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Non-adrenergic inhibition of the longitudinal muscle of rabbit distal colon may not be mediated by purinergic nerves

M. TONINI*, L. ONORI**, G. M. FRIGO, S. LECCHINI, L. D'ANGELO, A. CREMA, *Institute of Medical Pharmacology, University of Pavia, Piazza Botta 10, 27100 Pavia, Italy, ** Medical University of L'Aquila, Medical Clinic, Viale Duca degli Abruzzi 3A, 67100 L'Aquila, Italy*

Although ATP has been considered the most likely neurotransmitter of the non-adrenergic inhibitory response of several smooth muscles (Burnstock 1972, 1975), recent experimental data might argue against such role for ATP. In fact, unlike in the gut, no receptors for ATP seem to be present in the trachea of the guinea-pig (Christie & Satchell 1980). Moreover the anococcygeous muscle of the dog (Dehpour et al 1980) showed a variable response to ATP; when ATP relaxed the preparation, inhibition was not clearly dose-related and did not mimic the response to the inhibitory non-adrenergic nerves. In preparations which contracted in response to ATP, by a mechanism independent of prostaglandin biosynthesis, inhibitory nerve stimulation still relaxed the muscle. In the same animal species, intra-arterial injection of ATP in vascularly perfused isolated segments of small bowel (Northway & Burks 1980), caused mainly an intestinal stimulation through activation of cholinergic non-nicotinic receptors. Finally, Ferrero et al (1980) argued that another endogenous substance (bradykinin) appeared to be a serious contender for the transmitter role in the non-adrenergic nerves at least in both guinea-pig taenia caeci and the rat duodenum.

The present study was undertaken to examine the possible role of purinergic mechanisms in the electrically-induced non-adrenergic relaxation of the longitudinal coat of the distal colon of the rabbit. Due to the lack of availability of specific ATP antagonists (Kazic & Milosavljevic 1976; McKenzie et al 1977; Muller & Baer 1980), ATP receptor desensitization has been achieved by means of repeated exposures of the muscle to the drug. A similar procedure has been used by McKay & McKirdy (1972) to obtain ATP desensitization in rabbit rectum. In view of the high concentration of endogenous purines in the gastrointestinal wall (Pull & McIlwain 1972), high cumulative doses of ATP have been employed. We assume that desensitization to exogenous ATP was associated with the simultaneous development of desensitization to the endogenous putative mediator.

New Zealand White rabbits of either sex, 2200–2500 g, were killed by cervical dislocation and exsanguination. The distal colon was excised 1 cm above the pubis symphysis. Either a 5 cm long in toto preparation or a 0·3 cm wide longitudinal strip (2·5–3 cm in length) was suspended in an organ bath containing Tyrode solution gassed with 95% O_2 + 5% CO_2 and kept at 36 °C. The motility of the longitudinal musculature was recorded by an isotonic transducer in the whole piece and by an isometric transducer in the longitudinal strip. The tension applied was 0·8–2 g in both preparations. After 30–60 min had been allowed for incubation, electrical transmural stimulation was delivered by means of two silver electrodes placed 1 cm apart and parallel to the preparation. Square wave pulses of supramaximal voltage at 0·5–1–2–5–10 Hz, 0·5 ms of duration were applied, for 10–20 s, in the presence of hyoscine (2·2 μM), piperoxan (3·7 μM), propranolol (3·3 μM) and indomethacin (2 μM). The neurogenic nature of the electrically-induced relaxation was confirmed by the ability of tetrodotoxin (0·7 μM) to block the response.

Muscular desensitization to ATP, in the presence of the antagonists, was accomplished by repeating every 3–5 min(without washing) with increasing concentrations until the muscular response disappeared. In general, the ATP desensitization procedure was as follows: 5 applications of 10–20 μM were followed by 5 applications of 50 μM and finally by 2 applications of

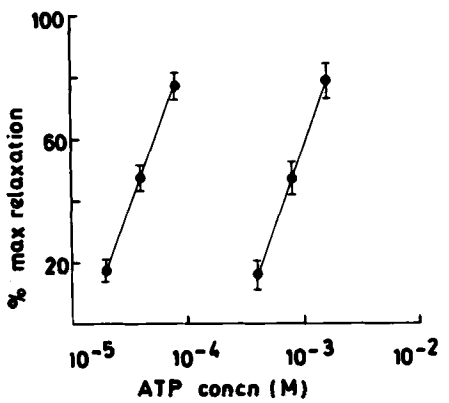

FIG. 1. Longitudinal muscle strip of rabbit distal colon. Concentration-response curves for ATP expressed as percentage of the inhibition caused by 200 μM of ATP, taken as 100% response. In each single strip only one concentration of ATP was tested initially (A), then desensitization was accomplished by adding cumulative concentrations of ATP up to 500 μM (see text). After desensitization, a dose approximately 20 times larger of ATP was necessary to mimic the initial response (B). Each point represents the mean ± s.e.m. of 5 experiments. Specimens not exhibiting spontaneous high tone were discarded (5 out of 20).

