Br. J. Pharmac. (1985), **86**, 405–416

Effects of tyramine on noradrenaline outflow and electrical responses induced by field stimulation in the perfused rabbit ear artery

Hiroko Miyahara & Hikaru Suzuki

Department of Pharmacology, Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

1 In the perfused rabbit ear artery the basal outflows of noradrenaline (NA) and 3,4-dihydroxyphenylglycol (DOPEG) were <1 ng g^{-1} and 1–2 ng g^{-1} wet weight of tissue respectively. Field stimulation increased outflows of NA and DOPEG in a frequency-dependent manner, and they reached the maximum value at frequencies over 5 Hz.

2 Tyramine (1×10^{-6}–1×10^{-4} M) increased basal outflow of NA and DOPEG, in a dose-dependent manner. This effect was not blocked by tetrodotoxin (TTX, 3×10^{-7} M), but was prevented by pretreatment with 6-hydroxydopamine (6-OHDA). Tyramine increased the field stimulation-induced outflow of NA but not that of DOPEG in a dose-dependent manner.

3 Cocaine (1×10^{-5} M) reduced the increased outflow of NA induced by tyramine at rest and during field stimulation, without modifying DOPEG-outflow. Guanethidine (5×10^{-6} M), increased outflows of NA and DOPEG at rest, and reduced the NA outflow induced by field stimulation.

4 Pretreatment with guanethidine (5×10^{-6} M) did not block the action of tyramine on NA and DOPEG basal outflows. Additional application of guanethidine during the presence of tyramine did reduce the outflow of NA induced by field stimulation, but did not modify the outflow of NA and DOPEG at rest.

5 Tyramine at concentrations over 1×10^{-5} M depolarized the smooth muscle membrane of the rabbit ear artery. After chemical denervation with 6-hydroxydopamine (6-OHDA) the depolarizing action of tyramine was reduced.

6 Tyramine-induced depolarization was attenuated by prazosin (5×10^{-6} M) or phentolamine (5×10^{-6} M), but not by guanethidine (5×10^{-6} M). In 6-OHDA-denervated tissues, tyramine-induced depolarization was attenuated by phentolamine but not by prazosin.

7 Field stimulation evoked excitatory junction potential (e.j.p.), slow depolarization and spike potential in the rabbit ear artery. Tyramine reduced, while guanethidine blocked these electrical responses. Tyramine did not alter the facilitation process of e.j.ps.

8 In tissues pretreated with guanethidine, tyramine evoked either no electrical response or a slow depolarization during field stimulation. The slow depolarization was blocked by prazosin.

9 Tyramine reduced the NA content of tissues in a dose-dependent manner (by 31% at 10^{-4} M). Guanethidine (5×10^{-6} M) reduced the NA content by 20%.

10 We conclude that in the rabbit ear artery, tyramine depolarizes the smooth muscle membrane indirectly by releasing neuronal NA which acts on α-adrenoceptors, and directly by an action on the smooth muscle cells. Two NA compartments (guanethidine-sensitive and tyramine-sensitive NA) could be identified. Field stimulation releases the former with associated generation of e.j.p. and slow depolarization whilst the release of the latter is not accompanied by e.j.p. generation.

Introduction

Tyramine has indirect and direct sympathomimetic actions on adrenergically innervated tissues: thus tyramine releases noradrenaline (NA) from adrenergic nerves and the released NA stimulates adrenoceptors distributing in the effector organ (Trendelenburg, 1972; Vanhoutte *et al.*, 1981), or tyramine stimulates α-adrenoceptors directly and produces muscle contractions in the dog cerebral arteries (Toda *et al.*,

1978). The release of NA by tyramine is reduced by cocaine which prevents tyramine from being taken up into the adrenergic nerves (Trendelenburg, 1972).

Stimulation of perivascular adrenergic nerves releases into the perfusate noradrenaline (NA) and its metabolites 3,4-dihydroxyphenylglycol (DOPEG), 3-methoxy-4-hydroxyphenylglycol (MOPEG), normetanephrine (NM) or 3-methoxy-4-hydroxymandelic acid (VMA) (Graefe & Henseling, 1983). Increased outflow of DOPEG during field stimulation is largely related to NA which had been taken up into the nerve terminal. Therefore, measurements of outflow of NA and DOPEG during field stimulation allow estimation of the amounts of released and taken up NA in vascular tissues (Mishima *et al.*, 1984).

In the rabbit ear artery, field stimulation evokes an excitatory junction potential (e.j.p.), a slow depolarization and a spike potential. α-Adrenoceptor blocking agents (prazosin or phentolamine) and Ca antagonists block the slow depolarization and the spike potential respectively, while guanethidine blocks all three electrical responses (Kajiwara & Casteels, 1983; Suzuki & Kou, 1983a).

It is not certain how the e.j.p. is related to the released NA in this tissue. Since tyramine releases NA from nerve terminals, it is of interest to compare outflows of NA and DOPEG induced by tyramine in relation to the generation of the e.j.p. and other effector responses.

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 central ear artery was dissected and fatty tissues and the connective tissues surrounding the vessel were removed in Krebs solution and at room temperature.

Measurement of outflow of noradrenaline and 3,4-dihydroxyphenylglycol

Segments of the ear artery (3–4 cm long) were used for measurements of NA outflow. A pair of Ag-AgCl wires (0.5 mm diameter, 5 cm long) was fixed vertically in parallel at a 1.5–2.0 mm distance, and the artery was mounted between the wires with cotton thread. Krebs solution (35°C) was superfused over the tissue at a constant flow rate of $1\,ml\,min^{-1}$ by use of a perfusion pump (Tokyo Rikakikai, PO-1). The tissue was incubated under such conditions for at least 1 h before starting the experiment. Electrical stimulation (square pulse of 0.2 ms duration, 50 V, 10 Hz and 600 stimuli) was applied 6 times at intervals of 25 min, from an electric stimulator (Nihon Kohden SEN 1101). The superfusates were collected in conical tubes at the bottom of the tissue, usually for 5 min before and after the field stimulation. Tyramine or guanethidine was added to the superfusate for 60 min, starting 15 min after the second stimulation. During application of tyramine or guanethidine, superfusates were collected every 5 min for the initial 20 min, then every 10 min. To each superfusate sample was added 50 μl perchloric acid (60%) and the solution was stored 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.

Perivascular adrenergic nerves were denervated chemically by use of 6-hydroxydopamine (6-OHDA) as described by Apligliano & Hermsmeyer (1976). Briefly, arteries were exposed to unbuffered electrolyte solution ($NaHCO_3$ and KH_2PO_4 were omitted) containing 6-OHDA ($750\,\mu g\,ml^{-1}$) and ascorbic acid ($0.2\,mg\,ml^{-1}$), adjusted to pH 4.3–4.95, for two 10 min periods with a 10 min interval. Then they were incubated in the Krebs solution for 1 h before experimentation.

NA and its metabolites in the samples were extracted by the alumina adsorption method (Oishi *et al.*, 1983), and 50 μl of the extracted samples was injected onto high-performance liquid chromatography (Yanagimoto MGF, Co L-2000L) with a Hamilton microsyringe. The detector was a thin layer voltammetric detector (VMD-101) with a glassy carbon working electrode vs a Ag-AgCl reference electrode with a detection limit of about 20 pg of NA or DOPEG.

