The distribution of γ -adrenoceptors and P_2 purinoceptors in mesenteric arteries and veins of the guinea-pig

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1 Membrane potential changes and contractions were recorded from mesenteric arteries and veins of the guinea-pig, during perivascular nerve stimulation or application of noradrenaline or adenosine triphosphate (ATP).

2 After α -adrenoceptor blockade, noradrenaline activated low affinity adrenoceptors (γ -adrenoceptors) causing depolarization and arterial contraction only in the presence of an inhibitor of catecholamine uptake.

3 Noradrenaline did not cause depolarization or contraction of the vein after α -adrenoceptor blockade even after catecholamine uptake was blocked.

4 Adenosine triphosphate caused depolarization and contraction of both arteries and veins. These responses were abolished by α -, β -,methylene adenosine triphosphate (Me-ATP).

5 Me-ATP abolished rapid excitatory junction potentials (e.j.ps) caused by perivascular nerve stimulation of arteries but had no effect on arterial responses mediated by γ -adrenoceptors.

6 In veins, perivascular nerve stimulation evoked slow e.j.ps which persisted in the presence of Me-ATP but were abolished after blockade of α -adrenoceptors.

7 The observations indicate that P_2 purinoceptors are present on both mesenteric artery and vein whilst γ -adrenoceptors are localized near the neuromuscular junction of the artery. However γ -adrenoceptors do not appear to be directly involved in the generation of arterial e.j.ps.

Introduction

In a number of arterial beds, a part of the response to sympathetic nerve stimulation persists in the presence of both α - and β -adrenoceptor antagonists. Examples are the neurally activated vasoconstrictions of rabbit mesenteric and saphenous arteries (Kügelgen & Starke, 1985; Burnstock & Warland, 1987), dog mesenteric arteries (Muramatsu, 1986) and the increase in peripheral resistance of rabbit hindlimbs (Hirst & Lew, 1987).

Sympathetic nerve stimulation initiates a rapid excitatory junction potential (e.j.p.) in most mammalian systemic arteries (Hirst, 1977; Holman & Surprenant, 1979; Surprenant, 1980; Kuriyama & Suzuki, 1981; Kuriyama & Suyama, 1983; Kreulen, 1986). If the e.j.p. is sufficiently large or if successive e.j.ps are summed together, the depolarization leads to the activation of voltage-dependent calcium channels and constriction follows (Hirst & Edwards, 1988). In all systemic arteries studied, e.j.ps have

been found to be resistant to α -adrenoceptor blockade but are abolished or reduced in amplitude by drugs interfering with neuronal release (Holman & Surprenant, 1979; 1980; Hirst & Neild, 1980; Cheung, 1982; Fujiwara *et al.*, 1982; Hirst *et al.*, 1982; Suzuki & Kou, 1983).

Two hypotheses have been suggested to explain the non α -adrenoceptor-mediated e.j.ps in systemic arteries. One suggests that released noradrenaline activates specialized junctional adrenoceptors termed γ -adrenoceptors which are located close to sympathetic nerve terminals (Hirst & Neild, 1980; 1981; Hirst *et al.*, 1982; Luff *et al.*, 1987). The other suggests that adenosine triphosphate (ATP), or a closely related compound, co-released with noradrenaline from the nerve terminal acts on P_2 purinoceptors to produce the rapid e.j.p. (Sneddon & Burnstock, 1984; Kügelgen & Starke, 1985; Ishikawa, 1985). This hypothesis is supported by the finding that α -, β -,methylene ATP (Me-ATP), a stable analogue of ATP desensitizes P_2 purinoceptor effects

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of ATP and blocks e.j.ps (Sneddon & Burnstock 1984; Ishikawa, 1985).

In contrast to arteries, rapid e.j.ps are not initiated by sympathetic nerve stimulation in veins, perhaps with the exception of dog mesenteric and rat saphenous veins (Cheung, 1981; Suzuki, 1984). Rather, repetitive sympathetic nerve stimulation typically produces a depolarization which is slow in onset and very long lasting (Suzuki, 1981; Kreulen, 1986). These slow e.j.ps are associated with contraction. Both the slow e.j.p. and the contraction are abolished by α -adrenoceptor antagonists (Suzuki, 1981; Kreulen, 1986).

The experiments described in this paper were designed to examine the distribution of γ -adrenoceptors and purinoceptors on mesenteric arteries and veins. The nature of the response to sympathetic nerve stimulation was also determined. It was found that, whereas γ -adrenoceptors were restricted to mesenteric arteries, P_2 receptors were present on both arterial and venous tissues. γ -Adrenoceptor responses in mesenteric arteries persisted in the presence of Me-ATP; γ -adrenoceptors are therefore unlikely to be involved in the initiation of e.j.ps.

