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Actions of α, β-methylene ATP and 6-hydroxydopamine on sympathetic neurotransmission in the vas deferens of the guinea-pig, rat and mouse: support for co-transmission

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1 α-Adrenoceptor antagonists (prazosin or phentolamine) reduced the contractile response to field stimulation of the isolated vasa deferentia of guinea-pig, rat and mouse. α, β-Methylene ATP (α, β-MeATP) reduced that portion of the contraction which was resistant to α-adrenoceptor blockade.

2 α, β-MeATP (1–800 μM) did not affect action potential conduction in the guinea-pig vas deferens nerves, and (up to 10 μM) did not reduce the stimulation-evoked overflow of [^{3}H]-noradrenaline from this tissue.

3 Spontaneous excitatory junction potentials (s.e. j.ps) in the majority of cells of guinea-pig, rat, and mouse vasa were abolished by α, β-MeATP (0.1–10 μM). In a small number of cells s.e.j.ps were resistant to the actions of α, β-MeATP (10 μM).

4 Excitatory junction potentials (e.j.ps) in the majority of cells in vasa of all species studied were abolished by α, β-MeATP (1–10 μM). E.j.ps elicited in some 'resistant' cells demonstrated marked facilitation characteristics.

5 It is concluded that α, β-MeATP inhibits s.e.j.ps and e.j.ps by a postjunctional action.

6 In all species pretreatment of animals with 6-hydroxydopamine produced a marked reduction in noradrenaline (NA) content (as determined by fluorescence histochemistry) and abolished e.j.ps, findings which suggest that e.j.ps originated from sympathetic nerves.

7 The results support the hypothesis that NA and ATP are co-transmitters in the sympathetic nerves of rodent vasa.

Introduction

The contractile response of the rodent vas deferens to field stimulation is biphasic, comprising a rapid phasic contraction followed by a slower tonic contraction. The first phase of the response seems to result from the summation of e.j.ps (Blakeley *et al.*, 1981). There is considerable doubt as to whether the e.j.ps and the initial contractile response are mediated by noradrenaline (NA). For example, they are neither mimicked by NA (Sneddon & Burnstock, 1984) nor blocked by α_1-adrenoceptor antagonists. Furthermore, these responses are not reduced by reserpine or enhanced by cocaine. (Ambache & Zar, 1971; Swedin, 1971; Sneddon & Westfall, 1984). The second contractile phase, however, is mediated by NA. It is mimicked by exogenous NA, blocked by α_1-adrenoceptor antagonists, reduced by reserpine, and enhanced by cocaine (Sneddon & Westfall, 1984). This phase is accompanied either by no detectable membrane potential change or by a small depolarization.

Several hypotheses have been put forward to explain these findings, namely that (1) e.j.ps and the first phase of contraction are not mediated by NA but by a second transmitter; (2) the failure of α_1-adrenoceptor antagonists to inhibit the e.j.ps and first phase of contraction may be due to failure of the antagonists to

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gain access to junctional α-adrenoceptors (Furness, 1974); (3) NA mediates the e.j.p. and initial contractile response but does so via an adrenoceptor pharmacologically different from the classical α-adrenoceptor, the γ-receptor, which may be present in a number of sympathetically innervated tissues (Hirst & Neild, 1980; 1984). Sneddon & Westfall (1984) have proposed that, in the guinea-pig vas deferens, the e.j.ps and first phase of the contractile response are mediated by ATP acting on P_2-purinoceptors. The fact that both the e.j.p. and the first phase of contraction were abolished by the 'ATP antagonists' α, β-methylene ATP (α, β-MeATP) and arylazido, aminopropionyl-ATP ($ANAPP_3$) led to the proposal that two transmitters are released from sympathetic nerves, ATP and NA (see Sneddon & Westfall, 1984; Sneddon & Burnstock, 1984) and that co-transmission may be a phenomenon common to many sympathetic nerves.

The purpose of the present study was to investigate further the mechanism of sympathetic neurotransmission. In particular, the effects of α, β-MeATP on action potential conduction, [^{3}H]-NA release and on e.j.ps were investigated to determine whether there was a prejunctional component to its action in blocking e.j.ps and contractions in rodent vasa deferentia.

Methods

Vasa deferentia from guinea-pigs (Duncan Hartley, 350–600 g), rats (Wistar, 250–350 g) and mice (Tucks Original or Porton, 30–50 g) were used.

Animals were killed by cervical dislocation and bled. In the guinea-pig the right vas deferens, the associated hypogastric plexus and the hypogastric nerve trunk up to a point 3 cm away from the vas deferens were removed. The vas deferens only was removed from mice and rats.

Electrophysiological experiments

Individual preparations were transferred to a 3 ml Perspex chamber, the base of which was covered by a thin layer of Sylgard (Dow Corning) and the vas deferens immobilized by gently stretching it longitudinally and pinning the connective tissue with fine stainless steel pins. The organ bath was perfused continuously at a rate of 2 ml min^{-1} with Krebs solution of the following composition (mM): NaCl 118.4, $NaHCO_3$ 25.0, NaH_2PO_4 1.13, $CaCl_2$ 1.8, KCl 4.7, $MgCl_2$ 1.3 and glucose 11.0 which was bubbled continuously with a mixture of 95% O_2 and 5% CO_2 to pH 7.4 and maintained at 36–37°C. Preparations could be stimulated preganglionically (guinea-pig only), by stimulating the hypogastric nerve at a point 3 cm away from the vas, or postganglionically by stimulating the vas deferens at its prostatic end, via Ag/AgCl ring electrodes (diameter 2 mm; separation 1.5mm).

Action potentials were recorded from small bundles of vas deferens nerves by use of a suction electrode as previously described (Cunnane & Stjärne, 1984). E.j.ps and s.e.j.ps were recorded intracellularly with glass micro-electrodes filled with 5 M potassium acetate (resistances 30–90 MΩ) essentially as previously described (Cunnane & Stjärne, 1984).

[^{3}H]-noradrenaline overflow experiments

Guinea-pig vasa deferentia were incubated at 37°C in 0.5 ml of Krebs solution that contained 12 μCi of [^{3}H]-NA for 30 min. After careful rinsing, the tissues were set up in a jacketed organ bath under a resting tension of approximately 1 g, and superfused at a rate of 1 ml min^{-1} at 37°C with Krebs solution which contained normetanephrine (10 μM), desipramine (0.6 μM), atropine (2.6 μM) and ascorbic acid (114 μM). Under these conditions, it has previously been shown that over 90% of the tritium present in the tissue after superfusion with Krebs solution is intact [^{3}H]-NA (Alberts *et al.*, 1981). The superfusate was collected in 2-min fractions. Tissues were stimulated at the prostatic end with Ag/AgCl ring electrodes (diameter 2 mm; separation 1.5 mm) (60 pulses, 0.5 ms pulse width, 60 V, 1–20 Hz). Contraction following field stimulation was measured with a Grass FT03 isometric transducer coupled to a Linseis pen recorder.

