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

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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

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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

used as the agent to induce raised vascular tone (20–50 mmHg above baseline) and the experiments described above repeated.

2.5. Effects of raising tone with endothelin-1 on contractile response to exogenous P2X-receptor agonists in rat perfused mesenteric vascular beds

After capsaicin pre-treatment, dose–response curves (in 3-fold increments) were generated to either ATP or α,β-methyleneATP under baseline perfusion conditions. Separate experiments were conducted to determine dose–response relationships for ATP and α,β-methyleneATP after raising tone with endothelin-1.

2.6. Isolated mesenteric small arteries

For these experiments, male Wistar rats were stunned by a blow to the cranium and killed by bleeding. After removal of the mesentery, segments of secondary mesenteric artery and vein were dissected and cleaned of adherent connective tissue. The proximal end of the mesenteric artery and vein was tied off and drawn into a suction stimulating electrode allowing excitation of perivascular nerves by electrical field stimulation. Intracellular recordings of membrane potential and excitatory junction potentials from smooth muscle cells were measured using sharp microelectrodes (120–200 MΩ) filled with 0.5 M potassium chloride and connected to an Axoclamp 2A (Axon instruments) as previously described (Brock and Van Helden, 1995). Membrane potential and excitatory junction potentials were measured in response to single pulse stimulation or trains of 5 stimuli at 0.5 and 5 Hz (15 V, 0.5 ms), in the presence and absence of endothelin-1 (2 nM). In all experiments, recordings were made from single cell impalements.

2.7. Drugs

Endothelin-1 (human, porcrine) was obtained from TOCRIS, Bristol USA. Prazosin, α,β-methyleneATP, 8-methyl-*N*-vanillyl-6-nonenamide (capsaicin), and U46619 were obtained from Sigma, St. Louis, USA. Capsaicin and U46619 were dissolved in ethanol and all other drugs were dissolved in distilled water.

2.8. Data analysis

Data are presented as mean ± S.E.M. Statistical comparisons between frequency response curves were made using 2-way analysis of variance (ANOVA) with a Bonferroni's post-hoc test to assess differences between individual points on the curve. Other comparisons were made using a Student's paired *t*-test. A value of $P < 0.05$ was taken to indicate statistical significance.

3. Results

3.1. Responses to nerve stimulation in the rat perfused mesenteric vascular bed under basal and raised tone conditions

Under basal tone conditions (baseline pressure = 22 ± 1.7 mmHg, $n = 18$), electrical field stimulation produced an increase in perfusion pressure that was frequency-dependent (Fig. 1). Pre-treatment with endothelin-1 (1.5–2 nM) significantly increased the perfusion pressure of preparations from 23 ± 1.3 mmHg to 51 ± 3.9 mmHg ($P < 0.05$, Student's paired *t*-test) ($n = 19$). Under these conditions, the response to electrical field stimulation was biphasic reflecting an initial pressor followed by a depressor response. Nonetheless, the size of the increase in perfusion pressure in response to sympathetic nerve stimulation of preparations after raising tone with endothelin-1 was significantly larger than those under basal tone conditions (Fig. 1). In capsaicin pre-treated preparations (endothelin-1-induced perfusion pressure = 47 ± 2.1 mmHg, $n = 18$) there was a larger response to electrical field stimulation when compared to responses with endothelin-1-induced raised tone alone and the depressor response was no longer apparent (Fig. 1).

3.2. Role of α_1-adrenoceptors and $P2X_1$-purinergic receptors in nerve-mediated contractile responses in rat perfused mesenteric vascular beds under basal conditions

Three consecutive frequency response curves to electrical field stimulation were reproducible under basal conditions ($n = 6$) (Fig. 2A). Under these conditions (baseline pressure = 17 ± 1.0 mmHg) ($n = 6$), prazosin (0.1 μM) almost abolished the pressor response to electrical field stimulation (10–40 Hz) ($P < 0.05$, ANOVA) (Fig. 2B) ($n = 6$). α,β-methyleneATP (1 μM) produced a transient increase in perfusion pressure, but had no effect on nerve induced increases in perfusion pressure in rat isolated mesenteric vascular beds (Fig. 2C).

