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Adrenergic transmission in the dog mesenteric vein and its modulation by α-adrenoceptor antagonists

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1 Adrenergic transmission was investigated in the dog mesenteric vein by recording electrical responses of single smooth muscle cells to perivascular nerve stimulation.

2 Perivascular nerve stimulation generated an excitatory junction potential (e.j.p.) and a slow depolarization of the membrane. The amplitude of the e.j.p. was increased by increasing the stimulus intensity, and at high intensity, a spike potential was generated.

3 Repetitive stimulation of the nerves showed facilitation of e.j.ps and enhanced the amplitude of slow depolarization. A linear relationship was observed between the amplitude of the e.j.p. and of slow depolarization.

4 The slow depolarization was inhibited by application of yohimbine or phentolamine, but not by prazosin. The amplitude of e.j.p. was increased by prazosin and was decreased by yohimbine. Both e.j.p. and slow depolarization were inhibited by guanethidine or tetrodotoxin.

5 Exogenously applied noradrenaline depolarized the muscle membrane and, in high concentrations ($> 10^{-7}$M), generated slow waves. These effects of noradrenaline were blocked by yohimbine. High concentrations of prazosin ($> 10^{-6}$M) showed weak inhibitory effects on the noradrenaline actions.

6 The amplitude of e.j.p. was decreased by exogenously applied noradrenaline in a dose-dependent manner. The inhibitory effect of noradrenaline on the e.j.p. was suppressed by yohimbine, but not by prazosin or phentolamine.

7 Phentolamine, but not prazosin, enhanced the facilitation process of e.j.ps. This effect was not suppressed by exogenously applied noradrenaline.

8 Application of neostigmine but not of atropine, reduced the e.j.p. amplitude without affecting the slow depolarization.

9 It was concluded that, in the dog mesenteric vein, perivascular nerve stimulation produced three types of electrical responses of the smooth muscle membrane, i.e., e.j.p., slow depolarization and spike potential. The slow depolarization was generated by activation of α_2-adrenoceptors. Exogenously applied noradrenaline reduced the e.j.p. amplitude through activation of prejunctional α_2-adrenoceptors, but the reduction may not involve α-autoinhibitory mechanisms.

Introduction

Most blood vessels are innervated by noradrenergic nerves (Bevan *et al.*, 1980), and the cellular responses of vascular smooth muscles to perivascular nerve stimulation are either an excitatory junction potential (e.j.p.) or a slow depolarization, or both. For example, perivascular nerve stimulation elicits an e.j.p. in the arteries of the uterus (Bell, 1969) or the mesenteric artery of guinea-pig (Hirst, 1977), a slow depolarization in the mesenteric vein (Suzuki, 1981) or the main pulmonary artery (Suzuki, 1983) of guinea-pig, or both e.j.p. and slow depolarization in the rat tail artery (Cheung, 1982). These nerve-mediated electrical responses may be differentiated by the use of α-adrenoceptor antagonists, since α-adrenoceptor antagonists can block the slow depolarization but not the e.j.p. (Holman & Surprenant, 1980; Cheung, 1982; Suzuki, 1983), and therefore the suggestion that a specific catecholamine receptor, i.e., γ-receptor, which is insensitive to α-adrenoceptor antagonists and is distributed in the junctional region, has been proposed (Hirst & Neild, 1980).

However, the e.j.p. is modified by α-adrenoceptor antagonists, for example phentolamine increases the amplitude of the e.j.p. and enhances the facilitation process of e.j.ps (Holman & Surprenant, 1980; Kou *et al.*, 1982. Kuriyama & Makita, 1983). Yohimbine reduces the amplitude of the first e.j.p. but enhances the facilitation process of e.j.ps in the guinea-pig mesenteric artery (Kuriyama & Makita, 1983). Prazosin does not affect the e.j.p. in the mesenteric artery of guinea-pig (Kuriyama & Makita, 1983) or dog (Kou *et al.*, 1982). The modulation by α-adrenoceptor antagonists of the amplitude of e.j.p. is attributed to suppression of prejunctional α-adrenoceptors which have inhibitory roles for transmitter release from the nerve terminals (Kuriyama & Makita, 1983), the idea being proposed originally from the measurements of catecholamine efflux (Langer, 1977).

In the guinea-pig mesenteric vein, stimulation of perivascular nerves produces slow depolarization of the membrane (Suzuki, 1981), with properties different from those recorded in the arteries, i.e., repetitive but not single stimuli are required for generation of the slow depolarization. The present experiments were undertaken to investigate the cellular responses of the dog mesenteric vein to perivascular nerve stimulation; the effects of α-adrenoceptor antagonists and agonists on neuromuscular transmission were also observed.

Methods

Mongrel dogs of either sex, weighing 10–15 kg, were anaesthetized by intravenous injection of pentobarbitone Na (40 mg/kg^{-1}) and the mesenteric vascular bed was excised. The tissue was kept in Krebs solution at room temperature. The mesenteric vein (0.3–0.5 mm in diameter) was excised and mounted in an organ bath which was made of Lucite plate with a volume of about 2 ml. The tissue was superfused with warmed (36°C) Krebs solution at a flow rate of 3 ml min^{-1}.

Perivascular nerves were stimulated by the point stimulation method (Suzuki & Fujiwara, 1982). A current pulse of 0.05–0.1 ms in duration and 15–100 V in intensity was supplied by an electric stimulator (NihonKohden SEN 7103). Electrical responses of the smooth muscle membrane of dog mesenteric vein were recorded by a glass capillary microelectrode filled with 3 M KCl. The electrode had a tip resistance of 40–80 MΩ, and penetrated the

