Dual contractile effects of ATP released by field stimulation revealed by effects of α,β -methylene ATP and suramin in rat tail artery

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1 The field stimulation-induced release of endogenous ATP and noradrenaline (NA) and contractile response in rat isolated tail artery were examined. The release of ATP was studied by extracellular electrophysiological recording and that of NA by a novel voltammetrical technique. The effects of the P_2 -purinoceptor antagonist, suramin, on these parameters were compared with those of α,β -methylene ATP, a P_{2X} -purinoceptor desensitizing agent.

2 Neither α,β -methylene ATP (10 μ M) nor suramin (100–500 μ M) had significant effects on the extracellularly recorded nerve terminal action potential but both abolished the ATP-induced excitatory junction current caused by stimulation at 0.1 Hz. Neither agent affected significantly the voltammetrically measured release of NA induced by 10 or 100 pulses at 20 Hz.

3 Combined blockade of both postjunctional α_1 - and α_2 -adrenoceptors by prazosin and yohimbine (both 0.1 μ M) profoundly depressed the contractile response to 10 pulses at 20 Hz. The small and fast residual contraction in the presence of these agents was abolished by α,β -methylene ATP (10 μ M) and inhibited by suramin in a concentration-dependent manner (10–500 μ M; IC_{50} 75 μ M) and was hence probably caused by ATP or a related nucleotide.

4 When added first, α,β -methylene ATP (10 μ M) or suramin (100–500 μ M) delayed the onset and enhanced the amplitude of the neurogenic contraction. This enhanced response was abolished by further addition of prazosin and yohimbine (both 0.1 μ M).

5 The K^+ channel blocker, tetraethylammonium (10 mM), dramatically enhanced the contractile response to 100 pulses at 1 Hz and caused it to become biphasic. Addition of α,β -methylene ATP (10 μ M) or suramin (100–500 μ M) abolished the large initial twitch component of this contraction and depressed the tonic phase.

6 Like α,β -methylene ATP, suramin (500 μ M) had no effect on the contraction caused by exogenous NA (10 nM–100 μ M) or KCl (60 mM); both agents almost abolished the contraction caused by ATP (100 μ M).

7 In conclusion, (i) the contractile response of rat tail artery to electrical field stimulation is mediated by both ATP and NA, and is thus an expression of ATP-NA co-transmission, (ii) the released ATP exerts two opposite effects via 'P_{2X}-like' purinoceptors, triggering the initial rapid phase of the neurogenic contraction and restricting the NA-mediated component of the contraction; and (iii) the source and possible physiological role of the ATP which causes the inhibitory effect are unknown at present.

Keywords: Adenosine 5'-triphosphate; α,β -methylene ATP; noradrenaline; P_{2X} -purinoceptors; neurogenic contraction; rat tail artery; suramin; sympathetic neurotransmission

Introduction

Adenosine 5'-triphosphate (ATP) was proposed as a transmitter in non-adrenergic, non-cholinergic nerves in the gut and bladder in the 1970s and later as a co-transmitter with noradrenaline (NA) in sympathetic nerves (see Burnstock, 1990). Some doubts or controversies in this regard still exist (Hirst & Neild, 1980; Fredholm *et al.*, 1982), but the view that sympathetic nerves in addition to NA also release ATP (Burnstock, 1990; von Kügelgen & Starke, 1991a) as a co-transmitter is now increasingly accepted. ATP mediates the 'fast' electrical response to sympathetic nerve stimulation, the excitatory junctional current (e.j.c.) and potential (e.j.p.), and also often triggers the twitch component of the neurogenic contraction (Burnstock, 1990; von Kügelgen & Starke, 1991a). Recently, ATP has also been recognized as a fast ionotropic neurotransmitter both in the peripheral and central nervous systems (Silinsky *et al.*, 1992; Evans *et al.*, 1992; Edwards *et al.*, 1992).