3.3. Effects of raising tone with endothelin-1 on nerve-mediated contractile response in the rat isolated mesenteric vascular bed

Under endothelin-1-induced raised tone conditions, three consecutive frequency response curves to electrical field stimulation were reproducible (data not shown) ($n = 6$). Under these conditions, prazosin (0.1 μM) produced a marked inhibition of the pressor response to sympathetic nerve stimulation at all frequencies ($P < 0.05$, ANOVA) (Fig. 3A). The small,

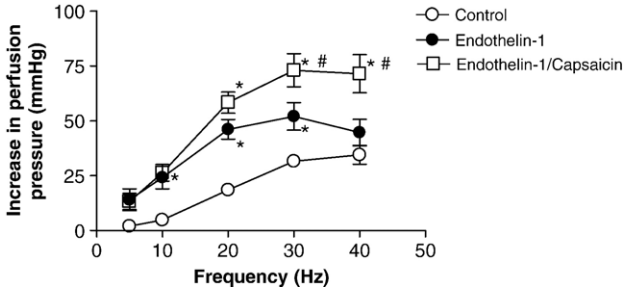

Fig. 1. Pressor responses to electrical field stimulation in the absence (control, $n = 5$) (5–40 Hz, 1 ms, 90 V, 30 s), and presence of endothelin-1 (1.5–2 nM)-induced tone ($n = 7$) and after inducing tone with endothelin-1 (1.5–2 nM) following capsaicin (1 μM) pre-treatment ($n = 6$) in rat isolated mesenteric vascular beds. Data are shown as mean ± standard error. $^*P < 0.05$ vs. control, $^{\#}P < 0.05$ vs. endothelin-1 group.

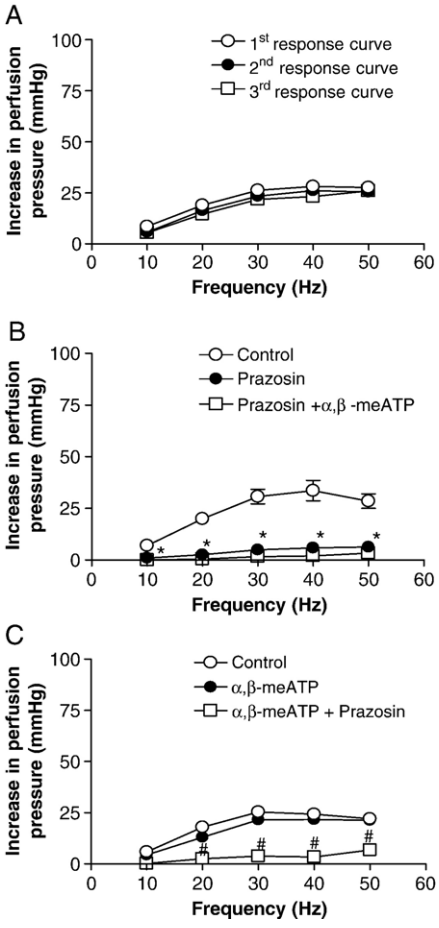

Fig. 2. Effects of A) time ($n=6$), B) the sequential addition of prazosin (0.1 μM), and α,β-methyleneATP (1 μM) ($n=6$) or C) the sequential addition of α,β-methyleneATP followed by prazosin ($n=6$) on pressor responses to electrical field stimulation (10–50 Hz, 1 ms, 90 V, 30 s) of rat isolated mesenteric vascular beds under basal tone conditions. Data are shown as mean± standard error. *$P<0.05$ vs. control, #$P<0.05$ vs. control and α,β-methyleneATP groups.

variable residual pressor response was further reduced in the presence of α,β-methyleneATP (1 μM) (Fig. 3A). α,β-methyleneATP alone caused a significant reduction in the increase in perfusion pressure to electrical field stimulation at frequencies between 10 and 30 Hz ($n=7$) ($P<0.05$, ANOVA) (Fig. 3B). The residual response to electrical field stimulation after α,β-methyleneATP was abolished by prazosin ($P<0.05$, ANOVA) (Fig. 3B).

Under endothelin-1-induced raised tone conditions and after capsaicin pre-treatment, prazosin (0.1 μM) reduced, but did not abolish, the pressor response to electrical field stimulation ($P<0.05$, ANOVA) (Fig. 4A) ($n=6$). The residual response was virtually abolished by α,β-methyleneATP (1 μM) ($n=6$) ($P<0.05$, ANOVA) (Fig. 4A). After capsaicin pre-treatment and under endothelin-1-induced tone conditions, responses evoked by electrical field stimulation were significantly attenuated by α,β-methyleneATP alone ($P<0.05$, ANOVA) (Fig. 4B). Prazosin blocked the residual response to nerve stimulation in the presence of α,β-methyleneATP ($P<0.05$, ANOVA) (Fig. 4B).