After 60 min perfusion, two trains of test stimuli (60 pulses at 10 Hz) were delivered to the tissues, which were then perfused for a further 30 min. Tissues were stimulated ten times in each experiment, at 12-min intervals. After the 4th control stimulus, the Krebs solution perfusing the tissue was replaced with Krebs solution containing α, β-MeATP at the required concentration. At the end of the experiment, the tissue was solubilised, and the radioactivity in the solubilisate and the superfusate samples was determined; 1 ml aliquots of sample were placed in 10 ml of scintillation fluid. The amount of radioactivity present in the samples was measured in a Packard liquid scintillation counter and was expressed as disintegrations per minute (d.p.m.) Corrections for counting efficiency were made by an automatic internal standard.

Pretreatment of animals with 6-hydroxydopamine

Rats and mice were pretreated as follows: the animals were anaesthetized with a mixture of 50% O_2 and 50% N_2O containing 2–4% halothane. Their tails were rubbed, close to the base with xylene, causing dilatation of the tail vein. The 6-hydroxydopamine (6-OHDA) was injected into the tail vein in volumes of less than 0.3 ml. On day 1, the rats and mice were injected (i.v.) with 6-OHDA 100 mg kg^{-1}, on day 2 the

dose of 6-OHDA was increased to 250 mg kg^{-1}. Animals were killed on day 3.

Guinea-pigs were pretreated with 6-OHDA according to the following protocol: the animals were anaesthetized with chloroform and then injected (i.p.) with 6-OHDA 150 mg kg^{-1} on day 1 and 250 mg kg^{-1} on day 2; the animals were killed on day 3.

Histochemistry

The extent of the degeneration of sympathetic nerves was assessed histochemically by a modification of the Falck-Hillarp technique (Gillespie & Kirpekar, 1966). Sections (6 μm) were cut and mounted in a drop of liquid paraffin. The fluorescing specimens were viewed and photographed on a Carl Zeiss ACM photomicroscope, equipped with a IV Fl epi-fluorescence system. The light source was an Osram HB50 mercury lamp; the filters used were; exciter-interference BP405/8; barrier-LP418; dichromatic beam splitter FT420. Photomicrographs were taken on Ektachrome ISO 160 film with a MC63A photomicrographic camera.

Drugs

Atropine sulphate (Sigma), desipramine (Sigma), α, β-methyleneadenosine 5'-triphosphate (Sigma), normetanephrine HCl (Sigma) phentolamine mesylate (Ciba laboratories), prazosin HCl (Pfizer), were made up as stock solutions in distilled water and serially diluted in Krebs solution as required. 6-Hydroxydopamine HBr (Sigma) was made up in 1 mg ml^{-1} ascorbic acid which had been gassed with nitrogen for at least 15 min. The 6-OHDA was made up immediately before use and was kept under nitrogen, on ice, to prevent oxidation. The solution was sonicated to ensure complete dissolution of the drug. (−)-[7, 8-^{3}H]-noradrenaline (Amersham U.K.) was reconstituted in distilled water that contained ascorbic acid 1 mg ml.$^{-1}$

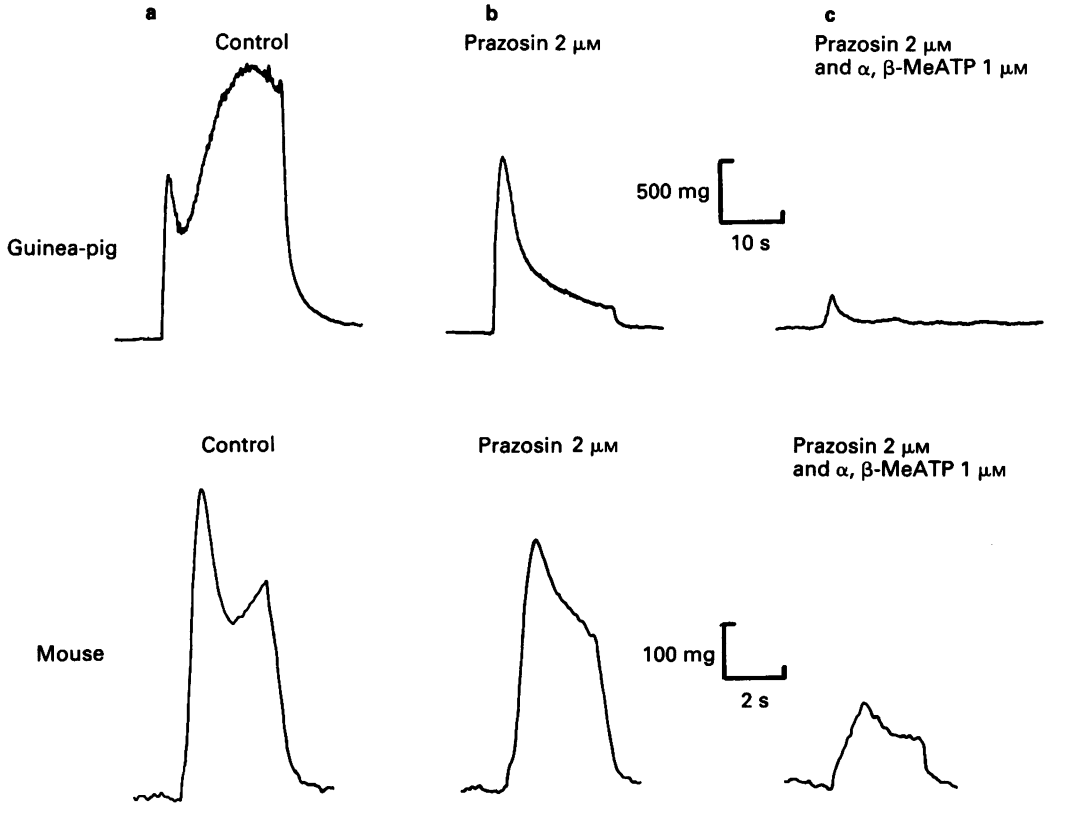

Figure 1 (a) Control contractile responses of the guinea-pig and mouse vasa deferentia to electrical field stimulation. (b) Contractile responses in the presence of prazosin 2 μM. (c) Contractile responses in the presence of α, β-methylene ATP (α, β-MeATP) 1 μM and prazosin 2 μM (0.5 ms pulses at 90 V; guinea-pig 200 pulses at 10 Hz, mouse 60 pulses at 20 Hz).

Mechanical responses

The effects of the α-adrenoceptor antagonists (phentolamine or prazosin), and α-adrenoceptor antagonists in combination with α, β-MeATP, on the electrically-evoked mechanical responses of three rodent vasa are shown in Figures 1 and 2. A combination of these agents, administered in either order, greatly reduced the contractile responses of guinea-pig, mouse and rat vasa evoked by field stimulation.