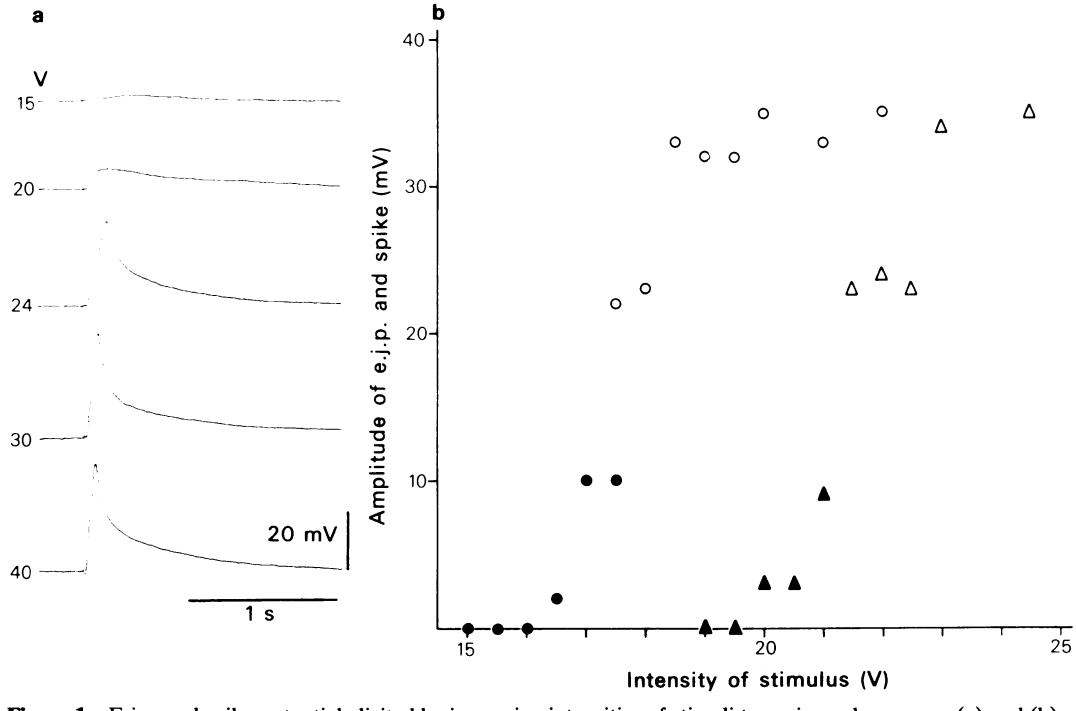

Figure 1 E.j.p. and spike potential elicited by increasing intensities of stimuli to perivascular nerves: (a) and (b) were recorded from different single cells. Stimulus duration, 0.05 ms in (a), 0.03 (▲△) and 0.05 ms (●○) in (b). Filled circles or triangles, e.j.p.; open circles or triangles, spike potential. Stimuli were applied at intervals of over 1 min.

cell through the mesenteric membrane. Electrical responses were displayed on a pen-writing recorder (NihonKohden Recticorder RJG 4024) and a tape-recorder (NihonKohden Data Recorder, RMG 5204).

A modified Krebs solution of the following ionic composition was used (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+}, 1.2, HCO_3^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134 and glucose 11.5. The solution was bubbled with 97% O_2 and 3% CO_2 and the pH was maintained at 7.2–7.3.

The following drugs were used at the molar concentrations described in the results; (−)-noradrenaline HCl (Sigma), prazosin HCl (Pfizer), phentolamine mesylate (Ciba-Geigy), yohimbine HCl, guanethidine sulphate (Tokyo Kasei), atropine sulphate (Merck) and neostigmine methyl sulphate (Shionogi).

Measured values were expressed as the mean ± s.d., and the statistical significance was assessed by Student's *t* test.

Results

Cellular responses of the dog mesenteric vein to perivascular nerve stimulation

The smooth muscle membrane of the dog mesenteric vein was electrically quiescent with no spontaneous electrical activity. The resting membrane potential was between − 65 and − 75 mV.

Application of an electric stimulus (0.05–0.1 ms duration and 16–20 V intensity) generated an excitatory junction potential (e.j.p.) followed by a slow depolarization of the membrane. The e.j.p. showed fast rising and slow falling phases with a duration of about 2 s. The amplitude of the e.j.p. was determined by the intensity and the duration of stimulus, and when a stimulus with long duration or strong intensity was applied, a spike potential was discharged. Figure 1a shows e.j.ps and spike potentials elicited by single stimuli with increasing intensities (pulse duration, 0.05 ms). An e.j.p. was elicited by 15 or 20 V