Measurement of content of noradrenaline and 3,4-dihydroxyphenylglycol in tissue

A pair of rabbit ear arteries was incubated for 1 h in Krebs solution; one artery was transferred to drug-containing solution and the other (control) to Krebs solution. After incubation, tissues were homogenized in 4 ml of ice cold 0.4 N perchloric acid with a glass homogenizer. The homogenate was centrifuged for 10 min at 1000 *g* and the supernatant was assayed for NA, according to the method mentioned above.

Recording of electrical activity

A segment of ear artery (1.5–2.0 cm in length) was mounted in a 2 ml chamber made of Lucite plate and superfused with Krebs solution(35.5°C) at a flow rate of $2–3\,ml\,min^{-1}$. The vessel was impaled from the outer surface with glass capillary microelectrodes filled with 3 M KCl, to record electrical activities of single smooth muscle cells. Electrical pulses were applied transmurally to stimulate perivascular nerves through a pair of Ag-AgCl wires (0.5 mm in diameter) placed at opposite sides of the artery. Square pulses of 0.05–0.1 ms duration and 20–50 V were supplied by an electric stimulator (Nihon-Kohden SEN 7103) and the responses of the smooth muscle cells were dis-

played on a pen-writing recorder (Nihon Kohden Recticorder RJG 4024).

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 $Na_2S_2O_5$ 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 tyramine, guanethidine sulphate, cocaine hydrochloride and tetrodotoxin (Sankyo), prazosin hydrochloride (Pfizer), phentolamine mesylate (Ciba-Geigy) and 6-hydroxydopamine (Sigma).

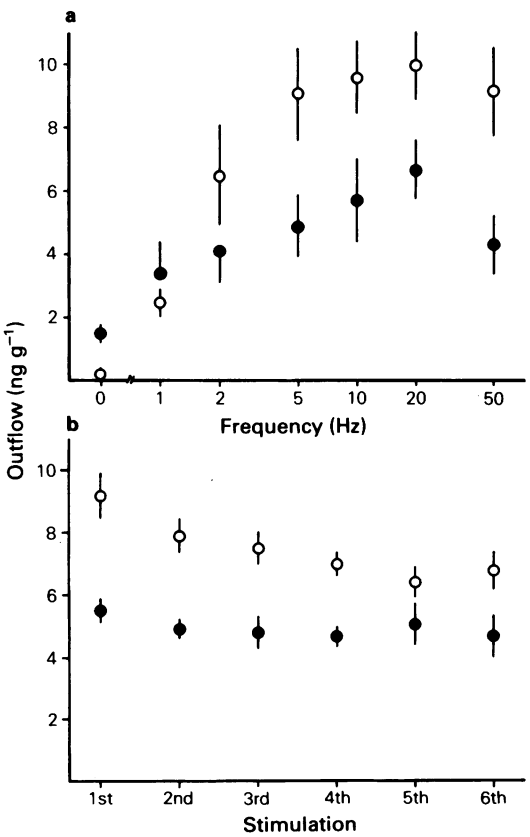

Figure 1 Outflow of noradrenaline (NA, O) and of 3,4-dihydroxyphenylglycol (DOPEG, ●) during field stimulation of the rabbit ear artery. (a) Field stimulation with 600 pulses at different frequencies. Frequency at zero means absence of field stimulation. Each value is mean from 6 experiments; vertical lines show s.e.mean. (b) Changes in NA and DOPEG outflow induced by 6 consecutive applications of field stimulation (600 stimuli at 10 Hz) at 25 min intervals, Values are mean with s.e.mean shown by vertical lines ($n = 16$).

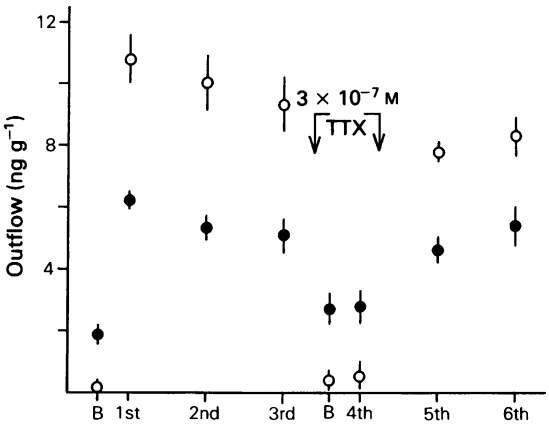

Figure 2 Effects of tetrodotoxin (TTX) on outflow of noradrenaline (NA, O) and 3,4-dihydroxyphenylglycol (DOPEG, ●) induced by field stimulation (600 pulses, 10 Hz every 25 min) in the rabbit ear artery. TTX (3×10^{-7} M) was applied for a period of 15 min at 10 min before application of the 4th stimulation. Outflows of NA and DOPEG are expressed as ng g^{-1} wet weight of tissue. B, basal outflow in the absence of field stimulation. Each point shows mean ($n = 6$) with vertical lines indicating s.e.mean.

Statistics

The experimental values were expressed by the mean ± s.d. or s.e. Statistical significance was determined by use of Student's t test ($P < 0.05$).

Results

Outflows of noradrenaline and 3,4-dihydroxyphenylglycol during field stimulation

At rest, the outflow of noradrenaline (NA) from the rabbit ear artery into the perfusate was less than 1 ng g^{-1} wet weight of tissue, and sometimes undetectable, while that of 3,4-dihydroxyphenylglycol (DOPEG) was 1–2 ng g^{-1}.

With field stimulation, outflows of both NA and DOPEG increased in proportion to the frequency or the number of stimuli. Figure 1a shows the relationship between the frequency of a fixed number of 600 stimuli and the amount of NA and DOPEG in the perfusate. The outflow of NA and DOPEG increased frequency-dependently, up to 5 Hz, and over 10 Hz they remained unchanged (5 Hz vs 10–50 Hz, $P > 0.05$). At 50 Hz DOPEG outflow decreased. In subsequent experiments perivascular nerves were stimulated with 600 stimuli at 10 Hz.

