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The effects of suramin on purinergic and noradrenergic neurotransmission in the rat isolated tail artery

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Abstract

Intracellular microelectrode recording was used to examine the effects of suramin, a P_2-purinoceptor antagonist, on the electrical responses evoked by sympathetic nerve stimulation in the rat isolated tail artery. Field stimulation (10 or 20 pulses at 0.5, 1 and 2 Hz) evoked a biphasic electrical response, consisting of fast, transient excitatory junctional potentials (e.j.p.s) and a slow, prolonged depolarisation. Suramin (100 μM) abolished the e.j.p.s and significantly increased the amplitude of the slow depolarisation at all frequencies. In contrast, phentolamine (2 μM) abolished the slow depolarisation, but had no effect on the magnitude of e.j.p.s. Neither drug altered the resting membrane potential of cells. The ability of suramin to inhibit e.j.p.s in rat tail artery is consistent with the proposal that it is a P_{2X}-purinoceptor antagonist and supports a role for ATP as an excitatory cotransmitter from the sympathetic nerves innervating this tissue. Suramin is also able to increase the α-adrenoceptor-mediated slow depolarisation by an unknown mechanism.

Keywords: Tail artery, rat; Sympathetic nerve stimulation; Purinergic transmission; P_{2X} purinoceptor; Suramin

1. Introduction

In many vascular and visceral smooth muscle tissues adenosine 5′-triphosphate (ATP) is released from sympathetic nerves as a cotransmitter with noradrenaline. In most of these tissues ATP mediates excitatory junction potentials (e.j.p.s) and a portion of the neurogenic contractile response by acting on P_{2X}-purinoceptors (see Burnstock, 1990; Von Kügelgen and Starke, 1991; Kennedy, 1993). Early evidence came from experiments using the potent, selective agonist α,β-methyleneATP (α,β-meATP), which produces a profound desensitisation of P_{2X}-purinoceptors (Kasakov and Burnstock, 1983; Meldrum and Burnstock, 1983; Sneddon and Burnstock, 1984; Kennedy et al., 1986) as selective antagonists were not available. However, it is preferable to study neurotransmission using selective antagonists and subsequently, competitive P_{2X}-purinoceptor antagonists, such as suramin and, more recently, pyridoxalphosphate-6-azophenyl-2′,4′-disulphonic acid (PPADS) (Lambrecht et al., 1992; McLaren et al., 1994), have been developed.

Dunn and Blakeley (1988) were the first to show that suramin, a trypanocidal drug which inhibits ATPase enzymes, could also antagonise P_{2X}-purinoceptors. In the mouse vas deferens suramin antagonised reversibly contractions to α,β-meATP, but had no effect on those to carbachol and noradrenaline. Suramin has since been found to antagonise contractions evoked by exogenous P_{2X}-purinoceptor agonists in a large number of smooth muscle preparations, including guinea-pig urinary bladder (Hoyle et al., 1990), rabbit ear artery (Leff et al., 1990) and rat vas deferens (Mallard et al., 1992). Subsequent studies showed that suramin is selective for P_2-purinoceptors over non-purinoceptors, but non-selective between P_2-purinoceptor subtypes (see Voogd et al., 1993).

Suramin antagonises neurogenic, purinergic contractions in a wide range of tissues, including the rat vas deferens (Mallard et al., 1992) and the rat tail artery (Bao and Stjärne, 1993) and neurogenic, purinergic inhibitory junction potentials and relaxation in the guinea-pig taenia coli (Den Hertog et al., 1989; Hoyle et al., 1990). However, the effect of suramin on purinergic e.j.p.s has been determined in only a few tissues (Sneddon, 1992; Nally and Muir, 1992; Jobling, 1994). We have now investigated the effect of suramin

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on the electrical responses evoked by sympathetic nerve stimulation in rat isolated tail artery. In this tissue nerve stimulation evokes e.j.p.s, which are thought to be mediated by ATP as they are abolished by desensitization of P_{2X}-purinoceptors by α,β-meATP (Sneddon and Burnstock, 1984) and a slow depolarisation which is due to noradrenaline acting on α-adrenoceptors (Cheung, 1982).

2. Materials and methods

2.1. Preparation and experimental design

Sprague Dawley male rats (100–200 g) were killed with CO_2 and dislocation of the neck. The ventral tail artery was dissected, mounted for recording in a 2 ml organ bath and allowed to equilibrate at 36°C for 1 h. A modified Krebs solution of the following composition was used throughout (mM): NaCl 118, KCl 5.4, NaH_2PO_4 1.16, $NaHCO_3$ 25, $MgSO_4$ 1.16, $CaCl_2$ 2.5 and glucose 11.1, bubbled with 95% O_2, 5% CO_2.

