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Short communication

Frequency dependent α_2-adrenoceptor mediated modulation of excitatory junction potentials in guinea-pig mesenteric artery

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Abstract

Excitatory junctional potentials (EJPs) elicited with brief duration (10 s) electrical field stimulation of guinea-pig mesenteric arteries were nearly abolished at all frequencies by pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS, 30 μM) but persisted following reserpinization. Suramin (100 μM) enhanced EJPs at 0.2–0.5 Hz responses and reduced them at 2–32 Hz. Phentolamine (1 μM) and yohimbine (0.1 μM) enhanced EJPs at 0.2–8 Hz but not at 16–32 Hz. Oxymetazoline (0.3 μM) reduced EJPs at 0.2–0.5 Hz but not at 1–32 Hz. Following reserpinization, EJPs were enhanced at 0.2–2 Hz but not at 4–32 Hz. Clonidine (0.1 μM) was without effect at all frequencies in control arteries but reduced EJPs at 0.2–2 Hz in reserpine-treated arteries. In conclusion, pre-junctional α_2-adrenoceptors modulate ATP release during low frequency, brief duration sympathetic nerve stimulation.

Keywords: Excitatory junctional potential; α_2-Adrenoceptors; Mesenteric artery

1. Introduction

Besides the well-known function that adenosine 5′-triphosphate (ATP) plays as an important intracellular constituent, it is now well accepted that this molecule plays a role as a sympathetic neurotransmitter along with the classic neurotransmitter noradrenaline (see Burnstock, 1990). The release of ATP and NA are not always modulated in parallel. For example, in the guinea-pig vas deferens prejunctional α_2 adrenoceptors primarily modulate the electrical field stimulation-evoked overflow of NA and to a much lesser extent ATP release (Driessen et al., 1993) or the overflow of total purines (Todorov et al., 1999). Likewise, α_2-adrenoceptor antagonists have no effect on EFS-induced release of ATP from rat hypothalamic slices (Sperlagh et al., 1998) or rat tail artery (Msghina et al., 1999). In these studies the preparations were subjected to electrical field stimulation for long periods of stimulation (i.e., 20–120 s duration, 2–20 Hz frequency) so that sufficient overflow of transmitters was present to evaluate release. Junctionally released ATP has been suggested to play a particularly important role during short bursts of neuronal activity (Burnstock, 1990). Thus, excitatory junction potentials (EJPs) that provide an indirect measure of junctionally released ATP can be recorded intracellularly in response to short bursts of stimuli or even in response to a single stimulus of electrical field stimulation (see Brock and Cunnane, 1993). A better understanding of the frequency dependence of pre-junctional modulation of ATP release by noradrenaline at short stimulation is important to our understanding of the regulation of purinergic transmission.

In the present study we have therefore used intracellular recording of EJPs to monitor the release of ATP elicited with short trains (10 s) of stimuli from sympathetic nerves supplying guinea-pig mesenteric arteries. In particular, this study has determined the frequency-dependence of α_2-adrenoceptor mediated modulation of EJPs over a broad range of stimulation frequency (i.e., 0.2–32 Hz).

2. Materials and methods

2.1. Tissue preparation

Male guinea-pigs (weighing 400–450 g) were killed by CO_2 overdose followed by exsanguination in keeping with

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protocols approved by the University of Nevada's Animal Care and Use Committee. Segments of first order branches of the inferior mesenteric artery (250–350 μm in diameter) were dissected out and bathed in regular Krebs solution while perfusing them with distilled water for 30 min to remove endothelium. Ring segments (7–10 mm long) were pinned in the sylgard bottom of a 2 ml recording chamber perfused with Krebs solution (3 ml/min; 37°C; aerated with 95% O_2/5% CO_2) of the following composition (mM): 120.2 NaCl; 3.0 KCl; 1.2 $MgCl_2$; 23.8 $NaHCO_3$; 1.2 KH_2PO_4; 11.0 dextrose; 2.5 $CaCl_2$. The solution routinely contained indomethacin (1 μM) and *N*ω-nitro-L-arginine (l-NNA, 100 μM) to block any residual effects of endothelium. Electrical field stimulation at supramaximal voltage with trains of square-wave pulses (0.1 ms pulse width, 13 V) was applied at 0.2–32 Hz for 10 s by means of two parallel platinum electrodes on both sides of the vessel connected to a Grass S48 stimulator. In some experiments, the animals were treated intraperitoneally with reserpine (1 mg/kg), 24 h prior to the experiment (Khoyi et al., 1988), in order to deplete NA.