Figure 1b shows that when field stimulation was

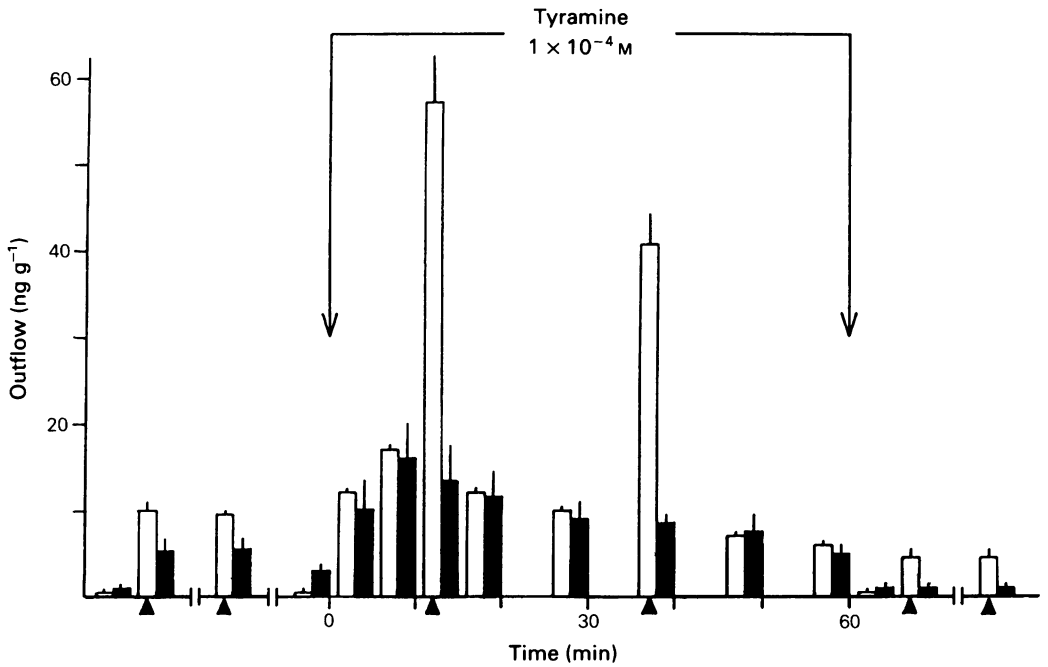

Figure 3 Effects of tyramine on outflow of noradrenaline (NA, open columns) and 3,4-dihydroxyphenylglycol (DOPEG, closed columns) from the rabbit ear artery. Tyramine (1×10^{-4} M) was applied for 60 min, between arrows. Amounts of NA and DOPEG contained in effluents which were collected during 5 min are shown as ng g^{-1} wet weight of tissue. Field stimulation (▲, 600 stimuli at 10 Hz) was applied every 25 min. Vertical lines represent s.e.mean ($n = 5$).

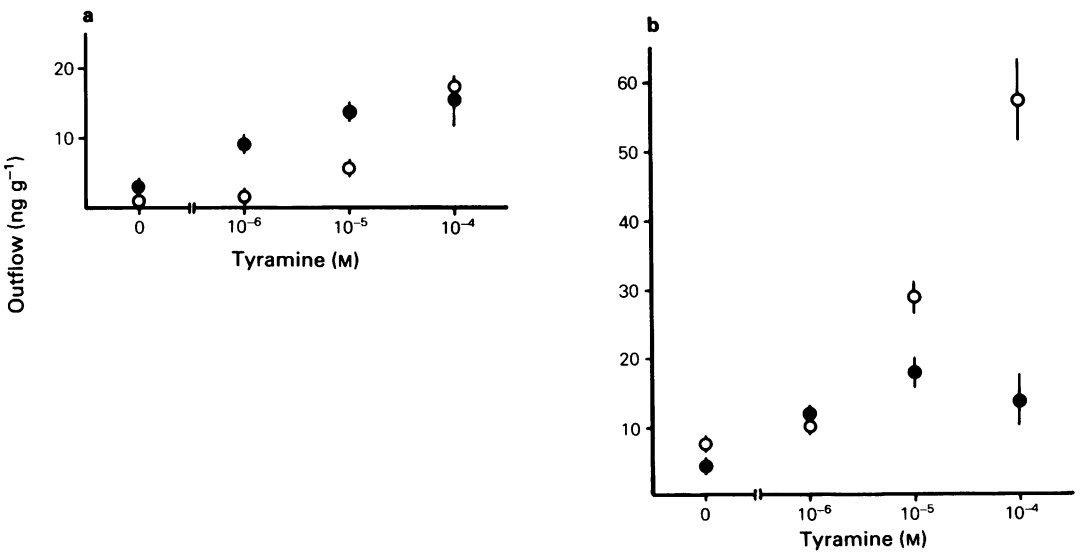

Figure 4 Dose-response relationship of the effects of tyramine on outflow from rabbit ear artery of noradrenaline (NA, ○) and of 3,4-dihydroxyphenylglycol (DOPEG, ●) measured in the absence (a) and presence (b) of field stimulation. Outflows of NA and DOPEG were measured between 5 and 10 min or between 10 and 15 min, after application of tyramine. Field stimulation (600 pulses at 10 Hz) was applied 10 min after exposure to tyramine. Each point shows mean ($n = 6$) with vertical lines indicating s.e.mean.

repeated at 25 min intervals, the NA outflow decreased gradually and reached an almost constant value at the 3rd stimulation,whilst DOPEG outflow was constant throughout (1st vs 6th, $P > 0.05$).

Tetrodotoxin (TTX, 3×10^{-7}M) applied before the 4th stimulation reversibly blocked the stimulation-induced increase in outflow of NA and DOPEG without altering the resting outflow (Figure 2). After denervation of adrenergic nerves by 6-hydroxydopamine (6-OHDA), outflow of NA and DOPEG was undetectable, in the presence or absence of field stimulation ($n = 5$).

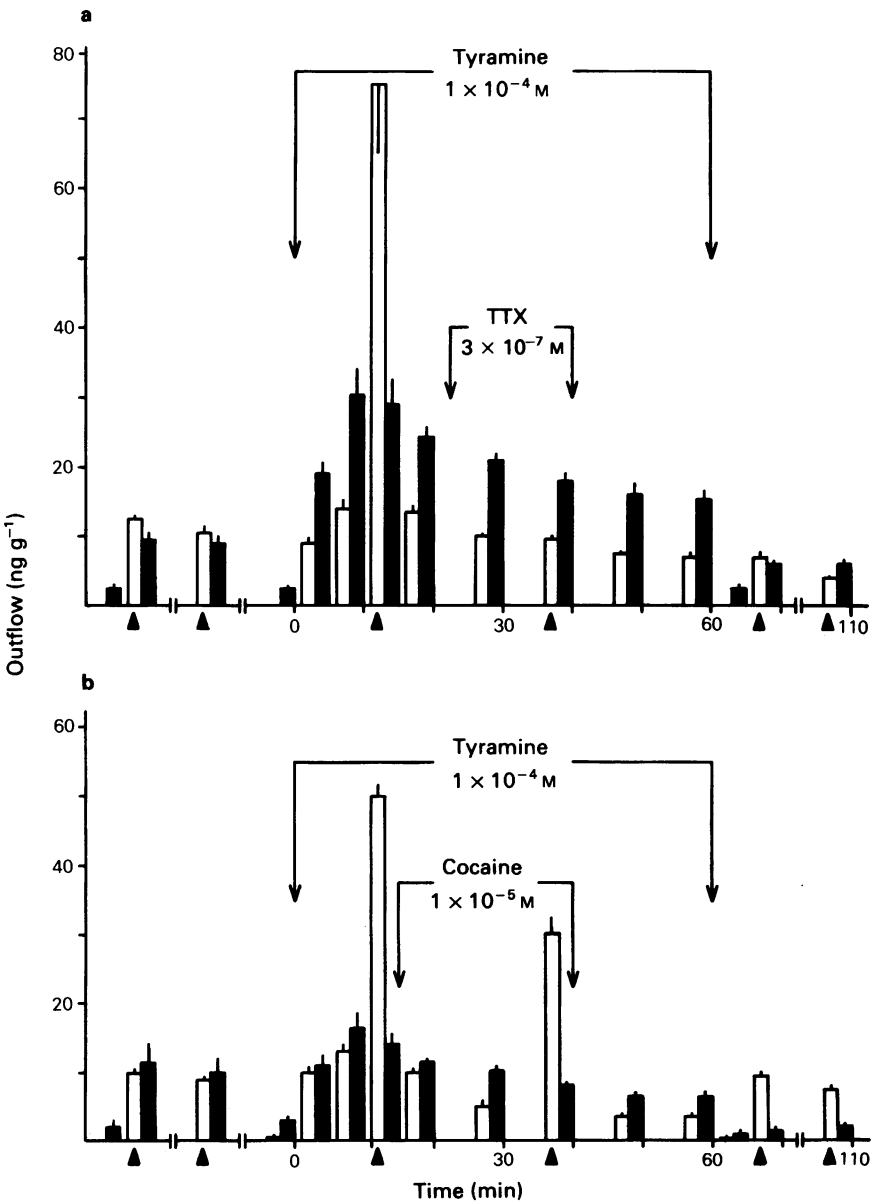

Figure 5 Effects of tetrodotoxin (TTX, a) or cocaine (b) on outflow of noradrenaline (NA, open columns) and 3,4-dihydroxyphenylglycol (DOPEG, closed columns) in the presence of tyramine from rabbit ear artery. TTX 3×10^{-7}M (a) or cocaine 1×10^{-5}M (b) was applied between arrows. Field stimulation (600 stimuli at 10 Hz) was applied at filled triangle. Vertical lines represent s.e.mean ($n = 3$).