Electrophysiological experiments were carried out using intracellular recording with glass microelectrodes of 30–50 MΩ resistance filled with 3 M KCl. The signal was recorded on a storage oscilloscope (Tektronix) and a tape recorder (Racal) via a preamplifier (Cell Explorer 800, Dagan). Impalements were accepted if the resting membrane potential maintained a stable level of at least −50 mV. E.j.p.s and slow depolarisations were evoked by field stimulation of sympathetic nerves at 0.5, 1 and 2 Hz for 10 pulses or 20 pulses, with a pulse width of 0.3 ms and at a voltage lower than that necessary to evoke a contraction. E.j.p. magnitude was measured as the additional rapid depolarisation above the developing slow depolarisation. When applied, suramin (100 μM) and phentolamine (2 μM) were allowed to equilibrate for at least 45 min. Suramin (Bayer, UK) and phentolamine (Sigma) were dissolved in distilled water and kept frozen as 100 mM and 1 mM stock solutions respectively.

2.2. Statistics

Values in the text refer to means ± S.E.M. Statistical comparison of the results was tested by Student's *t*-test for paired or unpaired data. Differences were considered significant when $P < 0.05$.

3. Results

3.1. Responses to nerve stimulation

The mean resting membrane potential of smooth muscle cells in the rat isolated tail artery was −68.3 ± 1.1 mV ($n = 29$). Stimulation of the sympathetic nerves (10 or 20 pulses at 0.5, 1 or 2 Hz) produced an electrical response with two distinct phases. Each stimulus evoked a rapid transient e.j.p. and as the train of pulses progressed a slow depolarisation developed (Fig. 1). The magnitude of the first e.j.p. in a train was 10.7 ± 0.8 mV ($n = 29$). Although the e.j.p.s were quite variable in magnitude over the length of the train, they showed no significant facilitation or depression at 1 Hz (10th e.j.p. = 9.4 ± 0.8 mV, $n = 24$) or 2 Hz (10th e.j.p. = 9.5 ± 0.7 mV, $n = 11$), but there was a slight depression of e.j.p. magnitude at 0.5 Hz (10th e.j.p. = 8.9 ± 0.7 mV, $n = 11$) ($P < 0.05$). The slow depolarisation, which was smaller than the e.j.p.s, reached a peak between 5 and 20 s after the last e.j.p. and decayed back to resting membrane potential within 60 s (Fig. 1). The magnitude of the slow depolarisation increased with the frequency of stimulation and with the number of pulses in the train. Stimulation frequencies above 2 Hz and train lengths greater than 10 pulses tended to cause smooth muscle contraction and ejection of the recording electrode. Therefore, in all subsequent experiments electrical responses to trains of 10 pulses at 0.5, 1 and 2 Hz were examined.

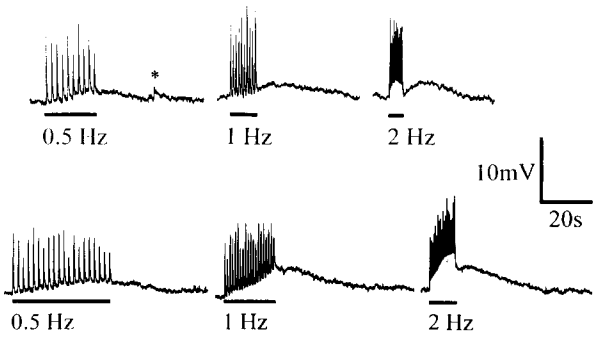

Fig. 1. E.j.p.s and slow depolarisation evoked by sympathetic nerve stimulation in the rat tail artery. Traces show e.j.p.s and slow depolarisations evoked by trains of 10 (upper panel) and 20 pulses (lower panel) at 0.5, 1 and 2 Hz. Solid bars indicate the time course of stimulation. All responses were obtained in a single cell of stable membrane potential. * Indicates a spontaneous e.j.p.

3.2. Effects of suramin

The P_2-purinoceptor antagonist suramin is slowly equilibrating in whole tissue preparations (Leff et al., 1990; Sneddon, 1992). Therefore, preliminary experiments were carried out to examine the time course of action of suramin at 100 μM, a concentration which virtually abolished ATP-mediated e.j.p.s in the guinea-pig vas deferens (Sneddon, 1992). Fig. 2 shows the time course of the effects of suramin (100 μM) on the e.j.p.s and slow depolarisation evoked by 10 pulses at 1 Hz in a single cell. Control e.j.p.s were 9–10 mV amplitude

