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NUCLEOTIDE PYROPHOSPHATASE ANTAGONIZES RESPONSES TO ADENOSINE 5′-TRIPHOSPHATE AND NON-ADRENERGIC, NON-CHOLINERGIC INHIBITORY NERVE STIMULATION IN THE GUINEA-PIG ISOLATED TAENIA COLI

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The enzyme nucleotide pyrophosphatase converted adenosine 5′-triphosphate (ATP) to adenosine 5′-monophosphate (AMP). In the isolated taenia coli of the guinea-pig it reduced the inhibitory responses to exogenously applied ATP. This could be explained on the basis that the ATP was rapidly converted to AMP which is less potent. The enzyme also reduced inhibitory responses to stimulation of non-adrenergic, non-cholinergic nerves but failed to reduce inhibitory responses to either perivascular sympathetic nerve stimulation or to noradrenaline. The results support the hypothesis that ATP is the transmitter released by non-adrenergic, non-cholinergic ('purinergic') inhibitory nerves.

Introduction Nucleotide pyrophosphatase (NP) hydrolyses nicotinamide adenine dinucleotide (NAD) to nicotinamide mononucleotide (NMN) and adenosine 5′-monophosphate (AMP). Adenosine 5′-triphosphate (ATP) is also a substrate and forms AMP. Since AMP is one thirtieth as potent as ATP in causing relaxation of the guinea-pig taenia coli (Satchell & Burnstock, 1975) it was considered that treatment of taenia coli strips with the enzyme might cause a reduction of responses to exogenously applied ATP. Under these conditions NP might also be expected to cause a reduction in the amplitudes of responses to non-adrenergic, non-cholinergic (NANC) inhibitory nerve stimulation of the taenia if the nerves release ATP as their neurotransmitter (Burnstock, Campbell, Satchell & Smythe, 1970; Burnstock, 1972).

In the present experiments, dose-response curves to ATP have been plotted in control preparations and in preparations treated with NP. Frequency-response curves to NANC inhibitory nerve stimulation were plotted under the same conditions. In order to test the specificity of the enzyme preparation, control experiments were carried out using dose-response curves to noradrenaline and frequency-response curves to perivascular sympathetic nerve stimulation.

Methods Taenia coli preparations were dissected from guinea-pigs of either sex weighing 500–1000 g and suspended in a modified Krebs solution (Maguire & Satchell, 1979). The solution was aerated with 95% O_2 and 5% CO_2 and maintained at 36°C. Tension (1.0 g) was applied to each preparation. Muscle activity was registered isometrically by means of Grass FT0 3 force transducers coupled to a Grass 7D polygraph with appropriate amplification.

Preparations were allowed to equilibrate for 60 min before exposure to drugs. Hyoscine (1.3×10^{-6} M) was present in all experiments. NANC inhibitory nerves were stimulated via platinum ring electrodes placed around the taenia at pulses up to 5 Hz. Maximum responses to NANC nerve stimulation were not reduced by treatment with guanethidine (3.5×10^{-6} M) demonstrating the lack of a sympathetic component in the response to field stimulation of that frequency. Perivascular sympathetic nerves were dissected according to the method of Burnstock, Campbell & Rand (1966) and were stimulated via platinum ring electrodes at frequencies ranging from 15–50 Hz. Responses at all frequencies were abolished by guanethidine (3.5×10^{-6} M).

Responses to drugs and nerve stimulation were plotted as the mean ± s.e. percentage of maximum response. Each response (both in control and treated preparations) was determined in preparations from at least 5 animals. ATP was obtained from E. Merck, Darmstadt, noradrenaline from Winthrop Laboratories, Sydney, and nucleotide pyrophosphatase from the Sigma Chemical Company, St. Louis (enzyme shipped during warm weather exhibited a loss of activity).

Chromatograms were run on Whatman No. 1 versene-washed paper in a solvent (1) composed of iso-butyric acid: water: 0.880 ammonia: 0.1 M versene (100 : 55.8 : 4.2 : 1.6) for 12 h ascending.

Results ATP and noradrenaline each caused concentration-dependent relaxations of strips of the guinea-pig taenia coli. NP (1 unit/ml: 1 unit hydrolyses 10^{-6} M of β-NAD to NMN and AMP per min at pH 7.4 and 37°C) caused a marked reduction of inhibitory responses to ATP producing a shift to the right of the dose-response curve (Figure 1a). Relaxa-

