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Inhibition of the excitatory junction potential in the guinea-pig saphenous artery by $ANAPP_3$

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Electrical stimulation of the perivascular nerves of the guinea-pig saphenous artery elicited excitatory junction potentials (e.j.ps) in the smooth muscle cells. These e.j.ps were inhibited by arylazido aminopropionyl adenosine triphosphate ($ANAPP_3$), a specific antagonist for the P_2-purinoceptor, suggesting that ATP may be the neurotransmitter.

Introduction Stimulation of the perivascular nerves in blood vessels elicited e.j.ps in the vascular smooth muscle cells. One peculiar feature of the e.j.ps is their resistance to blockade by α-adrenoceptor antagonists (Holman & Surprenant, 1980; Cheung, 1982). Hirst & Neild (1980) suggested that e.j.p. may be mediated by a new type of adrenoceptor, the γ-receptor. Unfortunately, this hypothesis could not be directly tested due to the lack of specific γ-adrenoceptor agonists and antagonists. Recently, ATP has been proposed as the transmitter since the e.j.p. and part of the neurally mediated vasoconstriction could be inhibited by desensitization with α, β-methylene-ATP, an ATP analogue (Sneddon & Burnstock, 1984; Ishikawa, 1985). However, direct demonstration of inhibition of the neural responses by a specific purinoceptor antagonist has not been performed. In the present study, we showed that $ANAPP_3$ (arylazido aminopropionyl adenosine triphosphate), a specific photoaffinity label for the P_2-purinoceptor which acts as an irreversible antagonist after photolysing (Hogaboom *et al.*, 1980), inhibited the e.j.p. in the guinea-pig saphenous artery.

Methods The experimental set-up and procedures were similar to those previously described (Cheung, 1984; 1985). Ring segments of saphenous artery from male guinea-pigs (Charles River) weighing about 300 g were suspended by two fine tungsten wires through the lumen. One wire was connected to a Narco 60 force transducer for tension recording. The other wire and a third wire running parallel to the preparation were used as stimulating electrodes. Pulses of 0.05 ms duration generated from a Grass S48 stimulator were used. The preparation was constantly superfused with physiological solution bubbled with 95% O_2 and 5% CO_2, containing (in mM): NaCl 120, $NaHCO_3$ 25, glucose 11, KCl 5, $CaCl_2$ 2.5, NaH_2PO_4 1, $MgSO_4$ 1, propranolol 1 μM and maintained at 36°C.

Fibre-filled glass micropipettes filled with 3 M KCl and of 40–70 MΩ resistance were used for intracellular recording. An equilibration period of at least 90 min was allowed before any recordings were made. The records were displayed on a Gould OS 1420 oscilloscope and stored on a TEAC cassette recorder. Student's *t* tests were used for statistical analysis and a *P* value of less than 0.05 was considered statistically significant.

$ANAPP_3$ was introduced directly into the recording chamber in the dark and the superfusion was stopped. After an incubation period of 6 min, the tissue was irradiated by two fibre-optic lights (Dolan-Jenner model 190, 3400°K) for 30 min. The solution was maintained at 36°C and bubbled with 95% O_2 and 5% CO_2 in the recording chamber. Spectrophotometric scans showed changes in the absorption spectrum of $ANAPP_3$ after irradiation, similar to that reported by Hogaboom *et al.* (1980). $ANAPP_3$ was kindly provided by Dr J.S. Fedan of the National Institute of Occupational Safety and Health, Morgantown.

Results The saphenous artery of the guinea-pig was electrically and mechanically quiescent with a resting membrane potential of -72.0 ± 0.7 mV (mean ± s.e., $n-5$). Stimulation of the perivascular nerves elicited e.j.ps (Figure 1a). The first e.j.p. of a train was always larger than the subsequent e.j.ps. When a threshold of -45.1 ± 0.9 mV was reached, an action potential was generated, triggering a fast phasic contraction (Figure 1b). After treatment with $ANAPP_3$ (1×10^{-4} M), the membrane potential did not change significantly (-71.3 ± 0.6 mV). However, the amplitude of the e.j.ps was significantly reduced to $54.6 \pm 4.6\%$ of control for the first e.j.p. and to $50.1 \pm 6.8\%$ for the last e.j.p. (Figure 1a). Only a small subthreshold e.j.p. was observed after $ANAPP_3$ with the same stimulus that was able to generate an action potential and a contraction under control conditions