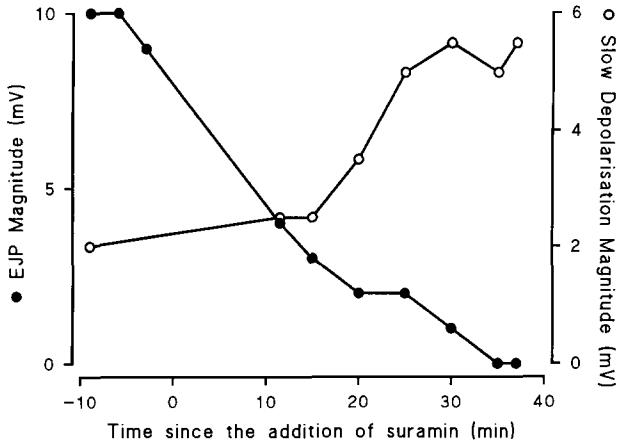

Fig. 2. The time course of the effect of suramin on e.j.p.s and the slow depolarisation in the rat tail artery. Plotted data shows the progressive inhibition of e.j.p.s (●, left axis) and increase in amplitude of the slow depolarisation (○), right axis) in the presence of suramin (100 μM). Note the different scales of the axes. The amplitude of the first e.j.p. in a train and the amplitude of the slow depolarisation in response to 10 pulses at 1 Hz, are shown. All data was obtained in a single cell of stable membrane potential.

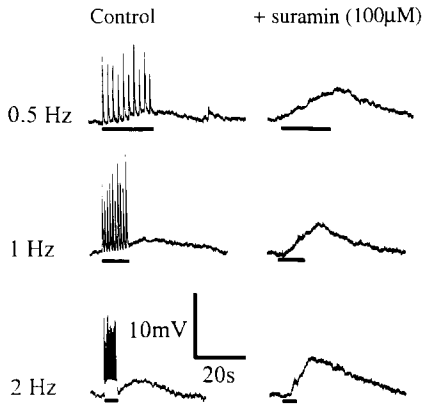

Fig. 3. The effects of suramin on e.j.p.s and slow depolarisations in a single cell. The traces show the abolition of e.j.p.s and the maximum potentiation of the slow depolarisation induced by suramin (100 μM). Neurogenic responses were evoked by 10 pulses at 0.5, 1 and 2 Hz. Solid bars indicate time course of stimulation. All data was obtained in a single cell of stable membrane potential.

and were slowly reduced following introduction of suramin, until they were abolished after 30–40 min. In contrast, the slow depolarisation increased in magnitude from an initial value of 2 mV to a maximum of 5–6 mV over the same period. These actions of suramin did not reverse even after 2 h washout. This shows that at this concentration suramin had a maximal effect within 45 min and therefore, this equilibration period was used in all subsequent experiments with suramin. In the same cell, suramin also abolished e.j.p.s and potentiated the slow depolarisation evoked at 0.5 and 2 Hz (Fig. 3)

Fig. 4 shows the mean data for the effects of suramin and phentolamine on e.j.p. and slow depolarisation amplitude. In all cells e.j.p.s were abolished by suramin (100 μM), but unaffected by phentolamine (2 μM) (Fig. 4a). Phentolamine abolished the slow depolarisation in all cells, but suramin significantly enhanced its magnitude at each frequency (Fig. 4b). The potentiation was most pronounced at 0.5 Hz and progressively less at 1 and 2 Hz. Note that in the absence of suramin the magnitude of the slow depolarisation increased with stimulation frequency, whereas in its presence it did not. Suramin and phentolamine together abolished all neurogenic responses in the rat tail artery (not shown). Neither drug had any significant effect on the resting membrane potential of the cells.