Effects of α, β-methylene ATP on nerve action potential conduction

In 7 experiments (4 postganglionic, 3 preganglionic), propagated action potentials were recorded from small axon bundles at the distal end of the guinea-pig vas deferens, which were exposed to increasing concentrations of α, β-MeATP (1–100 μM) by the cumulative addition of the drug to the Krebs solution. Action potentials evoked by trains of preganglionic stimuli at 0.5 Hz (10 μs, 3–20 V) before and after 15 min perfusion with 10 μM and 100 μM α, β-MeATP are shown in Figure 3. In one additional experiment, exposure to α, β-MeATP at a concentration of 800 μM did not affect action potential conduction in the vas deferens nerves.

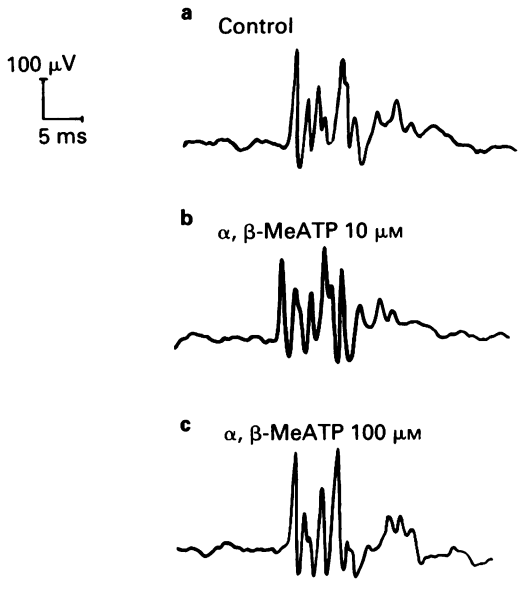

Figure 3 Action potentials recorded from a small axon bundle in the guinea-pig vas deferens following preganglionic stimulation of the hypogastric nerve with single pulses. (a) Control; (b) after perfusion with α, β-methylene ATP (α, β-MeATP) 10 μM for 15 min; (c) 15 min after α, β-MeATP 100 μM. Stimulation parameters: 10 μs, 3 V.

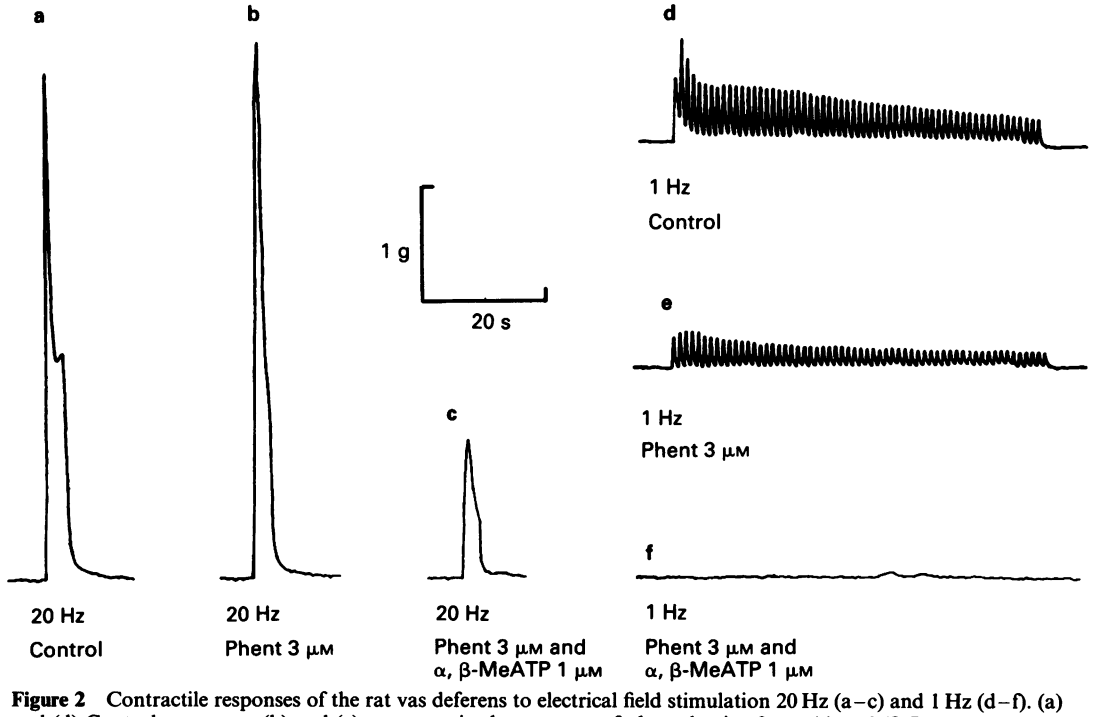

Figure 2 Contractile responses of the rat vas deferens to electrical field stimulation 20 Hz (a–c) and 1 Hz (d–f). (a) and (d) Control responses; (b) and (e) responses in the presence of phentolamine 3 μM. (c) and (f) Responses in the presence of α, β-methylene ATP (α, β-MeATP) 1 μM and phentolamine 3 μM (0.5 ms pulse width at 90 V, 60 pulses).