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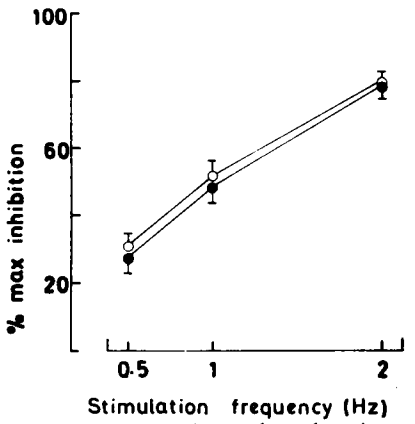

FIG. 2. Frequency-non-adrenergic relaxation curves of longitudinal muscle strip of rabbit distal colon. Electrically-induced relaxation is expressed as percentage of the inhibition caused by stimulation at 10 Hz, taken as 100% response. (○) Control; (●) after the pieces had been rendered tachyphylactic to ATP by adding cumulative concentrations of purine compound up to 500 μM. Each point represents the mean ± s.e.m. of 8 experiments. Specimens not exhibiting spontaneous high tone were discarded (7 out of 31).

100 μM. After desensitization had been achieved, response to electrical stimulation was tested and compared with a control response obtained before the beginning of the desensitization procedure.

In the whole preparation no clear relationship was observed between frequency of stimulation and inhibitory response, possibly due to the low tone exhibited by this kind of preparation (Tucker et al 1979). However, electrical stimulation at 1 and 5 Hz produced a relaxation, the magnitude of which was 3·72 ± 0·14 and 6·25 ± 0·23 mm respectively (mean ± s.e.m. of 11 experiments). When tachyphylaxis to the inhibitory effect of ATP was developed, the inhibitory responses to 1 and 5 Hz stimulation were 3·59 ± 0·16 and 6·15 ± 0·27 mm respectively, the difference being not significant. Frequency-related relaxations, on the contrary, were obtained by using longitudinal strips, which displayed a tone higher than that exhibited by the whole preparation. Since in some strips the relaxation brought about by ATP was followed by a contraction which could be partially prevented by indomethacin, the experiments for constructing dose-response curves for ATP were carried on in the presence of 2 μM of indomethacin. Fig. 1 shows the dose-response curves for ATP before and after desensitization obtained in the presence of 500 μM of purine cumulatively added to the bath. The relationship between frequency of stimulation and degree of relaxation before and after the strip had been desensitized up to about 20 times to ATP is shown in Fig. 2. Fig. 3 is a typical graph showing electrical inhibition before and after desensitization to ATP.

The finding that electrically-induced non-adrenergic inhibition was not significantly altered in the presence

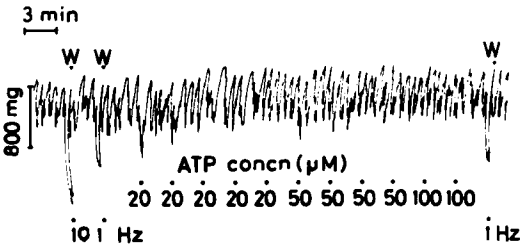

FIG. 3. Failure of ATP desensitization to inhibit the non-adrenergic relaxation in response to electrical stimulation applied for 20 s at 1 Hz frequency in the longitudinal muscle strip of rabbit distal colon. Maximal inhibition in response to stimulation at 10 Hz is shown for comparison purposes. ATP was added to the bath every 3 min up to a cumulative concentration of 500 μM. W indicates washout for 5 min. Vertical scale: tension of the isolated tissue in mg. Horizontal scale: time in min.

of acute desensitization to exogenous ATP suggests that muscular relaxation following stimulation may not be mediated by purinergic nerves. These results are in agreement with observations previously reported by Weston (1973) and by Ohga & Taneike (1977), who used a similar procedure to investigate the role of purinergic transmission in the rabbit duodenum and in the pig stomach respectively. However, since after ATP desensitization the amplitude of electrically-induced relaxation was often slightly reduced, we cannot exclude that ATP may accompany a still unidentified inhibitory transmitter (as happens in the adrenergic pathway; Kazic & Milosavljevic 1980) and play some role in inhibiting the musculature.

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