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Effects of desensitization to adenosine 5′-triphosphate and vasoactive intestinal polypeptide on non-adrenergic inhibitory responses of longitudinal and circular muscles in the rat ileum

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Non-cholinergic, non-adrenergic inhibitory nerves are widely distributed in peripheral tissues, particularly within the gastrointestinal tracts in various species of animals (Holman et al 1965). Although there is an abundance of evidence and observations supporting a role for ATP as an inhibitory transmitter (reviewed by Burnstock 1975) many experimental data argue against such a role for ATP (Ohga & Taneike 1977; Ambach et al 1977; Crema et al 1982).

Immunohistochemical techniques have demonstrated the occurrence of several biologically active polypeptides within gastrointestinal nerves, and their localization in the myenteric and submucous plexus (Besson et al 1978; Hökfelt et al 1980; Schultzberg et al 1980). In particular, vasoactive intestinal polypeptide (VIP) has been found to have potent inhibitory effects on a variety of smooth muscles including intestine (Piper et al 1970) and so it has been claimed that VIP, rather than ATP, may function as a non-adrenergic inhibitory neurotransmitter in some tissues (Fahrenkrug et al 1978; Goyal et al 1980; Matsuzaki et al 1980).

The present experiments were carried out to examine ATP and VIP as possible candidates for the neurotransmitter substance of non-adrenergic inhibitory nerve fibres in rat ileum. Because of the lack of specific and reliable antagonists to ATP (Baer & Frew 1979; Small & Weston 1979; Jenkinson 1981) and VIP (Bolton et al 1981) desensitization to these substances was achieved by means of continuous or repeated exposure of the preparations to the respective drugs. We assumed that desensitization to exogeneous agents may be associated with the simultaneous development of desensitization to the corresponding substances when they are released by nerve excitation.

Materials and methods

Male Wister rats, 200 to 300 g, were killed by a blow on the head and bled. The segment of ileum (2·5 to 3 cm in length) was suspended in an organ bath containing 20 ml Tyrode solution maintained at 37 °C and bubbled with 95% O_2–5% CO_2. After an equilibration period of 30 min, the longitudinal muscle contractions were recorded on a smoked drum by means of an isotonic lever (magnification 1:8, load 1 g). Transmural stimulation (TMS) of the rat ileum was with pulses of 0·5 ms width and maximal voltage given by two platinum electrodes, one placed in the lumen of the ileum and the other in the bath.

In other experiments the response of circular muscle layer was also checked because data on this tissue are not so abundant as those on longitudinal muscle and because of the role which circular muscle may have in physiological peristalsis. Ileal segments of 5 to 6 cm were mounted horizontally in a 20 ml organ bath with the mesenteric border sewn on an anchor at the bottom of the bath. TMS was applied by means of two platinum electrodes set up as described above. Relaxation of the circular muscle was recorded by connecting the serosa on the opposite side of the anchored point of the intestinal wall to an isotonic lever under a tension of 300 mg.

The neurogenic nature of the electrically-induced relaxation was confirmed by the ability of tetrodotoxin (1·5 μM) to block the response.

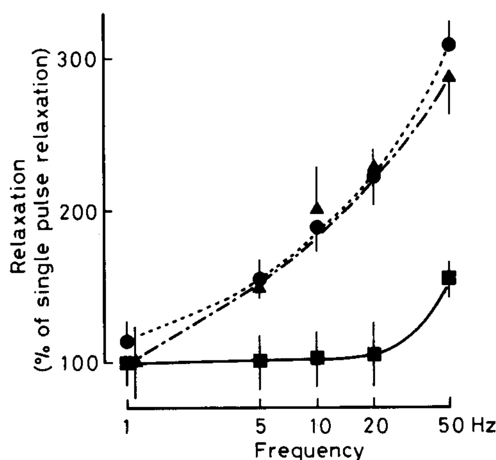

FIG. 1. Relationships between frequency of transmural stimulation (TMS) and amplitude of relaxation of the longitudial smooth muscle of rat ileum in the presence and the absence of atropine 0·5 μM or atropine 0·5 μM and guanethidine 4 μM. Ordinate: amplitude of relaxation as a percentage of the relaxation induced by a single pulse. Each point is the mean of 8 preparations. Vertical bars show s.e. means. Abscissa: frequency of TMS in a logarithm scale. ■——■ Control (in the absence of drug); ●- - -● in the presence of atropine; ▲— — — -▲ in the presence of atropine and guanethidine.

* Correspondence.

Results

Frequency-response relationships. Relationships between stimulation frequency (1–50 Hz) and responses of longitudinal or circular muscle were investigated by applying trains of pulses (0·5 ms duration) for 5 s every 5 min. TMS caused a rapid relaxation followed by a contraction of longitudinal muscle over the range of frequencies tested. The amplitude of the relaxation was about the same as that of a single pulse except at 50 Hz in normal Tyrode solution. In the presence of atropine or atropine and guanethidine, the amplitude of relaxations increased with an increase in frequency (Fig. 1). The amplitude of relaxation to single stimulus did not show any noticeable change to the drugs.

In circular muscle, TMS with a single pulse had no effect, or a relaxation sometimes, after contractions were obtained at frequencies less than 10 Hz. When the frequency was increased to over 20 Hz, the response changed to contraction. In the presence of atropine (5 μM) only a relaxation was obtained over the range of frequencies 1–50 Hz and the depth of relaxations increased with an increase in frequency until it reached a maximum at 50 Hz.

Even in the absence of atropine, the relaxations of longitudinal and circular muscle were clearly induced by single pulses or a train of pulses of 10 Hz for 2 s, respectively. Thus, the following experiments were carried out using these two kinds of stimulus.

