Br. J. Pharmac. (1986), **88**, 6–8

The effect of α,β-methylene ATP on the depolarization evoked by noradrenaline (γ-adrenoceptor response) and ATP in the immature rat basilar artery

N.G. Byrne & W.A. Large

Department of Pharmacology and Clinical Pharmacology, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE

Depolarizations evoked by noradrenaline that were resistant to α- and β-adrenoceptor antagonists were recorded in the rat basilar artery. These γ-adrenoceptor-mediated responses and the depolarizations to adenosine triphosphate (ATP) were blocked by pretreatment of the tissue with α,β-methylene ATP. These data are discussed with respect to the selectivity of α,β-methylene ATP.

Introduction It is well known that stimulation of motor sympathetic nerves to many tissues, including the vas deferens and many systemic arteries, elicits an excitatory junction potential (e.j.p.) that is resistant to α-adrenoceptor antagonists. At present there are two hypotheses to explain this observation. First, it has been suggested that the e.j.p. results from the action of noradrenaline on a specialized junctional receptor termed the γ-adrenoceptor (Hirst *et al.*, 1982). The second explanation is that the phentolamine-resistant e.j.p. results from the action of adenosine triphosphate (ATP) on a purinoceptor (e.g. Sneddon & Westfall, 1984). Recently persuasive evidence for the purinergic hypothesis has derived from experiments with the relative stable ATP analogue, α,β-methylene ATP. It has been demonstrated in the guinea-pig vas deferens that the contractile responses to ATP are reduced or abolished by pretreatment with α,β-methylene ATP due to a desensitizing mechanism. In contrast, the responses to noradrenaline and carbachol are not affected by α,β-methylene ATP (Meldrum & Burnstock, 1983). Also in the guinea-pig vas deferens α,β-methylene ATP reduced the e.j.ps and the depolarization to ATP, whereas the noradrenaline-induced depolarization was not altered (Sneddon & Burnstock, 1984). These data show that α,β-methylene ATP desensitizes the purinoceptor without affecting α-receptor responses. However, in the context of the present controversy, the hypothesis of Hirst and his colleagues is not that the e.j.p. is mediated by an α-receptor but rather by a γ-adrenoceptor. Thus it seemed worthwhile testing the selectivity of α,β-methylene ATP against responses mediated by γ-adrenoceptor activation.

Methods Depolarizations to noradrenaline and ATP were recorded from the isolated basilar artery of the rat as described previously (Byrne *et al.*, 1985). Briefly, the basilar artery was dissected from rats (aged 2–6 days) and superfused at room temperature (21–24°C) with Krebs solution containing (mM): NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. Experiments were carried out in the presence of phentolamine (10^{-6} M) and propranolol (10^{-7} M) to block any α- or β-receptor mediated responses (Byrne *et al.*, 1985). This solution was bubbled with 5% CO_2 and 95% O_2. Membrane potentials were recorded with intracellular microelectrodes filled with KCl 0.5 M with resistances of 100–150 MΩ. Noradrenaline and ATP were added to the preparation by micro-injection into the recording chamber as described previously (Byrne *et al.*, 1985). α,β-methylene ATP was added to the superfusion solution.

Solutions of noradrenaline contained ethylenediaminetetracetic acid (EDTA, 10 μg ml^{-1}) and ascorbic acid (50 μg ml^{-1}). Drugs were obtained from Sigma, except for (±)-propranolol HCl (ICI Ltd) and phentolamine mesylate (Ciba-Geigy).

