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Transmitter release modulated by α-adrenoceptor antagonists in the rabbit mesenteric artery: a comparison between noradrenaline outflow and electrical activity

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1 Effects of α-adrenoceptor blockers (prazosin, yohimbine, phentolamine and phenoxybenzamine) on the outflow of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) during perivascular nerve stimulation were observed in relation to electrical events in the rabbit mesenteric artery.

2 Cocaine or imipramine increased the NA outflow and reduced the DOPEG outflow induced by nerve stimulation. In the absence of stimulation, cocaine and imipramine did not significantly modify the NA and DOPEG outflows.

3 The α-adrenoceptor blockers we used enhanced the NA and DOPEG outflow during nerve stimulation, in a dose-dependent manner; the potency of the enhancement was higher for phentolamine and phenoxybenzamine than for prazosin and yohimbine. Higher concentrations (10^{-5}M) of yohimbine reduced the NA and DOPEG outflows induced by nerve stimulation.

4 Prazosin increased the DOPEG outflow in the absence of stimulation, and this effect was not inhibited by pretreatment with cocaine.

5 Guanethidine increased the NA and DOPEG outflow in the absence of stimulation, and the NA outflow was reduced during nerve stimulation. These effects of guanethidine were prevented by pretreatment with cocaine or imipramine.

6 Perivascular nerve stimulation evoked excitatory junction potentials (e.j.ps) and with high frequency stimulation, slow depolarization and spike potentials.

7 Application of phentolamine, phenoxybenzamine or yohimbine enhanced, and of prazosin had no effect, on the amplitude of the e.j.p. Spike potentials were not affected by these α-adrenoceptor blockers. Slow depolarization ceased in the presence of prazosin, phentolamine or phenoxybenzamine, and was slightly inhibited by yohimbine. Guanethidine blocked all of these electrical responses induced by perivascular nerve stimulation.

8 Application of prazosin, phentolamine or phenoxybenzamine did not alter the resting membrane potential of the smooth muscle cells. Depolarizations of smooth muscle membrane produced by exogenously applied NA were inhibited by prazosin, phentolamine or phenoxybenzamine. Yohimbine itself depolarized the membrane and the inhibitory effects on the NA-induced depolarization were weaker.

9 We conclude that the smooth muscle membrane of the rabbit mesenteric artery possesses α_1-adrenoceptors. Increase in NA outflow by α-adrenoceptor antagonists during nerve stimulation was not always consistent with increase in e.j.p. amplitude, presumably due to involvement of actions other than α-adrenoceptor blockade with each of these antagonists.

Introduction

α-Adrenoceptors located in blood vessels are classified into two subtypes, α_1- and α_2-receptors, based on their selectivity to α-adrenoceptor agonists and antagonists (Langer, 1977; Starke, 1977). Stimulation of the α_1-adrenoceptor causes most smooth muscle to contract, while that of the α_2-adrenoceptor

inhibits release of transmitter from the adrenergic nerves by activation of the α-autoinhibition mechanism (Langer, 1977; Starke, 1977; Bevan *et al.*, 1980).

Adrenergic transmission can also be investigated from responses of vascular smooth muscle membrane to perivascular nerve stimulation, a condition in which excitatory junction potentials (e.j.ps), slow depolarization, or both are observed (Kuriyama *et al.*, 1982). These electrical responses are probably generated by noradrenaline (NA) released from adrenergic nerves; however, the e.j.p. cannot be blocked by the α-adrenoceptor antagonists, presumably due to involvement of receptors different from traditional α-adrenoceptors in the junctional region (Hirst & Neild, 1980; 1981). However, the amplitude of e.j.ps can be modified by the α-adrenoceptor agonists and antagonists, e.g. clonidine or noradrenaline reduce and phentolamine or yohimbine enhance the e.j.p. amplitude (Holman & Surprenant, 1980; Kou *et al.*, 1982, Fujiwara *et al.*, 1982; Kuriyama & Makita, 1983), thus measurement of the e.j.p. amplitude would allow for estimation of pre-junctional regulation of adrenergic transmission (Kuriyama *et al.*, 1982). In the adrenergically innervated tissues, NA released from nerve terminals, is metabolized to many substances, i.e. 3,4-dihydroxyphenylglycol (DOPEG), 3,4-dihydroxymandelic acid (DOMA), normetanephrine (NM), 3-methoxy-4-hydroxyphenylglycol (MOPEG), or vanillylmandelic acid (VMA). Of these metabolites, the major ones present in the perfusate are DOPEG and MOPEG (Vanhoutte *et al.*, 1981), and DOPEG is readily released from tissue, in comparison with the other metabolites (Levin, 1974; Graefe *et al.*, 1977; Henseling *et al.*, 1978).

We investigated the relationship between NA and DOPEG released from perivascular adrenergic nerves and electrical responses of the postjunctinal smooth muscles cells. The amounts of NA and DOPEG released during perivascular nerve stimulation were measured by high-performance liquid chromatography (Oishi *et al.*, 1983) and electrical responses of the smooth muscle membrane were recorded by microelectrode techniques.

Methods

Albino rabbits of either sex, weighing 1.8–2.5 kg, were anaesthetized by injecting pentobarbitone Na (40 mg kg^{-1}) into the ear vein and were then exsanguinated. The mesenteric vascular beds were excised, the mesenteric artery was dissected and veins and fatty tissues were removed, in Krebs soulution and at room temperature.