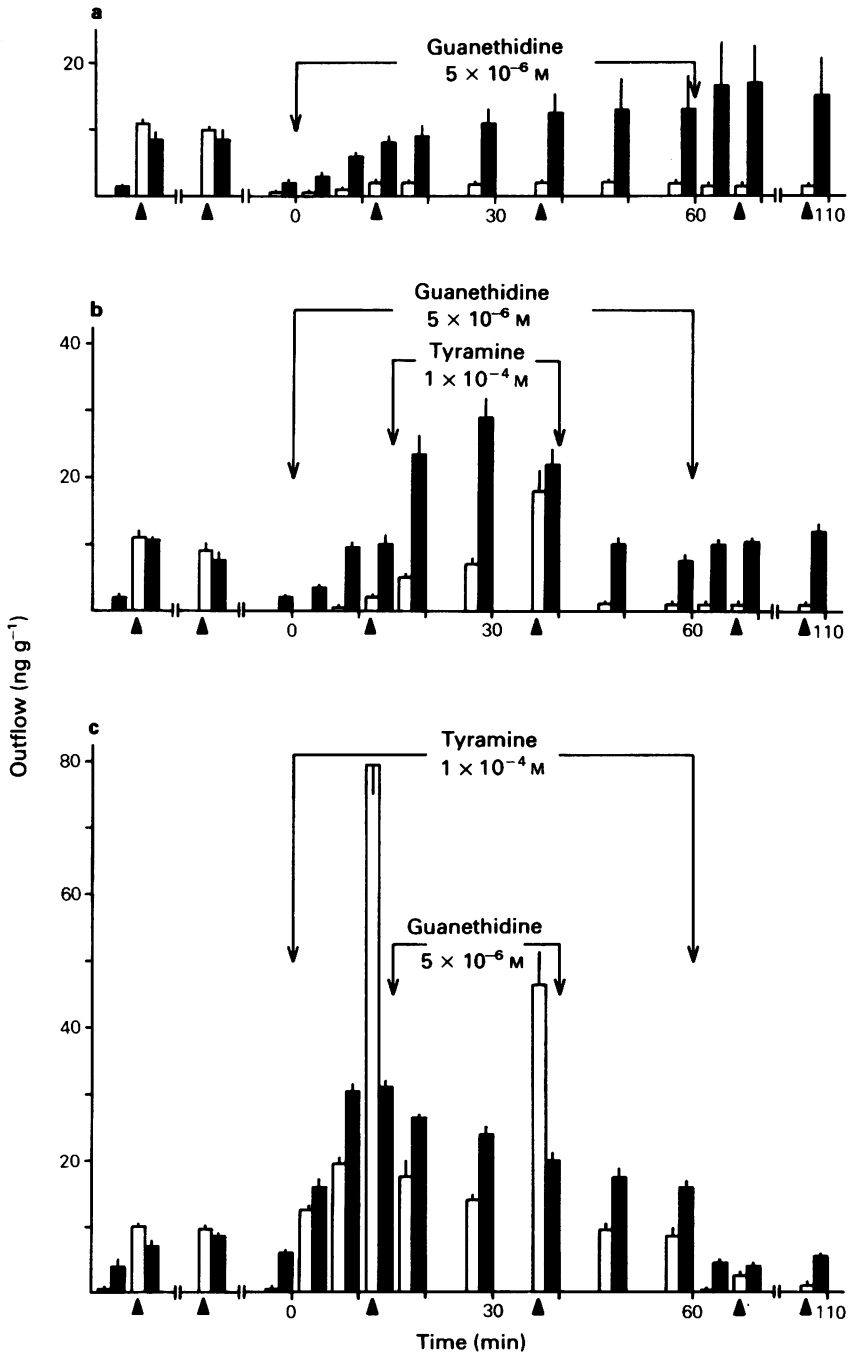

Figure 6 Effects of guanethidine and tyramine on outflow of noradrenaline (NA, open columns) and of 3,4-dihydroxyphenylglycol (DOPEG, closed columns) in the rabbit ear artery. Guanethidine (5×10^{-6}M) or tyramine (1×10^{-4}M) was applied between arrows. (a) Guanethidine was applied for 60 min. (b) Tyramine was applied for 25 min during presence of guanethidine. (c) Guanethidine was applied for 25 min during presence of tyramine. Field stimulation (600 stimuli at 10 Hz) at ▲. Each column shows mean value of 4 (a), or 3 (b and c) observations. Vertical lines represent s.e.mean.

Effects of tyramine on outflow of noradrenaline and 3,4-dihydroxyphenylglycol

Tyramine (1×10^{-6}–1×10^{-4}M) reversibly increased outflows of NA and DOPEG from the rabbit ear artery. Figure 3 shows a time course for outflows of NA and DOPEG during application of tyramine (1×10^{-4}M) for 60 min. Basal outflow of NA and DOPEG increased gradually and reached the maximum values at 10 min exposure to tyramine, and then decreased gradually. Field stimulation-induced outflow of NA was increased, while that of DOPEG remained unchanged in the presence of tyramine. Stimulation-induced outflows of NA and DOPEG decreased below the control values after washing out tyramine.

Figure 4 shows the dose-response relationship of the effects of tyramine on outflow of NA and DOPEG. Outflows of both substances between 5 and 10 min after application of tyramine (1×10^{-6}–1×10^{-4}M) were measured in the absence of field stimulation. Amounts of NA and DOPEG increased in a dose-dependent manner, and the outflow of DOPEG reached the maximum value with over 1×10^{-5}M tyramine (Figure 4a). Figure 4b shows the effects of tyramine on outflow of NA and DOPEG induced by field stimulation (600 stimuli at 10 Hz). The outflow of NA increased in a dose-dependent manner, while that of DOPEG remained unaltered from the basal outflow during application of 1×10^{-6}–1×10^{-4}M tyramine.

As shown in Figure 5a, application of TTX (3×10^{-7}M) in the presence of tyramine (10^{-4}M) blocked the increase in the outflow of NA induced by field stimulation. The basal outflows of NA and DOPEG induced by tyramine were not modified by TTX. Cocaine (1×10^{-5}M) reduced the basal and field stimulation-induced NA outflow in the presence of 10^{-4}M tyramine ($n = 3$, $P < 0.05$), without altering the outflow of DOPEG (Figure 5b).

Effects of guanethidine on tyramine actions

Guanethidine (5×10^{-6}M) increased basal outflow of both NA and DOPEG in the rabbit ear artery (Figure 6a), whilst reducing outflow of NA induced by field stimulation. In the presence of guanethidine, DOPEG outflow remained unchanged by field stimulation. These effects of guanethidine continued after the tissue had been rinsed for over 1 h.