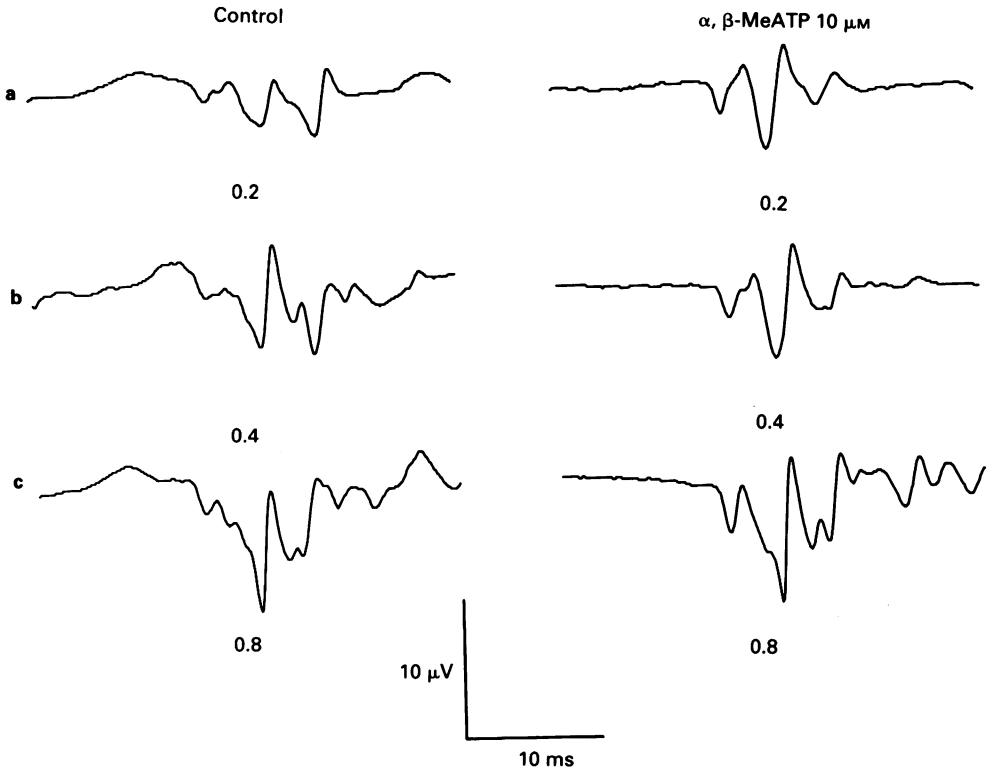

Figure 4 Action potentials in vas deferens nerve following field stimulation of the prostatic end of the vas deferens, before and in the presence of α, β-methylene ATP (α, β-MeATP) 10 μM. Each record is an average of 16 volleys at each stimulus intensity: (a) 0.2 ms (b) 0.4 ms and (c) 0.8 ms p.w., 15 V.

It was important to determine that α, β-MeATP had no effect on the initiation of action potentials in the vas deferens nerves. For strict comparison the stimulus parameters employed were similar to those used to evoke e.j.ps. The effects of α, β-MeATP (10 μM) on action potentials evoked with pulse widths of 0.2–0.8 ms following postganglionic stimulation of the vas deferens nerves are shown in Figure 4.

Effects of α, β-methylene ATP on transmitter release

Release of transmitter was assessed in two ways, (1) collection of [^{3}H]-NA; (2) measurement of s.e.j.ps and e.j.ps.

Effects of α, β-methylene ATP on [^{3}H]-noradrenaline overflow α, β-MeATP (0.1–10 μM) had no significant effect on the overflow of [^{3}H]-NA evoked by field stimulation of the guinea-pig vas deferens with trains of 60 pulses (0.5 ms, 60 V) at 1, 5, 10 and 20 Hz. The fractional release per applied stimulus in control tissues, and after 20–30 min exposure to α, β-MeATP 1 μM was not significantly different (Table 1). It is noteworthy, however, that in some experiments, α, β-MeATP caused an initial, transient rise in the background tritium efflux. In two additional experiments, 10 μM α, β-MeATP produced a large rise in the background efflux which continued for the duration of the experiment.

Effects of α, β-methylene ATP on s.e.j.ps S.e.j.ps were recorded from all control smooth muscle cells impaled. After 10 min perfusion with 0.1–10 μM α, β-MeATP, spontaneous activity in the majority of cells in guinea-pig, rat and mouse vasa deferentia was abolished (Figure 5b). In several cells, however, even in the presence of 10 μM α, β-MeATP, small s.e.j.ps were detected. In these cells there was no apparent change in s.e.j.p. frequency although this was not rigorously investigated. 6-OHDA pretreatment abolished s.e.j.ps in guinea-pig, rat and mouse vasa deferentia (Figure 5c), suggesting that they originated from sympathetic nerves.

Table 1 Fractional release per stimulus of [^{3}H]-noradrenaline from the guinea-pig vas deferens, evoked by field stimulation with trains of 60 pulses (0.5 ms, 60 V) at the frequencies indicated.

Stimulation frequency (Hz)	*Fractional release per applied stimulus*
Control	
1	9.5×10^{-5} ($\pm$ 2.25 $\times 10^{-5}$).
5	1.03×10^{-4} ($\pm$ 0.266 $\times 10^{-4}$).
10	1.14×10^{-4} ($\pm$ 0.248 $\times 10^{-4}$).
20	1.41×10^{-4} ($\pm$ 0.323 $\times 10^{-4}$).
α, β-MeATP 1 μM	
1	9.4×10^{-5} ($\pm$ 2.59 $\times 10^{-5}$).
5	9.97×10^{-5} ($\pm$ 2.94 $\times 10^{-4}$).
10	1.13×10^{-4} ($\pm$ 0.252 $\times 10^{-4}$).
20	1.41×10^{-4} ($\pm$ 0.318 $\times 10^{-4}$).

Values are mean $\pm$ s.e.mean; $n = 3$ for each value; $P > 0.05$.

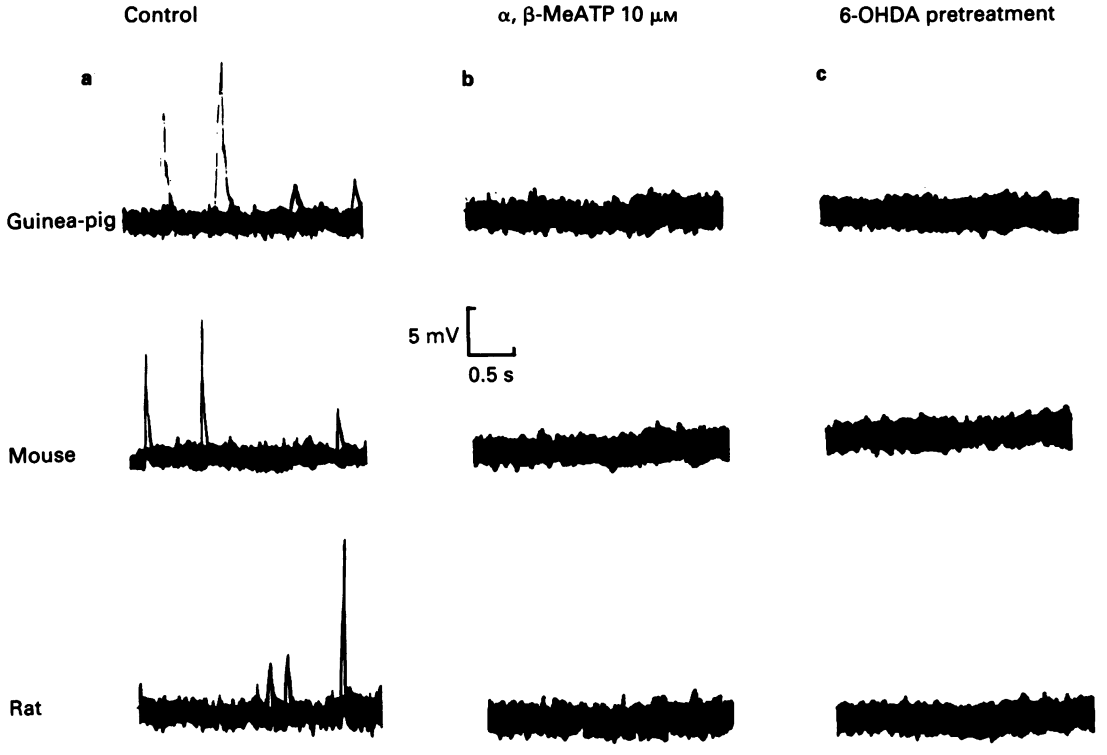

Figure 5 S.e.j.ps recorded from the guinea-pig, mouse and rat vasa deferentia. (a) Control tissues; (b) absence of s.e.j.ps after 15 min perfusion with α, β-methylene ATP (α, β-MeATP) 10 μM; (c) absence of s.e.j.ps in vasa from animals pretreated with 6-hydroxydopamine (6-OHDA). Records are photographs of at least ten superimposed sweeps of the storage oscilloscope.