Measurement of noradrenaline and its metabolites

A segment of the tissue (2–3 cm long, 0.5–1 mm diameter) was cut along the long axis of the artery and used for measurement of NA outflow. A pair of Ag–AgCl wires (0.5 mm diameter, 4 cm long) was fixed vertically in parallel, at a 1–1.2 mm distance, and the entire length of tissue was mounted between these wires, by means of cotton thread. Krebs solution (35 °C) was allowed to drip onto the tissue, at a rate of 1 ml min^{-1}, using a perfusion pump (Tokyo Rikakikai PO-1). The tissue was incubated under such conditions for at least 1 h and the solutions so perfused were collected in a conical test tube at the bottom of the tissue, usually 5 min before and after the nerve stimulation. Electrical stimulation (square pulse of 0.2 ms duration, 50 V intensity) was applied through the pair of Ag-AgCl wires. To the collected solution was added 50 μl perchloric acid (60%) and the preparation was stored in a freezer at −20 °C until the time of assay (usually the assay of catecholamine was done the next day). At the end of the experiments, the tissue was blotted and weighed.

NA and its metabolites in the samples were analysed by the alumina adsorption method (Oishi *et al.*, 1983): 50 μl of the extracted samples was injected for high-performance liquid chromatography (Yanagimoto MGF, Co., L-200L) with a Hamilton microsyringe. The content of catecholamines was expressed as ng g^{-1} wet weight of tissue.

Recording of electrical activity

A segment of the mesenteric artery (0.5–1 mm diameter, 1.5 cm long) was mounted in a chamber (volume 2 ml) made of Lucite plate, and warmed (35.5 °C) Krebs solution was superfused at a flow rate of 2–3 ml min^{-1}. The vessel was impaled from the outer surface with glass capillarly microelectrodes (40–80 MΩ), filled with 3 M KCl, to record the electrical activities of single smooth muscle cell. Electrical pulses were applied transmurally to stimulate perivascular nerves by the point stimulation method (Suzuki & Fujiwara, 1982). The pulses of 0.05–0.1 ms in duration and 50–100 V in intensity were supplied by an electric stimulator (Nihon Kohden SEN 7103) and the responses of the smooth muscle cells were displayed on a pen-writing recorder (Nihon Kohden Recticorder RJG4024).

Solution and drugs

The Krebs solution was of the following ionic composition (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.6, HCO_3^- 15.5, H_2PO_4 1.2, Cl^- 137, glucose 11.5 disodium ethylenediamine tetraacetate 0.03 and

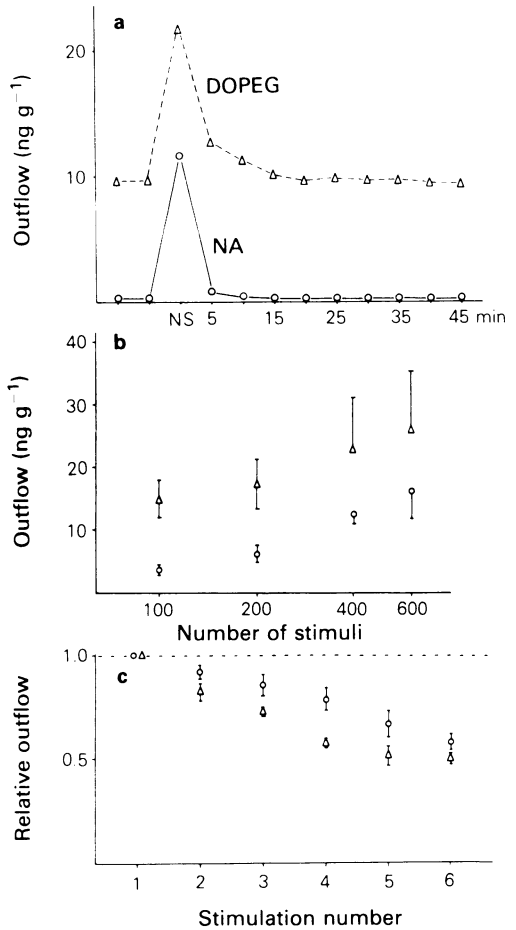

Figure 1 (a) Concentration of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) overflow into the perfusate. Amounts of NA (○) and DOPEG (△) during 5 min collection are expressed by ng g^{-1} wet weight of tissue. Nerve stimulation (NS) was applied for 1 min (600 stimuli at 10 Hz). (b) Effect of increasing the number of stimuli (at 10 Hz frequency) on NA and DOPEG outflow. Mean of 4 observations; vertical lines show s.e. (c) Decrease in NA and DOPEG outflow induced by nerve stimulation (600 stimuli at 10 Hz). The nerve stimulation was applied 6 times at 30 min intervals and concentrations of NA and DOPEG were expressed relative to the first. Mean of 5 observations; vertical lines show s.e.

$Na_2S_2O_5$ 0.5. The solution was bubbled with O_2 containing 3% CO_2, and the pH was kept at 7.2–7.3.

Drugs used were (−)-noradrenaline hydrochloride, tetrodotoxin (Sigma), guanethidine sulphate, yohimbine hydrochloride (Tokyo Kasei), prazosin hydrochloride (Pfizer), phentolamine mesylate (Ciba-Geigy), imipramine hydrochloride (Wako) and cocaine hydrochloride (Sankyo).

Statistics:

The experimental values were expressed at the mean ± standard deviation or standard error, and statistical significance was determined using Student's t test ($P<0.05$).

Results

Outflow of noradrenaline and 3,4-dihydroxyphenylglycol during nerve stimulation

In the absence of stimulation, the effluent contained small amounts of NA and DOPEG, the contents being 0.5–3 ng g^{-1} and 10–20 ng g^{-1} wet weight tissue, respectively. The spontaneous outflow of NA and DOPEG decreased gradually with time during the experiment (5–6 h) to about 50–60% of the initial.