Methods

Experiments were carried out on mesenteric arteries and veins arising from the superior mesenteric artery or vein of guinea-pigs (male or female, 150–300 g). Animals were stunned and exsanguinated. Sections of small intestine with attached arcades of mesenteric arteries and veins were rapidly removed and placed in a dissecting dish containing physiological saline (composition mM: NaCl 120, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 2.0, NaH_2PO_4 0.1, $NaHCO_3$ 25 and glucose, 11; gassed with 95% O_2 and 5% CO_2).

Electrical recordings

Lengths (1 cm) of artery and adjacent vein were removed from the arcade and pinned side by side in a recording chamber (volume 0.2 ml). To obtain rapid changes in bath composition a rapid-action two-way valve (Polyplan, Glen Waverly, Vic.) was placed adjacent to the bath (see Hirst *et al.*, 1982). In some experiments a larger volume chamber (1 ml, see Hirst, 1977) was used to enable a stimulating electrode to be placed around the proximal ends of the artery and vein. Perivascular nerves were stimulated at supramaximal parameters (20–60 V, 0.2–0.5 ms pulse width). Preparations were superfused at a rate of 6 ml min^{-1} with gassed physiological saline warmed to 37°C.

Intracellular recordings were made with micro-electrodes pulled from fibre-containing glass tubes (Clark electromedical, GC150F-15) with Brown-Flaming micropipette puller (model P.77, Sutter Instrument Co; San Francisco, California) and filled with 0.5 M KCl. Electrodes with resistances in the range 80–160 M Ω were used. Recordings were digitized and stored on disc for later analysis. Data acquisition and analysis were performed using programmes written under the DAOS software package (version 7.0, Laboratory Software Associates).

Mechanical recordings

Lengths of artery and vein of 5 mm were used for tension records. Vessels were attached to two perspex 'feet' by means of 50 μm tungsten wire (Goodfellow Metals, Cambridge) placed through the lumen. One foot was attached to a micromanipulator (Narashige model mm-3) whilst the other was attached to a Kistler-Morse deflection sensor (DSC-46.1003.01) for recording isometric changes in force. The voltage signal was amplified and filtered using a strain gauge conditioner (Analog devices model 2B31J) and the output was recorded on a pen recorder (Riken Denshi model SP-GC3). A resting tension of 500 mg was maintained on arteries and 200 mg on veins. Vessels were left to equilibrate for a period of 30 min before experiments were begun. Physiological saline, as used in the electrophysiological studies, was warmed to 35°C and flowed through a 5 ml bath at 7 ml min^{-1} .

Irreversible blockade of α -adrenoceptors was achieved by perfusing preparations with 10 μM benextramine (BNX) for 30 min followed by a 30 min wash period before recordings were made (Melchiorre *et al.*, 1978; Hirst & Lew, 1987).

Drugs used were: adenosine 5' triphosphate (ATP), α -, β -methylene adenosine 5' triphosphate (Me-ATP), benextramine hydrochloride, noradrenaline bitartrate ((-)-arterenol) (all obtained from Sigma chemicals), bretylium tosylate (Serva), desmethylinipramine (free base) (Ciba Geigy).

Results

Responses to sympathetic nerve stimulation recorded from mesenteric arteries and veins

The resting membrane potential of mesenteric arterial smooth muscle cells was $-66 \pm 0.6\text{ mV}$ ($n = 87$). When arterial perivascular nerves were stimulated, e.j.ps were initiated. Single supramaximal stimuli evoked an e.j.p. with a peak amplitude of $3.9 \pm 0.4\text{ mV}$, a rise time of $47.8 \pm 1.5\text{ ms}$ and a time constant of decay of $295 \pm 35.9\text{ ms}$ ($n = 6$) (Figure 1a). E.j.ps were not associated with a visible contrac-

Figure 1 E.j.ps recorded from a mesenteric artery following sympathetic nerve stimulation. The record shown in (a) illustrates the response to successive supra-maximal perivascular stimuli at a frequency of 1 Hz. The lower record (b) shows that the e.j.ps are abolished during prolonged superfusion with a solution containing $10\ \mu\text{M}$ Me-ATP. Calibration bars apply to both records.

tion. When short trains of stimuli (20 Hz) were applied, e.j.ps summed together and triggered an action potential. This in turn initiated arterial constriction. The irreversible α_1/α_2 -adrenoceptor antagonist benextramine ($10\ \mu\text{M}$) did not alter the

Figure 2 Depolarizations of mesenteric veins produced by stimulation of sympathetic nerves. In all three records, perivascular nerves were stimulated at a frequency of 20 Hz for 750 ms with supramaximal voltages. The record in (a) shows the effect of stimulation in control solution. The middle record (b) shows the depolarizations are little affected by prolonged incubation in $10\ \mu\text{M}$ Me-ATP. The lower record (c) shows that the response to nerve stimulation is abolished after incubation in $10\ \mu\text{M}$ benextramine (BNX).