In the presence of guanethidine (5×10^{-6}M), application of tyramine (1×10^{-4}M) further increased the outflow of NA and DOPEG (Figure 6b) but the increase was less than that seen with tyramine alone (Figure 3). In the presence of both guanethidine and tyramine, field stimulation increased the NA outflow, while it did not alter the DOPEG outflow, in comparison with that seen with guanethidine alone ($P > 0.4$).

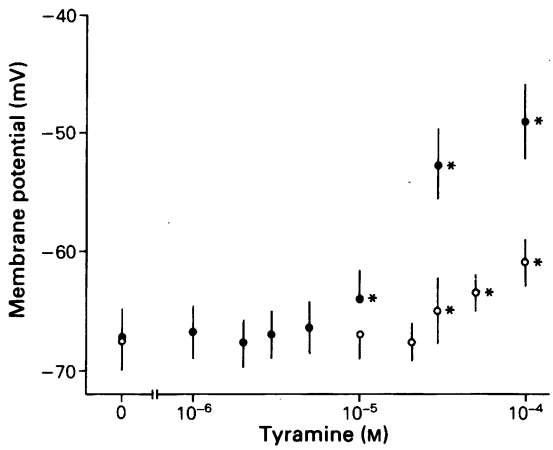

Figure 7 Dose-response relationship of the effects of tyramine on membrane potentials of smooth muscle cells of the rabbit ear artery. Membrane potentials were measured by successive impalements with electrodes of different cells in the intact (●, control) or the denervated (○) ear arteries, during application of tyramine for 30 min. Each point shows mean ($n = 16$–48) obtained from 9 intact and 3 denervated tissues; vertical lines show s.d.
*Significantly different from the membrane potential measured before application of tyramine ($P < 0.05$).

Table 1 Effects of guanethidine, prazosin or phentolamine on membrane depolarizations produced by 1×10^{-4}M tyramine in smooth muscle cells of the rabbit ear artery

	Membrane potential (mV) *Before Tyramine*	*Tyramine* (1×10^{-4}M)
(a) *Intact tissue*		
Control	−67.4 ± 3.5 (95)	−49.9 ± 3.2 (32)
Guanethidine 5×10^{-6}M	−63.9 ± 1.7 (15)*	−51.5 ± 2.3 (16)
Prazosin 5×10^{-6}M	−67.9 ± 1.9 (27)	−60.2 ± 2.3 (27)*
Phentolamine 5×10^{-6}M	−67.7 ± 2.4 (46)	−63.6 ± 2.5 (23)*
(b) *6-OHDA-denervated tissue*		
Control	−67.0 ± 3.7 (122)	−61.6 ± 2.2 (50)
Prazosin 5×10^{-6}M	−67.2 ± 3.6 (23)	−61.2 ± 3.5 (26)
Phentolamine 5×10^{-6}M	−66.6 ± 1.8 (15)	−63.7 ± 2.8 (32)*

Different cells were penetrated with microelectrodes while these drugs were applied for 20–40 min. Tyramine (1×10^{-4}M) was then applied for 30 min together with these drugs. Mean ± s.d. is shown. Number of observations obtained from 3–5 different tissues is shown in parentheses.
*Statistically significant difference from the control ($P < 0.05$).

The increase in outflow of NA was also observed in the presence of 5×10^{-6}M tyramine.

Figure 6c shows that application of guanethidine (5×10^{-6}M) in the presence of tyramine (1×10^{-4}M) did not alter basal outflow of either NA or DOPEG and decreased the outflow of NA during field stimulation ($P < 0.05$), in comparison with that observed during application of tyramine alone (Figure 3). Stimulation-induced outflows of NA and DOPEG decreased below the control values after washing out of guanethidine and tyramine.

Effects of tyramine on membrane potential of smooth muscle cells

Membrane potentials of smooth muscle cells of the rabbit ear artery were measured by impalements of different cells with microelectrode and various concentrations (1×10^{-6}–1×10^{-4}M) of tyramine were applied for over 30 min. Figure 7 shows the dose-response relationship of the effects of tyramine on membrane potentials recorded from intact and 6-OHDA-denervated tissues. In intact tissues, tyramine (over 10^{-5}M) depolarized the muscle membrane in a dose-dependent manner. In the denervated tissues, the minimum concentration of tyramine required to depolarize the membrane was increased to 3×10^{-5}M. The depolarization induced by tyramine was larger in the intact than in the denervated tissues.

Effects of guanethidine, prazosin or phentolamine on the tyramine-induced depolarization are summarized in Table 1. In the intact tissues, prazosin (5×10^{-6}M) and phentolamine (5×10^{-6}M), but not guanethidine (5×10^{-6}M), reduced the tyramine (1×10^{-4}M)-induced depolarization. Phentolamine was more potent than prazosin in inhibiting the depolarization at equimolar concentration ($P < 0.05$).

Denervation of adrenergic nerves by 6-OHDA did not alter the resting membrane potential of smooth muscle cells of the rabbit ear artery. In the denervated tissues, 1×10^{-4}M tyramine-induced depolarization was much smaller than that seen in the intact tissue, and the depolarization was attenuated by phentolamine but not by prazosin (Table 1b).

Effects of tyramine on adrenergic transmission

In smooth muscle cells of the rabbit ear artery, field stimulation evokes an excitatory junction potential (e.j.p.) with a slow depolarization and, in high-frequency stimulation, a spike potential superimposed on the e.j.p. (Suzuki & Kou, 1983a).

E.j.ps were evoked by single stimuli with intervals of over 30 s to exclude possible involvement of facilitation of e.j.ps in the effects of tyramine. The amplitude of e.j.ps was decreased by tyramine (1×10^{-6}–1×10^{-4}M) in a dose-dependent manner (Figure 8).

Repetitive application of field stimulation at low frequencies (1–0.1 Hz) produced e.j.ps with progressive increases in amplitude (facilitation phenomenon) in the rabbit ear artery. Figure 9 shows the effects of tyramine (5×10^{-6}M) on e.j.ps evoked by a train of 5 stimuli at 0.5 Hz frequency. In the control condition,

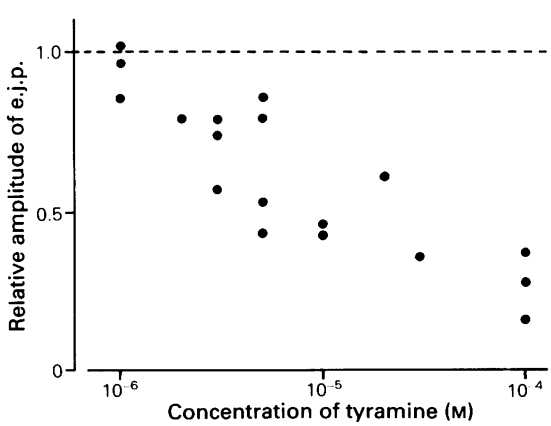

Figure 8 Effects of tyramine on amplitude of e.j.p. produced by field stimulation in the rabbit ear artery. Field stimulation was applied every 30 s, while tyramine was applied for 10–20 min. Amplitudes of e.j.ps relative to that evoked before application of tyramine are plotted, against concentration of tyramine. Each point is a mean value ($n = 7$–20) obtained from 7 tissues.