Figure 1a shows the content of NA and DOPEG in the effluents collected every 5 min, before, during and after (45 min) nerve stimulation (10 Hz, 600

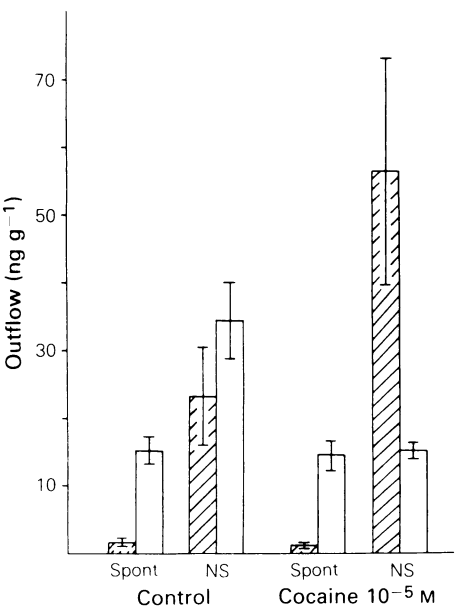

Figure 2 Effects of cocaine on the noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) outflow induced by nerve stimulation (600 stimuli at 10 Hz frequency). Effluents were collected 5 min before (Spont) and during the nerve stimulation (NS) Hatched columns: NA outflow; open columns: DOPEG outflow. Cocaine (10^{-5} M) was applied 10 min before the nerve stimulation. Mean of 4 observations for control and application of cocaine; s.e. indicated by vertical lines.

stimuli). The content of NA and DOPEG in each fraction increased during nerve stimulation and the outflow was reduced to the control level within 5 min after stimulation ceased. Hereafter, we collected each effluent every 5 min.

The relationship between number of stimuli (applied at a fixed frequency of 10 Hz) and amounts of NA and DOPEG outflow showed that the NA and DOPEG outflows were increased with increase in the number of stimuli (Figure 1b). Application of tetrodotoxin (TTX, 3×10^{-7} M) blocked the increase in the NA or DOPEG outflow, but did not alter the level of the spontaneous outflow (data not shown).

To observe changes in the outflow of NA and DOPEG, nerve stimulation (10 Hz, 600 stimuli) was applied repetitively every 30 min. As shown in Figure 1c, the outflow of NA and DOPEG decreased gradually with successive stimulations. In preliminary experiments, when intervals of nerve stimulations were varied between 10 and 60 min, the rate of decrease in the NA or DOPEG outflow was not modified with intervals of over 20 min. Therefore, in the present experiment, the nerve stimulation was applied at intervals of over 30 min.

Effects of cocaine (10^{-5} M) on the outflow of NA and DOPEG are shown in Figure 2. Cocaine was

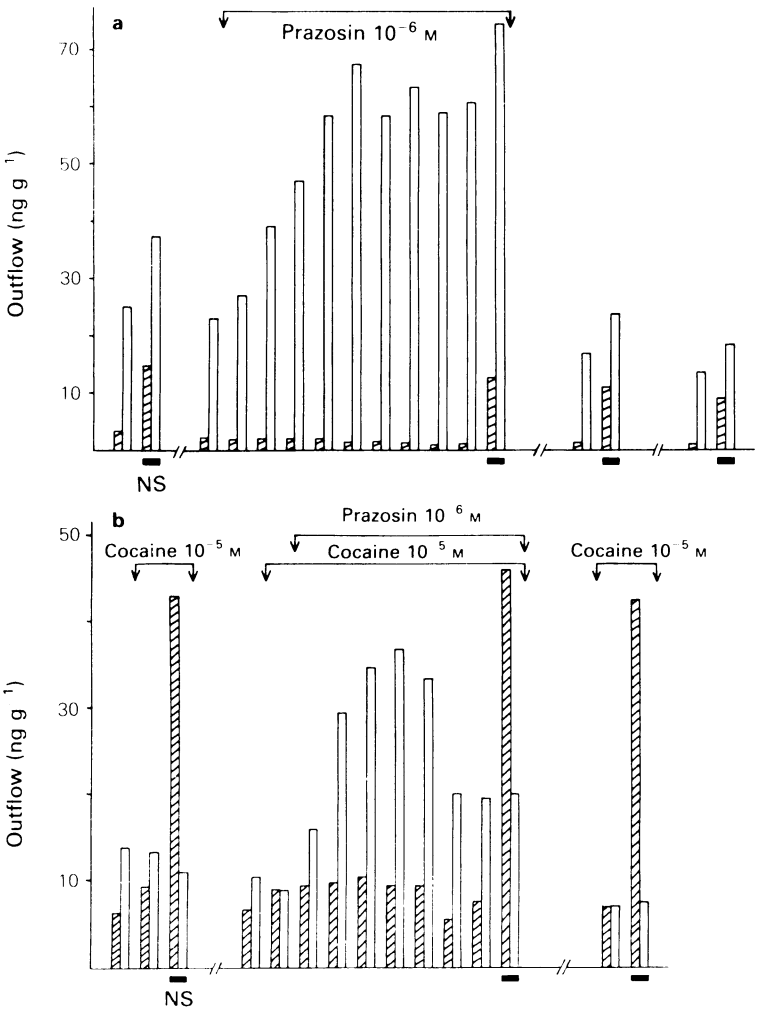

Figure 3 Effects of prazosin (10^{-6} M) on noradrenaline (NA, hatched columns) and 3,4-dihydroxyphenylglycol, (DOPEG, open columns) outflow in the absence (a) and presence (b) of 10^{-5} M cocaine. NS = nerve stimulation (600 stimuli at 10 Hz frequency) was applied every 30 min. Prazosin was applied between arrows. Effluents were collected every 5 min, in a series.

applied 10 min before nerve stimulation. In the absence of stimulation cocaine and imipramine did not significantly modify NA and DOPEG outflow. Nerve stimulation (600 stimuli at 10 Hz frequency) was applied twice, at 30 min intervals. Cocaine increased the NA outflow to more than double and decreased the DOPEG outflow by about half during the nerve stimulation. Imipramine (5×10^{-6} M) had much the same effect as cocaine.

