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EFFECTS OF 3, 4-DIHYDRO-8-(2-HYDROXY-3-ISOPROPYLAMINOPROPOXY)-3-NITROXY-2H-1-BENZOPYRAN (K-351) ON SMOOTH MUSCLE CELLS AND NEUROMUSCULAR TRANSMISSION IN THE CANINE MESENTERIC ARTERY

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1 The effects of K-351 on the electrical and mechanical responses were investigated in the canine mesenteric artery by isometric tension recording and the use of intracellular microelectrodes. The results were compared to the responses observed with other α-adrenoceptor blocking agents.

2 K-351 ($> 3 \times 10^{-7}$M) consistently inhibited the contraction evoked by perivascular nerve stimulation; however, K-351 had no effect on the contraction evoked by direct muscle stimulation, after pretreatment with 3×10^{-7}M tetrodotoxin.

3 Phentolamine enhanced and prazosin had no effect on the amplitude of contraction evoked by perivascular nerve stimulation at a high frequency (over 1.0 Hz). Pretreatment with phentolamine inhibited the contraction evoked by lower frequencies of perivascular nerve stimulation (below 0.5 Hz).

4 The potency for the inhibition of the response to perivascular nerve stimulation was in the order of K-351 > phentolamine > prazosin, while the contractions induced by exogenously applied noradrenaline (5×10^{-7}M) were inhibited in the order: prazosin > phentolamine > K-351.

5 K-351 ($< 3 \times 10^{-5}$M) did not modify the resting membrane potential or the membrane resistance, as estimated from the change in the amplitude of electrotonic potentials in the smooth muscle cell membranes.

6 K-351 ($> 3 \times 10^{-7}$M) inhibited the amplitude of the first e.j.p. and e.j.ps after completion of the facilitation process following stimulation at frequencies over 0.25 Hz.

7 K-351 ($< 3 \times 10^{-5}$M) did not modify the compound action potentials recorded from peripheral nerve bundles distributed on the mesenteric artery.

8 Phentolamine ($> 1 \times 10^{-8}$M) inhibited the first e.j.p. but this agent either inhibited or enhanced the amplitude of e.j.p. after completion of the facilitation process produced by repetitive stimulation below or over 1.0 Hz stimulus frequencies, respectively. Prazosin (1×10^{-6}M) had no effect on e.j.ps evoked by perivascular nerve stimulation, at any stimulus frequency applied.

9 These results indicate that K-351 inhibits the extra-junctional adrenoceptor with a slightly weaker potency than prazosin or phentolamine, but that this agent has a potent action as an intra-junctional adrenoceptor blocking agent. Phentolamine acts mainly on the extra-junctional adrenoceptors and also has weak actions on intra-junctional adrenoceptors, as a blocking agent. This agent also inhibits the presynaptic α_2-adrenoceptor. Prazosin inhibits only the extra-junctional α_1-adrenoceptor.

Introduction

A newly synthesized chemical agent, 3,4-dihydro-8 - (2 - hydroxy - 3 - isopropylaminopropoxy) - 3 - nitroxy-2H-1-benzopyran, K-351, has a long lasting antihypertensive action in spontaneously hypertensive rats (SKW) and DOCA/saline hypertensive rats (Uchida, Shimizu, Yamauchi, Ikuta & Nakamura, 1982). These investigators concluded that K-351 is a β-adrenoceptor blocking agent. In the guinea-pig mesenteric artery, *in vitro*, K-351 reduces the amplitude of the excitatory junction potential (e.j.p.) and the contraction evoked by exogenously applied noradrenaline, due to inhibition of intra- and extra-junctional α-adrenoceptors distributed on the muscle membranes. The extra-junctional adrenoceptor is blocked by phentolamine or prazosin, whereas the intra-junctional adrenoceptor is not affected by

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phentolamine or prazosin but is affected by K-351. Thus the action of K-351 differs from the actions of previously available α_1-adrenoceptor blocking agents (Asada, Nanjo, Itoh, Suzuki & Kuriyama, 1982).

Distribution of α_1- and α_2-adrenoceptors classified from actions of yohimbine, clonidine, and prazosin differ with region and species. For example, α_2-adrenoceptors are mainly distributed on the presynaptic nerve terminal as a regulator of noradrenaline release, and partly on the post-junctional muscle membrane. On the other hand, α-adrenoceptors distributed on the extra-junctional region are classified as the α_1-adrenoceptors because they are inhibited by application of prazosin (Rand, Story & McCulloch, 1975; Stjärne, 1975; Langer, 1977; Westfall, 1977; Starke, 1977; Vanhoutte, Verbeuren & Webb, 1981). An unidentified subtype of α-adrenoceptor distributed on the intra-junctional region is termed a γ-receptor by Hirst (1981) and, in the presence of prazosin or phentolamine, an e.j.p. is produced by perivascular nerve stimulation (Holman & Surprenant, 1980; Hirst & Neild, 1980; Kuriyama & Makita, 1982).