Figure 3 Noradrenaline (NA) responses in benextramine (BNX) treated mesenteric arteries and veins. Record (a) shows perfusion with NA after BNX treatment alone. The record in (b) shows that perfusion with the uptake inhibitor desmethylimipramine (DMI) reveals a depolarization in response to NA perfusion. Record (c) shows that incubation in Me-ATP has little effect on this depolarizing response to NA. Application of NA to veins in the presence of DMI (record d) produces little response. Perfusion times are shown by bars. Calibration bars apply to all records.

size of arterial e.j.ps (amplitude = $4.0 \pm 0.2\ \text{mV}$, $n = 5$), whilst the adrenergic neurone blocker bretylium ($10\ \mu\text{M}$) abolished e.j.ps after approximately 30 min.

Venous smooth muscle cells had a resting membrane potential of $-70 \pm 0.9\ \text{mV}$ ($n = 23$). A single stimulus applied to the perivascular nerves of veins failed to initiate either a rapid e.j.p or any other detectable depolarization. Repetitive nerve stimulation ($> 10\ \text{Hz}$) caused a slow e.j.p. which usually showed two components, an initial peak followed by a slower depolarization (Figure 2a). At fixed frequencies this slow e.j.p. increased in amplitude with increasing stimulus voltages until a maximum was reached. Further amplitude increases could be achieved by then increasing stimulus frequency. Stimulation at 20 Hz for 750 ms at supramaximal voltages initiated a slow e.j.p. in which the initial component was larger with an amplitude of $8.9 \pm 1.9\ \text{mV}$ ($n = 6$) (Figure 2a). The slow e.j.p. and contraction following venous nerve stimulation was abolished by $10\ \mu\text{M}$ benextramine ($n = 4$) (Figure 2c).

Distribution of γ -adrenoceptors and P_2 receptors on mesenteric arteries and veins

Noradrenaline ($1\text{--}10\ \mu\text{M}$), applied by superfusion, caused contraction of both arteries and veins. In both tissues these responses were prevented by incubating the tissues in benextramine. In either tissue, increasing the concentrations of noradrenaline up to $5\ \text{mM}$ failed to produce a detectable depolarization but occasionally produced a small hyperpolarization (Figure 3a). After the addition of the neuronal amine

Figure 4 Effect of adenosine triphosphate (ATP) on the membrane potential of mesenteric arteries and veins. The upper record (a) shows a rapid depolarization in response to $50 \mu\text{M}$ ATP. The middle record (b) shows that the response to 0.5 mM ATP is abolished by incubation in $10 \mu\text{M}$ Me-ATP. The lower record (c) shows a rapid depolarization to $50 \mu\text{M}$ ATP in a vein. Bars indicate perfusion time. Calibration bars apply to all records.

uptake blocker desmethylinipramine (DMI) ($1 \mu\text{M}$), noradrenaline ($>0.2 \text{ mM}$) caused arterial depolarization and contraction (Figure 3b). The amplitude of depolarization varied between preparations and electrodes were often dislodged due to contraction. The mean depolarization produced by noradrenaline (0.5 mM) was $12.6 \pm 1.4 \text{ mV}$ ($n = 12$).

Application of very high concentrations of noradrenaline to veins after benextramine treatment did not lead to a membrane potential change ($n = 4$). However, unlike arteries the addition of desmethylinipramine did not reveal an α -antagonist-resistant response (Figure 3d).

ATP ($>0.1 \text{ mM}$) caused substantial membrane depolarization in both arteries and veins (Figure 4a,c) with 0.5 mM ATP causing $18 \pm 4 \text{ mV}$ ($n = 5$) depolarization in arteries and $23 \pm 4 \text{ mV}$ ($n = 3$) for veins. These depolarizations often caused action potentials and were associated with rapid contractions which usually dislodged the electrodes.