In the tail artery of adult normotensive rats, the tissue examined in the present study, the e.j.cs and e.j.ps are caused

by ATP via P_{2X} -purinoceptors (Sneddon & Burnstock, 1984; Neild & Kotecha, 1986; Åstrand & Stjärne, 1989). The neurogenic contraction has been thought to be mediated exclusively by NA (Cheung, 1984; Medgett & Langer, 1984; Vidal *et al.*, 1986), but we have found that the initial phase of this response was inhibited by α,β -methylene ATP (α,β -MeATP), a P_{2X} -purinoceptor desensitizing agent (Kasakov & Burnstock, 1983; Sneddon & Burnstock, 1984), and hence was mediated by ATP (Bao *et al.*, 1989; 1990; 1993a). We have also reported that α,β -MeATP strongly increased the amplitude of the NA-mediated neurogenic contraction. The potentiating effect was more marked on the α_1 - than on the α_2 -adrenoceptor-mediated component of this response; during stimulation at 20 Hz it was inversely related to the train length (Bao *et al.*, 1990; 1993a). A similar potentiating effect of α,β -MeATP on the neurogenic contraction has been reported to occur in other vascular tissues and in vas deferens (Bulloch *et al.*, 1990; MacDonald *et al.*, 1992). We have proposed the hypothesis that the two opposite effects of α,β -MeATP may unmask a dual, excitatory as well as inhibitory mechanical effect normally exerted by ATP via 'P_{2X}-like' purinoceptors (Bao *et al.*, 1990). In the present

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paper we examined the validity of this hypothesis by comparing the effects of α,β -MeATP with those of suramin, a relatively selective P_2 -purinoceptor antagonist (Dunn & Blakeley, 1988).

Methods

Recording of contractile responses

The method for recording the contractile responses has been described in detail elsewhere (Bao *et al.*, 1990). Briefly, ring segments (about 3 mm in length) of the proximal central caudal artery from male normotensive Sprague-Dawley rats (200–300 g) were mounted, under a resting tension of 5 mN, in a 2 ml perfusion chamber and perfused (at 1 ml min^{-1} , 37°C) by a Gilson Minipulse II pump with Tyrode solution of the following composition (mM): NaCl 136.9, KCl 2.7, CaCl_2 1.8, MgCl_2 0.5, NaH_2PO_4 0.4, NaHCO_3 11.9 and D-glucose 5.6. The solution in the reservoir was bubbled with 93.5% O_2 and 6.5% CO_2 to give a pH of 7.3–7.4. Electrical field stimulation (0.05 ms, 50–60 V) was applied via a pair of platinum electrodes (separation: about 10 mm) on either side of the preparation. The contraction of the circular muscle was measured isometrically by a Grass FTO3C force-displacement transducer and recorded by a BBC Goerz Metrawatt SE 120 potentiometric recorder. When necessary, the chart speed was increased and the stimulus artifacts were recorded for better visualization of the kinetics of the contractile responses. The contractile response to stimulation with the parameters used was abolished by tetrodotoxin ($1 \mu\text{M}$) or guanethidine ($5 \mu\text{M}$) and hence, caused by action potential-induced release of transmitters from sympathetic nerves.

The perivascular nerves of the preparations were stimulated, in most cases with 10 pulses at 20 Hz at 1 min intervals and in some cases with 100 pulses at 1 Hz at 5–8 min intervals. Drugs to be tested were added to the reservoir proximally to the pump to the final concentrations indicated, after the responses to stimulation had become stable. They were added at intervals of 10–30 min depending on the time required for the effects of each addition to become maximal; the response at the end of this period was expressed as a % of that before addition of the drug. When effects of suramin on contractions caused by exogenously applied NA, ATP and KCl were studied, these vasoconstrictors were added directly to the bath to the final concentrations indicated under stop-flow conditions with the solution in the bath bubbled.

Electrophysiological and electrochemical analysis of the release of ATP and NA

The release of ATP and NA from sympathetic nerve terminals was studied by electrophysiological (for details, see Brock & Cunneane, 1988; Åstrand & Stjärne, 1989; Stjärne & Stjärne, 1989) and electrochemical (see Mermut *et al.*, 1990; Gonon *et al.*, 1993; Bao *et al.*, 1993b) techniques, respectively. Separate chambers were used but the conditions were kept as similar as possible. Segments (about 2 cm) from the proximal region of the artery were mounted in a 2–3 ml Perspex organ chamber and pinned to the Sylgard layer. The preparations were perfused (at 2 ml min^{-1} and 37°C) with Tris-Tyrode solution (electrophysiology) or phosphate-buffered solution (electrochemistry). The Tris-Tyrode solution had the following composition (mM): NaCl 136.9, KCl 2.7, CaCl_2 1.3, MgCl_2 0.5, D-glucose 5.6, Tris 20. In the phosphate-buffered solution, Tris was replaced by Na_2HPO_4 4.06 and KH_2PO_4 0.73. Prazosin ($0.1 \mu\text{M}$) was routinely present in the phosphate-buffered solution to prevent or minimize contractions which might disturb the electrochemical measurements. The solution in the reservoir was bubbled with 100% O_2 (pH 7.3–7.4). The proximal end of the preparation was drawn into a stimulating suction elect-

rode used to stimulate the perivascular sympathetic nerves with rectangular pulses (0.3 ms, 150–300 μA) at 0.1–20 Hz.