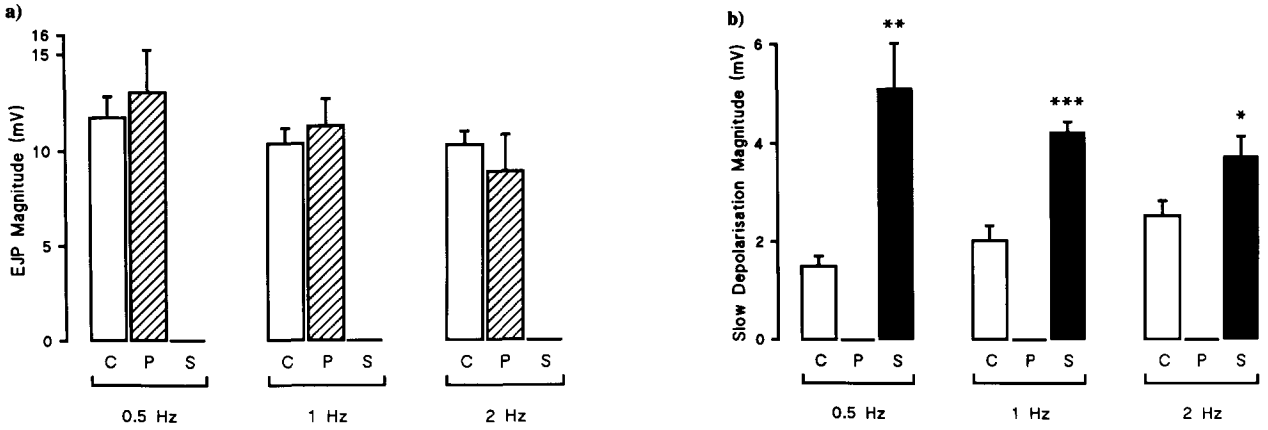

Fig. 4. The effect of suramin and phentolamine on e.j.p. and slow depolarisation magnitude in response to 10 pulses at 0.5, 1 and 2 Hz. (a) Columns show the mean amplitude of the first e.j.p. in a train of 10 pulses in the absence of drugs (C, open bars) and in the presence of phentolamine (2 μM) (P, cross-hatched bars) or suramin (100 μM) (S) ($n = 4$–24). (b) Columns show the mean amplitude of the slow depolarisation in the absence of drugs (C, open bars) and in the presence of phentolamine (2 μM) (P) or suramin (100 μM) (S, filled bars) ($n = 4$–24). Vertical bars show S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$.

4. Discussion

These results show that suramin inhibits e.j.p.s in the rat tail artery, confirming that ATP is an excitatory neurotransmitter in sympathetic nerves in this tissue. This supports the results of Sneddon and Burnstock (1984) who found that desensitisation of P_{2X}-purinoceptors in this artery with α,β-meATP significantly reduced e.j.p.s evoked at 0.5–4 Hz. Suramin was effective here and in the guinea-pig vas deferens (Sneddon, 1992) at 100 μM, which is 10 times lower than in the rabbit saphenous vein (Nally and Muir, 1992) and guinea-pig splenic arterioles (Jobling, 1994). The inhibition of e.j.p.s by suramin in the rat tail artery did not readily reverse, even after 2 h washing. A similar effect is seen in the guinea-pig vas deferens where inhibition of e.j.p.s by 100 μM suramin showed little or no reversal (Sneddon, 1992), although inhibition of purinergic contractions of the rat vas deferens by 30 μM suramin reversed after 40–60 min washout (Mallard et al., 1992).

In this study the neurogenic slow depolarisation was abolished by phentolamine, showing that it is due to noradrenaline acting at α-adrenoceptors. Studies using more selective antagonists such as yohimbine (Itoh et al., 1983) and idazoxan (Jobling and McLachlan, 1992) indicate the involvement of α_2-adrenoceptors (but note that Cheung (1984) reported that prazosin inhibited this response).

Suramin also caused a significant increase in the amplitude of the slow depolarisation in the rat tail artery, with a time course that was similar to that of the reduction of the e.j.p.s. The mechanism by which this occurs is unclear. In several tissues it has been claimed that prejunctional P_2-purinoceptors inhibit the release of ATP and noradrenaline (Kurz et al., 1993; Von Kügelgen et al., 1994). Thus, one possibility is that suramin antagonised this negative feedback via prejunctional P_2-purinoceptors in the rat tail artery, so increasing release of noradrenaline and increasing the amplitude of the slow depolarisation. However, this is unlikely to be the case as suramin has been reported to have no effect on the release of [^{3}H]noradrenaline in this tissue (Bao et al., 1993).

It is more likely that suramin acted at a postjunctional site to increase the amplitude of the slow depolarisation. Whilst suramin has been shown to inhibit the purinergic component of neurogenic contractions in the rat tail artery, it also potentiates the components mediated by α-adrenoceptors (Bao and Stjärne, 1993). The mechanism of potentiation was unclear but was suggested to be due to antagonism of an inhibitory P_{2Y}-purinoceptor present in the smooth muscle cells. An alternative possibility is that calcium ion influx through the P_{2X}-purinoceptor ion channel during an e.j.p. activates an outward potassium current which counteracts the slow depolarisation mediated by α-adrenoceptors. A further possibility is that inhibitory P_1-purinoceptors are present in the smooth muscle cells and that adenosine, produced by the breakdown of ATP by ectonucleotidase enzymes, acts at these receptors to depress α-adrenoceptor-mediated responses. Suramin inhibits the activity of ectonucleotidases (Hourani and Chown, 1989; Crack et al., 1994) and so could prevent production of adenosine and its inhibitory action. These possibilities have not been studied further.

In conclusion, these data show that suramin abolishes e.j.p.s in the rat isolated tail artery, confirming a cotransmitter role for ATP from sympathetic nerves in this tissue. Suramin also potentiated the noradrenergic slow depolarisation by a mechanism which is not yet fully explained.

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