2.2. Intracellular recordings

Intracellular measurements were made through the adventitia of the vessel, at a distance of 2–4 mm from the stimulating electrodes, with fiber-containing borosilicate electrodes filled with 3 M KCl and having resistance between 80 and 120 MΩ as previously described (e.g., Mutafova-Yambolieva and Keef, 1997). Intracellular measurements of membrane potential were made in the absence and the presence of drug tested, so that maximum of two frequency–response curves were generated in one tissue segment. EFS was applied every 2 min with increasing frequencies of stimulation. Only one drug was tested in a single preparation. Membrane potential changes were analyzed by AcqKnowledge 3.2.4 software (Biopac System).

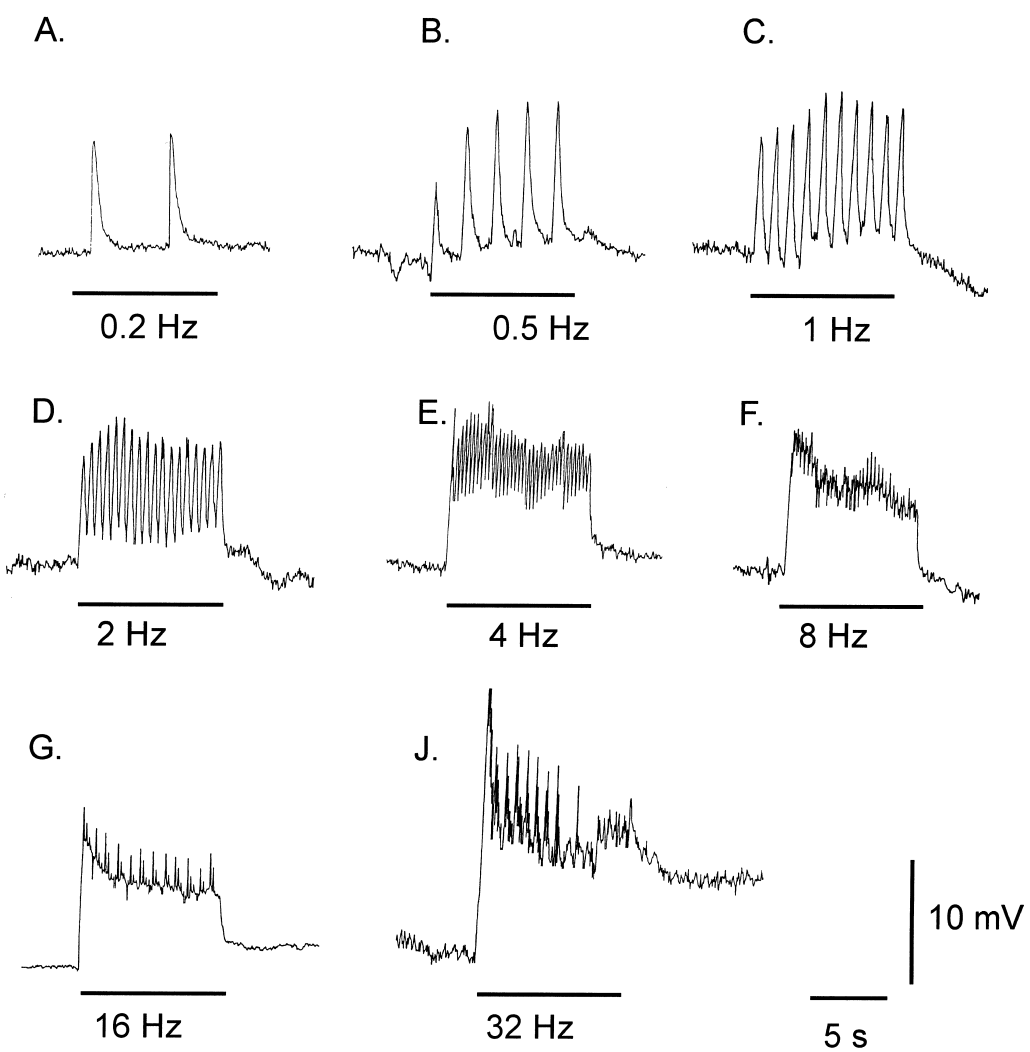

Fig. 1. Electrical field stimulation (EFS) evoked membrane depolarization in guinea-pig inferior mesenteric artery. Representative tracings showing the frequency-dependent response to EFS at 0.2–32 Hz. Tracings are representative of those obtained from six different preparations.

Guanethidine, oxymethazoline, phentolamine, pyridoxal-phosphate-6-azophenyl-2′,4′-disulphonic acid tetrasodium (PPADS), reserpine, suramin, tetrodotoxin, and yohimbine were purchased from Sigma–Aldrich, St. Louis, MO, USA. All drugs were dissolved in redistilled water except for reserpine (1 mM ascorbic acid) and further diluted in Krebs solution. EJPs were measured as area under the response curve for 10 s. Data were analyzed statistically by one-way analysis of variance (GraphPad Prizm v.3.0). A probability of less than 0.05 was considered significant. The results were obtained from 102 cells (n) of 35 animals (N).

3. Results

The mean resting membrane potential of guinea-pig inferior mesenteric arteries was -67.9 ± 1.1 mV ($n = 27$). Stimulation with single stimuli initiated EJPs in smooth muscle cells in all arteries examined. The mean EJP amplitude at 0.2 Hz of electrical field stimulation was 10 ± 2 mV and the area was 0.075 ± 0.004 mV/s ($n = 6$). Stimulation with increasing numbers of stimuli led to summation of the EJPs (Fig. 1) such that at frequencies > 2 Hz, the amplitudes of individual EJPs was difficult to resolve. Rather, the EJPs summed to give depolarization