Effects of α-adrenoceptor blockers on noradrenaline and 3,4-dihydroxyphenylglycol outflow

α-Adrenoceptor blockers, prazosin, phentolamine, phenoxybenzamine and yohimbine were used to observe the effects of these blockers on the outflow of NA and DOPEG from the rabbit mesenteric artery. Figure 3 shows the effects of prazosin (10^{-6} M) on NA and DOPEG outflow. Application of prazosin increased the spontaneous outflow of DOPEG to about twice that of the control, and the outflow showed a maximum value after 20–30 min. With the wash out of prazosin, the spontaneous outflow of DOPEG was reduced to 60–70% of the control. Pretreatment with cocaine (10^{-5} M) did not block these effects of prazosin (Figure 3b).

The spontaneous outflow of NA and DOPEG remained unchanged when phentolamine (10^{-8} – 10^{-5} M), phenoxybenzamine (10^{-8}–10^{-6} M) or yohimbine (10^{-8}–10^{-6} M) was applied for up to 60 min.

Figure 4 shows the dose-response relationship of the effects of these α-adrenoceptor blockers on the outflow of NA and DOPEG induced by nerve stimulation (600 stimuli at 10 Hz frequency). The nerve stimulation was applied twice at 30 min intervals and the amount of NA (Figure 4a) and DOPEG outflow (Figure 4b) induced by the second stimulation was expressed in relation to the first. Each of the α-adrenoceptor blockers was applied 10 min before and during application of the second stimulation. Outflow of NA and DOPEG was increased by application of these α-adrenoceptor blockers, in a dose-dependent manner, at concentrations over 10^{-7} M: the potency was higher for phentolamine and phenoxybenzamine than for yohimbine and prazosin. Increasing the concentration of yohimbine to 10^{-5} M reduced the NA and DOPEG outflow induced by nerve stimulation.

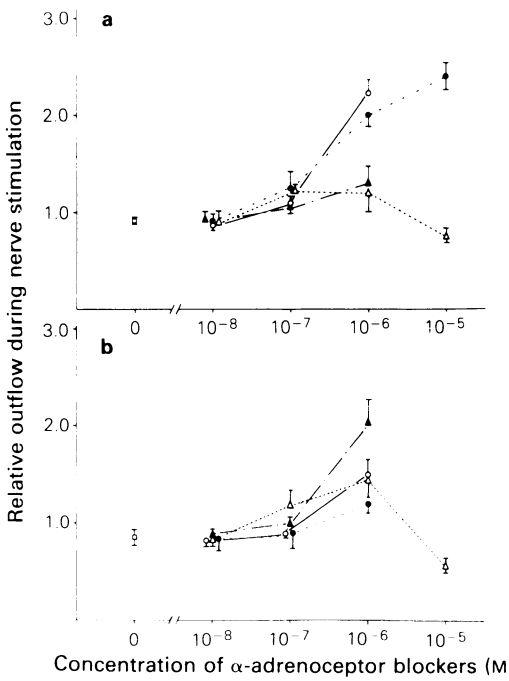

Figure 4 Dose-response relationship of the effects of α-adrenoceptor blockers on the outflow of (a) noradrenaline (NA) and (b) 3,4-dihydroxyphenylglycol (DOPEG) during nerve stimulation (600 stimuli at 10 Hz frequency). Each drug was applied 10 min before nerve stimulation: (○) phenoxybenzamine; (●) phentolamine; (△) yohimbine; (▲) prazosin. The nerve stimulation was applied twice, with a 30 min interval, and the amount of NA and DOPEG outflow produced by the second stimulation was expressed relative to the first. Mean values are shown; s.e. indicated by vertical lines. Number of observations was 3–5 for each agent.

Electrical responses of muscle membrane to perivascular nerve stimulation

Perivascular nerve stimulation elicited an excitatory junction potential (e.j.p.) in the smooth muscle of the rabbit mesenteric artery, the amplitude being 0.5–2 mV in response to a single stimulus. The response to a train of stimuli given at frequencies of 0.1–1 Hz showed both facilitation and depression, as in many vascular tissues (Kuriyama *et al.*, 1982). Figure 5 shows electrical responses of smooth muscle cells of the rabbit mesenteric artery induced by perivascular nerve stimulation for 1 min with three different frequencies (1, 3 and 5 Hz). With 1 Hz stimulation, e.j.ps with constant amplitude were observed, after the facilitation of e.j.ps was completed. Increasing the frequency of stimuli to 3 Hz produced a sustained depolarization due to summation of e.j.ps and also the development of a slow depolarization (Figure 5b). The latter component appeared more clearly when the frequency was increased to 5 Hz (Figure 5c).