A glass micropipette (inner tip diameter 150–250 μm) was used as a recording electrode which was internally perfused at a constant rate (Tang *et al.*, 1990) with Tris-Tyrode solution of the same composition as that perfusing the bath. The recorded electrical responses, the nerve terminal spike (NTS) which reflects the nerve terminal action potential, and the excitatory junction current (e.j.c.) which reflects ATP release, were amplified by an a.c. amplifier, stored on magnetic tape, digitized and analysed off-line on an IBM AT personal computer. Addition of α,β -MeATP is known to abolish the ATP-induced, P_{2X} -purinoceptor-mediated intracellularly recorded excitatory junction potential (e.j.p.) (Sneddon & Burnstock, 1984; Neild & Kotecha, 1986). Similarly, addition of α,β -MeATP inside the recording electrode abolishes the e.j.c. (Åstrand & Stjärne, 1989; Stjärne & Stjärne, 1989). In the present experiments, α,β -MeATP ($10 \mu\text{M}$) was continuously present in the external perfusion medium to block the positive-going e.j.cs caused by ATP released from sites outside the recording electrode. Under control conditions, the e.j.cs declined somewhat during the first 10 min of stimulation at 0.1 Hz but then remained stable for at least 1 h (not shown). The agents to be tested were added to the medium perfusing the electrode to the final concentrations indicated.

An electrochemically pretreated carbon fibre electrode (active part: 8 μm in diameter, 50–80 μm in length) was used to monitor, by differential pulse amperometry (DPA) once per second, the oxidation current caused by NA released by nerve stimulation (see Gonon *et al.*, 1993; Bao *et al.*, 1993b). The signal recorded at +120 mV, the oxidation potential of NA, reflects the rise in the local concentration of endogenous NA at the carbon fibre (CF) electrode and hence, is termed $\Delta[\text{NA}]_{\text{CF}}$. In these experiments, the preparations were stimulated with 10 or 100 pulses at 20 Hz every 2 min. After 2–3 control stimulations, the drugs to be tested were added to the reservoir proximally to the pump, to the final concentrations indicated, and their effects examined after they had been present for 20–30 min.

Analysis

The lag time of the contractile responses was measured from the onset of the stimulus artifact to the onset of contractions. Data are expressed as means $\pm$ s.e. mean; n refers to the number of preparations (= the number of animals). Means were compared by Student's unpaired t test, Mann-Whitney U-test or one way analysis of variance (ANOVA) followed by Fisher PLSD test, as appropriate. $P \leq 0.05$ was regarded as significant.

Drugs

α,β -Methylene adenosine 5'-triphosphate lithium salt, tetrodotoxin, yohimbine hydrochloride, and tetraethylammonium chloride (all from Sigma), guanethidine sulphate (Ciba-Geigy), prazosin hydrochloride (Pfizer) and suramin hexasodium salt (gift from Bayer) were used. Stock solutions of most drugs were prepared by dissolving them in distilled water but tetraethylammonium was dissolved directly in saline and prazosin in 50% ethanol. Control experiments showed that ethanol at its final concentration (0.0005%) did not *per se* affect the nerve stimulation-induced electrochemical and contractile responses.

Results

Effects of α,β -MeATP and suramin on e.j.cs

When present only in the external perfusion medium, α,β -MeATP ($10 \mu\text{M}$) for at least 1 h had no significant effect on the nerve terminal spike (not shown) or the e.j.c. (Figure 1a).

Figure 1 Effects of adding to the medium perfusing the recording electrode suramin (200 and 500 μM) on the nerve terminal spike (NTS) and the peak amplitude of the excitatory junction current (e.j.c.) evoked by stimulation at 0.1 Hz, in rat isolated tail artery. In (a), the e.j.c. peak amplitude is plotted against the stimulus number; initial parts of the e.j.c. responses are omitted. In (b) each trace is the average of 30 consecutive sweeps; traces 1–3 represent control, suramin 200 and 500 μM , respectively. (a) and (b) are from the same experiment. In all experiments, 10 μM α, β -methylene ATP was present in the external perfusion medium to block the positive-going e.j.cs caused by ATP released from sites outside the recording electrode. Four similar experiments were performed. Note that suramin did not affect the NTS but concentration-dependently inhibited the e.j.cs.