Effects of α, β-methylene ATP on e.j.ps In preliminary experiments, it was shown that α, β-MeATP produced a concentration dependent inhibition (10 nM – 10 μM) of e.j.ps in the guinea-pig vas deferens at a time when the resting membrane potential was not significantly different from controls, confirming the results of Sneddon & Burnstock (1984). In the majority of experiments in the present study, therefore, concentrations of α, β-MeATP which were known to block the e.j.p in the guinea-pig vas (1–10 μM) were chosen for comparison between species.

E.j.ps were evoked by single pulses or by trains of stimuli at 0.5 to 10 Hz. In the rat and mouse vas deferens, the e.j.p. evoked by a single electrical stimulus normally initiated a muscle action potential and contraction. In the guinea-pig vas deferens the initiation of a muscle 'spike' in response to a single pulse was not observed. The effects of 10 μM α, β-MeATP on e.j.ps evoked by single pulses in the rat, mouse and guinea-pig vas deferens are shown in Figure 6. After 10 min perfusion with 10 μM α, β-MeATP, stimulus parameters identical to or much greater than those used in control cells did not evoke an e.j.p. (Figure 6b). Thus, in all three species the e.j.p. and 'spike' potential were usually abolished.

On reaching the bath, 1–10 μM α,β-MeATP produced a transient depolarization of the smooth muscle cell membrane. In the guinea-pig, the membrane repolarized to its original value (64.3 ± 6.8 mV, $n = 202$) (mean ± s.d.) after 7–10 min perfusion with 10 μM α, β-MeATP; in the mouse, the time taken for repolarization varied from preparation to preparation

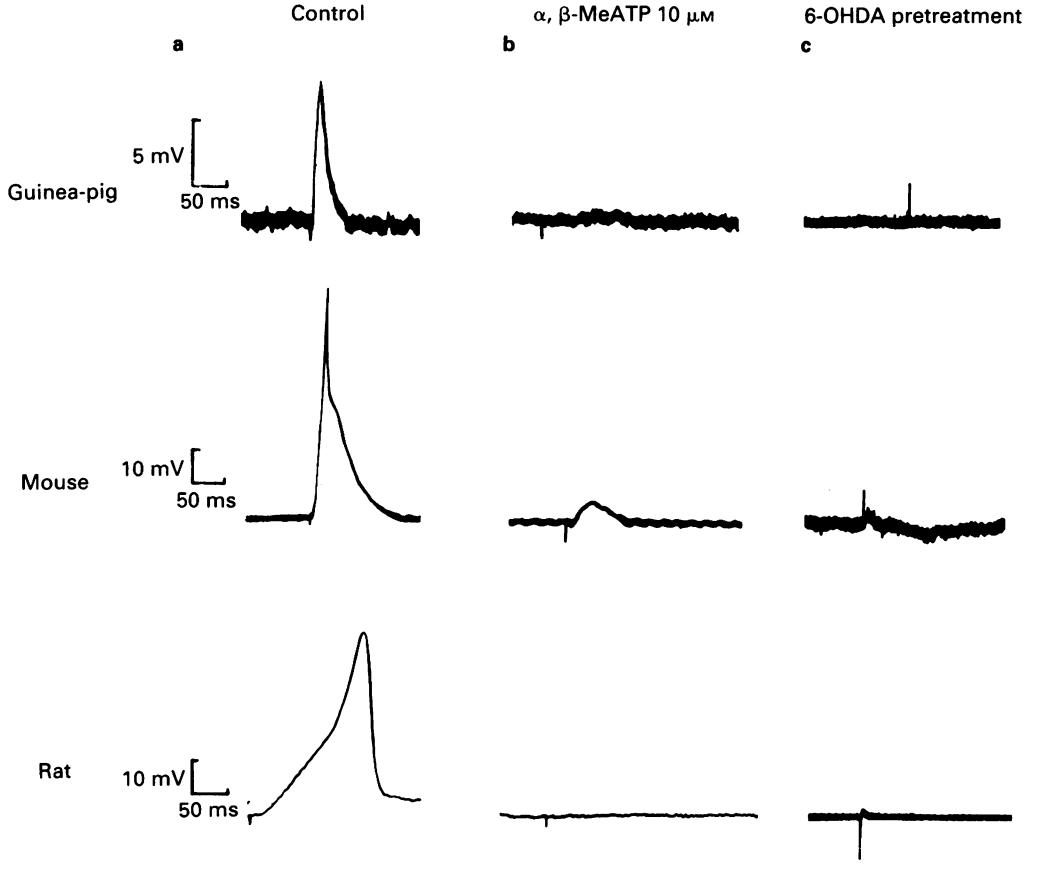

Figure 6 (a) Electrical activity recorded from the guinea-pig, mouse and rat vasa deferentia evoked by single pulses (indicated by stimulus artifacts). (Pulse parameters; guinea-pig 90 μs, 15 V; mouse 90 μs, 50 V; rat 100 μs, 10 V). (b) Responses evoked after 10 min perfusion with α, β-methylene ATP (α, β-MeATP) 10 μM (pulse parameters as for controls). (c) Failure to evoke e.j.ps in vasa deferentia from animals pretreated with 6-hydroxydopamine (6-OHDA) (pulse parameters similar to or greater than those used in the control tissues).

but, in general, the membrane potential returned to its original value after 5–15 min. During the period of depolarization, it was difficult to maintain penetration of individual smooth muscle cells. Figure 7b and c shows the responses of smooth muscle cells of guinea-pig vas deferens to trains of pulses (identical stimulus parameters to control cells) after 7 and 28 min perfusion (respectively) with 10 μM α, β-MeATP. In the majority of cells, e.j.ps could not be evoked.

'Resistant cells'

In only a small number of cells of the guinea-pig vas deferens (7 cells, 3 preparations) were e.j.ps resistant to the actions of α, β-MeATP. It was possible to evoke e.j.ps of a similar magnitude to those in control cells (see Figure 7d and e). This effect was not due to α, β-MeATP having had insufficient time to act, as similar cells were found (in the same preparations) after 30 min perfusion with the high concentration of 10 μM α, β-MeATP (Figure 7e).

