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Theophylline-sensitive modulation of non-cholinergic excitatory neurotransmission in the guinea-pig ileum

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Atropine-resistant longitudinal contractions of the guinea-pig ileum due to field stimulation were greatly augmented by theophylline (150 μM). Adenosine exerted an opposite effect. Theophylline did not potentiate contractions evoked by exogenous substance P. It is suggested that a theophylline-sensitive inhibitory mechanism (possibly mediated by adenosine or a related substance) controls transmitter release from enteric non-cholinergic excitatory neurones.

Introduction Theophylline increases the stimulation-evoked release of neurotransmitters in many experimental situations; among others it facilitates the release of acetylcholine in the myenteric plexus (for review see Vizi, 1979; Fredholm, 1980). The aim of the present study was to test the effect of theophylline on atropine-resistant longitudinal contractions of the field-stimulated, isolated ileum of the guinea-pig (Ambache & Freeman, 1968). These responses are most probably mediated by a substance P-like peptide, released from intrinsic neurones of the myenteric plexus (cf. Furness & Costa, 1980; Barthó & Holzer, 1985). The effect of adenosine, the most likely endogenous activator of theophylline-sensitive (P_1) purinoceptors (Burnstock, 1978), was also investigated.

Methods Segments of guinea-pig ileum of approximately 2 cm length were suspended in Krebs-Henseleit solution of the following composition (mM): NaCl 118.3, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2 and glucose 10. The solution was kept at 37°C and aerated with 5% CO_2 and 95% O_2. Longitudinal contractions were recorded with the aid of an isotonic transducer (low-friction tuning capacitor system; signal: 1–100 mV) connected to a compensographic recorder (MTA-KUTESZ, Hungary). The load on the tissue was 0.005 N (0.5 g). Naloxone (0.5 μM) and guanethidine (5 μM) were also added to the organ bath to prevent opioid and sympathetic inhibition of non-cholinergic excitatory neurotransmission (cf. Barthó & Holzer, 1985). Field stimulation was applied through a pair of longitudinal platinum electrodes. Parameters of stimulation were: supramaximal voltage, 0.1 ms impulse width; 150 impulses were delivered at a frequency of 1, 5 or 20 Hz. In each experiment only one of these frequencies was used and stimulations were separated by 10-min intervals. Drug effects were investigated only when uniform control responses had been obtained. Contact time for adenosine (Reanal), theophylline (Sigma) and tetrodotoxin (Sankyo) was 3 min. In some experiments strips of myenteric plexus-longitudinal muscle were used; they were prepared according to the method of Paton & Vizi (1969). Contractions were expressed as % of the maximal response evoked by histamine (5 μM) given at the end of the experiment. Data are expressed as mean ± s.e.mean. Student's one-sample t test was used for assessing the statistical significance of differences.

Results Atropine-resistant ileum contractions evoked by field stimulation were potently enhanced by theophylline (150 μM). Smaller concentrations of theophylline (15 and 50 μM) had similar effects; however, an increase in responses to field stimulation was still evident when the concentration of theophylline was raised from 50 to 150 μM ($n = 5$). Half-maximal contractions produced by substance P (1–3 nM) were slightly but significantly reduced by theophylline (150 μM; Figure 1). Adenosine inhibited non-cholinergic contractions; this effect was prevented by theophylline (Figure 1) in a surmountable fashion. Theophylline (150 μM) caused an apparently parallel shift to the right of the concentration-response curves for adenosine. Estimated EC_{50} values of adenosine on contractions due to 5 Hz stimulation were 6.9 ± 0.8 μM in the absence and 46.8 ± 4.0 μM in the presence of theophylline (150 μM; $n = 9$). The effects of both theophylline and adenosine were readily reversible by washing. Adenosine (up to 50 μM) did not inhibit the action of exogenous substance P which was used in concentrations (1–3 nM) producing approximately half-maximal contractions. In the presence of adenosine (50 μM) these contractions reached 99 ± 3.7% of those in the absence of adenosine ($n = 10$). Theophylline (150 μM) also enhanced and adenosine (15 μM) also inhibited non-cholinergic contractions due to field stimulation (5 Hz) of myenteric plexus-