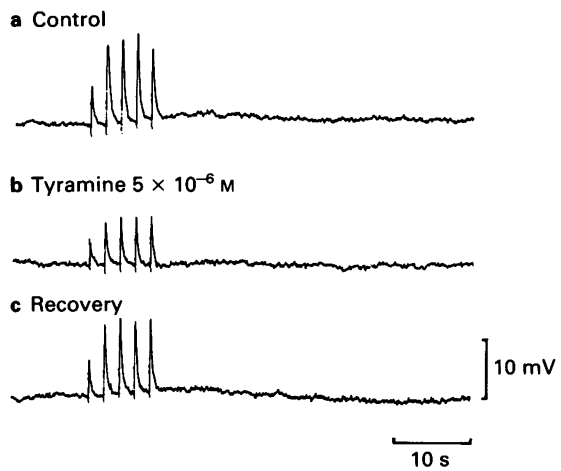

Figure 9 Effects of tyramine on e.j.ps and slow depolarization produced by field stimulation in the rabbit ear artery. Field stimulation (0.05 ms pulse width, 30 v) was applied 5 times at 0.5 Hz: (a) control; (b) tyramine 5×10^{-6}M (15 min); (c) recovery at 20 min after washing out tyramine. All the responses were recorded from the same cell.

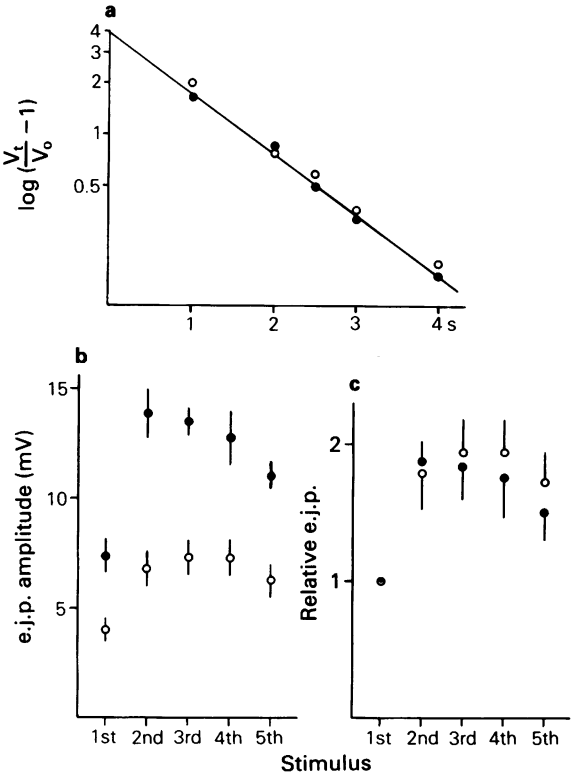

Figure 10 Effects of tyramine on e.j.ps recorded from the rabbit ear artery. (a) Two stimuli were applied at different intervals (1–4 s), and relative amplitude of the second e.j.p. expressed as (Vt/Vo − 1) was plotted on a logarithmic scale as a function of time between the two stimuli, where Vo and Vt were amplitudes of the first and the second e.j.ps (Mallart & Martin 1967). (●) Control; (○) tyramine 5×10^{-6}M (5–60 min). Straight line in the figure is given by $f = 3.9e^{-0.8t}$, where f = the e.j.p. amplitude determined by (Vt/Vo − 1) and t = time between the two stimuli. (b) Amplitudes of e.j.ps evoked by 5 stimuli at 0.5 Hz. (●) Control ($n = 5$); tyramine 5×10^{-6}M (5–60 min, $n = 8$). Mean ± s.d. is shown. (c) Amplitudes of e.j.ps evoked by 5 stimuli at 0.5 Hz were expressed as relative value to the first of each train of stimulation. (●) Control; (○) tyramine 5×10^{-6}M (5–60 min). Mean values are shown with s.d. indicated by straight lines.

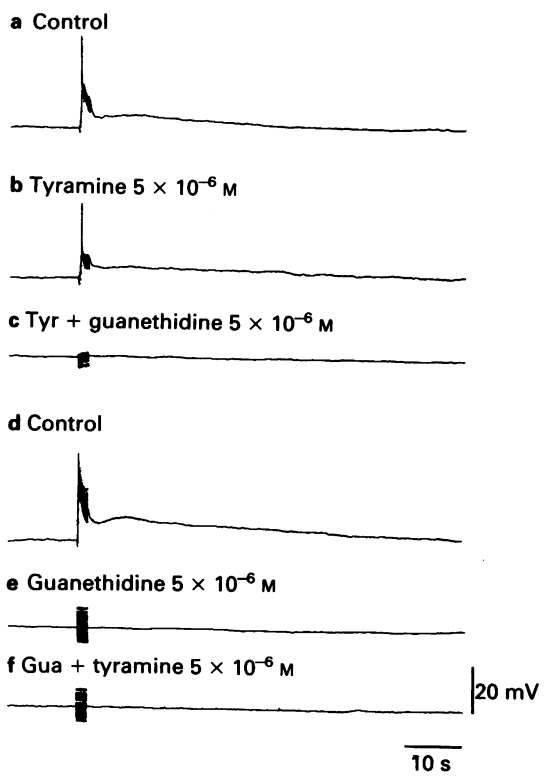

Figure 11 Effects of tyramine or guanethidine on electrical responses produced by field stimulation (20 stimuli at 10 Hz) in the rabbit ear artery. (a) Control; (b) tyramine 5×10^{-6}M (10 min); (c) tyramine 5×10^{-6}M plus guanethidine 5×10^{-6}M (20 min); (d) control; (e) guanethidine 5×10^{-6}M (15 min); (f) guanethidine 5×10^{-6}M plus tyramine 5×10^{-6}M (12 min). a–c and d–f were recorded from different tissues.

the field stimulation evoked e.j.ps superimposed on a slow depolarization of about 2 mV in amplitude. In the presence of 5×10^{-6}M tyramine (5–30 min), the amplitude of e.j.ps decreased and the slow depolarization was also decreased below detectable amplitude.

A pair of field stimulation pulses separated by different intervals (1–4 s) was applied to the rabbit ear artery, before, and during application of tyramine (5×10^{-6}M). The amplitude of e.j.ps evoked by the second stimulus was expressed as (Vt/Vo − 1), where Vo and Vt were the first and the second e.j.p. amplitudes respectively, and these values were plotted on a logarithmic scale against the interval between the two stimuli (Mallart & Martin, 1967). As shown in Figure 10a, the relationship was linear, and was not modified by tyramine (5×10^{-6}M). Figure 10b shows that amplitude of e.j.ps evoked by a train of 5 stimuli at a frequency of 0.5 Hz decreased by application of tyramine (5×10^{-6}M). However, the facilitation phenomenon of e.j.ps was still observed in the presence of tyramine. Figure 10c shows relative amplitude of e.j.ps, in which the amplitude of e.j.ps is expressed as relative value to the first of each train. The facilitation process of the e.j.ps was not modified by tyramine (5×10^{-6}M).

Field stimulation (50 stimuli at 10 Hz) evoked a spike potential on the summed e.j.ps and then a slow depolarization in the rabbit ear artery (Figure 11a, d).

Application of 5×10^{-6}M tyramine reduced the amplitudes of these electrical responses produced by field stimulation, without change in the membrane potential (Figure 11b). Additional application of guanethidine (5×10^{-6}M) blocked all these electrical responses to field stimulation (Figure 11c).