Nor did α, β -MeATP, when further added to the medium internally perfusing the electrode, affect the nerve terminal spike but now rapidly abolished the spontaneous and evoked e.j.cs (not shown). Similarly, addition of suramin (100–500 μM) to the medium perfusing the recording electrode did not significantly affect the nerve terminal spike but concentration-dependently inhibited the e.j.cs (Figures 1a, 1b).

Effects of α, β -MeATP and suramin on $\Delta[\text{NA}]_{\text{CF}}$

The effects of α, β -MeATP and suramin on the nerve stimulation-induced rise in the concentration of endogenous NA at the carbon fibre electrode ($\Delta[\text{NA}]_{\text{CF}}$) was used to study their effects on NA release. The $\Delta[\text{NA}]_{\text{CF}}$ response to stimulation at 20 Hz with 10 or 100 pulse trains every 2 min was unaffected by 10 μM α, β -MeATP or 10–500 μM suramin (not shown).

Effects of α, β -MeATP and suramin on the contractile responses to electrical field stimulation, exogenous ATP, NA and KCl

Field stimulation with 10 pulses at 20 Hz at 1 min intervals produced reproducible, monophasic contractile responses (Figure 2). The contraction started after a delay of 374 ± 20 ms, continued to grow for 2.6 ± 0.2 s after the end of stimulation

Figure 2 (a) Original recordings representative of 6 experiments in rat isolated tail artery, showing the effects of cumulative addition of prazosin, yohimbine (both 0.1 μM) and suramin (500 μM) on the contractile responses to electrical field stimulation with trains of 10 pulses at 20 Hz at 1 min intervals. The stimulation was discontinued for 20–30 min after addition of suramin; the intervals between addition of other drugs were 10–20 min. The bottom trace indicates the stimulus artifact. (b) Superimposed original recordings, same condition as in (a), on a faster time base to visualize the kinetics. Arrows in the bottom trace indicate the beginning and end of stimulation. Note that the contraction started after a delay (420 ms in this preparation) and continued to grow for several seconds (3.3 s) after the end of stimulation and that block of the NA-mediated contractions by prazosin and yohimbine in combination unmasked the small and fast, ATP-mediated component of the contraction.

Figure 3 Concentration-dependence of the suramin-induced inhibition of the contractile response to 10 pulses at 20 Hz in the presence of prazosin and yohimbine (both 0.1 μM). Effects of suramin were evaluated after it had been present in the bath for 20–30 min. Contractions were expressed as a % of 'control' before addition of suramin (i.e. mean of 3–5 stable responses in the presence of prazosin and yohimbine). $n = 4-6$. *** $P < 0.001$ all vs control, one way ANOVA followed by Fisher PLSD test.

and had a peak amplitude of 0.9 ± 0.1 mN ($n = 14$). Combined addition of the α_1 - and α_2 -adrenoceptor antagonists, prazosin and yohimbine (both 0.1 μM), did not change the lag time but profoundly reduced the amplitude of the contraction (by $87.5 \pm 2.9\%$, $n = 10$; $P < 0.001$) and shortened its duration (Figure 2b). Subsequent addition of α, β -MeATP

(10 μM) itself caused a contraction (peak value 6.5 ± 0.3 mN, $n = 51$) which subsided within 20–30 min. Now the small residual neurogenic contraction in the presence of prazosin and yohimbine was almost abolished (not shown, but see Figure 5 in Bao *et al.*, 1993a). Unlike α, β -MeATP, suramin (10–500 μM) did not change the resting tension, but like α, β -MeATP, concentration-dependently reduced the residual contractile response in the presence of prazosin and yohimbine (Figures 2,3); the IC_{50} for this effect was 75 μM (Figure 3).

