), which was similar in height to the contraction induced by the field stimulation (20 Hz, for 5 s), was not inhibited by PGE_1 and PGE_2 which abolished field stimulation. Therefore, the inhibitory effect of PGE series was due predominantly to the prevention of transmitter release from nerve terminals. It was reported that PGE_1 inhibited the intestinal motility of rabbits (Suzuki, Hashikawa, Takano & Hayashi, 1975) and dogs *in vivo* (Shehadeh, Price & Jacobson, 1969). These results may be due to the presynaptic inhibitory action of PGE series on the circular muscle.

Indomethacin potentiated the response of circular muscle to field stimulation, indicating that the endogenous prostaglandin may act prejunctionally to control the release of ACh. However, indomethacin did not affect the response of longitudinal muscle to field stimulation. It was reported that non-steroidal anti-inflammatory drugs, including indomethacin, inhibited the response of longitudinal intestinal muscle to field stimulation in guinea-pig and that this inhibition was reversed by the administration of prostaglandins (Ehrenpreis, Greenberg & Belman, 1973; Famaey, Fontaine & Reuse, 1977). Furthermore, prostaglandin-induced contraction of the longitudinal muscle of the guinea-pig small intestine was partially mediated via the release of ACh (Bennett, Eley & Stockley, 1975; Nakahata, Suzuki, Mashiko & Watanabe, 1977). These reports and the present study, suggest that there are species differences in the effects of prostaglandin on the intestinal muscle. Kadlec, Masek & Seferna (1974) considered that the potentiating effect of prostaglandin on the contraction induced by nerve stimulation in the longitudinal muscle of guinea-pig ileum was due to inhibition of adrenergic nerves which normally inhibit cholinergic transmission.

It was reported that prostaglandin was released from the stomach by cholinergic nerve stimulation (Coceani, Pace-Asciak, Volta & Wolfe, 1967; Bennett, Friedmann & Vane, 1967). There is the problem of whether the source of prostaglandin is the nerve terminal or the smooth muscle itself. In the adrenergic system, Greenberg (1978) suggested that prostaglandins released from rabbit portal vein by field stimulation were of neuronal origin since release was not decreased when the contractile response was inhibited by adrenergic blocking agents. Gilmore, Vane & Wyllie (1968), however, showed that prostaglandins released from the spleen by adrenergic stimulation were not of neural origin. In the present experiments, the release of prostaglandin from circular muscle was increased by application of ACh (10 μg/ml) or by field stimulation at 20 Hz (total of 1000 pulses) but not at 2 Hz (total of 2400 pulses). Therefore, prostaglandins were probably released from smooth muscle accom-

panying contraction. In conclusion, a PGE-mediated negative feedback mechanism of excitatory transmission may be present in the circular but not the longitudinal muscle of canine small intestine.

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