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Excitatory P_1-purinoceptors on pre- and post-ganglionic cholinergic nerve terminals in the chick oesophagus

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Abstract—The mechanism of action of ATP and the purinoceptors involved have been investigated on the chick oesophagus. The susceptibility of the excitatory responses to ATP/adenosine to tetrodotoxin indicates that their action is neurally mediated. Blockade of ATP/adenosine responses by atropine suggests the involvement of endogenous acetylcholine. ATP action depends on breakdown to AMP/adenosine, since theophylline blocks ATP/adenosine responses. The inhibition of ATP responses by pentolinium implies the involvement of preganglionic fibres.

Adenosine and adenine nucleotides have been reported to inhibit the release of transmitters from both adrenergic and cholinergic nerve fibres (Hayashi et al 1978; Su 1978; Gustafsson et al 1981; Moody & Burnstock 1982). However, ATP, a purine nucleotide, has been observed to produce contraction of the chick oesophagus through a cholinergic mechanism (Bartlet 1974). The present investigation was undertaken to determine the site(s) of action of ATP and the nature of purinoceptors involved in mediating its response in the chick oesophagus.

Materials and methods

Pre-crop oesophagus, obtained from White Leghorn chicks (1–2 wks) of either sex, was used as described by Mishra & Raviprakash (1980). The oesophagus was stimulated electrically by transmural stimulation (TMS) as described by Paton (1955). The stimulation consisted of monophasic square-wave pulses at a frequency of 1 Hz of 0·5 ms duration delivered at a supramaximal voltage of 5 V for 15 s. The distance between the plantinum wire electrodes placed within and outside the lumen was 8 mm. An interval of 10 min was maintained between two successive doses of drugs/stimuli. The tissues were exposed to an antagonist for 15–30 min before the responses to agonists/stimuli were elicited.

The drugs used were ATP, adenosine, acetylcholine chloride (ACh), theophylline bromide, quinidine sulphate, atropine sulphate, pentolinium tartrate and tetrodotoxin (TTX).

Results

ATP ($3{\cdot}61 \times 10^{-6}$M) and adenosine ($2{\cdot}99 \times 10^{-6}$M) produced contractions of the chick oesophagus and the responses were consistent when the agents were administered at an interval of 10 min. Similarly, ACh ($5{\cdot}5 \times 10^{-7}$M) and TMS induced contractions on this tissue. TTX ($1{\cdot}57 \times 10^{-7}$M) abolished the responses induced by ATP, adenosine and TMS, without significantly affecting those to ACh (Fig 1A). Atropine ($1{\cdot}44 \times 10^{-7}$M) markedly inhibited the responses to ATP, adenosine, ACh and TMS (Figs 1B, 2B). Theophylline ($3{\cdot}86 \times 10^{-5}$M) produced a significant inhibition of the contractions induced by ATP and adenosine without significantly affecting the responses to ACh or TMS (Figs 1C, 2A). Quinidine ($1{\cdot}07 \times 10^{-5}$M) did not alter the responses of ATP, ACh or TMS (not shown). Pentolinium ($7{\cdot}42 \times 10^{-6}$M) significantly inhibited (by about 75 per cent) the ATP response without affecting those of ACh or TMS (Fig. 1D).

Discussion

Since ATP breaks down to AMP/adenosine in the tissues, the present study was conducted using both ATP and adenosine. The ATP/adenosine-induced excitatory responses on the chick oesophagus are purely neurogenic since these responses are abolished by TTX. This, therefore, indicates that purine nucleotides (ATP/adenosine) stimulate impulse propagation in nerves associated with the chick oesophagus. Furthermore, marked inhibition of these responses by atropine implies participation of a cholinergic mechanism. Since the purine agonists are equipotent on a molar basis (adenosine, $1{\cdot}84 \times 10^{-4}$M = AMP, $1{\cdot}84 \times 10^{-4}$M = ADP, $1{\cdot}87 \times 10^{-4}$M and ATP $1{\cdot}97 \times 10^{-4}$M: Bartlet 1974) on the chick oesophagus, it appears that the neuromodulatory role of ATP could be attributed to its hydrolysed products AMP or adenosine which act on the prejunctional P_1-purinoceptors as suggested by De Mey et al (1979) and Moody & Burnstock (1982). This is further substantiated by the fact that theophylline, a selective P_1-purinoceptor antagonist (Burnstock & Meghji 1981) significantly inhibited its responses.