Results In the presence of phentolamine (10^{-6} M) and (±)-propranolol (10^{-7} M) the cells were electrically quiescent and had a mean resting membrane potential (E_M) of -47.1 ± 1.6 mV (mean ± s.e.mean, $n = 16$). Noradrenaline (0.05–3 μmol and ATP (2–500 nmol) each evoked rapid depolarizations which could reach 19 and 22 mV, respectively. The responses to both agonists were similar in time course and peaked within 0.4 to 4s (Figure 1a and b). A small, slow phase of depolarization which peaked within 30–40 s sometimes followed the fast response to ATP (Figure 1b). Perfusion of the tissue with Krebs solution containing α,β-methylene ATP (10^{-6} M) depolarized the cells and this was followed by a partial or complete repolarization over a 30–40 min period in

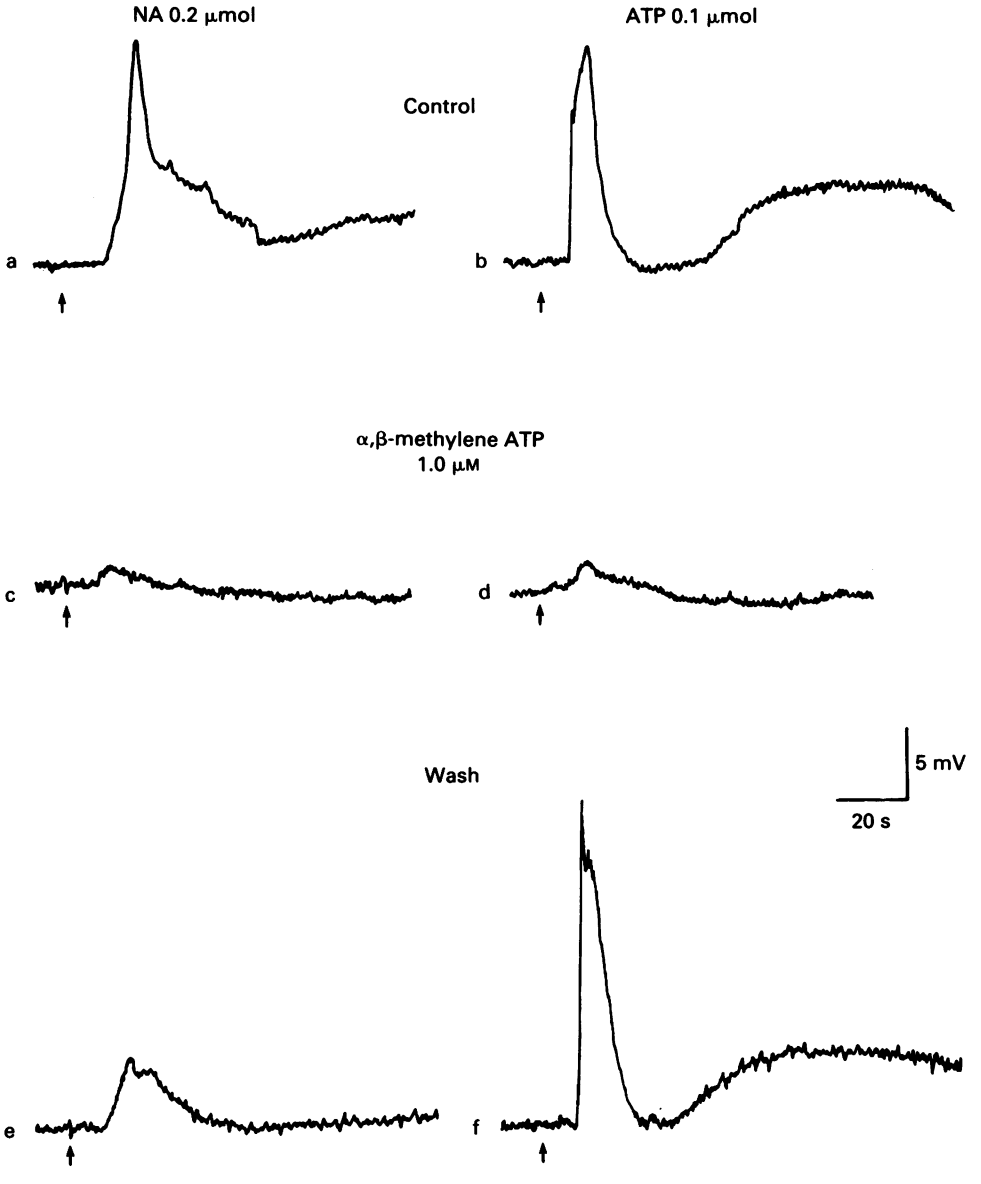

Figure 1 Effect of α,β-methylene ATP on the depolarizations evoked by the injection of noradrenaline (NA) and adenosine triphosphate (ATP) in the immature rat (6 day old) basilar artery. (a–b) were recorded from the same cell ($E_M = -43$mV); (c–d) in the presence of α,β-methylene ATP (10^{-6} M for 35 min) and (e–f) after 10 min washing out α,β-methylene ATP. (c–f) were recorded from the same cell ($E_M = -40$ mV). Phentolamine (10^{-6} M) and propranolol (10^{-7} M) were present throughout.

the continued presence of the drug. After the recovery of the membrane potential, the depolarizations to ATP and noradrenaline were reduced greatly (Figure 1c and d) or sometimes converted to a hyperpolarization. The blocking effect of α,β-methylene ATP on the rsponses to ATP and noradrenaline persisted initially (2–5 min) following removal of α,β-methylene ATP from the bath. The depolarization to noradrenaline

recovered partially and that to ATP recovered completely after washing out the α,β-methylene ATP for 30–40 min (Figure 1e and f). Similar results were obtained from arteries taken from adult animals (6 weeks old).

Discussion The present study shows clearly that the depolarizations evoked by noradrenaline and ATP were reduced or abolished in the presence of α,β-methylene ATP. One explanation is that in the presence of α,β-methylene ATP the membrane conductance is increased even though the resting membrane potential has almost returned to control values (Ishikawa, 1985). An increase in membrane conductance would attentuate a drug-induced depolarization, which itself resulted from