3.4. Effects of raising tone with U46619 on nerve-mediated contractile response in the rat isolated mesenteric vascular bed

In capsaicin pre-treated preparations, U46619 (50–70 nM) significantly increased perfusion pressure (from 26±0.8 to 65±3.1 mmHg, $P<0.05$, Student's paired t-test) ($n=18$). The size of the pressor response to nerve stimulation was significantly greater than under basal tone conditions (at 30 Hz from 27±1.7 to 56±5.6 mmHg ($P<0.05$, ANOVA followed by Bonferroni's post-hoc test) ($n=18$). Responses were reproducible for three consecutive frequency response curves ($n=6$) (data not shown). Prazosin significantly reduced the pressor response to nerve stimulation after inducing tone with U46619 ($P<0.05$, ANOVA) ($n=6$). The small residual response was further reduced by α,β-methyleneATP (Fig. 5A). α,β-methyleneATP alone caused a significant reduction in the pressor response evoked by electrical field stimulation at 20 and 30 Hz ($n=6$) ($P<0.05$, ANOVA). The residual response to nerve stimulation was abolished by prazosin ($P<0.05$, ANOVA) (Fig. 5B).

3.5. Effect of raising tone with endothelin-1 on responses to exogenous ATP and α,β-methyleneATP

Under basal tone conditions (perfusion pressure=15.9± 2.3 mmHg), exogenous ATP produced dose-dependent vasoconstrictor responses ($n=4$). Endothelin-1 infusion significantly increased perfusion pressure (from 16.4±2.3 to 61.5±5.7 mmHg, $n=6$, $P<0.05$, Student's paired t-test). Under these conditions ATP produced a biphasic response, an initial pressor response followed by a depressor response. The pressor response to ATP

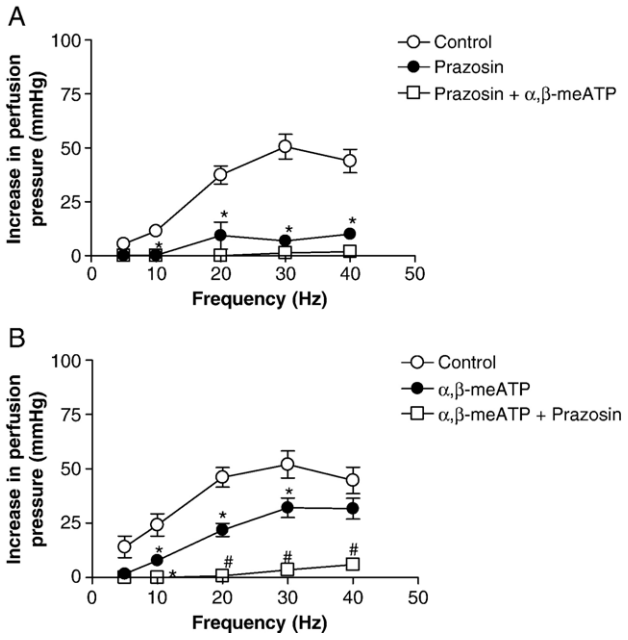

Fig. 3. Effects of sequential addition of A) prazosin (0.1 μM) and α,β-methyleneATP (1 μM) ($n=6$) or B) α,β-methyleneATP followed by prazosin ($n=7$) on pressor responses to electrical field stimulation (5–40 Hz, 1 ms, 90 V, 30 s) of rat isolated mesenteric vascular beds under endothelin-1 (1.5–2 nM)-induced raised tone conditions. Data are shown as mean±standard error. *$P<0.05$ vs. control, #$P<0.05$ vs. control and α,β-methyleneATP groups.