In the presence of guanethidine (5×10^{-6}M for 30–60 min), field stimulation produced no detectable change in membrane potential (Figure 11e). In such tissues, additional application of 5×10^{-6}M tyramine depolarized the smooth muscle membrane by about 4 mV (guanethidine alone, -65.2 ± 2.3, $n = 18$; guanethidine plus tyramine, -61.1 ± 2.0, $n = 11$, $P < 0.05$), and electrical response was not evoked (Figure 11f). In 2 out of 7 tissues, field stimulation evoked slow depolarization. The amplitude of this slow depolarization was small (0.5–2 mV vs 5–8 mV in the control). By adding prazosin (10^{-6}M) to the tyramine-containing solution, the slow depolarization was abolished.

After the depolarization induced by tyramine was blocked by phentolamine, field stimulation during application of 1×10^{-4}M tyramine and guanethidine (5×10^{-6}M) did not evoke e.j.p..

Effects of tyramine on tissue content of noradrenaline

The content of NA in the rabbit ear artery, measured after homogenizing the tissue with a glass homogenizer, varied between tissues (700 ng g^{-1} to 2000 ng g^{-1} wet weight of tissue). A pair of ear arteries was excised from each animal, one serving as control whilst the other was given tyramine or guanethidine. Table 2 summarizes the effects of 60 min incubation with tyramine (1×10^{-6}, 1×10^{-5} or 1×1^{-4}M) or guanethidine (5×10^{-6}M) on tissue content of NA. Contents of NA are shown as relative to those of the control. Tyramine reduced the tissue content of NA in a dose-dependent manner and about 69% of NA still remained in the tissue after 1×10^{-4}M tyramine had been applied for 60 min. Guanethidine (5×10^{-6}M) reduced the tissue content of NA to the level similar to that treated by 1×10^{-5}M tyramine.

Table 2 Noradrenaline (NA) content of the rabbit ear arteries incubated with tyramine or guanethidine for 60 min

	NA content (ng g^{-1} wet wt)
Tyramine 10^{-6}M	0.88 ± 0.16 ($n = 3$)*
Tyramine 10^{-5}M	0.78 ± 0.05 ($n = 4$)*
Tyramine 10^{-4}M	0.69 ± 0.10 ($n = 5$)*
Guanethidine 5×10^{-6}M	0.80 ± 0.04 ($n = 4$)*

Tissues were homogenized and centrifuged, and NA content in the supernatant was measured. A pair of ear arteries was taken form the same animal, and the arteries were incubated either in the drug-containing or drug-free (control) solution. Content of NA is expressed relative to the control value (mean ± s.d., n = number of tissue). Mean content of NA in the control tissue was 1751 ± 177 ng g^{-1} wet weight of tissue (mean ± s.e., $n = 26$).
*different from control ($P < 0.05$).

Discussion

Tyramine increased basal outflows of NA and DOPEG from the rabbit ear artery. These effects were not modified in the presence of TTX indicating that they were not mediated through excitation of perivascular nerves. Cocaine reduced the basal outflow of NA induced by tyramine, presumably by inhibiting the uptake of tyramine into nerve terminals (Trendelenburg, 1972; Vanhoutte *et al.*, 1981). Guanethidine also increased basal outflow of both NA and DOPEG, an effect seen also in rabbit mesenteric artery where it was blocked by cocaine (Mishima *et al.*, 1984). Thus, both tyramine and guanethidine may be taken up into nerve terminals (Mitchell & Oates, 1970), and this process is thought to be impaired by cocaine. Guanethidine increased basal outflow of DOPEG more than that of NA as did the low concentration of tyramine (1×10^{-6}M). However, the high concentration (1×10^{-4}M) of tyramine increased basal outflows of NA and DOPEG to a similar extent. The potentiating effect of tyramine on NA outflow was reduced but not blocked by guanethidine, while that of guanethidine was not evident in the presence of tyramine. These observations suggest that tyramine is taken up by the guanethidine-sensitive sites and also by sites different from them in the nerve terminal (Trendelenburg, 1972; Vanhoutte *et al.*, 1981).

During inhibition by guanethidine of electrical responses produced by field stimulation, the content of NA in the tissue decreased to 80% of the control. This suggests that less than 20% of NA stored in the nerves is available for transmission in the rabbit ear artery. Histochemical study shows that NA is present in all the processes of adrenergic nerves (Bevan *et al.*, 1980). Therefore the above value of NA may be underestimated. Tyramine reduced the content of NA in the tissue more than guanethidine. However, in such conditions, field stimulation still released NA in the rabbit ear artery. Thus, tyramine facilitates outflow of NA during nerve excitation, in addition to increase in the basal outflow of NA. These observations suggest that the NA compartment sensitive to tyramine is larger than that sensitive to guanethidine. NA released from these two compartments could stimulate α-adrenoceptors in the postjunctional membrane, but may be different in the releasing mechanism, i.e., the release of the guanethidine-sensitive NA is accompanied by, while that of the tyramine-sensitive NA is not accom-

panied by e.j.p. generation. In the absence of guanethidine, tyramine increased NA outflow while the e.j.p. amplitude decreased in a dose-dependent manner, although decrease in e.j.p. amplitude in higher concentrations of tyramine (over 1×10^{-5}M) may be partly due to depolarization of the membrane. Thus, tyramine may also have an effect on the release of the guanethidine-sensitive NA.

In the rabbit ear artery, tyramine increased NA outflow (de la Lande & Waterson, 1968) and depolarized the smooth muscle membrane. Application of α-adrenoceptor antagonists (prazosin or phentolamine) attenuated the tyramine-induced depolarization. In this artery, endogenous and exogenously applied NA depolarizes the smooth muscle membrane through activation of α_1-adrenoceptors, and the depolarization is blocked by prazosin or phentolamine (Suzuki & Kou, 1983a). These observations confirmed the previous observation that the depolarizing effect of tyramine on smooth muscle of the rabbit ear artery is mainly produced indirectly by NA released from perivascular adrenergic nerves, through activation of α-adrenoceptors (Trendelenburg, 1972).

A direct sympathomimetic action of tyramine on α-adrenoceptors was reported for dog cerebral and mesenteric arteries by Toda *et al.* (1978). In the denervated rabbit ear artery, tyramine depolarized the smooth muscle membrane, without increase in NA outflow, and this depolarization was attenuated by phentolamine but not by prazosin. Both phentolamine and prazosin, at concentrations employed in this experiment (5×10^{-6}M), could block all the α-adrenoceptors (α_1- and α_2-subtypes) found in vascular tissues (Vanhoutte *et al.*, 1981), and moreover phentolamine has inhibitory actions on receptors other than α-adrenoceptors in vascular tissues (e.g. histamine receptors in the guinea-pig main pulmonary artery, Suzuki & Kou, 1983b). Therefore, depolarizations induced by tyramine in smooth muscles of the denervated rabbit ear artery may be due to direct action, and phentolamine has weak inhibitory effects on the depolarization induced by tyramine in this ear artery.

Electrical responses of smooth muscle cells of the rabbit ear artery to field stimulation are an e.j.p., a slow depolarization and a spike potential, among which the slow depolarization, but not the e.j.p. and the spike potential, is blocked by prazosin or phentolamine (Suzuki & Kou, 1983a). Depletion by reserpine of NA stores in perivascular nerves abolishes the slow depolarization but not the e.j.p. in the rabbit ear artery (Suzuki *et al.*, 1984). Therefore, the slow depolarization may be generated by NA through activation of α_1-adrenoceptors, whereas the e.j.p. may be elicited by substances other than NA, possibly by ATP which is released with NA on nerve stimulation (Suzuki *et al.*, 1984).