Since both pre- and post-ganglionic cholinergic fibres are located in the myenteric plexus, it was of interest to see whether P_1-purinoceptors are located in preganglionic fibres besides as well as in post-ganglionic cholinergic nerve terminals. Marked inhibition of ATP responses by pentolinium, a ganglionic nicotinic receptor blocker, indicates that the breakdown products of ATP (AMP or adenosine) might be diffusing to the ganglia to interact with P_1-purinoceptors located on the preganglionic cholinergic fibres to release acetylcholine which subsequently activates the post-ganglionic fibres. However, a direct action of these break-down products of ATP on post-ganglionic nerve fibres is still evident since responses appear even

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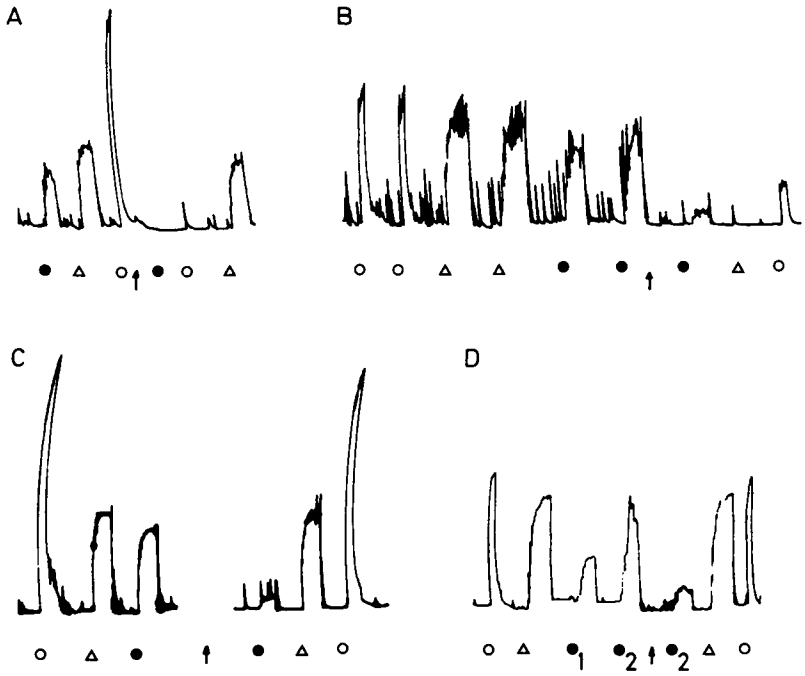

FIG. 1. A. The effect of tetrodotoxin (↑ $1{\cdot}57 \times 10^{-7}$M) on the responses of ATP (● $3{\cdot}61 \times 10^{-6}$M), ACh (△ $5{\cdot}5 \times 10^{-7}$M) and TMS (○ 1 Hz; 0·5 ms PD; 5V) on chick oesophagus (n = 4). B. The effect of atropine (↑ $1{\cdot}44 \times 10^{-7}$M) on the responses of TMS (○), ACh (△) and ATP (●) on chick oesophagus (n = 4). C. The effect of theophylline (↑ $3{\cdot}86 \times 10^{-5}$M) on the responses of TMS (○), ACh (△) and ATP (●) on chick oesophagus (n = 6). D. The effect of pentolinium (↑ $7{\cdot}42 \times 10^{-6}$M) on the responses of TMS (○), ACh (△) and ATP ($●_2$ $3{\cdot}61 \times 10^{-6}$M) on chick oesophagus. Note the graded responses of ATP at the doses of $1{\cdot}8 \times 10^{-6}$M ($●_1$) and $3{\cdot}61 \times 10^{-6}$M ($●_2$) (n = 5)

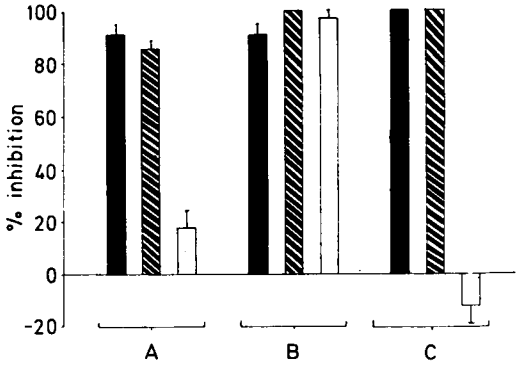

FIG. 2. The effect of (A) theophylline ($3{\cdot}86 \times 10^{-5}$M), (B) atropine ($1{\cdot}44 \times 10^{-7}$M) and (C) tetrodotoxin ($1{\cdot}57 \times 10^{-7}$M) on the responses of (■) ATP ($3{\cdot}61 \times 10^{-6}$M), (▨) adenosine ($2{\cdot}99 \times 10^{-6}$M) and (□) ACh ($5{\cdot}5 \times 10^{-7}$M) on chick oesophagus; vertical bars indicate the standard error of the mean (n = 5–6).

after ganglionic blockade. Though it is difficult to distinguish the actions of theophylline with respect to its P_1-purinoceptor antagonism at pre- and post-ganglionic sites, it is possible that the purinoceptors at the ganglionic level are also antagonized. As the ganglia involved are not simple nicotinic relays, the involvement of a cholinergic interneuron with excitatory P_1-purinoceptors on its cell body cannot be ruled out.

In general, the purines are known to inhibit neurotransmitter release from presynaptic nerve terminals acting on the P_1-purinoceptors located on them (Su 1983). However, the present study on chick oesophagus reveals the presence of excitatory P_1-purinoceptors on the cholinergic fibres associated with this smooth muscle.

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