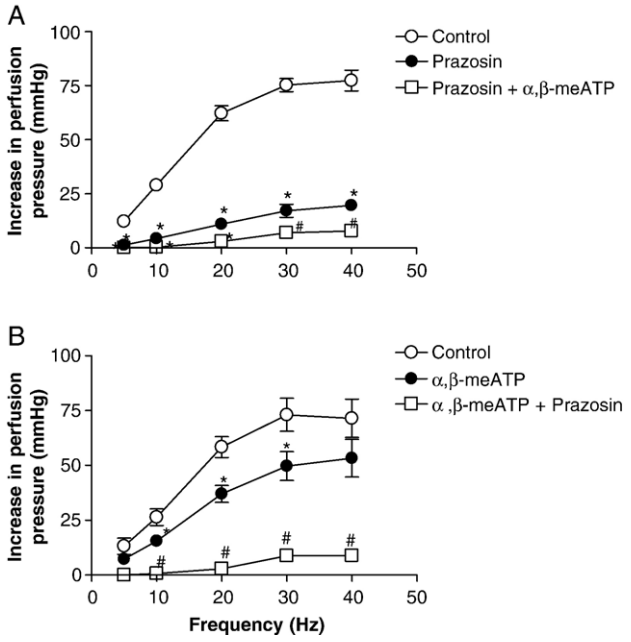

Fig. 4. Effects of sequential addition of A) prazosin (0.1 μM) and α,β-methyleneATP (1 μM) ($n=6$) or B) α,β-methyleneATP followed by prazosin ($n=6$) on pressor responses to electrical field stimulation (5–40 Hz, 1 ms, 90 V, 30 s) of rat isolated mesenteric vascular beds under endothelin-1 (1.5–2 nM)-induced raised tone conditions and after capsaicin (1 μM) pre-treatment. Data are shown as mean±standard error. *$P<0.05$ vs. control, #$P<0.05$ vs. control and α,β-methyleneATP groups.

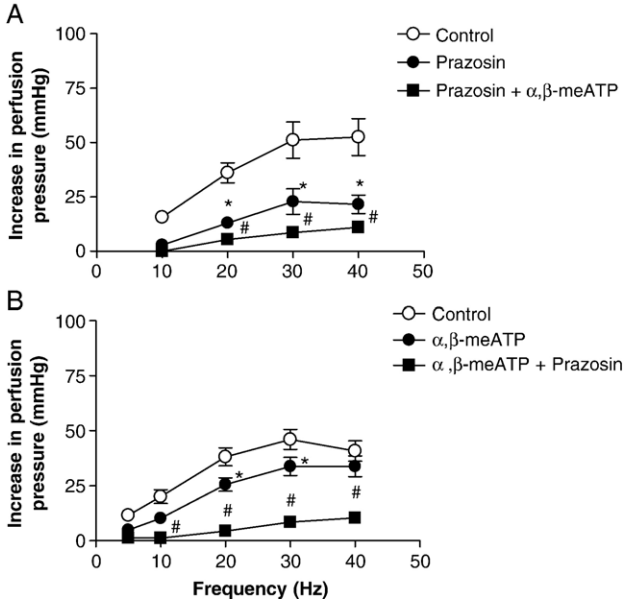

Fig. 5. Effects of the sequential addition of A) prazosin (0.1 μM) and α,β-methyleneATP (1 μM) ($n=6$) or B) α,β-methyleneATP followed by prazosin ($n=6$) on pressor responses to electrical field stimulation (5–40 Hz, 1 ms, 90 V, 30 s) of rat isolated mesenteric vascular beds under U46619 (50–70 nM)-induced raised tone condition and after capsaicin (1 μM) pre-treatment. Data are shown as mean±standard error. *$P<0.05$ vs. control, #$P<0.05$ vs. control and α,β-methyleneATP groups.

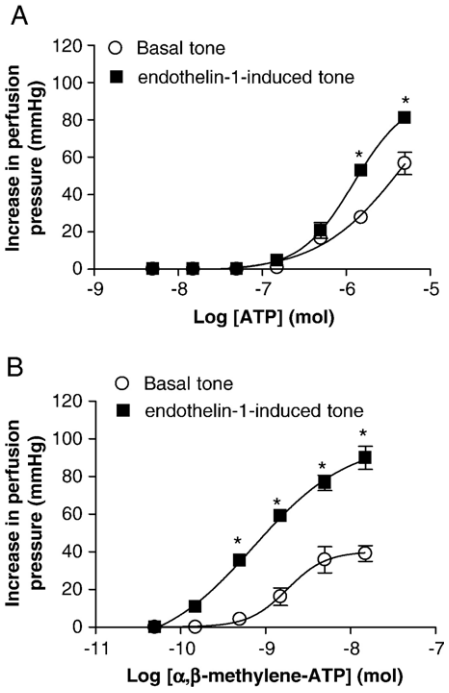

Fig. 6. Effects of raising tone with endothelin-1 on responses to exogenous application of (A) ATP ($n=4$) or (B) α,β-methyleneATP ($n=4$–5). Data are shown as mean±standard error. *$P<0.05$ vs. control.

was enhanced in the presence of endothelin-1-induced tone (Fig. 6A). α,β-methyleneATP also produced a dose-dependent pressor response after raising tone with endothelin-1 (perfusion pressure=62.4±8.7 mmHg, $n=5$) that was significantly bigger than those produced under basal tone conditions (perfusion pressure=17.3±1.5 mmHg, $n=4$, $P<0.05$, ANOVA) (Fig. 6B).