Mechanical responses

Contraction of arteries to exogenous noradrenaline had a threshold of $5 \mu\text{M}$ (Figure 5a). After benextramine treatment in the presence of desmethylinipramine, arteries proved unresponsive to even high concentrations (1 mM) of noradrenaline if the concentration was allowed to rise slowly. Rapid contractions were produced if the bath concentration of noradrenaline was rapidly increased to its final concentration (0.5 – 2 mM) by microinjection into the bath (Figure 5a) (cf. Byrne & Large, 1986). Contractions

produced by 1 mM noradrenaline were $1099 \pm 252 \text{ mg}$, ($n = 6$). ATP (1 mM) also produced a rapid contraction although never as large as that to noradrenaline ($678 \pm 135 \text{ mg}$, $n = 5$) (Figure 5a).

Veins contracted with much less force than arteries. The maximal contraction caused by increasing the potassium concentration to 70 mM was $254 \pm 26 \text{ mg}$, $n = 8$ (Figure 5b). Noradrenaline (0.1 mM) caused a contraction of $127 \pm 18 \text{ mg}$ ($n = 7$) (Figure 5b). This contraction was abolished by incubation in $10 \mu\text{M}$ benextramine. After incubation in benextramine and in the presence of DMI, noradrenaline in concentrations of up to 2 mM added by microinjection, failed to produce a detectable contraction (Figure 5b). ATP (1 mM) caused a contraction in all venous preparations, the mean peak tension ($247 \pm 41 \text{ mg}$, $n = 8$) was similar to that produced during a maximal potassium contraction (Figure 5b).

Effects of Me-ATP

Perfusing the preparation with $10 \mu\text{M}$ Me-ATP caused a rapid depolarization of arteries and veins. After 30 min the vessels had repolarized to control values. Measurements of tension showed that Me-ATP 10 and $100 \mu\text{M}$ produced a rapid transient contraction which soon returned to a stable resting tension (Figure 5a). In the presence of Me-ATP, sympathetic nerve stimulation now failed to produce an e.j.p. in the artery. However, the slow e.j.p. detected in veins after sympathetic nerve stimulation was little affected (mean peak amplitude $7.6 \pm 2.9 \text{ mV}$, $n = 5$) (Figure 2b).

The benextramine-resistant depolarization of arteries, (γ -adrenoceptor response), was unaffected by the presence of $10 \mu\text{M}$ Me-ATP, whilst the response to ATP (0.5 mM) was abolished ($n = 4$) (Figure 4b).

Tension measurements in arteries showed that the benextramine-resistant peak contraction to noradrenaline was unchanged ($1393 \pm 99 \text{ mg}$, $n = 3$) whilst the response to ATP was greatly attenuated $31.75 \pm 31 \text{ mg}$ ($n = 3$) (Figure 5).

Discussion

This study compared the membrane potential changes that occur in mesenteric arteries and veins during sympathetic transmission. The distributions of P_2 purinoceptor and γ -adrenoceptor types on these vessels were also determined. As has been shown previously, rapid e.j.ps were initiated in mesenteric arteries (Kuriyama & Suzuki, 1981; Suzuki, 1981; Ishikawa, 1985; Kreulen, 1986). Rapid e.j.ps persisted after treatment with the irreversible α -adrenoceptor antagonist, benextramine, but were abolished by bretylium, which prevents the release of

Figure 5 The effect of noradrenaline (NA) and ATP on tension in mesenteric arteries and veins. The upper trace (a) is of an artery showing that the contraction produced by NA after benextramine (BNX) treatment requires a relatively high NA concentration and is little affected by incubation in 0.1 mM Me-ATP, whilst the response to ATP is almost abolished. The preparation was continuously perfused with 1 μM desmethylinipramine (DMI) following BNX treatment. Continuous perfusion with Me-ATP caused an initial contraction which relaxed to resting levels. The lower trace (b) is of a vein showing the presence of α -adrenoceptors and P₂ purinoceptors but an absence of response to NA after BNX treatment. The preparation was continuously perfused with DMI following BNX treatment. Bars indicate perfusion times. Arrow heads ($\blacktriangle$) indicate microinjection into the organ bath. The arrows below the trace indicate continuous perfusion of Me-ATP or DMI.

noradrenaline from sympathetic nerves (Bowman & Rand, 1980). In contrast, rapid e.j.sps were not recorded from mesenteric veins following sympathetic nerve stimulation, rather repetitive nerve stimulation initiated a slow long lasting membrane depolarization. This slow e.j.p. was associated with constriction and was prevented by benextramine. Similar observations have been made from a number of venous preparations (Suzuki, 1981; Kreulen, 1986; van Helden, 1988).