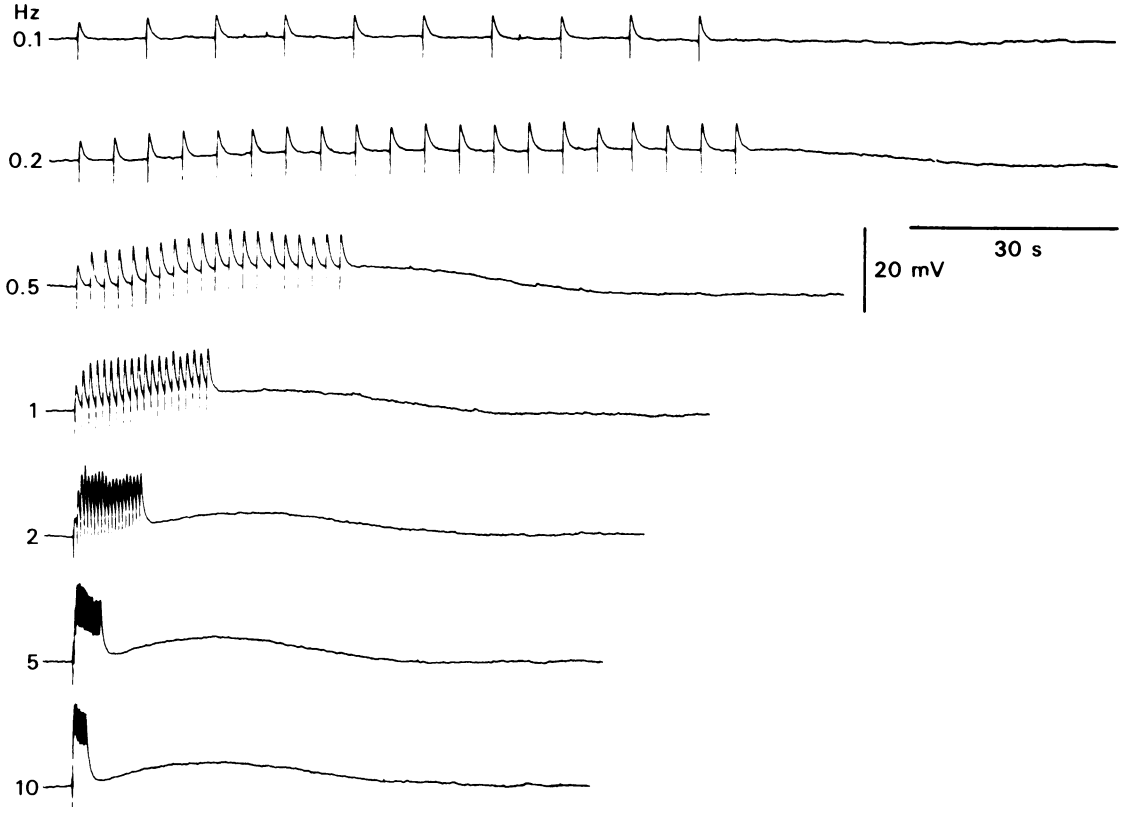

Figure 2 E.j.ps and slow depolarizations elicited by perivascular nerve stimulation. A train of 10 stimuli (0.1 Hz) or 20 stimuli (0.2–10 Hz) was applied at various frequencies (0.1–10 Hz). All the responses were recorded from a single cell.

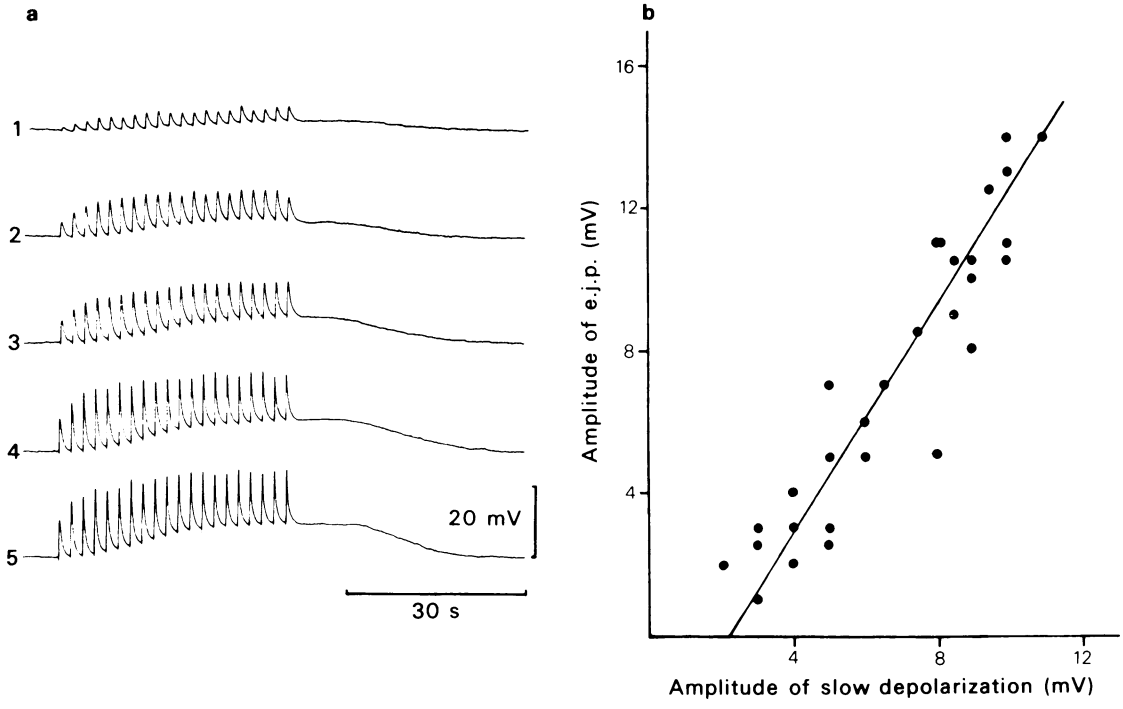

Figure 3 E.j.ps and slow depolarizations produced by nerve stimulation. (a) A train of 20 stimuli was applied at a frequency of 0.5 Hz, and stimuli of various intensities were applied. The responses were recorded from the same tissue. (b) Relationship between amplitude of the first e.j.p. of a train and that of the slow depolarization produced by 20 stimuli at 0.5 Hz. Responses recorded from 5 cells in the same tissue were plotted. A regression line in the figure is given by $Y = 1.6X - 3.54$ (Y, e.j.p. amplitude; X, slow depolarization, $r = 0.90$, n = 29, $P < 0.05$).

stimulus, and increasing the intensity to over 24 V generated a regenerative spike potential on the e.j.p. Relationship between intensity of stimulus and the amplitude of e.j.p. or spike potential obtained from a different single cell is shown in Figure 1b, in which increasing intensities of stimuli with two different pulse durations (0.03 and 0.05 ms) were applied at intervals of over 1 min. With a pulse duration of 0.03 ms, the minimum stimulus intensity required to produce an e.j.p. was 20 V, and increasing the intensity increased the amplitude of e.j.ps by two steps. Spike potentials with two different amplitudes were generated by stimulation of over 21.5 V in intensity. Increasing the stimulus duration to 0.05 ms resulted in a reduction of threshold intensity for the generation of e.j.p. to 16.5 V and of spike potential to 17.5 V. With both pulse durations, increasing the intensity increased the amplitude of e.j.ps or spike potentials in a stepwise fashion. The amplitude of each potential was much the same for both stimulus durations.

The slow depolarization was generated with some delay (3–5 s) and reached a peak amplitude of up to 2 mV at about 25 s after a single nerve stimulation. The amplitude of the slow depolarization was increased by repetitive application of nerve stimulation. Figure 2 shows examples of electrical responses of a smooth muscle cell of the dog mesenteric vein to repetitive stimulation of perivascular nerves. A train of 10 or 20 stimuli was applied with increasing frequencies (0.1–10 Hz). Stimulations below 0.5 Hz elicited an e.j.p. to each stimulus, superimposed on the slow depolarization of the membrane. The facilitation phenomenon of the e.j.p. amplitude was observed with successively applied stimuli, in the initial 3–5 e.j.ps of a train. The slow depolarization was generated 4–5 s after initiation of the first e.j.p. and reached a peak amplitude at 20–30 s. Stimulation with higher frequencies (> 2 Hz) showed summation of e.j.ps and the maximum amplitude of e.j.ps was increased in a frequency-dependent manner. The slow depolarization developed further and reached a peak amplitude after cessation of stimuli.