Stimulation of nerves with frequencies over 10 Hz generated a spike potential superimposed on the summed e.j.ps and this was followed by a slow depolarization (Figure 5d). The spike potential of

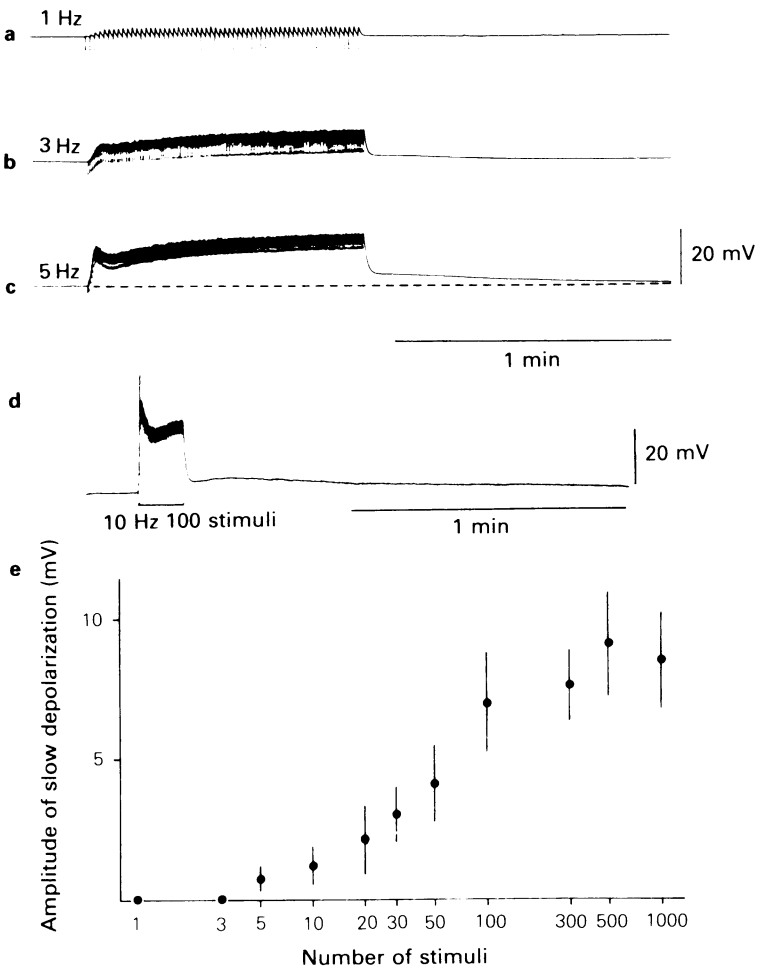

Figure 5 Electrical responses of smooth muscle membrane of the rabbit mesenteric artery to perivascular nerve stimulation: (a) 60 stimuli at 1 Hz frequency; (b) 180 stimuli at 3 Hz frequency; (c) 300 stimuli at 5 Hz frequency; (d) 100 stimuli at 10 Hz frequency. (e) Relationship between number of stimuli applied at 10 Hz frequency and the amplitude of slow depolarization. Mean of 4–9 observations is shown; s.d. shown by vertical lines. (a–c) and (d–e) were recorded from different tissues.

30–45 mV in amplitude appeared once at the beginning of a train of stimuli, regardless of the number of stimuli (over 3–5 stimuli) Figure 5e shows the relationship between the number of stimuli applied at a fixed frequency of 10 Hz and the amplitude of the slow depolarization. The amplitude of slow depolarization increased with increase in the number of stimuli and reached a maximum amplitude of about 8 mV by over 100 stimuli.

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

Application of phentolamine (10^{-7}–10^{-5} M), phenoxybenzamine (10^{-7}–10^{-6} M) or yohimbine (10^{-7}–10^{-6} M), but not prazosin (10^{-7}–10^{-5} M), increased the amplitude of e.j.ps in the rabbit mesenteric artery, as has been noted in many vascular tissues (Kuriyama *et al.*, 1982). Figure 6a is an example of the effects of yohimbine on the e.j.ps elicited by perivascular nerve stimulation for a period of 10 s with three different frequencies (0.5, 1 and 2 Hz). Application of yohimbine (10^{-6} M) increased the e.j.p. amplitude and inhibited the depression process of e.j.ps observed at 1 and 2 Hz frequency stimulations, in the control condition.

The amplitude of e.j.ps elicited by a single stimulus was usually too small (0.5–2 mV) to measure accu-