3.6. Electrophysiology

Under control conditions, the resting membrane potential of the smooth muscle cells was −56±1 mV ($n=6$). In the presence of endothelin-1 (2 nM) the resting membrane potential was significantly more positive compared to control (−48±1 mV, $n=6$, $P<0.05$, Student's paired t-test).

Stimulation of perivascular sympathetic nerves evoked excitatory junction potentials with a duration of approximately 1 s. In the presence of endothelin-1, the amplitude of excitatory junction potentials evoked by trains of five stimuli at 0.5 and 5 Hz was significantly reduced (Fig. 7, $P<0.05$, $n=6$, Student's paired t-test). The time course of excitatory junction potentials was not affected at either frequency.

4. Discussion

The main findings of the present study are that inducing tone with either U46619 or endothelin-1, uncovered a purinergic response to nerve stimulation that was absent under basal conditions in the rat perfused mesenteric vascular bed. Further investigations showed that endothelin-1 enhanced postjunctional activation of P2X receptors but reduced the amplitude of excitatory junction potentials produced as a consequence of the

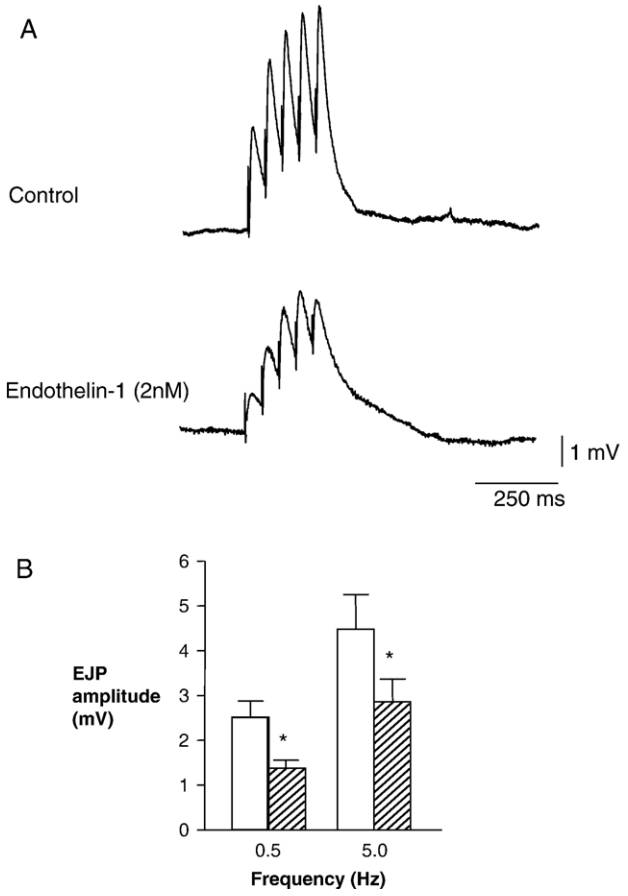

Fig. 7. The effect of endothelin-1 on excitatory junction potential amplitude in smooth muscle cells of the rat mesenteric artery. (A) Excitatory junction potentials produced by trains of 5 pulses at 5 Hz in the presence and absence of endothelin-1 (2 nM). (B) The effect of endothelin-1 (2 nM) on the amplitude of the 5th excitatory junction potential produced in a train of 5 excitatory junction potentials at 0.5 and 5 Hz. *$P<0.05$, significantly different to control ($n=6$). Control (open bars), endothelin-1 (hatched bars).

release of ATP from sympathetic nerve varicosities. These observations suggest that under conditions of partial vasoconstriction, similar to prevailing conditions in vivo, ATP acts as a functional sympathetic neurotransmitter in the rat mesentery.

