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Adenosine deaminase antagonizes inhibitory responses to adenosine and non-adrenergic, non-cholinergic inhibitory nerve stimulation in isolated preparations of guinea-pig trachea

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In guinea-pig isolated tracheal tube preparations treated with dipyridamole, adenosine deaminase antagonized inhibitory responses to adenosine and non-adrenergic, non-cholinergic nerve stimulation. Inhibitory responses to vasoactive intestinal peptide (VIP) were unaffected and hyoscine-sensitive excitatory responses to field stimulation were not reduced. The evidence supports a role of adenosine as an inhibitory neurotransmitter in non-adrenergic, non-cholinergic nerves in trachea.

Introduction In the present experiments dipyridamole-treated preparations have been used to test the effects of adenosine deaminase on inhibitory responses to adenosine and non-adrenergic, non-cholinergic (henceforth NANC) nerve stimulation in guinea-pig tracheal preparations. Such an experiment can be used to examine the suggestion (Satchell, 1982) that NANC nerves in guinea-pig trachea release adenosine as their neurotransmitter.

Methods Tracheae were dissected from guinea-pigs (weights 600–900 g) of either sex and suspended as whole preparations in organ baths recording intraluminal pressure (Coleman, 1976) with Gould Statham P23 pressure transducers coupled to a Grass 7D polygraph. Organ baths contained a Krebs solution (Satchell, 1982), gassed with 95% O_2 and 5% CO_2 and maintained at 36.5°C. Preparations were allowed to equilibrate for 60 min before stimulation or exposure to drugs.

Hyoscine (1.3 μM), dipyridamole (0.5 μM) and guanethidine (3.5 μM) were present except where stated. NANC and cholinergic nerves were stimulated via coaxial electrodes (Coleman, 1976) at pulses up to 30 Hz. Responses to drugs and nerve stimulation were plotted as the mean ± s.e. percentage of maximum response. Each response was determined in preparations from at least 5 animals. Guanethidine was obtained from Ciba-Geigy. Hyoscine, dipyridamole, vasoactive intestinal peptide (VIP), adenosine and adenosine deaminase (type 111) were obtained from the Sigma Chemical Company. When an aliquot of enzyme preparation was placed in the organ bath, the Krebs solution was 15.5 mM with respect to added glycerol and the K^+ level was increased from 4.65 to 4.67 mM; the pH was not changed.

Results Adenosine caused dose-dependent falls in intraluminal pressure in tracheal tube preparations. The concentration-effect curve of this compound is shown in Figure 1a. Following treatment with adenosine deaminase (5 units ml^{-1}) the concentration-effect curve of adenosine was displaced to the right by four orders of magnitude and the maximal response was reduced by 50%. VIP also caused a fall in intraluminal pressure and the concentration-effect curve for this compound was not significantly altered by pretreatment with adenosine deaminase (5 units ml^{-1}) (Figure 1b).

Field stimulation of NANC nerves in the presence of hyoscine (1.3 μM), and guanethidine (3.5 μM) caused a reduction in intraluminal pressure. A frequency-response curve is plotted in Figure 1c. The responses to NANC nerve stimulation were reduced in the presence of adenosine deaminase (5 unit ml^{-1}). The reductions were significant at frequencies ranging from 6 to 30 Hz. When the experiment described above was repeated in the absence of hyoscine, the response to field stimulation was biphasic in the form of an increase in pressure preceding a decrease. It seemed likely that the initial increase was due to field stimulation of cholinergic nerves preceding the decrease in response to NANC nerve stimulation. The effects of adenosine deaminase (5 units ml^{-1}) were determined on the initial increase in pressure to see whether this enzyme affected the response to stimulation of nerves other than those of the NANC type.

Adenosine deaminase failed to cause a reduction in the increases in pressure in response to field stimu-