If K-351 acts as an inhibitor of intra- and extra-junctional adrenoceptors, this agent may be a useful tool in clarifying the nature of the adrenoceptors distributed in the post-junctional region.

Thus, we attempted to clarify the action of K-351 on neuromuscular transmission, particularly in relation to the post-junctional adrenoceptor on the canine mesenteric artery. Since species differences in drug actions are remarkable, it has to be determined whether this agent possesses the same action on the canine mesenteric artery as that observed on the guinea-pig mesenteric artery or possesses the properties of a β-adrenoceptor blocking agent. We conclude that K-351 inhibits the extra-junctional α-adrenoceptor with a slightly weaker potency than prazosin, but that it inhibits the intra-junctional adrenoceptor to a greater extent than previously available α_1-adrenoceptor blockers.

Methods

Mongrel dogs, weighing 15–20 kg, were anaesthetized by intramuscular injections of pentobarbitone Na (40 mg/kg) and the mesentery was excised from jejunal regions. The tissue was kept in cold Krebs solution bubbled with 97% O_2 and 3% CO_2. The mesenteric artery was excised under a binocular microscope, and the tissue was cut in a helical direction (0.5–1 mm in width and 10 mm in length).

To stimulate the perivascular nerves (0.5–1 ms pulse duration, 100 V in intensity) and the muscle (10 ms, 1 s, 10–100 V in intensity), the partition stimulating method (Abe & Tomita, 1968) was used. To record the action potential from perivascular nerve fibres, a suction recording electrode was used, and a pair of stimulating electrodes (silver wires; 0.1 mm in diameter) was placed on the nerve fibres distributed in the vascular tissues (Kuriyama & Suzuki, 1981).

The resting membrane and electrotonic potentials were recorded from vascular muscle cells by means of a glass capillary electrode filled with 3 M KCl. The resistance of the electrode ranged between 60–80 MΩ. The microelectrode was inserted from the outer layer. The tissue was superfused with Krebs solution at 35–36°C with a flow rate of 2.0 ml/min.

To measure the contraction isometrically, a preparation was placed in an organ bath, and one end of the tissue was fixed at the bottom of the bath with silk thread and the other end connected to a mechanotransducer (Nihon Kohden, FD pick-up, TB 612T). The stimulating electrode was placed in parallel to the preparation in a longitudinal direction. The preparation was superfused with Krebs solution at 35–36°C with a flow rate of 2.0 ml/min in a 1.0 ml organ bath. All these procedures have been described elsewhere (Kuriyama & Suzuki, 1981).

Krebs solution (Bülbring, 1954) contained (mM): Na^+ 137.4, K^+ 5.9, Ca^{2+} 2.5, Mg^{2+} 1.2, HCO_2^- 15.5, $H_2PO_4^-$ 1.2, Cl^- 134.0 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; 3,4-dihydro-8-(- 2 - hydroxy - 3 - isopropylaminopropoxy) - 3 - nitroxy-2H-1-benzopyran (K-351; Kowa), prazosin (Pfeizer), phentolamine (Ciba-Geigy), (−)-noradrenaline (Sigma) and tetrodotoxin (TTX; Sigma). The solutions were freshly prepared for each experiment.

Values of the measured parameters of muscle membrane and mechanical responses were expressed as the mean value ± s.d. (n = number of penetrations by the microelectrode or number of preparations for the mechanical response). Statistical significance was assessed using Student's t test.

Results

Effects of K-351 and other adrenoceptor blocking agents on the mechanical responses evoked by various procedures

The mechanical response evoked from the canine mesenteric artery could be elicited by either direct muscle stimulation, nerve stimulation or chemical stimulation. Figure 1 shows the effects of K-351 and tetrodotoxin (TTX) on the mechanical response evoked by either perivascular nerve stimulation