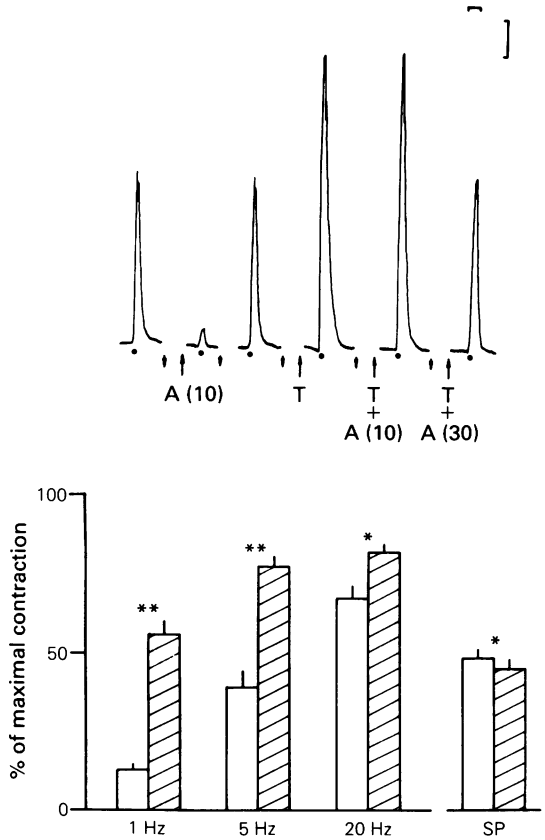

Figure 1 (a) Atropine-resistant longitudinal contractions of the guinea-pig ileum due to field stimulation (dots; 150 impulses at 5 Hz). The effect of adenosine (A) in the absence and in the presence of theophylline (T; 150 μM). Concentrations of adenosine (μM) are indicated by the numbers in parentheses. At downward arrows the bath fluid was changed three times. Calibrations: 10% of the maximal contraction produced by 5 μM of histamine (vertical); 1 min (horizontal). (b) Effect of theophylline on atropine-resistant contractions due to field stimulation at different frequencies ($n = 8$ in each group) or in response to substance P (SP; 1–3 nM, $n = 10$). Open columns: control response. Hatched columns: responses in the presence of theophylline (150 μM). Mean values are given with s.e.mean shown by vertical lines. Significant changes are indicated by asterisks; $*P<0.05$, $**P<0.001$.

longitudinal muscle preparations ($n = 5$). On segments of whole ileum, half-maximally contracted by histamine, transmural stimulation (single pulses at 0.1 Hz or 5 pulses at 5 Hz) produced rapid and short-lived relaxations. These responses were not influenced by theophylline (150 μM, $n = 5$). Tetrodotoxin (0.5 μM) prevented all of the above responses to field stimulation ($n = 4$ each).

Discussion These findings provide pharmacological evidence for the presence of a theophylline-sensitive modulation of transmitter release from non-cholinergic excitatory neurones of the myenteric plexus. The stimulant action of theophylline on the atropine resistant contractile responses is unlikely to be explained by a post-junctional potentiation of smooth muscle contraction or by the elimination of a nerve-mediated non-adrenergic relaxation mechanism since contractions due to exogenous substance P were not enhanced and non-adrenergic relaxations due to field stimulation were not inhibited by theophylline. Likewise, in the guinea-pig taenia caeci, theophylline does not inhibit the non-adrenergic relaxation due to field stimulation (Small & Weston, 1979). We suggest that theophylline enhances contractions of the ileum to field stimulation by eliminating the action of an endogenous inhibitory substance and thus enhancing the release of the non-cholinergic excitatory mediator. Our findings with adenosine, in conjunction with those of others (Burnstock, 1978; Vizi, 1979) may indicate that the inhibitory substance is adenosine or another adenine derivative. EC_{50} values for adenosine (in the absence and in the presence of theophylline) are similar to those reported by Sawynok & Jhamandas (1976) on cholinergic 'twitch' responses of the guinea-pig ileum. The present results support the view (Barthó & Holzer, 1985) that the pharmacology of the enteric release of acetylcholine and substance P is similar.

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