Under basal tone conditions, we showed that responses to nerve stimulation were susceptible to α_1-adrenoceptor blockade by prazosin, but resistant to P2X-receptor desensitization with α,β-methyleneATP, indicating that they were solely mediated by activation of postjunctional α_1-adrenoceptors (see also Eikenburg, 1984; Kong et al., 1994; Williams and Clarke, 1995). Raising tone with endothelin-1 produced a biphasic response to nerve stimulation resulting in an initial pressor followed by a depressor response. Capsaicin pre-treatment eliminated the depressor response and augmented the size of pressor responses to electrical field stimulation. This data is consistent with the well established observation that mesenteric arteries are supplied by sensory nerves expressing TRPV1 receptors for capsaicin, and their activation causes vasorelaxation, mediated by calcitonin gene-related peptide (Maggi and Meli, 1988; Kawasaki et al., 1988), and that pre-treatment with capsaicin augments the pressor response to electrical field stimulation (Li and Duckles, 1992, Ralevic and Kendall, 2002). The pronounced increase in the size of pressor responses to nerve stimulation produced by endothelin-1 in the rat mesentery has been shown previously (Tabuchi et al., 1990). These authors concluded that this was a consequence of postjunctional enhancement of responses to noradrenaline, and that this occurred despite endothelin-1 causing a decrease in noradrenaline release by a pre-junctional mechanism (Tabuchi et al., 1989, 1990). We have shown additionally, that after inducing tone with endothelin-1, part of the response to sympathetic nerve activation was susceptible to the desensitizing action of α,β-methyleneATP and hence was mediated via activation of P2X receptors. Our data are consistent with a postjunctional enhancement of purinergic responses produced by endothelin-1 since this agent increased the size of the pressor response to exogenous ATP and of α,β-methyleneATP. A similar postjunctional enhancement of P2X-receptor function, produced by endothelin-1, has been reported in non-vascular smooth muscle preparations (Lau et al., 1995; Luciano et al., 1998).

The postjunctional enhancement of purinergic nerve-mediated responses occurred despite the fact that endothelin-1 decreased the amplitude of the excitatory junction potentials obtained to single pulse stimulation and to trains of 5 pulses at 0.5 and 5 Hz. The reduced excitatory junction potential amplitude may reflect a decreased release of ATP, or an alteration in the transfer of charge through the ionotropic P2X receptor. The fact that endothelin-1 acts to decrease the release of NE (Tabuchi et al., 1989, 1990) suggests that the release of the co-transmitter ATP from sympathetic nerves in the rat mesentery may also be decreased, by a pre-junctional action.

The mechanism whereby raising vascular tone uncovers a nerve-mediated purinergic response is unknown. However, the effect is not specific to endothelin-1. Raising tone with the thromboxane receptor agonist, U46619 (Shaw et al., 2004), produced a very similar profile to the effects of raising tone with endothelin-1, i.e. larger pressor responses to electrical field stimulation and greater susceptibility of the response to the actions of α,β-methyleneATP. This raises the possibility that any mechanism that increases vascular smooth muscle excitability will increase the prominence of ATP as a functional sympathetic neurotransmitter. Both endothelin-1 (present study and see also: Van et al., 1988; Rubanyi and Polokoff, 1994) and U46619 (Shaw et al., 2004, Crane and Garland, 2004) have been shown to cause vascular smooth muscle depolarization and to increase intracellular calcium levels. Thus, in the presence of endothelin-1 or U46619, resting membrane potential is more positive than under basal conditions, such that when ATP activates P2X receptors, the associated further depolarization increases the open probability of voltage-sensitive Ca^{2+} channels to allow contractions that were not evident when membrane potential was more negative. Alternatively, endothelin-1 also increases the production of a number of second messengers principally increasing the levels of inositol 1,4,5-triphosphate, diacylglycerol and protein kinase C activity which may alter the sensitivity of the contractile machinery and allow responses mediated via P2X receptors to become apparent (Rubanyi and Polokoff, 1994). Whatever the

mechanism, our observations suggest that raising mesenteric perfusion pressure levels closer to those experienced in vivo, increases the physiological importance of ATP as a sympathetic neurotransmitter.

In summary, we found that only α_1-adrenoceptors mediate pressor responses to sympathetic nerve stimulation under basal tone conditions in the rat mesenteric arterial bed. Pre-treatment with endothelin-1 or U46619, which increased the perfusion pressure, showed that ATP can act as a functional neurotransmitter in the perfused rat mesenteric vasculature.

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