When added alone (Figure 4), α, β -MeATP delayed the onset (from 374 ms to 673 ± 48 ms, $n = 4$; $P < 0.001$; Figure 4b) but increased the amplitude (by 244% on average, Figure 4c) of the neurogenic contraction. The enhanced contraction

Figure 4 Effects of cumulative addition of α, β -methylene ATP (α, β -MeATP, mATP, 10 μM), prazosin (Pra) and yohimbine (Yoh) (both 0.1 μM) on the contractile responses to field stimulation with trains of 10 pulses at 20 Hz at 1 min intervals. Original recordings from one representative experiment shown at normal (a) and fast chart speed (b). The stimulation was discontinued for 20–30 min after addition of α, β -MeATP; other drugs were added at 10–20 min intervals. The bottom traces denote stimulus artifacts (the arrows indicate the beginning and end of the stimulation). Note that α, β -MeATP delayed the onset (from 430 to 770 ms in this experiment) but increased the amplitude of the contraction. (c) Summary of 4 experiments identical to that shown in (a). Contractions are expressed as a % of control (Con), i.e. the mean of 3–5 stable responses before addition of drugs. ** $P < 0.01$ and *** $P < 0.001$, one way ANOVA followed by Fisher PLSD test.

was abolished by further addition of prazosin and yohimbine (both 0.1 μM) (Figure 4). The effects of adding suramin alone varied somewhat with the experimental protocol. In one series of experiments in which the stimulation was discontinued for 20–30 min after each change in the concentration of suramin, to mimic the protocol in α, β -MeATP experiments (Figure 5), 10 μM suramin was without effect but 100 μM suramin delayed the onset (from 374 ms to 858 ± 18 ms, $n = 3$; $P < 0.001$) and tended to reduce slightly the amplitude of the neurogenic contraction (Figure 5). At a higher concentration (200–500 μM) suramin did not further change the lag time but concentration-dependently (by up to 207%) enhanced the amplitude of the contraction (Figure 5c). In another series in which stimulation was applied at fixed

Figure 5 (a) Original recordings showing the effects of cumulative addition of suramin (10–500 μM), prazosin and yohimbine (both 0.1 μM) on the contractile responses to trains of 10 pulses at 20 Hz at 1 min intervals. The bottom traces denote the stimulus artifacts. The intervals between addition of drugs were 10–30 min. In this series of experiments, the stimulation was discontinued for 20–30 min after each addition of suramin before the next stimulation period was resumed. (b) Superimposed contractile responses, same condition as in (a), recorded at fast chart speed (the arrows indicate the beginning and end of the stimulation). Tracings 1–6 denote control, suramin 10 μM , 100 μM , 200 μM , 500 μM and further addition of prazosin and yohimbine (both 0.1 μM), respectively. Note (i) that 10 μM suramin was without effect, (ii) that 100 μM suramin delayed the onset (from 260 to 840 ms in this experiment) and slightly depressed the amplitude, and (iii) that 200–500 μM suramin increased the amplitude of the neurogenic contraction. (c) Summary of 3 experiments similar to that in (a) showing the concentration-dependent effects of suramin (10–500 μM) and the effects of further addition of prazosin (Pra) and yohimbine (Yoh) (both 0.1 μM). '0' concentration indicates control, i.e. the mean of 3–5 stable responses before addition of drugs. Contractions were expressed as a % of control. * $P < 0.05$ and ** $P < 0.01$ all vs control; one way ANOVA followed by Fisher PLSD test.

intervals, 10 μ M suramin did not change the lag time or the amplitude of the contraction (the reduction by $37.5 \pm 11.2\%$, $n = 6$, was not significant), 100 μ M suramin delayed the onset (from 374 ms to 840 ± 26 ms, $n = 4$; $P < 0.001$) and further reduced the amplitude (by $43.0 \pm 9.6\%$, $n = 6$; $P < 0.05$) while higher concentrations (200–500 μ M) did not further delay the onset but increased the amplitude of the neurogenic contraction (by up to $168 \pm 75\%$, $n = 6$; $P < 0.01$). The potentiating effect of suramin developed slowly and was maximal after about 20 min (not shown). The effect was slowly reversible by prolonged washing (for at least 30 min). Further addition of prazosin and yohimbine abolished the contraction in the presence of suramin (see Figure 5).

Addition of the K^+ channel blocker, tetraethylammonium (10 mM), accelerated the rate of rise and dramatically (by about 100 fold, $n = 14$) increased the peak amplitude of the contractile responses to 100 pulses at 1 Hz, and often caused the contraction to become diphasic (Figure 6). Spontaneous twitch contractions occurred frequently under the conditions (not shown). Further addition of prazosin (1 μ M) had little effect on the rising phase of the contraction but substantially reduced the later phase (by $66.2 \pm 4.9\%$, $n = 8$; $P < 0.01$) and always unmasked the diphasic nature of the contraction (Figure 6a). The initial twitch phase was abolished and the second phase strongly (by $78.0 \pm 3.6\%$, $n = 8$; $P < 0.01$) depressed by 10 μ M α, β -MeATP. The small residual response was profoundly reduced or abolished by further addition of 0.1–1 μ M yohimbine (Figure 6a). Addition of 10 μ M α, β -MeATP before prazosin abolished the initial rapid phase and slightly reduced the second phase; the latter was totally suppressed by further addition of prazosin and yohimbine (both 1 μ M, Figure 6b). Similarly, suramin (100–500 μ M) abolished the initial rapid phase of the contraction in the presence of tetraethylammonium but often caused the tension to oscillate (Figure 7). The oscillation was not studied in detail in the present paper.