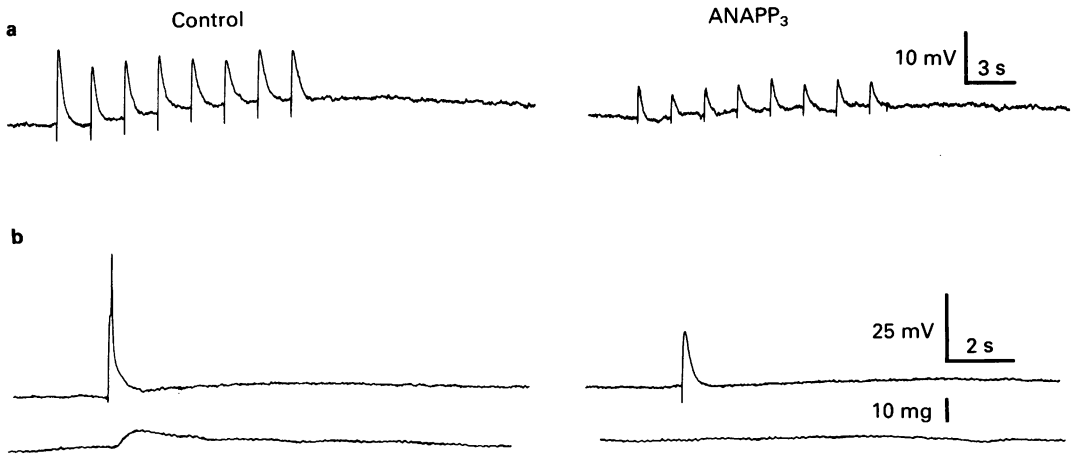

Figure 1 Effect of $ANAPP_3$ on the neural responses of the guinea-pig saphenous artery. (a) Stimulation at 40 V, 0.05 ms pulse duration and a frequency of 0.5 Hz elicited e.j.ps in the saphenous artery. After treatment with $ANAPP_3$ (1×10^{-4} M), the amplitude of the e.j.ps was significantly reduced (right panel). (b) Stimulation at 55 V and 0.05 ms elicited an e.j.p. and an action potential. A contraction was triggered by the action potential (bottom trace). After treatment with $ANAPP_3$, the e.j.p. became subthreshold. Consequently, no action potential or contraction was elicited (right panel).

(Figure 1b). When the e.j.p. was subthreshold, no contraction was elicited.

Discussion Recently there have been indications that ATP and noradrenaline may be co-transmitters in the sympathetic nervous system. In the rat tail artery, neural stimulation elicited two types of electrical response, an e.j.p. and a slow depolarization (Cheung, 1982). The slow depolarization but not the e.j.p. was inhibited by α-adrenoceptor antagonists (Cheung, 1982; 1984). Conversely, the e.j.p. but not the slow depolarization was selectively antagonized by α,β-methylene-ATP (Sneddon & Burnstock, 1984). Since α,β-methylene-ATP supposedly acts by desensitizing the P_2-purinoceptor, it was suggested that ATP may be the transmitter for the e.j.p. (Sneddon & Burnstock, 1984).

In the vas deferens, a more direct domonstration of ATP as the transmitter for the e.j.p. was achieved by use of the irreversible antagonist $ANAPP_3$, which binds to the P_2-receptor covalently after photoactivation (Hogaboom *et al.*, 1980; Sneddon *et al.*, 1982). In the present study, we demonstrated that $ANAPP_3$ could also inhibit the e.j.p. in the saphenous artery. If $ANAPP_3$ were selective only for the P_2-purinoceptor as suggested (Hogaboom *et al.*, 1980), and had no effect on transmitter release (Fedan *et al.*, 1981), our data on the saphenous artery would be consistent with ATP being the transmitter for the e.j.p. in blood vessels.

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References

CHEUNG, D.W. (1982). Two components in the cellular response of rat tail arteries to nerve stimulation. *J. Physiol.*, **328**, 461–468.

CHEUNG, D.W. (1984). Neural regulation of electrical and mechanical activities in the rat tail artery. *Pflugers Arch.*, **400**, 335–337.

CHEUNG, D.W. (1985). An electrophysiological study of α-adrenoceptor mediated excitation-contraction coupling in the smooth muscle cells of the rat saphenous vein. *Br. J. Pharmac.*, **84**, 265–271.

FEDAN, J.S., HOGABOOM, G.K., O'DONNELL, J.P., COLBY, J. & WESTFALL, D.P. (1981). Contribution by purines to the neurogenic response of the vas deferens of the guinea pig. *Eur. J. Pharmac.*, **69**, 41–53.

HIRST, G.D.S. & NEILD, T.O. (1980). Noradrenergic transmission. *Nature*, **288**, 301–302.

HOGABOOM, G.K., O'DONNELL, J.P. & FEDAN, J.S. (1980). Purinergic receptors: photoaffinity analog of adenosine triphosphate is a specific adenosine triphosphate antagonist. *Science*, **208,** 1273–1276.

HOLMAN, M.E. & SURPRENANT, A.M. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71,** 651–661.

ISHIKAWA, S. (1985). Actions of ATP and α,β-methylene ATP on neuromuscular transmission and smooth muscle membrane of the rabbit and guinea-pig mesenteric arteries. *Br. J. Pharmac.*, **86,** 777–787.

SNEDDON P. & BURNSTOCK, G. (1984). ATP as a co-transmitter in rat tail artery. *Eur. J. Pharmac.*, **106,** 149–152.

SNEDDON, P., WESTFALL, D.P. & FEDAN, J.S. (1982). Co-transmitters in the motor nerves of the guinea-pig vas deferens: electrophysiological evidence. *Science*, **218,** 693–695.

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