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Raised tone reveals purinergic-mediated responses to sympathetic nerve stimulation in the rat perfused mesenteric vascular bed

Poungrat Pakdeechote, Nicole M. Rummery, Vera Ralevic, William R. Dunn *

Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, University of Nottingham, Nottingham, NG7 2UH, United Kingdom

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Abstract

Noradrenaline and ATP are sympathetic co-transmitters. In rat isolated mesenteric small arteries, activation of sympathetic nerves can produce a vasoconstrictor response mediated by ATP. In contrast, the rat perfused mesenteric bed displays vasoconstrictor responses that are blocked solely by α_1-adrenoceptor antagonists. This study assessed the effect of raising tone with a vasoconstrictor on purinergic and noradrenergic responses to sympathetic nerve stimulation in the rat perfused mesentery. Rat mesenteric vascular beds were perfused with physiological salt solution and responses to nerve stimulation, or P2X-receptor agonists, were determined under basal conditions and after raising tone with endothelin-1. The contribution of noradrenaline and ATP to sympathetic nerve-mediated responses was assessed using the α_1-adrenoceptor antagonist, prazosin and the P2X-receptor desensitizing agent, α,β-methyleneATP. The effect of endothelin-1 on excitatory junction potentials generated in response to nerve stimulation in isolated mesenteric arteries was also assessed. Under baseline conditions, responses to nerve stimulation were mediated solely by activation of α_1-adrenoceptors. After raising perfusion pressure with endothelin-1 or the thromboxane mimetic 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U44619), sympathetic nerve-mediated responses were larger than under basal conditions and the response was partly sensitive to P2X-receptor desensitization. Responses to exogenous P2X-receptor agonists were enhanced after treatment with endothelin-1, while endothelin-1 decreased the amplitude of excitatory junction potentials. These results indicate that ATP acts as an important, functional, sympathetic neurotransmitter in the perfused mesentery under raised tone conditions, where the perfusion pressure is closer to that found in vivo. This effect is due to a postjunctional enhancement of purinergic function.

Keywords: Sympathetic nerve; Noradrenaline; ATP; P2X receptor; α_1-adrenoceptor; Perfused mesentery

1. Introduction

ATP and noradrenaline are co-stored and co-released from sympathetic neurons and can cause vasoconstriction by activating vascular P2X receptors and α_1-adrenoceptors respectively (Burnstock and Kennedy, 1985, Brock and Cunnane, 1999). There is now a substantial body of evidence indicating that both noradrenaline and ATP act as functional co-transmitters in many isolated large arteries including rabbit isolated mesenteric arteries (von Kugelgen and Starke, 1985), rat tail artery (Sneddon and Burnstock, 1984) and rabbit proximal saphenous artery (Burnstock and Warland, 1987).

The potential role for ATP as a functional sympathetic neurotransmitter in the resistance vasculature appears to depend upon the experimental methodology employed to study nerve-mediated responses, or the prevailing experimental conditions. In isometrically-mounted rat small mesenteric arteries, a small purinergic response to nerve stimulation was first suggested by Angus et al. (1988), although the major contribution (>90%) was provided by noradrenaline, acting via postjunctional α_1-adrenoceptors. Subsequently, Sjoblom-Widfeldt et al. (1990) showed that purinergic responses were more evident at low frequencies of nerve stimulation. More recently, Gitterman and Evans (2001) have provided evidence for ATP involvement in sympathetic nerve-mediated responses in Mg^{2+}-free modified physiological buffer. They suggested an increased role for activation of P2X receptors as the size of the mesenteric arteries decreased. Similarly, Luo et al. (2003) have shown that ATP is the principal

* Corresponding author. Tel.: +44 115 823 0188; fax: +44 115 823 0142.
E-mail address: William.dunn@nottingham.ac.uk (W.R. Dunn).

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sympathetic neurotransmitter in superfused small mesenteric arteries (<200 μm) in which changes in diameter in response to nerve stimulation were monitored using a video-tracking system.

While it is of interest to determine the involvement of ATP as a sympathetic neurotransmitter in isolated small arteries, it is also important to determine which sympathetic neurotransmitters are responsible for controlling vascular resistance in the whole organ. Most studies that have examined responses to sympathetic nerve stimulation in the isolated, perfused, mesenteric vascular bed have shown that the response is almost completely sensitive to α-adrenoceptor antagonists (Eikenburg, 1984; Kong et al., 1994; Williams and Clarke, 1995). Donoso et al. (1997) proposed a modest role for ATP as a sympathetic neurotransmitter in this vascular bed, while Yamamoto et al. (1992) showed that cooling could uncover a purinergic response to periarterial nerve stimulation. Given the available evidence indicating a role for P2X-receptor activation after nerve stimulation in rat isolated small mesenteric arteries, it is surprising that it has been relatively difficult to demonstrate a purinergic response in the perfused mesenteric vascular bed, which contains both small and large arteries.