Similarly to 10 μ M α, β -MeATP (see Bao *et al.*, 1990), suramin did not even at the highest concentration used (500 μ M) affect the contraction caused by KCl (60 mM, $n = 6$) or exogenous NA (10 nM–100 μ M, $n = 6$), but profoundly inhibited the response to exogenous ATP (100 μ M) ($n = 4$, not shown).

Discussion

The present results support our hypothesis that ATP released by field stimulation has a dual effect in the rat tail artery, i.e. triggers *per se* the early component of the contraction but restricts its later, NA-mediated component (Bao *et al.*, 1990). We will briefly discuss the usefulness of α, β -MeATP and suramin, then the main findings.

Usefulness of suramin

Although certain effects of α, β -MeATP have been claimed to be unrelated to P_{2X} -purinoceptors (Byrne & Large, 1986), this agent is generally considered to desensitize these receptors reasonably specifically (Bao *et al.*, 1990; Fedan & Lampert, 1990).

Suramin, a structurally different compound, has been shown to block specifically the contraction caused by ATP, but not by NA, KCl or acetylcholine (Dunn & Blakeley, 1988; Evans & Surprenant, 1992), and to block the inward currents induced by ATP or α, β -MeATP but not by 5-HT, acetylcholine or the nicotinic agonist, cytosine (Evans *et al.*, 1992; Edwards *et al.*, 1992; Silinsky *et al.*, 1992). Suramin has therefore been widely used for pharmacological analysis of the role of ATP in neurotransmission (von Kgelgen *et al.*, 1989a; Evans *et al.*, 1992; Edwards *et al.*, 1992; Silinsky *et al.*, 1992). Suramin is not selective for P_{2X} -purinoceptors, but also blocks P_{2Y} -purinoceptors in some smooth muscle preparations, e.g. mouse vas deferens (Blakeley *et al.*, 1991) and P_{2Z} -purinoceptors in platelets (Hourani *et al.*, 1992). These effects probably do not interfere with the neurogenic contraction in rat tail artery, however, because the P_{2Y} -purinoceptor antagonist reactive blue 2 (Burnstock & Warland, 1987) did not at 10–50 μ M concentrations significantly affect the neurogenic contraction of rat tail artery caused by 10 pulses at 20 Hz, or the enhancing effect of α, β -MeATP on the contraction ($n = 3$, not shown), and because P_{2Z} -purinoceptors do not seem to exist on smooth muscle cells (see Gordon, 1986). The following findings in the present study indicate that the effects of suramin were due to block of the P_{2X} -purinoceptor-mediated ATP effects. (i) Like α, β -MeATP, suramin did not significantly change the nerve stimulation-induced release of

Figure 6 Original recordings representative of 8 (a) and 6 (b) experiments, showing the effects on the contractile responses to 100 pulses at 1 Hz (marked by bars) at 5–8 min intervals caused by cumulative addition of (a) tetraethylammonium (TEA, 10 mM), prazosin (Pra, 1 μ M), α, β -methylene ATP (α, β -MeATP, mATP, 10 μ M) and yohimbine (Yoh, 1 μ M), or (b) TEA, α, β -MeATP, Pra and Yoh. The intervals between addition of drugs were 10–15 min. Note the different time bars in (a) and (b) and the different amplitude bars in (b). Note also that α, β -MeATP abolished the twitch phase or the initial rapid phase of the contraction in the presence of TEA and prazosin, and TEA alone, respectively.