Experiments were designed to see whether the e.j.p. has any causal relationship with the slow depolarization in the dog mesenteric vein. Various stimulus intensities were applied for 40 s at a frequency of 0.5 Hz. The amplitude of the first e.j.p. of the train increased with an increase in stimulus intensity, and at any given intensity the amplitudes of the

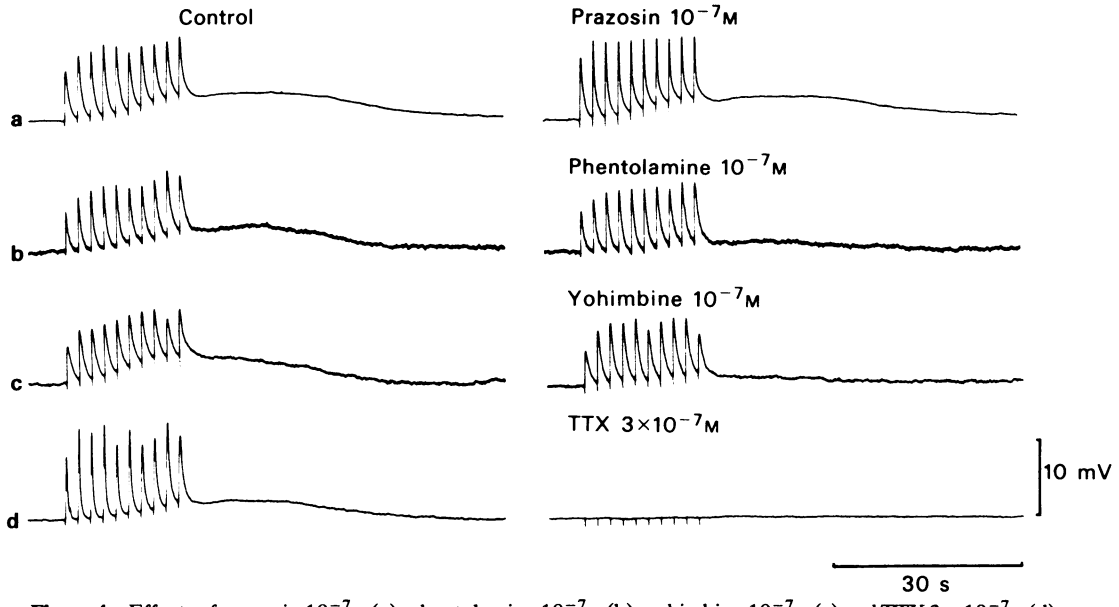

Figure 4 Effects of prazosin 10^{-7}M (a), phentolamine 10^{-7}M (b), yohimbine 10^{-7}M (c) and TTX 3×10^{-7}M (d) on the electrical responses produced by perivascular nerve stimulation. Perivascular nerves were stimulated by a train of 10 stimuli at 0.5 Hz. Stimulus, 0.05 ms duration and 20 V in intensity. Control, response recorded before application of drugs. Each set of responses was recorded from single cells in different tissues.

initial several e.j.ps increased progressively (facilitation phenomenon) and then fell to a steady value together with simultaneous development of the slow depolarization (Figure 3a). The slow depolarization developed rather slowly and reached a steady amplitude 24–26 s after starting a train of stimuli The steady depolarization was maintained for 8–10 s after cessation of stimuli. Many factors may be responsible for determination of the amplitude of the e.j.ps elicited by repetitive stimuli, such as e.j.ps facilitation, activation of α-autoinhibitory mechanisms (Langer, 1977) or changes in the membrane potential due to generation of the slow depolarization. Therefore, the amplitude of the first e.j.p. of a train was chosen as an indicator, and the relationship between amplitude of the first e.j.p. and that of the slow depolarization produced by 20 stimuli at 0.5 Hz frequency was plotted. Figure 3b shows the relationship obtained from the same tissue. The responses were elicited by stimuli which did not generate spike potentials. The relationship was found to be linear, and the regression line calculated using the least squares method was given by $Y = 1.6X - 3.5$, where Y and X are the amplitudes of the first e.j.p. and of the slow depolarization, respectively. The coefficient of correlation of the relationship, $r = 0.90$ ($n = 29$), and the relationship was statistically significant ($P < 0.05$).

The same experiment was repeated in different tissues (by applying stimuli of various intensities) to observe the relationship between the amplitude of the slow depolarization and that of the e.j.p. or the spike potential. The amplitude of the first e.j.p. of a train varied between 1 and 16 mV, and that of the spike potential varied between 20 and 44 mV. The relationship was linear as long as a spike potential was not generated. When the spike potential was generated, the amplitude of the slow depolarization was much the same, independent of the amplitude of the spike potential (mean amplitude of the slow depolarization was 12.6 ± 2.4 mV, $n = 22$).

Effects of α-adrenoceptor antagonists on the e.j.p. and slow depolarization

The pharmacological properties of the e.j.p. and the slow depolarization were investigated using the α-adrenoceptor antagonists, prazosin, phentolamine and yohimbine. Figure 4 shows effects of these antagonists on membrane responses produced by perivascular nerve stimulation with a train of 10 stimuli at 0.5 Hz frequency. Stimulus intensity was chosen so that an e.j.p. but not a spike potential was generated by the first stimulus of a train. Nerve stimulation generated e.j.ps and slow depolarization; the former showed the facilitation phenomenon to the initial 3–5 stimuli, while the latter was generated 8–12 s after the first stimulus and developed further after cessation of stimuli. Pretreatment (3–10 min)

with prazosin $1-3 \times 10^{-7}$M increased the amplitude of e.j.ps, without modifying the slow depolarization. When the concentration of prazosin was increased to 10^{-6}M, the amplitude of the slow depolarization was reduced (control, 9.8 ± 2.5 mV, $n=8$; in 10^{-6}M prazosin, 6.6 ± 1.8 mV, $n=8$). Application of 10^{-7}M phentolamine reduced the amplitude of slow depolarization to $20.0 \pm 9.9\%$ ($n=5$) of the control value and slightly increased the amplitude of e.j.ps. Yohimbine (10^{-7}M) completely blocked the generation of slow depolarization, without significant effects on the amplitude of the first e.j.p. of the train. The effects of prazosin, phentolamine or yohimbine remained after washing out, and the responses recovered partially after washing out these antagonists for up to 2 h.