In the guinea-pig vas, the mean amplitude of fully facilitated e.j.ps at 1 Hz in control cells was 9.9 mV ± 0.9 (mean ± s.e.mean, $n = 110$). After 10 min perfusion with 10 μM α, β-MeATP, mean e.j.p. amplitude was markedly reduced to 0.2 mV ± 0.1 ($P < 0.001$, $n = 42$). In the resistant cells, α, β-MeATP failed to produce a significant reduction in the mean e.j.p. amplitude ($P > 0.05$), the mean e.j.p. amplitude (7.7 ± 0.9 mV, $n = 7$) was similar to that of the control.

Likewise, in the majority of cells in the mouse vas deferens, α, β-MeATP produced a statistically significant ($P < 0.001$) decrease in the mean e.j.p.

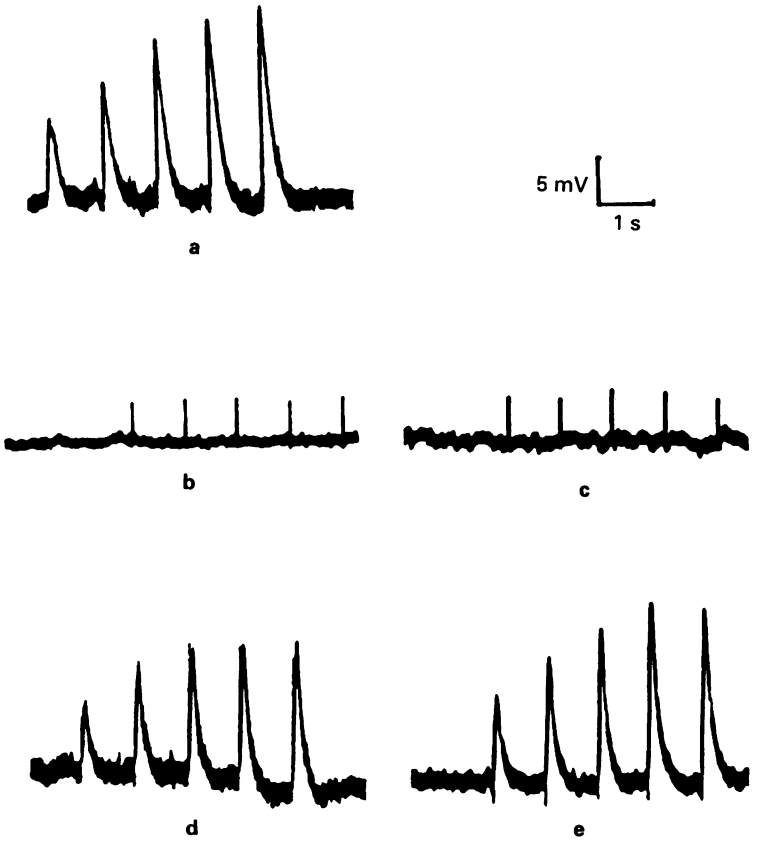

Figure 7 Effect of α, β-methylene ATP (α, β-MeATP) 10 μM on e.j.ps evoked in response to a train of pulses (5 pulses at 1 Hz, 5 μs, 15 V) in the guinea-pig vas deferens. (a) Control; (b) and (c) the lack of response of different cells to a train of pulses (same parameters as before) after 7 and 28 min (respectively) perfusion with α, β-MeATP; (d) and (e) the responses of different 'resistant' cells to the same stimulation parameters, after 8 and 30 min perfusion with the drug.

amplitude. However, the mean e.j.p. amplitude of the resistant cells was significantly less than that of the control (18.2 mV cf. 41.3 mV control, $P < 0.01$). Figure 8 shows the mean results for all resistant cells recorded in the mouse vas deferens (5 cells) plotted against the mean amplitude of a series of control e.j.ps, and a series of e.j.ps readily blocked by α, β-MeATP in a single preparation (10 stimuli at 0.5 Hz, $n = 7$). It is important to emphasise that resistant e.j.ps are the exception rather than the rule and that in the majority of cells, e.j.ps were readily abolished.

Effect of stimulation strength and paired stimuli in 'resistant' cells

In control cells in the mouse vas, e.j.ps were graded with stimulus strength and a single pulse readily evoked an e.j.p. and muscle spike. In resistant cells, however, increasing the stimulus intensity more than 10 fold had little effect on the e.j.p. elicited by a single pulse (see Figure 9 a, b, c). However, if a second test stimulus was applied, a large e.j.p. could be evoked, the magnitude of which depended on the interval between the paired pulses (Figure 9d). Figure 10 shows (in mouse vas deferens) e.j.ps evoked in response to double pulses at the frequencies indicated, before and after perfusion with 10 μM α, β-MeATP. In the control, the stimulus parameters were deliberately adjusted so

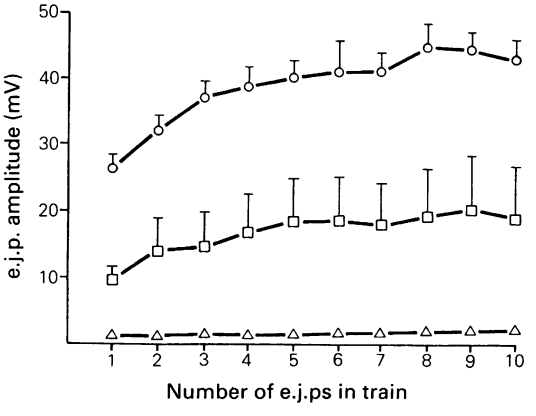

Figure 8 Effects of α, β-methylene ATP (α, β-MeATP) 10 μM on the mean amplitude of e.j.ps evoked by trains of pulses at 0.5 Hz in the mouse vas deferens. E.j.ps were evoked before ('control', $n = 13$ (○)) and after perfusion with α, β-MeATP. In most cells ($n = 7$) the e.j.ps were significantly reduced or abolished after 5–15 min perfusion with the drug ($P < 0.001$): these were said to be 'non-resistant' (△). In a few cells s.e.j.ps and e.j.ps could still be recorded, and the reduction in mean e.j.p. amplitude was less significant ($n = 5$, $P > 0.01$). The latter were designated 'resistant' cells (□).