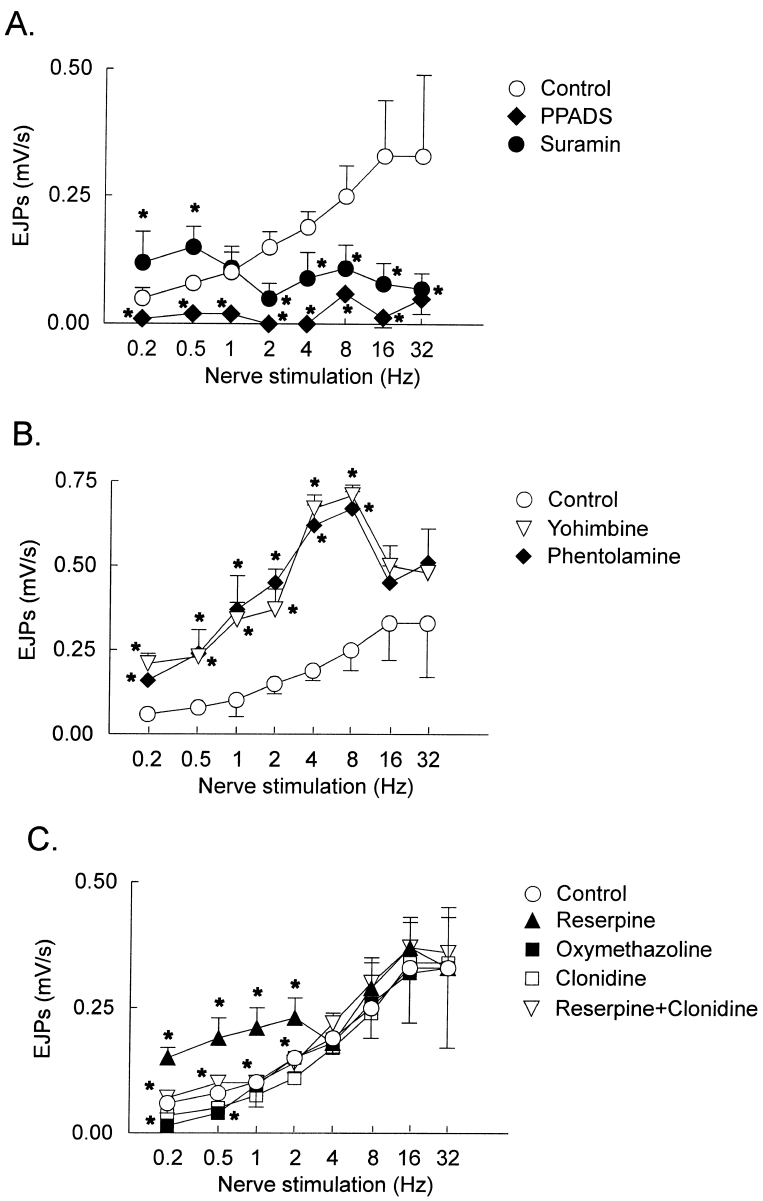

Fig. 2. Effects of P2 receptor antagonists suramin (100 μM) and PPADS (30 μM) (A), α-adrenoceptor antagonists phentolamine (1 μM) and yohimbine (0.1 μM) (B), and reserpine (1 mg/kg i.p. 24 h prior to experiment), clonidine (0.1 μM) and oxymethazoline (0.3 μM) (C), on EFS-evoked membrane depolarization in guinea-pig mesenteric artery. Asterisks denote significant difference from corresponding controls ($P < 0.05$). Each point represents an n of four to six cells; S.E. are presented by error bars.

that peaked 10–20 mV above the resting membrane potential. Therefore, we analyzed the area under the response for the duration of the stimulation (10 s) as a measure of the membrane potential changes evoked during the EJPs. In most arteries, EJPs were not followed by a slower depolarization until 32 Hz of EFS. In some experiments, blood vessels were exposed either to the fast Na^+ channel blocker tetrodotoxin (1 μM) or the sympathetic neuronal blocker guanethidine (10 μM). Both drugs virtually abolished EJPs at all stimulation frequencies (data not shown). These findings suggest that the EJPs elicited by electrical field stimulation depend on activation of sympathetic neurons.

The EJPs produced by all frequencies of electrical field stimulation in control tissues (Fig. 2A) and reserpinized tissues ($n = 4$; data not shown) were nearly abolished by PPADS (30 μM). Interestingly, suramin (100 μM) reduced the responses to 2–32 Hz while it facilitated the responses to 0.2 and 0.5 Hz (Fig. 2A).

Both the nonselective α_1/α_2-adrenoceptor antagonist phentolamine (1 μM) as well as the selective α_2-adrenoceptor antagonist yohimbine (0.1 μM) enhanced the responses to 0.2–8 Hz but did not affect the responses to 16 and 32 Hz (Fig. 2B). In tissues isolated from reserpine-treated animals, EFS-elicited EJPs were potentiated at 0.2–2 Hz and not affected at 4–32 Hz (Fig. 2C). Oxymethazoline (0.3 μM), a selective α_2-adrenoceptor agonist, reduced the responses to 0.2 and 0.5 Hz but not to 1–32 Hz. Clonidine (0.1 μM), an α_2-adrenoceptor agonist, did not affect the responses to all frequencies of stimulation in control arteries (Fig. 2C), whereas clonidine reduced the EJPs produced by 0.2–2 Hz electrical field stimulation in reserpine-treated tissues.