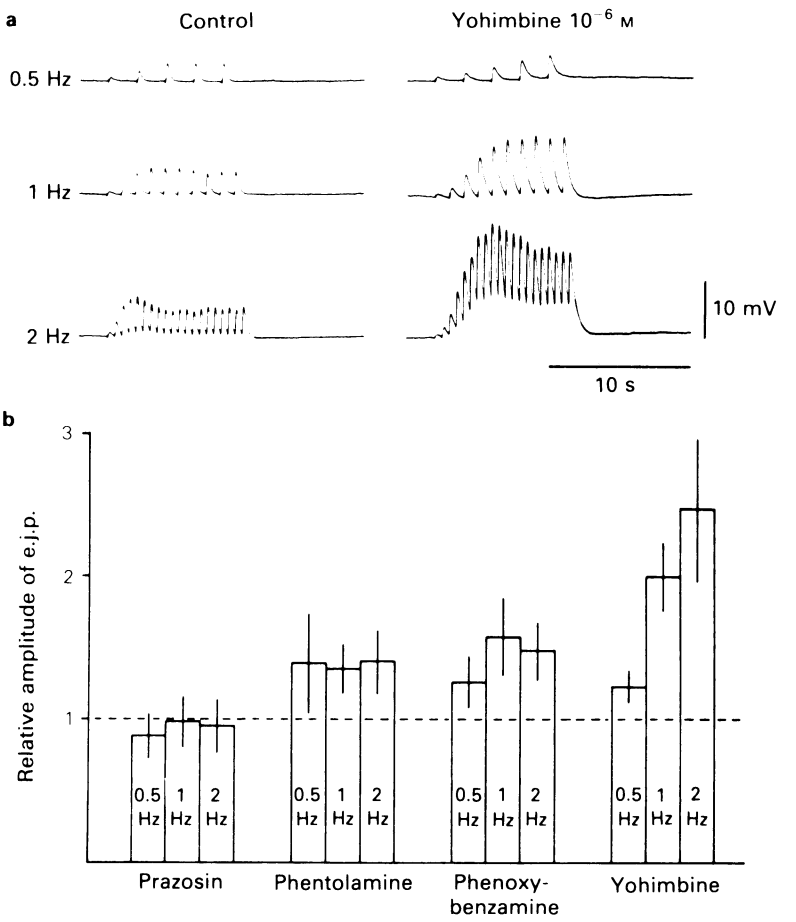

Figure 6 (a) Effects of yohimbine (10^{-6} M) on e.j.ps produced by perivascular nerve stimulation at 0.5 Hz (5 stimuli), 1 Hz (10 stimuli) and 2 Hz (20 stimuli) frequencies. Yohimbine was applied 5 min before recording these responses. All the responses were recorded from a single cell. (b) Effects of prazosin (10^{-6} M), phentolamine (10^{-6} M), phenoxybenzamine (10^{-6} M) and yohimbine (10^{-6} M) on the maximum amplitude of e.j.p. observed during perivascular nerve stimulation at 0.5, 1 and 2 Hz frequencies. Mean amplitudes of e.j.p. observed in each condition were compared in 4–6 tissues, and they are expressed as relative values (± s.d.).

rately, therefore the nerves were stimulated for a period which was sufficient to complete the facilitation process (10 s). However, because of the facilitation and depression phenomena, the amplitude of the e.j.ps, was not constant throughout the train of stimulation. To quantify the effects of α-adrenoceptor blockers on the e.j.p. amplitude, the maximum amplitude of e.j.ps elicited during 10 s stimulation was measured. Figure 6b shows the effects of prazosin, phentolamine, phenoxybenzamine and yohimbine, at a concentration of 10^{-6} M, on the maximum amplitude of e.j.ps elicited during perivascular nerve stimulation for a period of 10 s with 0.5, 1 and 2 Hz frequencies. The amplitude of e.j.p. is shown relative to the control value (=1.0). Prazosin did not affect the amplitude of e.j.ps ($P>0.05$). Phentolamine, phenoxybenzamine or yohimbine enhanced the e.j.p. amplitude, yohimbine being the most potent.

Application of prazosin (10^{-6} M) or phentolamine (10^{-6} M), but not of yohimbine (10^{-6} M) suppressed the generation of the slow depolarization but not of the e.j.ps (Figure 7). Phenoxybenzamine had the same effect as phentolamine in different experiments.

Effects of exogenously applied noradrenaline

Figure 8 shows the effects of exogenously applied NA (10^{-7}–10^{-5} M) on the membrane potential of the smooth muscles of the rabbit mesenteric artery. Each

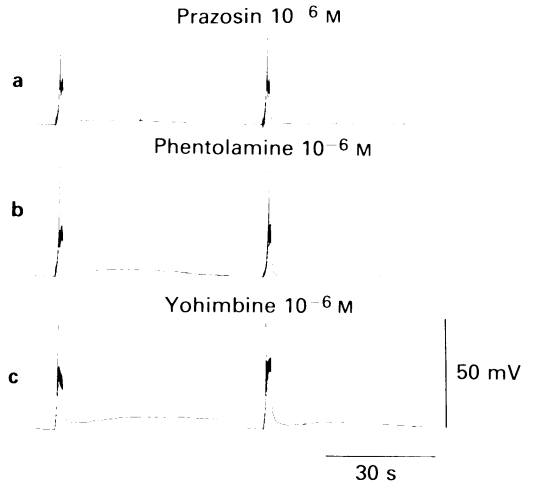

Figure 7 Effects of (a) prazosin (10^{-6}M), (b) phentolamine (10^{-6}M) or (c) yohimbine (10^{-6}M) on the electrical responses produced by perivascular nerve stimulation (20 stimuli at 10 Hz frequency). Each of these drugs was applied 5–7 min before recording these responses. Each response was recorded from a different tissue.

concentration of NA was applied for 10 min, and the membrane potentials were measured by impalement of different cells (usually 3–5 cells in each tissue) by the electrode. Application of NA depolarized the smooth muscle membrane at concentrations of over 5×10^{-7}M ($P<0.01$).

Figure 8 also shows the effects of NA on the membrane potential in tissues pretreated (5–10 min) with prazosin (10^{-6}M), phentolamine (10^{-6}M) or yohimbine (10^{-6}M). The resting membrane potential remained unchanged by application of prazosin or phentolamine, but was depolarized by application of

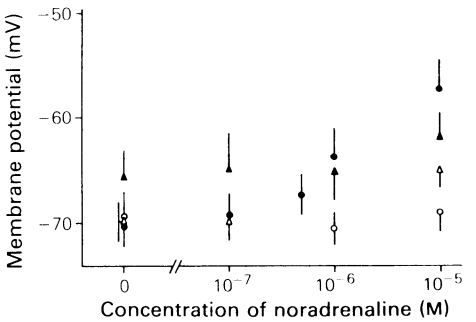

Figure 8 Dose-response relationship of the effects of prazosin (10^{-6}M, ○) phentolamine (10^{-6}M, △) or yohimbine (10^{-6}M, ▲) on the membrane depolarization produced by exogenously applied noradrenaline (10^{-7}–10^{-5}M); (●) = control. Mean of 12–54 observations is shown; s.d. indicated by vertical lines.