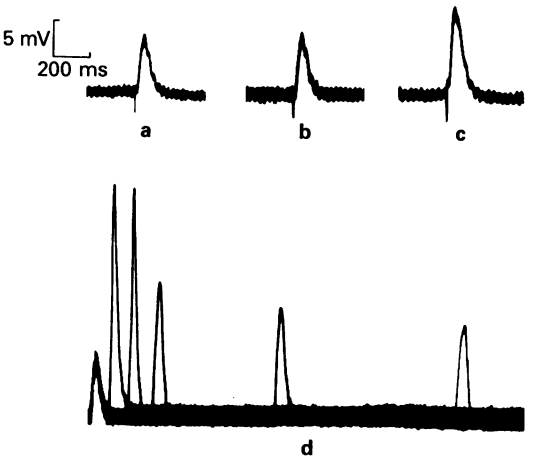

Figure 9 Effect of increasing stimulus intensity on e.j.ps evoked by single pulses in a 'resistant' cell in the mouse vas deferens: (a) 50 μs; (b) 200 μs; (c) 500 μs (15 V). Note that e.j.ps were not markedly graded with stimulus strength, unlike control cells. (d) Effects of paired pulses at 10, 5, 3, 2 and 1 Hz in the same cell (pulse width 50 μs).

that the e.j.ps elicited were small, to allow direct comparison of the 'normal' magnitude of facilitation for e.j.ps of comparable size to those recorded in the presence of 10 μM α, β-MeATP.

In control cells, the average magnitude of facilitation between the first and second pulse (expressed as amplitude 2nd e.j.p./amplitude 1st e.j.p. × 100) was 0% at 1 Hz; 29% at 5 Hz and 44% at 10 Hz; after 15 min perfusion, with 10 μM α, β-MeATP the percentage facilitation, in a 'resistant' cell, between the first and second pulse was 78% at 1 Hz; 287% at 5 Hz and 329% at 10 Hz. Thus, facilitation was apparently greater in this 'resistant' cell. Limited data preclude further analysis of this interesting observation.

Reversibility of the effects of α, β-methylene ATP

The effects of α, β-MeATP were reversible in all three species but this was dependent on the time of exposure to the drug. Prolonged exposure to high concentrations (10 μM) resulted in poor recovery of e.j.ps and s.e.j.ps in the guinea-pig vas. In the mouse, e.j.ps of a similar magnitude to those obtained in control cells could be evoked 30 min after washing with fresh Krebs solution.

Effect of 6-hydroxydopamine pretreatment

Figure 11 shows fluorescence photomicrographs of transverse sections of control and 6-OHDA-pretreated guinea-pig vas deferens. Pretreatment with

6-OHDA produced complete loss of NA from the sympathetic neurones innervating the longitudinal muscle layer of the guinea-pig, mouse and rat vas deferens (mouse and rat not shown). Characteristically, some sympathetic neurones residing in the inner, circular smooth muscle layer, especially in the epididymal region, were resistant to the effects of 6-OHDA. It is possible that the 6-OHDA-pretreatment schedule was insufficient to produce complete denervation perhaps because 6-OHDA is less able to gain access to these neurones.

Sympathetic denervation was accompanied by complete abolition of s.e.j.ps in the mouse, guinea-pig and rat vas deferens (see Figure 5), a reduced uptake of [^{3}H]-NA and abolition of electrically evoked [^{3}H]-NA release. (Allcorn & Cunnane, unpublished observations). Furthermore, in the vasa deferentia of 6-OHDA-pretreated animals, e.j.ps could not be evoked in response to a single pulse with stimulus parameters similar to or much greater than those used to elicit e.j.ps in control cells (Figure 6c). Small e.j.ps could be evoked by trains of stimuli and occasional s.e.j.ps were also recorded in the rat vas. The mean amplitudes of the fully facilitated e.j.p. in control vasa of the guinea-pig and mouse were 41 and 10 mV respectively. In vasa deferentia from 6-OHDA pretreated animals, the mean e.j.p. amplitude was less than the noise level of the recording system in all species.

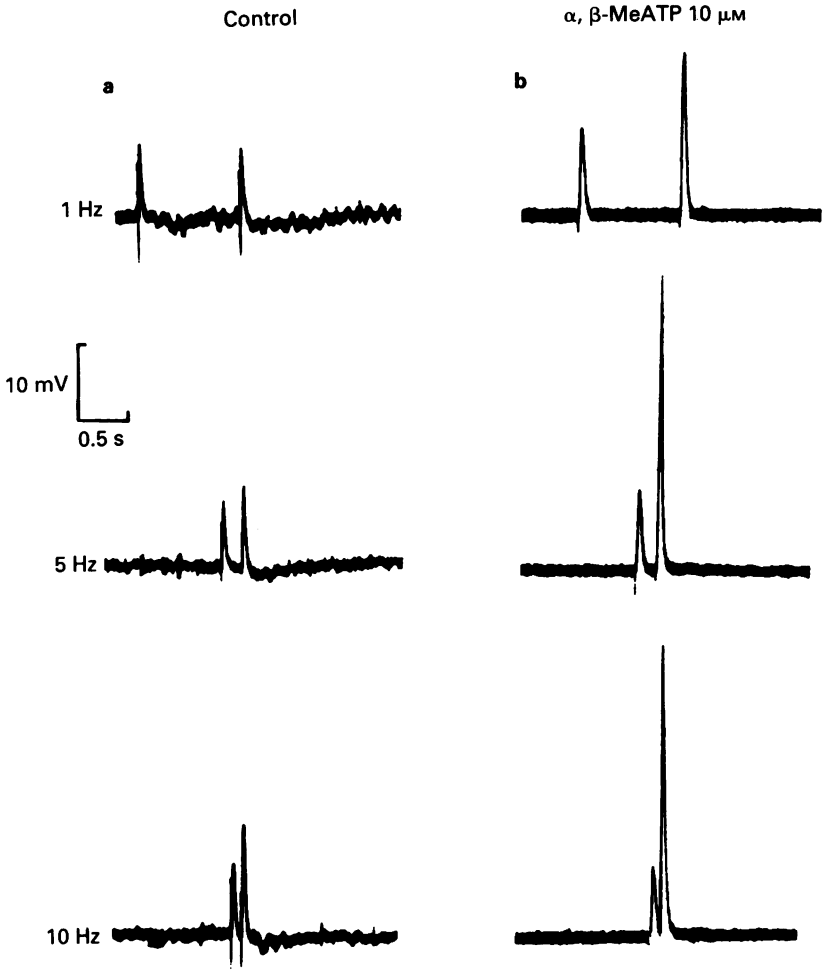

Figure 10 E.j.ps recorded from a smooth muscle cell of the mouse vas deferens to a pair of stimuli delivered at the frequencies indicated (pulse parameters 40 μs, 15 V). (a) Control cell; (b) e.j.ps evoked in a 'resistant' cell using identical stimuli, after 15 min perfusion with α, β-methylene ATP 10 μM.