Studies with ATP or VIP desensitization. Muscular desensitization to ATP was achieved by exposing the preparation to a high dose of ATP (50 μM) for 30 min without washing. After this procedure the preparation returned to its original tone and was insensitive to the initial dose of ATP (0·5 μM) which produced the same degree of relaxation as TMS in the fresh preparation; however, the relaxation to TMS remained without change (Fig. 2A). The relaxation to TMS was also not influenced after desensitization to adenosine which was

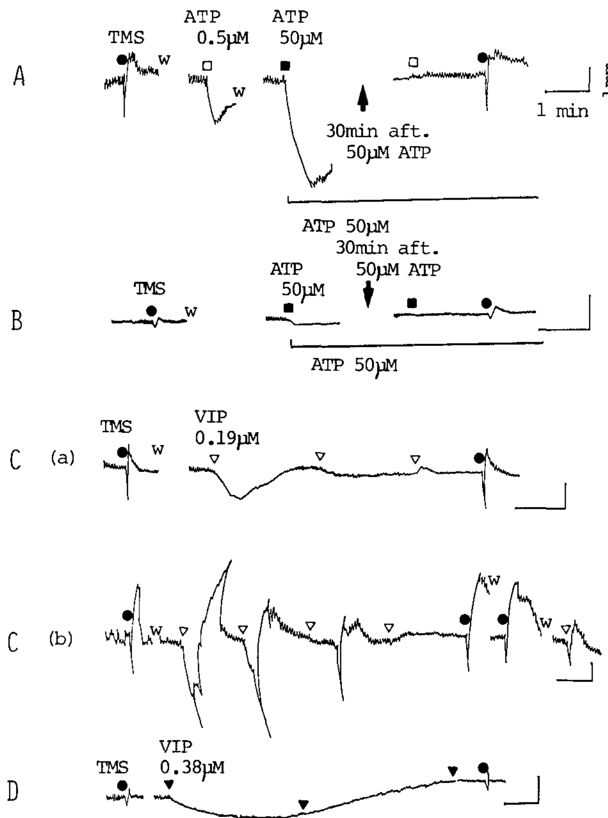

FIG. 2. Effects of desensitization to ATP (0·5 μM □, or 50 μM ■) and VIP 0·19 μM ▽, or 0·38 μM ▼) on the relaxation induced by TMS (●). A high dose of ATP (50 μM) was added into the bath and remained thereafter as indicated. VIP was added to the bath every 2 to 4 min until its effect had disappeared. A, C: longitudinal muscle response. B, D: circular muscle response. C(a): *typical traces showing the case in which* VIP produced slow relaxation. C(b): typical traces showing VIP produced rapid relaxation followed by a contraction. W indicates washout for 5 min. The horizontal bar shows the scale for 1 min and the vertical bar the scale for 1 mm.

accomplished by repeatedly adding a dose of adenosine (100 μM) every 2 min without washing until the response to the drug had disappeared.

VIP (0·19 μM) produced a slow relaxation (5 out of 10 experiments) or a rapid relaxation followed by a contraction (5 out of 10) in longitudinal muscle (Fig. 2C(a), (b)) and slow relaxation in circular muscle (Fig. 2D). The rapid relaxation of longitudinal muscle, which mimicked that seen with TMS, usually occurred with latency of about 12 s. The effect of VIP invariably faded away within 90 s and the initial tone and the spontaneous activity of the preparation was restored even in the presence of VIP. When 0·19 μM VIP was repeatedly added to the bath within 5 min without washing, desensitization to VIP was consistently achieved usually without modification of the baseline tone. Complete muscular unresponsiveness to VIP was usually obtained within 4 consecutive administrations of VIP. In such a desensitized preparation relaxation to TMS was not affected (Fig. 2C(a), (b)). The desensitization to VIP was abolished by prolonged washing.

Similarly, the inhibitory responses of circular muscle to TMS were clearly observed in both the preparations densitized to ATP or VIP (Fig. 2B, D).

Discussion

The present experiments show that both the longitudinal and the circular muscle of the rat ileum are innervated with intrinsic cholinergic excitatory and non-adrenergic inhibitory nerves, and that the non-adrenergic neurons have a lower stimulation threshold than the excitatory neurons. In contrast with guinea-pig ileum, significant relaxation was observed after TMS even in the absence of atropine. This indicates the possibility that non-adrenergic inhibitory responses have a significant role in the physiological condition in the rat ileum.

Desensitiazation to purine compounds has been shown to be relatively specific to the purinoceptor since the response of the circular muscle of rabbit colon to isoprenaline was not affected, and the condition could be overcome by higher concentrations of purinoceptor agonists (Crema et al 1982). The present finding that the TMS-induced non-adrenergic inhibitory response was not significantly altered in preparations acutely desensitized to exogenous ATP and VIP suggest that the muscular relaxation after stimulation may not be mediated by these substances. The results are consistent with previous observations by Ohga & Taneike (1977) in the pig stomach, Crema et al (1982) in the rabbit colon and Bartlett et al (1979) in the rat ileum, who argued against the possibility of ATP or related compounds as a non-adrenergic transmitter.

According to Huizinga et al (1981) relaxation of guinea-pig stomach muscle evoked by field stimulation can be explained by assuming that ATP is released from the nerves, with subsequent degradation to adenosine causing relaxation of the muscle. However, this is unlikely in rat ileum since the TMS-induced relaxation was also resistant to adenosine desensitization.

In conclusion, the present experiments have confirmed that the non-adrenergic inhibitory nerve is unlikely to be purinergic or VIPergic in nature at least in rat ileum. Negation of VIP as a non-adrenergic inhibitory transmitter in guinea-pig taenia has been reported by Mackenzie & Burnstock (1980).

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