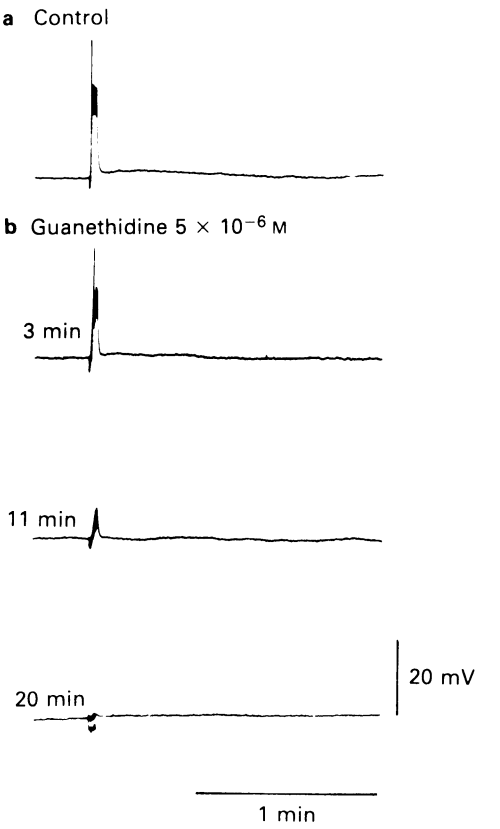

Figure 9 Changes in electrical responses produced by perivascular nerve stimulation (20 stimuli at 10 Hz frequency) after application of guanethidine (5×10^{-6}M). All the responses were recorded from a single cell.

yohimbine. Pretreatment with prazosin or phentolamine blocked the NA-induced depolarization, in doses up to 10^{-5}M. The yohimbine-induced depolarization was unaltered by TTX (3×10^{-7}M) or guanethidine (10^{-6}M–5×10^{-6}M). The amplitude of depolarizations produced by NA (10^{-6}M–10^{-5}M) was decreased by yohimbine ($P<0.05$). In different experiments, application of phenoxybenzamine (10^{-6}M) blocked the NA-induced depolarization of the membrane.

Effects of guanethidine on noradrenaline and 3,4-dihydroxyphenylglycol outflow and on electrical responses

Figure 9 shows the effects of guanethidine 5×10^{-6}M on the electrical responses produced by nerve stimulation (20 stimuli at 10 Hz frequency). This stimulation produced a spike potential superimposed on summed e.j.ps and a slow depolarization. Application of guanethidine reduced the amplitudes of the

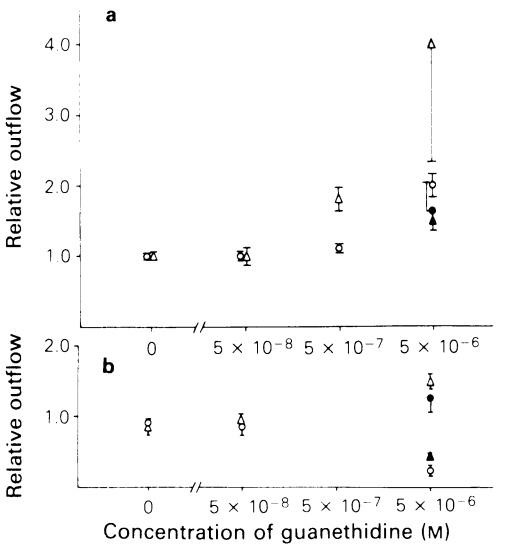

Figure 10 Effects of guanethidine on noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) outflow in unstimulated (spontaneous, a) and during nerve stimulation (600 stimuli at 10 Hz frequency, b). Guanethidine was applied 30 min before application of the nerve stimulation. Effluents were collected for 5 min before and during the nerve stimulation in control and during application of guanethidine. Amount of NA or DOPEG outflow is expressed relative to the first. Cocaine (10^{-5} M) was applied together with guanethidine (5×10^{-6} M) 30 min before the nerve stimulation. (○) NA outflow; (●) Na outflow with cocaine 10^{-5} M; (△) DOPEG outflow; (▲) DOPEG outflow with cocaine 10^{-5} M. Mean of 5 observations for control, 3 observations for guanethidine and 3 observations for guanethidine and cocaine; s.e. shown by vertical lines.

spike potential, the e.j.ps and the slow depolarization, and at 11 min, only the e.j.ps with reduced amplitude appeared. At 20 min, all these electrical responses induced by the nerve stimulation ceased. These effects of guanethidine were observed in 4 tissues, and the nerve-induced electrical responses disappeared in 15–40 min.

Application of guanethidine (5×10^{-6} M) increased the outflow of DOPEG and NA, in the absence of stimulation ($P<0.05$). Increase of the DOPEG outflow reached a maximum at 20–30 min after application of guanethidine. Guanethidine also blocked the increase in the outflow of NA induced by nerve stimulation ($P<0.01$). These effects of guanethidine were blocked by application of cocaine (10^{-5} M) or imipramine (10^{-5}).

The dose-response relationships of the effects of guanethidine on the spontaneous and nerve-stimulation evoked outflow of NA and DOPEG are shown in Figure 10. With no stimulation (Figure 10a), guanethidine (over 5×10^{-7} M) increased both the NA and DOPEG outflows, and cocaine (10^{-5} M) blocked the DOPEG outflow ($P<0.05$), but not the NA outflow measured in the presence of guanethidine. During nerve stimulation (Figure 10b), the NA outflow was decreased ($P<0.01$) and DOPEG outflow was increased ($P<0.01$) by pretreatment with guanethidine (5×10^{-6} M). Pretreatment with cocaine (10^{-5} M) reversed the effects of guanethidine, i.e., NA outflow was increased and DOPEG outflow was decreased. Imipramine (10^{-5} M) had much the same effect as cocaine on the NA and DOPEG outflow whether spontaneous or during electrical stimulation.