4. Discussion

It is well established that ATP serves as a cotransmitter with noradrenaline in the sympathetic neuroeffector junction in numerous systemic arteries including mesenteric artery (e.g., Burnstock, 1990). EJPs are blocked by various purinergic antagonists and hence it is generally believed that they are mediated by ATP released from nerves (Brock and Cunnane, 1993). Multiple EJPs can sum to initiate an active membrane response associated with the opening of voltage-operated calcium channels and smooth muscle contraction (Hirst et al., 1992). Therefore, modulation of ATP release from sympathetic nerves appears to play an important role in autonomic neurovascular control. The release of noradrenaline from sympathetic nerves is generally thought to be modulated pre-junctionally by autoinhibitory α_2-adrenoceptors. The issue of whether the release of ATP is also subject to modulation through α_2-adrenoceptors is still controversial. Thus, whereas some studies have shown such modulation at sympathetic postganglionic junctions (Brock and Cunnane, 1987; Brock et al., 1990), other studies have suggested that the α_2-adrenoceptors have either little (Driessen et al., 1993; Todorov et al., 1999) or no influence on the release of ATP (Sperlagh et al., 1998; Msghina et al., 1999) in different systems. Our experiments provide electrophysiological evidence that α_2 receptor-mediated modulation of ATP release occurs with short duration, low frequency nerve stimulation but not with high frequency nerve stimulation. Therefore, the presence of pre-junctional α_2-adrenoceptor modulation of ATP release might go undetected or underestimated when only high frequencies and/or long durations of nerve stimulation are employed.

In the present study, electrical field stimulation elicited frequency-dependent membrane depolarization consisting primarily of EJPs that were inhibited by PPADS at all stimulation frequencies, confirming the concept that EJPs are mediated by P2 receptors and hence are caused by ATP released during nerve stimulation. Suramin also reduced the responses to 1–32 Hz but the reduction was smaller than with PPADS. Interestingly, EJPs obtained at lower frequencies (i.e., 0.2–0.5 Hz) were actually facilitated by suramin. In addition to the known ability of suramin to antagonize purinergic receptors, this drug has also been reported to block ATP hydrolysis (Hourani and Chown, 1989). Thus, suramin has two opposing actions that can counteract one another. Indeed, in a previous study, we suggested that this was the case since suramin did not significantly affect the contractile response to exogenous ATP in guinea-pig mesenteric artery (Mutafova-Yambolieva et al., 2000). Interpretation of results with suramin is therefore complicated. It is possible that at lower stimulation frequencies the inhibitory effect of suramin on ecto-ATPases prevails whereas at higher frequencies the inhibitory effect on the P2 receptors by suramin predominates.

Both phentolamine and yohimbine increased the EJPs in response to 0.2–8 Hz, suggesting that presynaptic α_2 adrenoceptors limit the amount of ATP release that occurs at these stimulation frequencies. Interestingly, oxymethazoline, a selective α_2-adrenoceptor agonist, reduced EJPs only at very low frequencies of stimulation (i.e., 0.2 and 0.5 Hz). Furthermore, clonidine, another α_2-adrenoceptor agonist, did not affect responses at all frequencies tested. These observations taken together suggest that the amount of noradrenaline released at low frequencies of nerve stimulation is sufficient to activate the majority of α_2-adrenoceptors, limiting additional activation by α_2-adrenoceptor agonists. Likewise, the fact that responses to low frequency nerve stimulation were enhanced following reserpinization suggests that endogenous noradrenaline limits ATP release. One might expect therefore the α_2 adrenoceptor agonists to reduce the nerve evoked EJPs when NA stores are depleted. In the present study, clonidine indeed reduced the responses to 0.2–1 Hz in reserpine-treated preparations, whereas the responses to 2–32 Hz remain unaffected. It should be noted that responses

above 2 Hz are not potentiated in the reserpinized preparations as they are in control preparations with yohimbine. This could be due to incomplete depletion of NA stores during reserpine treatment. The results of our experiments with antagonists of α_2-adrenoceptors demonstrate the existence of heteroinhibition of ATP release by noradrenaline during low frequencies of nerve stimulation. However, as the frequency of nerve stimulation increases, this pathway no longer appears to play a role. Similarly, it is generally recognized that pre-junctional autoinhibition of noradrenaline release is more effective at lower frequencies of nerve stimulation (Langer, 1997).

In conclusion, the prejunctional modulation of ATP release by noradrenaline appears to be an important feedback mechanism at lower frequencies of stimulation. At higher frequencies (as might be expected during stress), this kind of regulation does not appear to play a significant role.

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