In the present study, we have assessed the potential role of ATP as a sympathetic neurotransmitter in the rat mesenteric vascular bed under conditions where the baseline perfusion pressure was increased to a more physiological level using either endothelin-1 or the thromboxane mimetic, U46619.

2. Materials and methods

2.1. Mesenteric vascular bed preparations

Male Wistar rats (225–250 g) (Charles River Laboratory; Kent, UK) were killed by CO_2 overdose followed by exsanguination. The abdominal cavity was opened and the superior mesenteric artery was identified, cleaned of connective tissue and cannulated with a blunted hypodermic needle (No. 21). The superior mesenteric vein was cut and the preparation flushed with 0.5 ml Krebs' solution. The mesenteric vascular bed was separated from the gut by carefully cutting close to the intestinal wall. The preparation was then placed on a stainless steel grid (7×5 cm) in a humid chamber and perfused at a constant flow rate of 5 ml/min, using a peristaltic pump (model 7554–30, Cole-Parmer Instrument, Chicago, IL). Krebs' solution was composed of the following (mM): NaCl 118, $NaHCO_3$ 25, KCl 4.8, KH_2PO_4 1.2, $MgSO_4 \cdot 7H_2O$ 1.2, $CaCl_2$ 1.25, and glucose 11.1. The solution was maintained at 37 °C and continually gassed with a 95% O_2/5% CO_2 gas mixture.

Mesenteric vascular responses were detected as changes in perfusion pressure (mmHg). This was monitored continuously by way of a pressure transducer (model P23XL; Viggo-Spectramed, Oxnard, CA) and recorded using a powerlab (ADInstruments, Pty Ltd., Castle Hill, Australia). The preparation was allowed to equilibrate for 30 min before experimentation. Electrical field stimulation (5–50 Hz, 90 V, 1 ms for 30 s at 8 min intervals) was applied with a Grass SD9 stimulator, which passed a current between the hypodermic needle with which the preparation was cannulated and the wire grid on which the preparation rested.

2.2. Responses to electrical field stimulation in the rat perfused mesenteric vascular bed under basal tone conditions

After an initial 30 min equilibration period, responses to electrical field stimulation (10–50 Hz, 90 V, 1 ms for 30 s) were determined at 8 min intervals. A second frequency response curve was generated after a further 30 min and a third frequency response curve after a subsequent 30 min. These experiments acted as the time control. In some experiments prazosin (0.1 μM), an α_1-adrenoceptor antagonist, was added after the first frequency response curve, while the combination of prazosin plus α,β-methyleneATP (1 μM), a $P2X_1$ purinoceptor agonist and desensitizing agent, was applied after the second frequency response curve. α,β-methyleneATP acts at both $P2X_1$ and $P2X_3$ receptors, but there is a differential expression of these receptors in different tissues; $P2X_1$ is the main subtype causing vasocontraction of vascular smooth muscle, and $P2X_3$ is expressed on sensory nerves (Ralevic and Burnstock, 1998). In the rat mesenteric arterial bed, contractile responses to α,β-methyleneATP desensitize, and this blocks contractile responses to ATP, indicating desensitization of $P2X_1$ receptors (Ralevic and Burnstock, 1988). In other experiments the order of exposure to prazosin or α,β-methyleneATP was reversed.

2.3. Effects of raising tone with endothelin-1 on contractile response to electrical field stimulation in rat perfused mesenteric vascular beds

Endothelin-1 (1.5–2 nM) was added to raise the tone in each preparation (20–50 mmHg above baseline) prior to generating nerve-mediated contractile responses to electrical field stimulation in each group. After raising tone, three consecutive frequency response curves were obtained, separated by 30 min. These provided time control data. In a further two groups of experiments the sequential effects of (a) prazosin followed by prazosin plus α,β-methyleneATP or (b) α,β-methyleneATP followed by prazosin plus α,β-methyleneATP were investigated in preconstricted mesenteric arterial beds after obtaining an initial control frequency response curve.

2.4. Effects of capsaicin treatment on neurogenic responses under raised tone conditions

Because a biphasic response to electrical field stimulation was obtained under endothelin-1-induced raised tone conditions, preparations were pre-treated with capsaicin (1 μM) for 20 min, followed by a 15 min washout period, to deplete the sensory nerves of their neurotransmitters (Dunn et al., 2003). Thereafter endothelin-1 was added to induce tone and contractile responses to electrical field stimulation of the rat isolated mesenteric vascular bed were obtained under the following conditions: (a) prazosin followed by prazosin plus α,β-methyleneATP or (b) α,β-methyleneATP followed by α,β-methyleneATP plus prazosin or (c) time control. In addition, in a separate series of the experiments, 9,11-dideoxy-11α,9α-epoxymethanoprostaglandin $F_{2\alpha}$ (U46619; 50–70 nM) was