Discussion

In the rabbit mesenteric artery, the outflow of NA and DOPEG during nerve stimulation increased in parallel with increase in number of stimuli, and application of cocaine or imipramine enhanced the NA outflow and decreased the DOPEG outflow, close to levels seen in case of spontaneous outflow. Similar findings were apparent in human veins (Janssens & Verhaeghe, 1983). These observations suggest that most of the DOPEG in the effluent during the nerve stimulation is of presynaptic origin, as a result of oxidative deamination of NA after being taken up at the nerve terminal.

The spontaneous release of DOPEG from the rabbit mesenteric artery was increased by application of guanethidine. Inhibition by cocaine or imipramine of this effect of guanethidine suggests that guanethidine may cause the depletion of NA stored in the nerve terminal after being taken up into the nerve terminal (Mitchell & Oates, 1970; Kirpekar & Furchgott, 1972; Shand *et al.*, 1973). Application of guanethidine increased the DOPEG outflow and decreased the NA outflow during the nerve stimulation. Electrical responses of the smooth muscle membrane to perivascular nerve stimulation were completely blocked by application of guanethidine. Thus, blockade of adrenergic transmission by this drug is presumably due to a reduction in the amount of NA released from the nerve terminal. DOPEG does not produce electrical responses in the smooth muscle membrane of the rabbit mesenteric artery.

Our experiments revealed that the smooth muscle membrane of the rabbit mesenteric artery possesses α-adrenoceptors, as determined from the evidence that the depolarization produced by exogenously applied NA was blocked by prazosin, phentolamine or phenoxybenzamine, drugs which possess α_1-adrenoceptor blocking action (Langer, 1977; Starke, 1977). Moreover the NA-induced depolarization was partly blocked by yohimbine.

Perivascular nerve stimulation elicited an e.j.p., a spike potential and a slow depolarization in the rabbit mesenteric artery. These three cellular responses are elicited by nerve stimulation in the rat tail artery (Cheung, 1982), the rabbit ear artery (Suzuki & Kou, 1983) or the dog mesenteric vein (Suzuki, 1984). The α-adrenoceptor blockers blocked only the slow depolarization in these tissues. In each of these tissues, the depolarizations produced by exogenously applied NA are also blocked by α-adrenoceptor blockers which are specific for block of the slow depolarization in each tissue. In the rabbit mesenteric artery, the slow depolarization was blocked by prazosin, phentolamine or phenoxybenzamine but not by yohimbine, suggesting that the slow depolarization in the rabbit mesenteric artery is generated mainly through activation of α_1-adrenoceptors. On the other hand, none of the α-adrenoceptor blockers used in our experiment blocked the e.j.p., as observed in many vascular tissues (Kuriyama *et al.*, 1982). This may be due to involvement of different types of noradrenoceptors located in the junctional region (Hirst & Neild, 1980; 1981).

The effect of α-adrenoceptor blockers on the NA outflow were not always consistent with the change in e.j.p. amplitude in the rabbit mesenteric artery. Yohimbine enhanced the e.j.p. amplitude over twice that of the control, but the NA outflow was increased only by 10–20% of the control. In the rabbit ear artery, yohimbine depolarized the smooth muscle membrane and produced contraction, and the effects were unaltered by tetrodotoxin or guanethidine (Suzuki & Kou, 1983). The smooth muscle membrane of the rabbit mesenteric artery was also depolarized by yohimbine. Therefore, the enhancement by yohimbine of the amplitude of the e.j.p. may involve direct effects on the smooth muscle membrane, in addition to increases in the NA release from nerve terminal. In the rabbit mesenteric artery, the increasing effects of yohimbine on the NA outflow during nerve stimulation, were weak in comparison to those of phentolamine or phenoxybenzamine and, at a high concentration (10^{-5} M), the NA and DOPEG outflow decreased. In the rabbit pulmonary artery, yohimbine increased NA overflow during nerve stimulation (Starke *et al.*, 1975). Thus, the effects of yohimbine differ with the tissue, as has been noted in the case of phentolamine, i.e., this drug does not increase the NA outflow in the guinea-pig atrium (Angus & Korner, 1980; Angus *et al.*, 1984), in contrast to what is seen in other tissues (Langer, 1977; Starke, 1977). In the guinea-pig atrium, the increasing effects of phenoxybenzamine on NA outflow depend on frequency and duration of neuronal activity, and the α-autoinhibition mechanism may not operate when nerves are stimulated at extremely high or low frequencies (Angus & Korner, 1980; Story *et al.*, 1981). We examined the effects of α-adrenoceptor antagonists on NA outflow during nerve stimulation at 10 Hz whereas those on the e.j.p. amplitude were studied at 0.5–2 Hz. The difference in frequency could be relevant to the different effects of yohimbine on the e.j.p. amplitude and on the NA outflow.

Prazosin blocked the generation of slow depolarization and the depolarization produced by exogenously applied NA in the rabbit mesenteric artery. These effects of prazosin may be due to blockade of α_1-adrenoceptors located in the smooth muscle membrane. The amplitude of e.j.p. was not modulated by application of prazosin. However, the spontaneous outflow of DOPEG, but not of NA, was drastically increased by application of prazosin, and this effect was not blocked by cocaine or imipramine. Preliminary observations indicate that the tissue content of NA is decreased by application of prazosin in the rabbit mesenteric artery (S. Mishima, unpublished observation). Presumably, prazosin facilitates leakage of NA stored in the nerve terminal (Anderson *et al.*, 1979), and penetration of prazosin into the nerve terminal may occur by mechanisms other than uptake of catecholamines or guanethidine.

The present experiments showed that in the rabbit mesenteric artery, electrical responses recorded from the smooth muscle cells do not always show changes in parallel with the NA outflow, presumably due to actions other than α-adrenoceptor blockade with each drug and also due to heterogeneity of α-adrenoceptors in the pre- and postjunctional membranes.

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