Figure 4 also shows the effect of tetrodotoxin (TTX) on the e.j.p. and slow depolarization. Application of TTX 3×10^{-7}M completely inhibited the generation of e.j.p. and slow depolarization, this effect being reversible. In different tissues, application of guanethidine $10^{-6}-5 \times 10^{-6}$ gradually reduced the amplitude of e.j.p. and slow depolarization and, after 30 min, blocked the generation of nerve-mediated electrical responses.

Effects of exogenously applied noradrenaline

Electrical responses of single smooth muscle cells of the dog mesenteric vein to exogenously applied noradrenaline are shown in Figure 5a. A low concentration of noradrenaline (5×10^{-8}M) produced a sustained depolarization of the membrane. Application of 10^{-7}M noradrenaline produced membrane depolarization with small-amplitude oscillatory potentials which were enhanced by further increase in noradrenaline concentration to 10^{-6}M. Application of yohimbine (10^{-6}M) completely blocked the 10^{-6}M noradrenaline-induced electrical responses. In the presence of yohimbine (10^{-6}M), application of 10^{-5}M noradrenaline depolarized the membrane and generated small-amplitude oscillatory potentials.

Figure 5b shows the dose-response relationship of the effect of exogenously applied noradrenaline on the membrane potential of the dog mesenteric vein. The maximum value of the membrane potential was measured by impalements of the electrode into different cells. Noradrenaline at concentrations above 3×10^{-8}M depolarized the membrane in a dose-dependent manner. The relationship shifted to the right in the presence of 10^{-6}M yohimbine.

Figure 6 shows effects of noradrenaline on the e.j.ps and slow depolarization produced by 20 stimuli at 0.5 Hz frequency, in the presence of prazosin, phentolamine or yohimbine. The intensities of the stimuli were chosen to generate e.j.p. but not spike potentials. In the control condition, application of noradrenaline (5×10^{-8}M) reduced the amplitude of e.j.p. and slow depolarization. Application of prazosin (10^{-6}M) enhanced the e.j.p. amplitude and re-

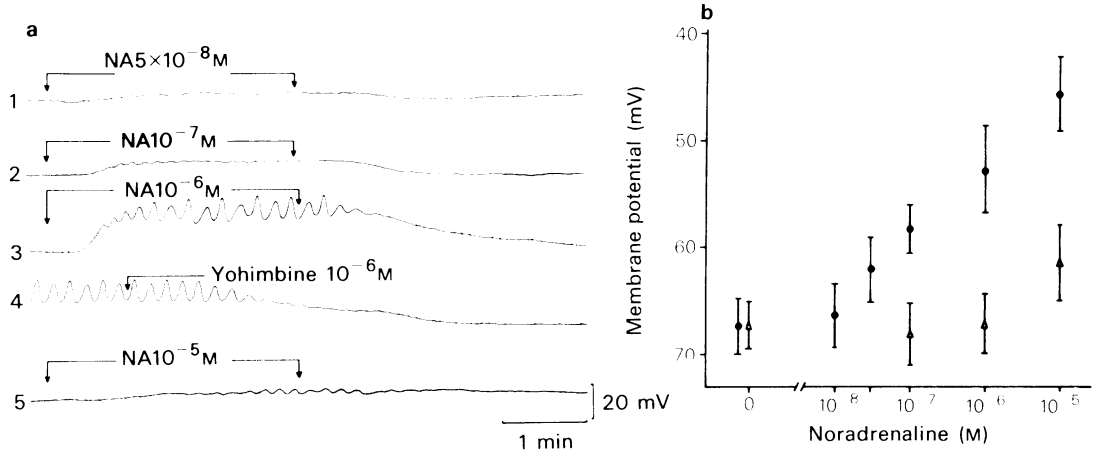

Figure 5 (a) Electrical responses of the smooth muscle cells of dog mesenteric vein to exogenously applied noradrenaline. All the responses were recorded from the same tissue. Noradrenaline (NA) at concentrations of 5×10^{-8}M (1), 10^{-7}M (2) or 10^{-6}M (3) was applied between arrows; (4) pretreatment with 10^{-6}M noradrenaline generated slow waves and, in addition 10^{-6}M yohimbine was applied at an arrow; (5) in the presence of 10^{-6}M yohimbine, 10^{-5}M noradrenaline was applied between arrows. (b) Dose-response relationship of the effect of exogenously applied noradrenaline on the membrane potential. Membrane potentials were measured by successive impalements of different cells. Increasing concentrations of noradrenaline were applied for 10 min in the absence (control, ●) or in the presence of 10^{-6}M yohimbine (△), and membrane potentials obtained from 3–5 different tissues were shown as mean ± s.d. ($n=16-35$). Abscissae, concentration of noradrenaline on a logarithmic scale.