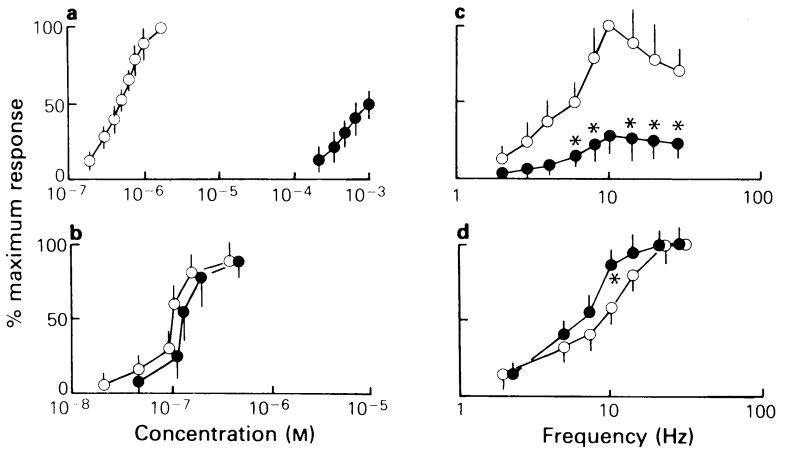

Figure 1 Log-concentration-effect curves plotted for decreases in intraluminal pressure to: (a) adenosine; (b) vasoactive intestinal peptide (VIP). Frequency-response curves for (c) decreases in intraluminal pressure to field stimulation of NANC nerves. (d) Hyoscine-sensitive increases in intraluminal pressure as the initial component of the biphasic response to field stimulation. (○) Control; (●) after incubation for 15 min with adenosine deaminase (5 units ml^{-1}). Hyoscine (1.3 μM) was present in all experiments except (d). Dipyridamole (0.5 μM) and guanethidine (3.5 μM) were present in all experiments. Responses to adenosine and VIP were plotted as a percentage of the maximal response to adenosine. Responses to field stimulation were plotted as a percentage of each control maximal response in (c) and (d).

* Denotes significant difference at the frequency ($P < 0.05$) between control and treated preparations. In (c) the large difference between control and treated curves at 10 Hz is not asterisked since the control response at this frequency was the reference 100% response for which s.e.means were not plotted.

lation in the absence of hyoscine (Figure 1d). In the figure it can be seen that responses at frequencies from 5 to 15 Hz in the presence of adenosine deaminase were greater than those in the absence of the enzyme although this finding was significant at 10 Hz only.

Discussion The finding that adenosine deaminase caused a shift to the right of the concentration-effect curve of adenosine can be explained by the rapid formation of inosine in the presence of the enzyme and by the low efficacy of inosine in dipyridamole-treated preparations (Satchell, 1984). VIP served as a control and adenosine deaminase caused no significant change in the concentration-effect curve for this substance. This demonstrated that the reduction in the responses to adenosine in the presence of adenosine deaminase was not a non-specific effect of the enzyme preparation.

The reduction in responses to NANC nerve stimulation by adenosine deaminase could be explained on the basis that the nerves release adenosine as their neurotransmitter.

The failure of adenosine deaminase to reduce the initial component of the biphasic response to field stimulation in the absence of hyoscine could suggest that the enzyme preparation does not reduce responses to cholinergic nerve stimulation. However, increases in pressure as the response to this form of stimulation were significantly greater at 10 Hz in the presence of the enzyme. An interpretation of this finding is that the initial component of the biphasic response is not purely cholinergic but is masking an opposing response due to the start of the second phase, i.e. the NANC response. The abolition or reduction of this component by adenosine deaminase could then cause the apparent enhancement of the response at 10 Hz. Thus, an accurate determination of the effects of adenosine deaminase on the response to cholinergic nerve stimulation cannot be made but it seems unlikely that the enzyme preparation caused a large reduction in responses to cholinergic nerve stimulation.

Earlier studies with dipyridamole demonstrated both in guinea-pig isolated tracheal tube preparations (Coleman, 1976) and in transverse strips of the trachea (Kinnaird, 1973) that responses to adenosine and to NANC nerve stimulation were both potentiated by dipyridamole. These findings taken together with the present experiments provide evidence favouring a role of adenosine as a neurotransmitter in guinea-pig trachea. ATP is unlikely to be the neurotransmitter in NANC nerves in this tissue

since inhibitory receptors for ATP appear to be lacking (Christie & Satchell, 1979; Satchell, 1984).

VIP has been suggested as the inhibitory neurotransmitter released by NANC nerves in guinea-pig trachea (Matsuzaki *et al.*, 1980). Further experiments are necessary to evaluate the roles of the putative neurotransmitters adenosine and VIP in the airways of different species. The possibility of a co-transmitter role of the two substances should also be examined.

References

CHRISTIE, J. & SATCHELL, D.G. (1980). Purine receptors in the trachea: Is there a receptor for ATP? *Br. J. Pharmac.*, **70,** 512–514.

COLEMAN, R.A. (1976). Effects of some purine derivatives on the guinea-pig trachea and their interaction with drugs that block adenosine uptake. *Br. J. Pharmac.*, **51,** 51–57.

KINNAIRD, C. (1973). Non-adrenergic inhibitory responses of guinea-pig trachealis smooth muscle. *Master of Science Thesis, University of Melbourne.*

MATSUZAKI, Y., HAMASAKI, Y. & SAID, S. (1980). Vasoactive intestinal peptide: A possible transmitter of non-adrenergic relaxation of guinea-pig airways. *Science,* **210,** 1252–1253.

SATCHELL, D.G. (1982). Non-adrenergic, non-cholinergic nerves in mammalian airways: Their function and the role of purines. *Comp. Biochem. Physiol.*, **72C,** 189–196.

SATCHELL, D.G. (1984). Purine receptors in trachea: Studies with adenosine deaminase and dipyridamole. *Eur. J. Pharmac.*, (in press).

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