Both arteries and veins contracted when low concentrations of noradrenaline were applied. These responses were abolished by benextramine treatment. Neither arterial nor venous preparations responded to high concentrations of noradrenaline after benextramine treatment. However when the tissues were further incubated with DMI, an inhibitor of neuronal uptake, the arteries alone responded to noradrenaline. At these concentrations of noradrenaline, Uptake₁ should be saturated (Lightman & Iversen, 1969) and inhibition of uptake would be

expected to have no effect on the concentration of noradrenaline reaching the receptor. The simplest explanation of this is that the γ -adrenoceptors activated by exogenous noradrenaline lie next to an uptake site which is not saturated. This could occur if γ -adrenoceptors are localised near the sympathetic neuromuscular junction and diffusional access to noradrenaline is limited by a narrow junctional gap. This suggestion is in accord with the observation that γ -adrenoceptors are restricted to regions of the artery near sympathetic varicosities (Hirst & Neild, 1981; Luff, 1987).

The lack of response of veins to noradrenaline after α -adrenoceptor blockade even in the presence of DMI suggests that they are devoid of γ -adrenoceptors. Tension studies on a variety of rabbit blood vessels found that non- α -adrenoceptors were restricted to systemic arteries but not veins (Laher *et al.*, 1986). The distribution of γ -adrenoceptors, present in arteries but not veins, is therefore correlated with the presence of α -antagonist resistant

neuronal responses. A similar finding has been made in rat middle cerebral arteries where γ -adrenoceptors were found in proximal innervated arterioles but not in the distal arterioles which never receive an innervation (Edwards *et al.*, 1989).

Both arteries and veins responded to ATP. Therefore the distribution of P_2 purinoceptors shows no correlation with the ability of sympathetic nerves to initiate an e.j.p. If sympathetic nerves did indeed release appreciable amounts of ATP, then non- α -adrenoceptor neuronal responses should be detected in veins unless the junctional cleft width is large or innervation is sparse. Where measured, the junctional cleft width in veins has been shown to be similar to that of arteries and often narrow (Rowan & Bevan, 1983). Mesenteric veins of the guinea-pig have been shown to be well innervated and although they appear less dense in whole mounts than arteries (Furness, 1971), the medial layer is much thinner and nerve density may be equivalent. Given a similar neuromuscular junction to the artery, either the sympathetic nerves which innervate veins must fail to release ATP, or P_2 purinoceptors may be located away from points of transmitter release. P_2 purinoceptors are relatively low affinity receptors and may only be activated in a junctional position where the local concentration of transmitter is high.

The slow e.j.p. in veins was not seen until stimulation frequency was increased. Similarly, at the low stimulus parameters required to activate arterial e.j.ps, no responses attributable to α -adrenoceptors were found. However, relatively high frequency stimulation has been reported to activate α -adrenoceptors in these arteries (Hottenstein & Kreulen, 1987). Such a dependence on high frequencies could indicate an extrajunctional position for α -adrenoceptors.

In rat basilar arteries, Me-ATP has been shown to be an antagonist to both γ -adrenoceptors and P_2 purinoceptors (Byrne & Large, 1986). This clearly is not the case for mesenteric vessels. Me-ATP abolished the responses produced by ATP in both

arteries and veins but did not abolish the responses produced by γ -adrenoceptor activation in mesenteric arteries. The different sensitivity of γ -adrenoceptors in the rat basilar artery (Byrne & Large, 1986) and those of the guinea-pig mesenteric artery to Me-ATP, suggests that more than one class of γ -adrenoceptors may exist. The proposal of more than one γ -adrenoceptor has been previously suggested for the rat basilar artery where a biphasic depolarization to exogenous noradrenaline exists, neither component being due to α -adrenoceptor activation (Byrne *et al.*, 1986). The first component of this depolarization was easily desensitized and was only seen if noradrenaline was micro-injected into the bath. That is, if the bath concentration rose quickly (Byrne *et al.*, 1986). No biphasic γ -adrenoceptor response could be detected in this study even though the bath concentration would have risen quickly (see Methods).

Since Me-ATP abolished the rapid e.j.ps recorded from arteries, it seems unlikely that the activation of γ -adrenoceptors can underlie the initiation of an e.j.p. Thus arteries have a population of adrenoceptors that are located near sympathetic nerve terminals and are only activated by high concentrations of noradrenaline. It has already been pointed out that arteries generate muscle action potentials during neuromuscular transmission. Since the ability of arterioles to support inward calcium movement is related to the density of sympathetic nerves (Hill *et al.*, 1986), it could be that γ -adrenoceptors are closely linked to voltage-dependent calcium channels. This suggestion has been previously made by Benham & Tsien (1987), who found that calcium entry into single isolated arterial cells was increased in the presence of noradrenaline, the receptor involved was neither an α - nor a β -adrenoceptor.

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