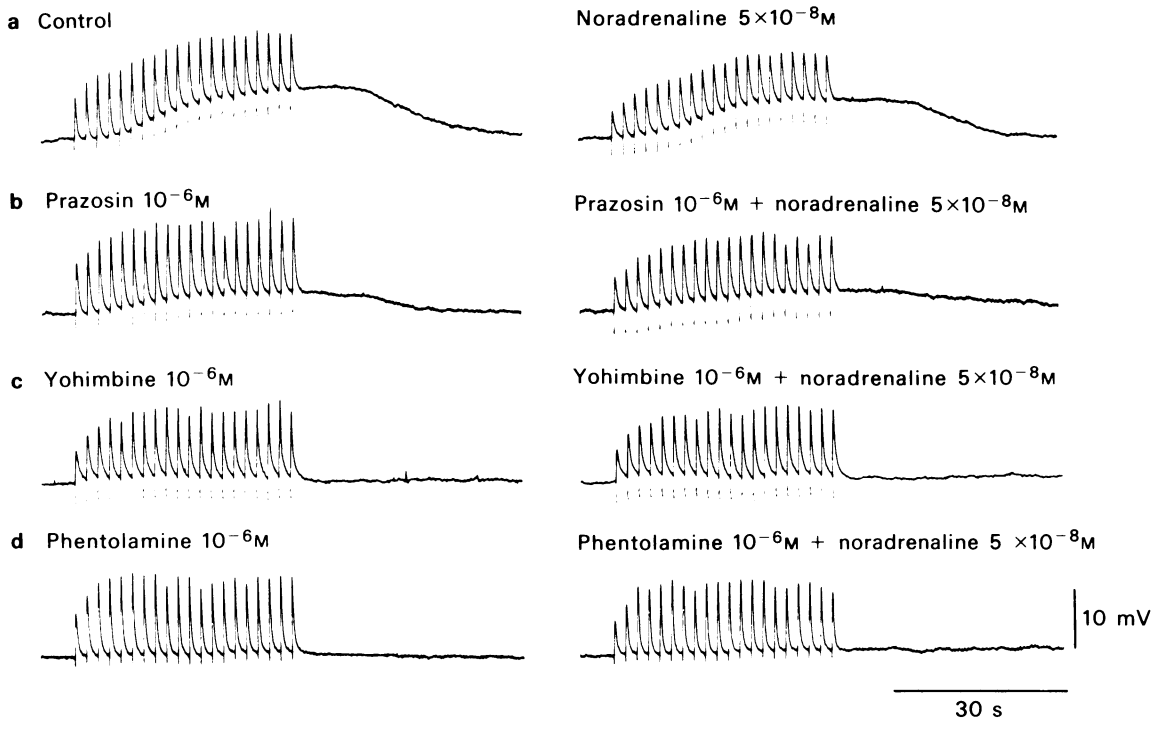

Figure 6 Effects of exogenously applied noradrenaline on the e.j.ps and slow depolarizations produced by nerve stimulation before (control) and during the application of prazosin 10^{-6}M (b), yohimbine 10^{-6}M (c) or phentolamine 10^{-6}M (d). Perivascular nerves were stimulated by a train of 20 stimuli at 0.5 Hz, at intervals of over 3 min. Responses before (left) and during the application of 5×10^{-8}M noradrenaline (right) are shown. Each set of responses was recorded from the same cell in different tissues.

duced the amplitude of slow depolarization. Application of phentolamine (10^{-6}M) inhibited the generation of slow depolarization. Addition of noradrenaline to prazosin or phentolamine reduced the amplitude of e.j.ps. Yohimbine (10^{-6}M) reduced the amplitude of e.j.ps and inhibited the generation of slow depolarization, but additional application of noradrenaline did not show any detectable change in the e.j.p. amplitude.

The dose-response relationship of the effect of noradrenaline on the amplitude of e.j.p. is shown in Figure 7, in which the amplitude of e.j.p. elicited by a single stimulus is expressed relative to the control value. Exogenously applied noradrenaline reduced the amplitude of e.j.ps in a dose-dependent manner. This effect of noradrenaline was completely inhibited by yohimbine, but not by prazosin or phentolamine. In the presence of 10^{-6}M yohimbine, the amplitude of e.j.p. decreased to $70.7 \pm 9.3\%$ ($n = 7$) of the control value, but additional application of noradrenaline (10^{-8}–10^{-7}M) failed to reduce further the e.j.p. amplitude.

Although the amplitude of e.j.ps was modified by

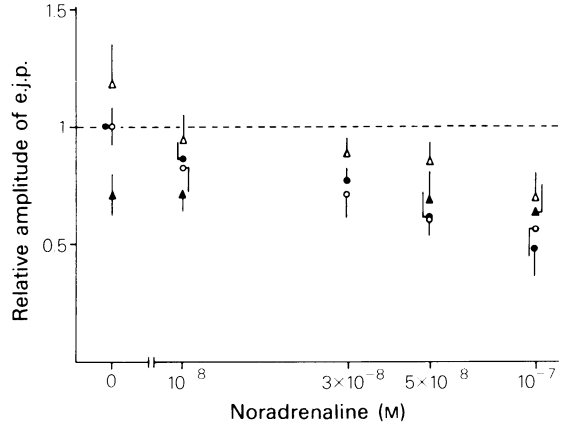

Figure 7 Effect of exogenously applied noradrenaline on the amplitude of e.j.p. elicited by single stimuli. Amplitude of e.j.p. relative to the control (= 1.0) is shown by mean ±s.d. ($n = 7$–15). Effect of noradrenaline was observed in the absence (●) or in the presence of prazosin 10^{-6}M (△), phentolamine 10^{-6}M (○) or yohimbine 10^{-6}M (▲).

noradrenaline or α-adrenoceptor antagonists, repetitive stimulation of perivascular nerves showed facilitation of e.j.ps in all cases. When the nerves were stimulated at 0.5 Hz frequency, the initial 4 or 5 e.j.ps of the train were generated before onset of the slow depolarization, and the remainder were generated on the slow depolarization (Figure 6). Therefore the effects of α-blockers or noradrenaline on the facilitation process of e.j.ps were observed for the initial five e.j.ps of a train, to rule out the possible involvement of change in the membrane potential on the amplitude of e.j.ps. Figure 8 shows effects of prazosin and phentolamine on the amplitude of e.j.ps produced by 0.5 Hz stimulation. Prazosin (10^{-6}M) but not phentolamine (10^{-6}M) increased the e.j.p. amplitude. To observe the effects of prazosin or phentolamine on the facilitation process of e.j.ps, the amplitude of e.j.ps was expressed as a relative value to the first