Figure 7 Original recordings representative of 4 experiments, showing the effects on the contractile responses to 100 pulses at 1 Hz (marked by bars) at 5–8 intervals caused by cumulative addition of tetraethylammonium (TEA, 10 mM) and suramin (100–500 μ M). Similar results were obtained whether or not the stimulation was discontinued for 20–30 min after each addition of suramin. Note (i) the different amplitude bars in (a) and (b); amplitude bar in (b) also applies to (c) and (d), and (ii) that suramin abolished the initial rapid phase of the contraction in the presence of TEA and caused the contraction to oscillate.

endogenous NA, in line with the reported lack of effects of suramin on [^{3} H]-NA overflow in the mouse vas deferens (von Kügelgen *et al.*, 1989b). (ii) Like α,β -MeATP, suramin did not affect the nerve terminal spike but concentration-dependently blocked the e.j.c.s. The effect was thus not due to blockade of nerve impulse conduction, in agreement with the finding of Evans & Surprenant (1992) in guinea-pig submucosal neurones, that suramin did not affect the threshold, amplitude or duration of the nerve action potential. (iii) Like α,β -MeATP, suramin almost abolished the contractile response to exogenous ATP but did not affect those to NA or KCl. It seems therefore reasonably safe to use suramin as a P_{2X} -purinoceptor antagonist under our experimental conditions.

Excitatory effect of ATP

We have previously reported that α,β -MeATP blocked the usually small, fast initial component of the neurogenic contractile response of the tail artery of normotensive rats (Bao *et al.*, 1989; 1993a). The demonstration in the present paper that suramin, like α,β -MeATP, delayed the onset of the neurogenic contraction (Figures 4b, 5b) and blocked (IC_{50} 75 μ M) the small residual contractile response to nerve stimulation with short trains at 20 Hz in the presence of prazosin and yohimbine (Figures 2, 3), is further evidence that ATP indeed mediates a component of the neurogenic contraction in the tail artery of adult normotensive rats. Due to the relatively short incubation time used in the present study for suramin, especially at low concentrations (e.g. 10 μ M), the calculated IC_{50} value for this effect may be overestimated (see Leff *et al.*, 1990).

Addition of the K^+ -channel blocker, tetraethylammonium, greatly amplified the ATP- as well as NA-induced components of the neurogenic contraction, probably by dramatically increasing the release of both NA and ATP (Mermet *et al.*, 1990; Stjärne *et al.*, 1990) and the excitability of the smooth muscle (Haeusler & Thorens, 1980). In the presence of tetraethylammonium, α,β -MeATP or suramin not only abolished the early twitch phase but also depressed its tonic phase (Figures 6, 7) revealing a cooperative depolarizing effect of ATP-NA co-transmission, which helps lower the threshold of the cells for firing the muscle action potential. If all observed effects of α,β -MeATP and suramin were due to desensitization or block, respectively, of ATP receptors, these results then imply that ATP released by electrical field stimulation indeed triggers one component of the neurogenic contraction in rat tail artery. We have shown elsewhere that this neurogenic ATP-mediated contraction was abolished by nifedipine and thus probably caused by activation of L-type Ca^{2+} channels (Bao *et al.*, 1993b) and that the ATP-mediated contractile response faded rapidly during long high frequency trains (Bao *et al.*, 1993b), probably as a result of decline in per pulse release of ATP (Msghina & Stjärne, 1993).

Inhibitory effect of ATP

Our finding that α,β -MeATP (10 μ M) substantially amplified the NA-mediated neurogenic contraction is not unique to the rat tail artery; a similar effect of α,β -MeATP has also been reported in other tissues (Bullock & McGrath, 1988; Bullock *et al.*, 1990; MacDonald *et al.*, 1992). Initially we considered the possibility that this effect of α,β -MeATP might be due to enhanced release of NA, as a result of block of prejunctional P_{2X} -purinoceptors (Bao *et al.*, 1989). This was clearly not the case, however, because α,β -MeATP did not significantly affect the [^{3} H]-NA overflow (Bao *et al.*, 1990) or $\Delta[NA]_{CF}$ (Bao *et al.*, 1993a and the present study) responses to nerve stimulation. We have therefore proposed a different explanation, namely that α,β -MeATP enhanced the NA-mediated contraction by blocking an inhibitory effect normally exerted by ATP released by field stimulation (Bao *et al.*, 1990). This hypothesis is strengthened by the present finding that suramin had a similar dual effect, i.e. blocked the ATP-mediated component of the neurogenic contraction but also amplified the NA-mediated contractions in rat tail artery. An alternative explanation, namely that α,β -MeATP may enhance the neurogenic contraction by depolarizing the smooth muscle, has been proposed, based on the finding that a similar degree of depolarization by KCl also amplified the neurogenic contraction (Neild & Kotecha, 1986). In our hands, however, 10–25 mM K^+ increased the amplitude of the contractile response to 10 pulses at 20 Hz by only 45% ($n=2$), i.e. much less strongly than α,β -MeATP or suramin. The fact that the effects of α,β -MeATP were mimicked by suramin, which did not contract the rat tail artery and thus probably did not depolarize the smooth muscle, also argues against this explanation. It seems reasonable to assume, therefore,