Differences in effects between tyramine and guanethidine were also noted on outflow of NA during field stimulation. During field stimulation, outflow of NA was reduced in the presence of guanethidine, while it increased in the presence of tyramine. Field stimulation evoked the e.j.p. in the presence of tyramine but not in the presence of guanethidine. Both tyramine and guanethidine also increased the outflow of DOPEG. DOPEG does not produce electrical responses in the vascular smooth muscle membrane (Mishima *et al.*, 1984). Thus, field stimulation-induced outflow of NA seems to correlate with generation of e.j.p. in the rabbit ear artery. However, after generation of e.j.p. had been blocked by guanethidine, application of tyramine caused either no electrical responses or, in some tissues, generation of slow depolarization by field stimulation, with associated increase in NA outflow. These observations again suggest that NA does not produce the e.j.p. but does cause the slow depolarization in the rabbit ear artery.

In the presence of tyramine, the e.j.p. amplitude decreased while the facilitation process of the e.j.p. remained unchanged. Tyramine increased the outflow of NA, thereby increasing the concentration of NA around the nerve terminal. This NA would stimulate the α-autoregulation mechanism present in the nerve terminals (Starke, 1977; Langer, 1977). Stimulation of the α-autoregulation mechanism decreases the e.j.p. amplitude and depresses the facilitation process of e.j.ps in the guinea-pig mesenteric artery (Kuriyama & Makita, 1983). However, in the rabbit saphenous or ear arteries (Holman & Surprenant, 1980) and in the dog mesenteric vein (Suzuki, 1984), the decrease in e.j.p. amplitude during NA application is not prevented by α-adrenoceptor antagonists which possess higher selectivity for the α_2-subtype. Thus, the decrease in e.j.p. amplitude during application of tyramine may not be due to stimulation of α-autoinhibition mechanism in the rabbit ear artery.

It is concluded that in the rabbit ear artery, tyramine depolarizes the smooth muscle membrane by direct and indirect actions; the indirect action is mediated by release of neuronal NA which acts on α_1-adrenoceptors. Two NA compartments are identified, i.e. guanethidine-sensitive and tyramine-sensitive ones. Field stimulation releases the guanethidine-sensitive NA, and this NA-release is accompanied by generation of e.j.p. and slow depolarization. Release of NA from the tyramine-sensitive compartment is not related to generation of e.j.p. Whether differences in NA compartments in relation to the generation of e.j.p. are due to localization of NA in the nerve terminals (Rapoport *et al.*, 1981) remains to be determined.

The authors are grateful to Prof. H. Kuriyama for his pertinent advice and critical comments. Prazosin was a gift from Pfizer Taito, Co.

References

APLIGLIANO, O. & HERMSMEYER, K. (1976). *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmac. exp. Ther.*, **198,** 568–576.

BEVAN, J.A., BEVAN, R.D. & DUCKLES, S.P. (1980). Adrenergic regulation of vascular smooth muscle. In *Handbook of Physiology*, sec. II., vol. 2. ed. Bohr, D., Somlyo, A.P. & Sparks, H.V. pp. 515–566. Bethesda: American Physiological Society.

DE LA LANDE, I.S. & WATERSON, J.G. (1968). A comparison of the pharmacology of the isolated rabbit ear and its central artery. *Aust. J. exp. Biol. med. Sci.*, **46,** 739–745.

GRAEFE, K,-H. & HENSELING, M. (1983). Neuronal and extraneuronal uptake and metabolism of catecholamines. *Gen. Pharmac.*, **14,** 27–33.

HOLMAN, M.E. & SURPRENANT, A. (1980). An electrophysiological analysis of the effects of noradrenaline and α-receptor antagonists on neuromuscular transmission in mammalian muscular arteries. *Br. J. Pharmac.*, **71,** 651–661.

KAJIWARA, M. & CASTEELS, R. (1983). Effects of Ca-antagonists on neuro-muscular transmission in the rabbit ear artery. *Pflugers Arch.*, **396,** 1–7.

KURIYAMA, H. & MAKITA, Y. (1983). Modulation of noradrenergic transmission in the guinea-pig mesenteric artery: an electrophysiological study. *J. Physiol.*, **335,** 609–627.

LANGER, S.Z. (1977). Presynaptic receptors and their role in the regulation of transmitter release. *Br. J. Pharmac.*, **60,** 481–497.

MALLART, A. & MARTIN, A.R. (1967). An analysis of facilitation of transmitter release at the neuromuscular junction of the frog. *J. Physiol.*, **193,** 679–694.

MISHIMA, S. MIYAHARA, H. & SUZUKI, H. (1984). Transmitter release modulated by α-adrenoceptor antagonists in the rabbit mesenteric artery: a comparison between noradrenaline outflow and electrical activity. *Br. J. Pharmac.*, **83,** 537–547.

MITCHELL, J.R. & OATES, J.A. (1970). Guanethidine and related agents. I. Mechanism of the selective blockade of adrenergic neurons and its antagonism by drugs. *J. Pharmac. exp. Ther.*, **172,** 100–107.

OISHI, R., MISHIMA, S. & KURIYAMA, H. (1983). Determination of norepinephrine and its metabolites released from rat vas deferens using high-performance liquid chromatography with electrochemical detection. *Life Science*, **32,** 933–940.

RAPOPORT, R.M., TAKIMOTO, G.S. & CHO, A.K. (1981). Compartmental analysis of tyramine-induced norepinephrine depletion. *Pharmacology*, **22,** 235–242.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmac.*, **7,** 1–124.

SUZUKI, H. (1984). Adrenergic transmission in the dog mesenteric vein and its modulation by α-adrenoceptor antagonists. *Br. J. Pharmac.*, **81,** 479–489.

SUZUKI, H. & KOU, . (1983a). Electrical components contributing to the nerve-mediated contractions in the smooth muscles of the rabbit ear artery. *Jap. J. Physiol.*, **33,** 743–756.

SUZUKI, H. & KOU, K. (1983b). Direct and indirect effects of histamine on the smooth muscle cells of the guinea-pig main pulmonary artery. *Pflugers Arch.*, **399,** 46–53.

SUZUKI, H., MISHIMA, S. & MIYAHARA, H. (1984). Effects of reserpine on electrical responses evoked by perivascular nerve stimulation in the rabbit ear artery. *Biomed. Res.*, **5,** 259–266.

TODA, N., HAYASHI, S. & HATTORI, K. (1978). Analysis of the effect of tyramine and norepinephrine in isolated canine cerebral and mesenteric arteries. *J. Pharmac. exp. Ther.*, **205,** 382–391.

TRENDELENBURG, U. (1972). Classification of sympathomimetic amines. In *Handbook of Experimental Pharmacology*, ed. Blaschko, H. and Muscholl, E. pp. 336–362. Springer Verlag: Berlin.

VANHOUTTE, P.M., VERBEUREN, T.J. & WEBB, R.C. (1981). Local modulation of adrenergic neuroeffector interaction in the blood vessel wall. *Physiol. Rev.*, **61,** 151–247.

(Received March 8, 1985.
Revised June 4, 1985.
Accepted June 10, 1985.)