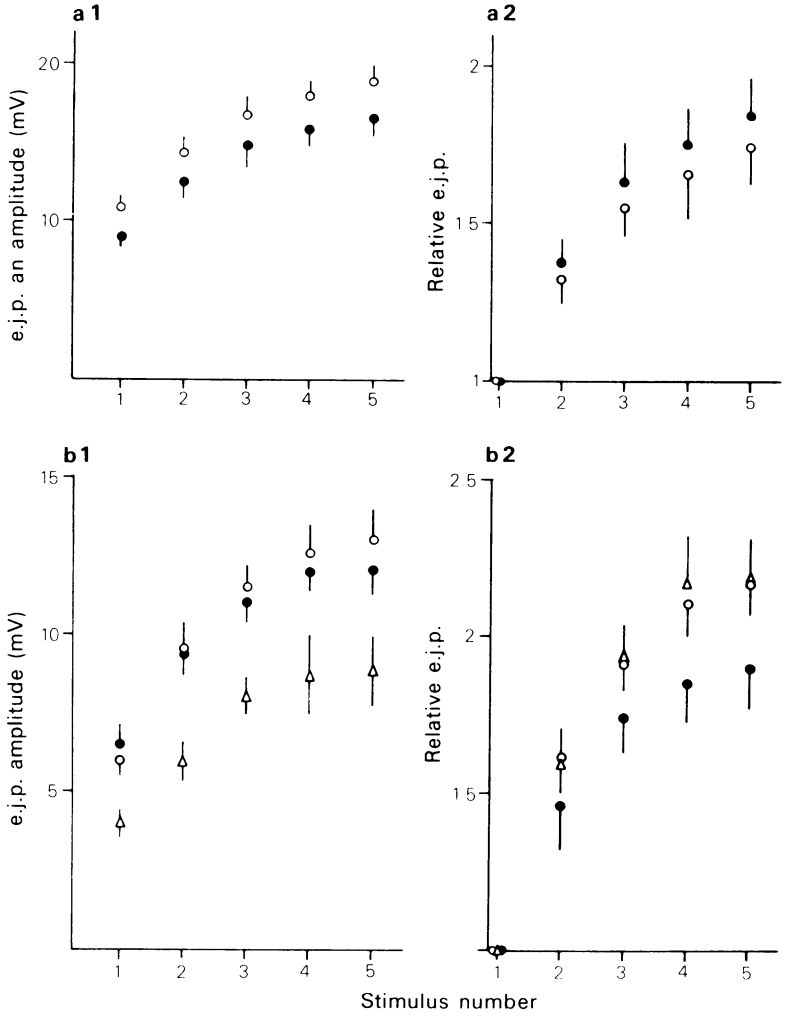

Figure 8 Effects of prazosin (a) or phentolamine (b) on the facilitation of e.j.ps produced by 5 stimuli at 0.5 Hz frequency. (a1) amplitude of e.j.ps before (control, ●) and during the presence of prazosin 10^{-6}M (○). (a2) Results shown in (a1) were plotted as relative amplitude of the first e.j.p. of the train (amplitude of the first e.j.p. = 1.0). (b1) Amplitude of e.j.ps before (●), during application of phentolamine 10^{-6}M (○) and during application of phentolamine 10^{-6}M plus noradrenaline 3×10^{-8}M (△). (b2) Results shown in (b1) were plotted as relative amplitude of the first e.j.p. of a train in each condition. Abscissa scale, stimulus number. Mean ± s.d. of 5–12 observations is shown. (a) and (b) were obtained from the same cell in different tissues.

e.j.p. of the train. Unlike phentolamine (Figure 8 b2), prazosin did not enhance the facilitation process of e.j.ps (Figure 8 a2). Yohimbine (10^{-6}M) reduced the amplitude of e.j.ps but the facilitation process of e.j.ps was enhanced, as with phentolamine. Application of phentolamine plus noradrenaline (3×10^{-8}M) reduced the amplitude of e.j.ps (Figure 8 b1), but the facilitation process of e.j.p. was not different from that seen in the presence of phentolamine alone. Application of noradrenaline plus yohimbine did not further modify the enhanced facilitation process of e.j.ps produced by yohimbine (Figure 6).

Effects of neostigmine and atropine on e.j.p. and slow depolarization

Possible involvement of vasomotor cholinergic nerves in the dog mesenteric vein was investigated. The amplitude of e.j.p. was decreased in 3 out of 4 tissues examined after application of neostigmine (1.5×10^{-6}M) without change in the membrane potential. The slow depolarization was not changed by application of neostigmine. The decreased amplitude of e.j.p. induced by neostigmine did not recover after washing out the drug for up to 20 min. Subsequently applied atropine (10^{-6}M) did not change the membrane potential and the amplitude of e.j.p. or slow depolarization. In different experiments, application of atropine without previous application of neostigmine also showed no detectable effect on the nerve-mediated electrical responses.

Discussion

Perivascular nerve stimulation generated an e.j.p. and a slow depolarization in the smooth muscle cells of dog mesenteric vein. The pharmacological properties of the slow depolarization are different between tissues, i.e., the slow depolarization observed in the rabbit ear artery (Suzuki & Kou, 1983) or the guinea-pig main pulmonary artery (Suzuki, 1983) is sensitive to prazosin, while this potential is sensitive to yohimbine in the rat tail artery (Itoh *et al.*, 1983). The slow depolarization observed in the dog mesenteric vein was sensitive to yohimbine or phentolamine but not to prazosin. Exogenously applied noradrenaline depolarizes the smooth muscle membrane of these vascular tissues: this effect is suppressed by prazosin in the rabbit ear artery and in the guinea-pig main pulmonary artery (Suzuki, 1983; Suzuki & Kou, 1983) and is suppressed by yohimbine in the rat tail artery (Itoh *et al.*, 1983) and in the dog mesenteric vein. Phentolamine inhibits the depolarization produced by endogenous and exogenous noradrenaline in all of these tissues (Suzuki, 1981; Cheung, 1982; Suzuki, 1983; Suzuki & Kou, 1983). The α-adrenoceptors are classified pharmacologically into two subtypes, α_1- and α_2-receptors: prazosin and yohimbine are selective antagonists of these receptors respectively, and phentolamine is an antagonist of both α_1- and α_2-receptors (Langer, 1977). Therefore, the α-receptors of the smooth muscles in the dog mesenteric vein could be mainly categorized as α_2-receptors, and both endogenous and exogenous noradrenaline depolarize the smooth muscle membrane through the α_2-receptor. On the other hand, a linear relationship was observed between the amplitude of e.j.p. and of the slow depolarization in the dog mesenteric vein. The e.j.p. could also be generated after the slow depolarization had been blocked by yohimbine or phentolamine. Application of TTX or guanethidine inhibited the generation of e.j.p. and slow depolarization. These observations suggest that each component of the nerve-mediated electrical responses is produced by substances released from perivascular adrenergic nerves. In arteries, a catecholamine receptor insensitive to α-adrenoceptor antagonists seems to be present in the junctional region and is called the γ-receptor (Hirst & Neild, 1981). If this theory is applicable to the present experiments, the smooth muscle membrane of dog mesenteric vein would then possess both α_2-adrenoceptors and γ-receptors.