that both the excitatory and inhibitory effects of α,β -MeATP and suramin were due to block of effects normally exerted by ATP. It should be kept in mind, however, that the effects of α,β -MeATP and suramin observed in this study were not identical. Suramin did not change the resting tension while α,β -MeATP *per se* caused a large transient contraction. Suramin (10–100 μ M) tended to depress the amplitude of the neurogenic contraction under some experimental conditions while α,β -MeATP did not. Furthermore, suramin caused the tension in the presence of tetraethylammonium to oscillate, while α,β -MeATP did not.

Sources of ATP

The excitatory effect is almost certainly caused by ATP released exocytotically from the nerves as a sympathetic co-transmitter. After its release, ATP is rapidly (within 100 ms) metabolized by ecto-ATPase (Cunnane & Manchanda, 1988) and hence, probably exerts its effects locally, near the sites of release. However, the source of the ATP which inhibits the NA-mediated neurogenic contraction is less clear. It may be non-neuronal or neuronal. In favour of the former alternative, more than 90% of the ATP released by field stimulation has been claimed to be derived from non-neuronal sources (Sedaa *et al.*, 1990), and furthermore, α,β -MeATP-labelled P_{2X} -purinoceptors have been reported to be distributed throughout the media of blood vessel (Bo & Burnstock, 1989). In preliminary experiments we found that removal of the endothelium did not prevent the α,β -MeATP-induced enhancement of the neurogenic contraction ($n=2$, not shown). An intriguing possibility is that the ATP restricting field stimulation-induced NA-mediated neurogenic contraction was released from smooth muscle and that the effect was exerted by preventing the electrotonic spread, perhaps via gap junctions, of the NA-mediated excitation. However, we have so far failed to find evidence supporting this possibility. Several gap junction inhibitors, e.g. endosulfan and (4 β)phorbol 12-myristate 13-acetate (Fransson-Steen & Wärngård, 1992; Seseke *et al.*, 1992), failed to affect the neurogenic contraction in this vessel (unpublished data). Furthermore, neither α,β -MeATP nor suramin affected the contractile response to exogenous NA. Thus, the ATP inhibiting the

neurogenic NA-mediated contraction was not released from smooth muscle by NA via postjunctional α -adrenoceptors (see Seddaa *et al.*, 1990; Katsuragi *et al.*, 1990; von Kügelgen & Starke, 1991b). The possibility remains, however, that ATP was released from smooth muscle by neurally released ATP (Katsuragi *et al.*, 1991; von Kügelgen & Starke, 1991b).

Alternatively, both the excitatory and inhibitory effect may be exerted by ATP released as a sympathetic co-transmitter. In support of this possibility, the enhancing effect of α,β -MeATP on the neurogenic contractile response at 20 Hz (Bao *et al.*, 1990) paralleled the decline in the per pulse release of ATP at this frequency (Msghina & Stjärne, 1993); both were inversely related to the length of the stimulus trains. As the excitatory effect probably involves activation of L-type Ca^{2+} channels and an increase in the intracellular Ca^{2+} concentration (Bao *et al.*, 1993b), the inhibitory effect of ATP may be due to hyperpolarization of smooth muscle secondary to activation of Ca^{2+} -activated K^{+} channels. In direct support of this idea, ATP effects via P_2 -purinoceptors have recently been reported to both increase the intracellular Ca^{2+} level and subsequently activate Ca^{2+} -activated K^{+} channels in rat cultured aortic smooth muscle cells (von der Weid *et al.*, 1993). This would explain why α,β -MeATP and suramin did not enhance the neurogenic contraction in the presence of the K^{+} channel blocker tetraethylammonium (Figures 6, 7).

In conclusion, the present results show that ATP released by electrical field stimulation exerts via ' P_{2X} -like' purinoceptors dual excitatory and inhibitory effects in rat isolated tail artery, triggering the initial component of the neurogenic contraction but also restricting its later, NA-mediated component. The source and possible physiological role of the ATP inducing the inhibitory effect remain to be established in future studies.

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