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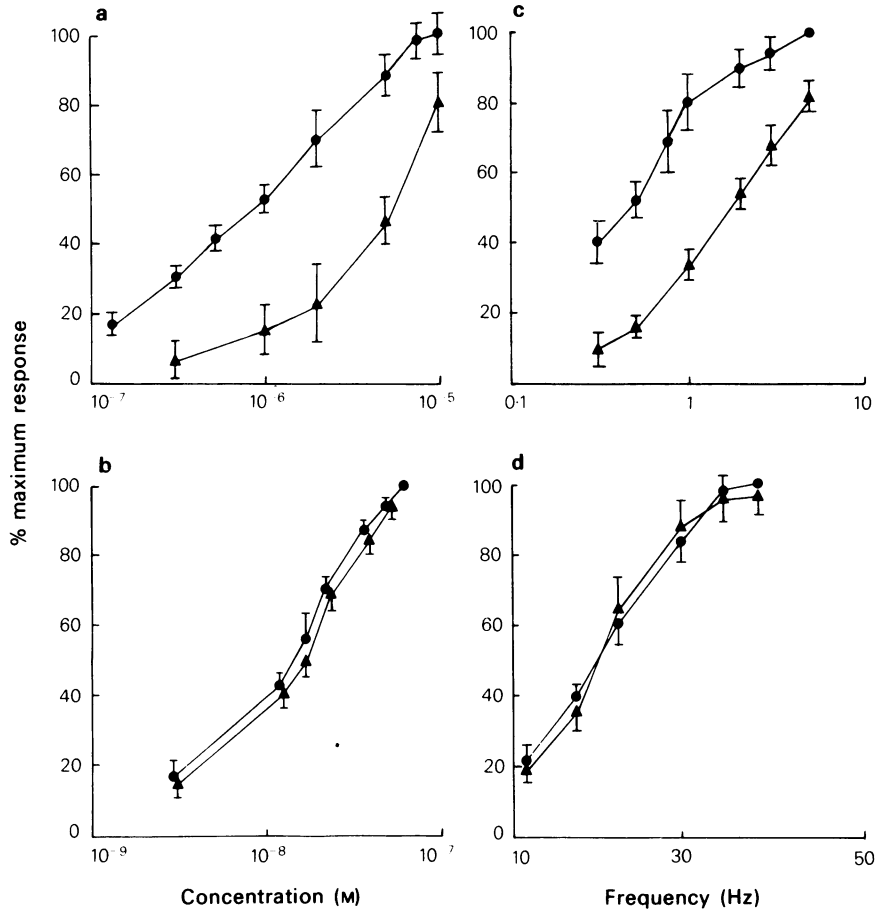

Figure 1 Log dose-response curves to (a) ATP; (b) noradrenaline. Frequency-response curves to (c) field stimulation of purinergic nerves (d) stimulation of perivascular sympathetic nerves. (●) Control; (▲) after incubation for 15 min with nucleotide pyrophosphatase 1 unit/ml. Hyoscine (1.3×10^{-6} M) was present in all experiments.

tions to the lower concentrations of ATP were antagonized more effectively than those to the higher concentrations. This is reflected in the change in shape of the dose-response curve in the presence of NP. Relaxations to noradrenaline were not affected by NP treatment (Figure 1b). Relaxations to field stimulation of NANC nerves were reduced following treatment with NP (1unit/ml). Responses to the lower frequencies of stimulation were antagonized more effectively than the responses to the higher frequencies. This is reminiscent of the effects of NP on responses to ATP and is also reflected in the change in shape of the frequency-response curve following NP treatment (Figure 1c). Relaxations in response to perivascular sympathetic nerve stimulation were unaffected by NP (Figure 1d). NP (1 unit/ml) which had been boiled for 2 min was ineffective in causing reductions in responses to either ATP (3×10^{-7} M) or to NANC inhibitory nerve stimulation (0.3 Hz).

When 2×10^{-7} M of ATP was incubated with 2.85 units of enzyme for 5 min at 35°C it yielded a purine which on chromatography in solvent (1) had an R_F value of 0.63 which was the same as that of authentic AMP.

Discussion The finding that NP caused a reduction in the amplitudes of inhibitory responses to ATP can be explained on the basis that the enzyme readily converted ATP to the less active AMP. Moreover, in the presence of NP the dose-response curve to ATP was not only shifted to the right but became curved over the 20 to 50% response range. The dose-

response curve to AMP is markedly curved over this range (Satchell & Burnstock, 1975). The observation that NP also caused a reduction in the inhibitory responses to field stimulation of NANC nerves could be explained on the basis that the nerves release ATP as their neurotransmitter. Under these conditions the released ATP, like the exogenously applied ATP, could also be rapidly converted to the less active AMP.

The findings that NP failed to affect inhibitory responses to either perivascular sympathetic nerve stimulation or noradrenaline and that its effects on the ATP response were abolished by boiling are consistent with the view that an adenine nucleotide specific enzyme is the active fraction of the NP preparation.

Evidence has accumulated both in favour and against the hypothesis that ATP is the transmitter released by the NANC inhibitory nerves of the gut and other sites (the 'purinergic nerve hypothesis'). This evidence has recently been reviewed (Maguire & Satchell, 1981). While drugs have been found which specifically potentiate responses to NANC nerve stimulation and to ATP in the taenia (Satchell, Lynch, Bourke & Burnstock, 1972), no antagonists that are specific for the inhibitory response to ATP have been found. The present findings would appear to augment the evidence supporting the purinergic nerve hypothesis.

While the evidence is explained on the basis that NP is the active fraction of the enzyme preparation causing the reduction of responses to ATP and purinergic nerve stimulation, the possibility has not been discounted that a second active heat-labile fraction could also be present.

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