The spike potential was not an 'all or none' type, but the amplitude increased in a stepwise manner by increasing the stimulus intensity. The amplitudes of the e.j.p. increased in a stepwise manner with increase in the stimulus intensity, suggesting the contribution of many nerves with different thresholds for excitation to generate an e.j.p. in the dog mesenteric vein. In the guinea-pig mesenteric vein, noradrenaline depolarizes the smooth muscle membrane and increases the membrane resistance (Suzuki, 1981). Therefore, increasing the amount of released noradrenaline, as a result of excitation of an increasing number of nerves, would be responsible for the generation of spike potentials with larger amplitudes.

Noradrenaline decreases and phentolamine or yohimbine increases the efflux of [^{3}H]-noradrenaline induced by nerve stimulation in tissues which are innervated by adrenergic nerves, indicating that a prejunctional α-adrenoceptor with an inhibitory role for transmitter release from the nerves is involved (Langer, 1977). Kuriyama & Makita (1983) showed that in the guinea-pig mesenteric artery the amplitude of the e.j.p. is decreased by exogenously applied noradrenaline or clonidine and is increased by phentolamine. In this tissue, application of yohimbine decreases the amplitude of e.j.p. elicited by single stimuli but enhances the facilitation process of e.j.ps produced by repetitive stimuli. These effects of adrenoceptor agonists and antagonists on the amplitude of e.j.ps are explained by the α-autoinhibition

mechanism, i.e., a prejunctional α-adrenoceptor activated by noradrenaline causes reduction of the transmitter release (Kuriyama & Makita, 1983). In the dog mesenteric vein, exogenously applied noradrenaline showed effects similar to those seen in the guinea-pig mesenteric artery, namely noradrenaline reduced the amplitude of e.j.p. in a dose-dependent manner. Stimulation of perivascular nerves at 0.5 Hz frequency produced slow depolarization which reached a maximum amplitude of about 12 mV in the dog mesenteric vein. A similar amplitude of depolarization was produced by exogenously applied noradrenaline at a concentration of about 10^{-7}M which was high enough to reduce the e.j.p. amplitude to about a half of the control value. However, endogenous noradrenaline did not elicit such an inhibitory effect. The differences between the effects of endogenous and exogenous noradrenaline were also noted in the mouse and guinea-pig vas deferens (Blakely *et al.*, 1982).

In the dog mesenteric vein, the amplitude of e.j.p. generated by single stimuli was increased by application of prazosin, decreased by yohimbine, and unchanged by phentolamine. The facilitation of e.j.ps produced by repetitive stimulation was enhanced by phentolamine or yohimbine, but not by prazosin. These observations suggest that there might be an α_2-adrenoceptor at the prejunctional membrane regulating transmitter release. Enhancement by prazosin of the e.j.p. amplitude may not be due to suppression of the prejunctional α-receptor, but may possibly be due to non-specific action. However, exogenously applied noradrenaline reduced the amplitude of e.j.p. even in the presence of phentolamine, with no change in the facilitation of e.j.ps. Furthermore, yohimbine blocked these inhibitory effects of exogenously applied noradrenaline on the e.j.ps. Thus, these results question the view that α-autoreceptors have a role in the inhibition of transmitter release at the nerve terminal (similar doubts were also raised for the neuromuscular transmission in rabbit muscular arteries; Holman & Surprenant, 1980). Evidence shows that both phentolamine and yohimbine increase the release of [^{3}H]-noradrenaline (Langer, 1977). Presumably these α-adrenoceptor blockers possess other actions on sites distinct from α-receptors on adrenergic nerves.

The amplitude of e.j.p. or amount of [^{3}H]-noradrenaline efflux is highly dependent on the concentration of external calcium ions in the adrenergic nerves (Alberts *et al.*, 1981; Kuriyama & Makita, 1983). On the other hand, on rat sympathetic neurones, exogenously applied noradrenaline inhibits a voltage-dependent calcium current (Horn & McAfee, 1980). This may be the reason for noradrenaline-induced inhibition of e.j.p. in the dog mesenteric vein. Nevertheless, the inhibitory effect of noradrenaline on the calcium current is suppressed by phentolamine in rat sympathetic neurones (Horn & McAfee, 1980), but not in the dog mesenteric vein. These observations suggest differences in the actions of α-adrenoceptor antagonists between nerve axons and nerve terminals. Phentolamine may facilitate action potential propagation toward the distal side of the varicosites with consequent enhancement of transmitter release, as proposed for the adrenergic nerves of the vas deferens (Alberts *et al.*, 1981).

Cholinergic innervation of vascular tissues has been suggested on the basis of observations that acetylcholinesterase is densely distributed in the wall of cerebral arteries (Florence & Bevan, 1979). However, exogenously applied acetylcholine shows no effects on the dog cerebral artery (Fujiwara *et al.*, 1982). Hyperpolarization by acetylcholine of the smooth muscle membrane is reported in the rabbit mesenteric artery (Kuriyama & Suzuki, 1978). In the dog vena cava, stimulation of the greater splanchnic and vagus nerves in the presence of α-adrenoceptor blockers produces vasoconstriction, and the constriction is enhanced by neostigmine and suppressed by atropine (Nakazato *et al.*, 1982). Although exogenously applied acetylcholine reduces the e.j.p. amplitude in the guinea-pig mesenteric artery through muscarinic receptors (Kuriyama & Suzuki, 1981), in the present experiments, neostigmine reduced but atropine did not increase the amplitude of e.j.p. Thus, cholinergic innervation was apparently not detected in the dog mesenteric vein. Reduction by neostigmine of the e.j.p. amplitude might have been caused by a non-specific inhibitory action.

In conclusion, perivascular nerve stimulation elicits an e.j.p. and a slow depolarization in the dog mesenteric vein. Exogenously applied noradrenaline reduced the e.j.p. amplitude through yohimbine-sensitive receptors, not necessarily involving an α-autoinhibition mechanism. The slow depolarization and the depolarization produced by exogenously applied noradrenaline were generated through a similar type of pharmacologically identified adrenoceptor, the α_2-receptor.

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