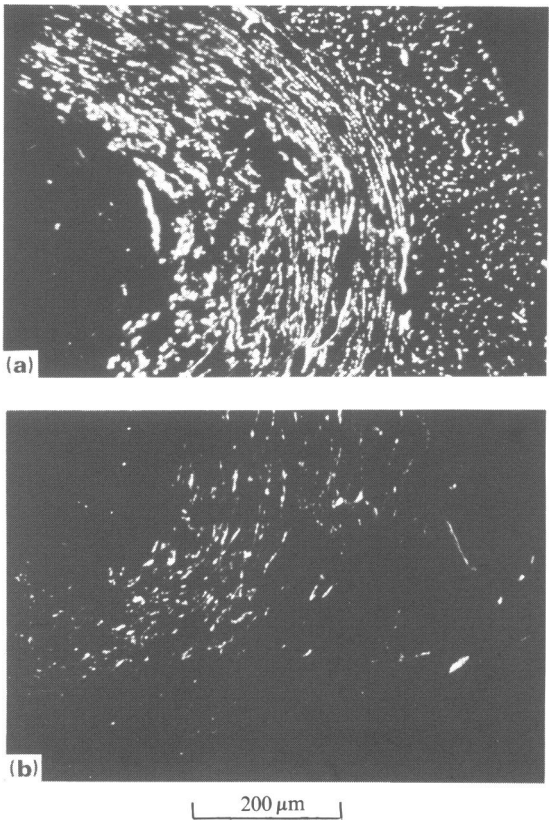

Figure 11 Fluorescence photomicrographs of transverse sections of the guinea-pig vas deferens. (a) Control tissue (b) tissue from animal pretreated with 6-hydroxydopamine.

Discussion

The smooth muscle cells of the guinea-pig vas deferens are densely innervated by sympathetic nerve fibres containing high levels of NA (Hukovič, 1961; Sjöstrand, 1965; Burnstock, 1970). In spite of this dense noradrenergic innervation, however, neither the e.j.ps nor the initial contractile response appear to be mediated by NA (see introduction).

Whilst Hirst & Neild (1980, 1984) have proposed the existence of a third type of adrenoceptor, the γ-receptor, others have presented evidence which supports an alternative hypothesis, namely, that a second neurotransmitter is involved and that ATP, acting on P_2-purinoceptors, mediates both the e.j.p. and the initial contractile phase in the guinea-pig vas deferens (Fedan *et al.*, 1981; Sneddon & Burnstock, 1984; Sneddon & Westfall, 1984).

The absence of separate 'purinergic nerves' in the vas deferens (Burnstock & Costa, 1975), the knowledge that ATP is contained within the storage vesicles of sympathetic nerves (ratio 50:1 NA:ATP) and the fact that noradrenergic neurone blocking drugs abolish e.j.ps in the guinea-pig vas deferens, led Westfall *et al.*, (1978) to propose that the neuronally-released ATP is liberated from sympathetic nerve terminals.

In the present study, we have largely confirmed the results of Sneddon & Burnstock (1984) that α, β-MeATP blocks e.j.ps and s.e.j.ps in the guinea-pig vas deferens. In addition, we have shown that α, β-MeATP does not block e.j.ps by impairing nerve impulse propagation in either the vas deferens or hypogastric nerve fibres; this agent, therefore, does not interrupt cholinergic transmission across the hypogastric ganglion. Furthermore since α, β-MeATP does not reduce [^{3}H]-NA overflow from the guinea-pig or mouse vas deferens (see also Stjärne & Åstrand, 1984), a prejunctional effect on NA release is unlikely to be involved. We have further shown that α, β-MeATP abolished s.e.j.ps and e.j.ps evoked by a single pulse or train of pulses in both mouse and rat vas deferens and inhibits a component of the electrically-evoked contractile response, findings which suggest that ATP may be a transmitter in the vas deferens of these species, also.

To test the proposal that ATP and NA were co-transmitters in the vas deferens, rats, mice and guinea-pigs were pretreated with 6-OHDA, the rationale being that, if ATP did mediate e.j.ps in the vas deferens and, if ATP and NA were co-transmitters and stored in sympathetic terminals, then pretreatment with 6-OHDA would greatly reduce or abolish e.j.ps. 6-OHDA produces a specific degeneration of sympathetic nerve endings (Malmfors & Thoenen, 1971); its specificity resides in the fact that it is selectively accumulated in sympathetic nerves via a neuronal uptake mechanism (Wakade, 1979).

The extent of sympathetic denervation was estimated by the use of histochemistry, [^{3}H]-NA release and by measurement of e.j.ps. In the mouse, rat and guinea-pig vasa deferentia, 6-OHDA pretreatment produced almost complete loss of endogenous NA, indicating that 6-OHDA had successfully destroyed the sympathetic nerve terminals. Sympathetic denervation was accompanied by complete abolition of s.e.j.ps from the rat, mouse and guinea-pig vasa deferentia. Futhermore, in vasa deferentia from 6-OHDA pretreated animals, e.j.ps could not be evoked in response to either a single pulse or trains of pulses. Thus, it would seem that both phases of motor transmission in the vas deferens are dependent on sympathetic nerves.

Whilst the results presented so far are in agreement with those of Westfall and his colleagues, and support the hypothesis that ATP and NA are co-transmitters in the vas deferens, certain anomalous results prevent

the whole-hearted dismissal of the γ-receptor hypothesis.

In both the guinea-pig and mouse vas deferens, a small population of cells were found which were resistant to the inhibitory actions of α, β-MeATP. Possible explanations for the existence of 'resistant' cells include (1) α, β-MeATP could not gain access to these cells and thus transmission was unaffected, or that (2) ATP is not the transmitter mediating the e.j.ps and s.e.j.ps in these cells. Indeed, the possibility that the resistant e.j.ps were mediated by NA acting on α-adrenoceptors cannot be ruled out because the effects of α-adrenoceptor antagonists on these 'resistant' cells were not investigated. It is interesting that α-adrenoceptor-mediated e.j.ps in the rat anococcygeus muscle were indeed, resistant to the actions of α, β-MeATP (Cunnane & Muir, unpublished observations) providing further evidence for the specificity of this agent. The functional significance of these cells in transmission is unclear, since the electrically-evoked contraction of vasa deferentia in all three species was virtually abolished by a combination of α-adrenoceptor and P_2-purinoceptor antagonists. The 'resistant' cells may provide an explanation for the small residual contraction which persists under these conditions (see Figures 1 and 2).

In conclusion, the results were examined bearing in mind two current hypotheses regarding neurotransmission in sympathetic nerves. The results obtained are consistent with the hypothesis that ATP and NA are co-transmitters, not only in the guinea-pig vas deferens but also in the rat and mouse vasa. However, the existence of a small number of cells 'resistant' to α, β-MeATP, suggests that an adenine nucleotide may not be the transmitter for these cells and may provide evidence for the existence of a transmitter other than ATP, or indeed another receptor type. It would seem from electrophysiological and mechanical studies that the bulk of transmission can be accounted for by the co-transmission hypothesis, with ATP acting on P_2-purinoceptors and NA acting on α-adrenoceptors. However, it is important to remember that the possibility that α, β-MeATP blocks the e.j.ps by interacting with the specialised postjunctional adrenoceptor (the γ-receptor) has not been fully eliminated.

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