an increase in conductance. Other explanations for the blocking effect of α,β-methylene ATP include the following. It is possible that the depolarizations to ATP and noradrenaline are mediated by different pharmacological receptors but utilize the same ionic channel. In this situation desensitization of one receptor type may prevent the opening of the channel by activation of the other receptor and thus lead to cross-desensitization. Secondly, in the rat basilar artery, noradrenaline and ATP may stimulate the same receptor which is desensitized by α,β-methylene ATP. Since ATP is more potent than noradrenaline, pharmacological convention would militate that the receptor be termed a purinoceptor. The present data do not help to identify the transmitter responsible for the phentolamine-resistant e.j.p. in the rat basilar artery. However, the evidence indicates that α,β-methylene ATP might abolish the e.j.p. whether it was produced by the action of ATP on a purinoceptor or by noradrenaline on a γ-adrenoceptor (or even purinoceptor). There are no α- or β-adrenoceptors in the adult rat basilar artery so if the e.j.p. were produced by ATP, there would be no obvious function for the noradrenaline present in the neurones (as indicated by fluorescence histochemistry, Hirst *et al.*, 1982).

This work was supported by the M.R.C.

References

BYRNE, N.G., HIRST, G.D.S. & LARGE, W.A. (1985). Electrophysiological analysis of the nature of adrenoceptors in the rat basilar artery during development. *Br. J. Pharmac.*, **86**, 217–227.

HIRST, G.D.S., NEILD, T.O. & SILVERBERG, G.D. (1982). Noradrenaline receptors on the rat basilar artery. *J. Physiol.*, **328**, 351–360.

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.

MELDRUM, L.A. & BURNSTOCK, G. (1983). Evidence that ATP acts as a cotransmitter with noradrenaline in sympathetic nerves supplying the guinea-pig vas deferens. *Eur. J. Pharmac.*, **92**, 161–163.

SNEDDON, P. & BURNSTOCK, G. (1984). Inhibition of excitatory junction potentials in guinea-pig vas deferens by α,β-methylene-ATP: further evidence for ATP and noradrenaline as cotransmitters. *Eur. J. Pharmac.*, **100**, 85–90.

SNEDDON, P. & WESTFALL, D.P. (1984). Pharmacological evidence that adenosine triphosphate and noradrenaline are co-transmitters in the guinea-pig vas deferens. *J. Physiol.*, **347**, 561–580.

(Received December 30, 1985.
Accepted January 29, 1986.)