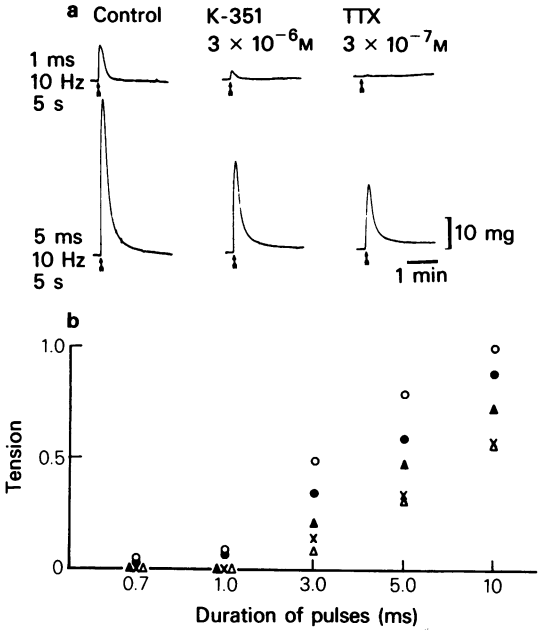

Figure 1 Effects of K-351 and tetrodotoxin (TTX) on the contraction evoked by either perivascular nerve stimulation or direct muscle stimulation. (a) Contractions evoked by perivascular nerve stimulation (1 ms pulse duration, 100 V intensity, 10 Hz, 5 s) and direct muscle stimulation (5 ms pulse duration, 100 V intensity, 10 Hz, 5 s). K-351 3×10^{-6} M and TTX 3×10^{-7} M were applied 10 min before application of electrical stimulation. Arrow indicates application of electrical stimulation. (b) Effects of various concentrations of K-351 and 3×10^{-7} M TTX on the contraction evoked by electrical stimulation with various durations (10 Hz and 5 s). The amplitude of contraction induced by 10 ms pulse stimulation in the absence of K-351 is shown, as a relative value of 1.0 (3–6 preparations). (○) Control responses obtained before application of K-351; (●), (▲) and (×), responses obtained during application (5–30 min) of 3×10^{-7} M, 3×10^{-6} M and 3×10^{-5} M K-351, respectively; (△) responses obtained during application (5–30 min) of 3×10^{-7} M TTX.

(1 ms pulse duration) or direct muscle stimulation (5 ms pulse duration). With application of 3×10^{-6} M K-351, the mechanical response evoked by perivascular nerve stimulation was inhibited to a greater extent than that induced by direct muscle stimulation. The mechanical response evoked by perivascular nerve stimulation was blocked by application of 3×10^{-7} M TTX, while the response evoked by direct muscle stimulation was reduced to half the amplitude of the contraction evoked in Krebs solution. Figure 1(b) shows the effects of K-351 and TTX on the mechanical response and stimulus duration. The contraction evoked by application of 1 ms pulse duration (10 Hz and 10 s stimulus duration) ceased with application of K-351 or TTX. However, contractions evoked by prolonged pulse duration (over 1 ms) were only partly inhibited by both K-351 and TTX. However, inhibition of the contraction by application of K-351 was dose-dependent. The results suggest that K-351 acts on the nerve-mediated mechanical response but not on that evoked by direct muscle stimulation.

To confirm the action of K-351 on the contraction evoked by direct muscle stimulation, the effects of various concentrations of K-351 on the mechanical response evoked by single stimuli (1 s pulse duration) during treatment with 3×10^{-7} M TTX were observed. Application of K-351 ($< 3 \times 10^{-5}$ M) did not alter the amplitude of the mechanical response.

Figure 2 shows the effects of phentolamine and K-351 on the mechanical response evoked by perivascular nerve stimulation (1 ms pulse duration; 100 V stimulus intensity and 0.1 Hz stimulus frequency). Application of phentolamine 1×10^{-7} M slightly inhibited the amplitude of contraction, and with application of 1×10^{-5} M, the amplitude of contraction was reduced to two-thirds that of the control. Application of K-351 3×10^{-6} M reduced the amplitude of contraction to one-tenth that of the control. Furthermore, after application of K-351, restoration of the amplitude of the contraction to the control level required more than 30 min. In contrast with the effects of K-351, TTX completely and rapidly blocked the mechanical response evoked by the same stimulus conditions. However, the effects of phentolamine varied between different stimulus conditions. When the mechanical response was evoked by high frequencies of stimulation (> 1.0 Hz) (Figure 3), application of phentolamine ($> 1 \times 10^{-9}$ M) enhanced the amplitude of contraction, whereas increased concentrations of phentolamine ($> 1 \times 10^{-5}$ M) decreased the amplitude of contraction. With application of K-351 in concentrations over 3×10^{-7} M, the amplitude of the mechanical response was consistently reduced. Prazosin ($10^{-7} - 10^{-5}$ M) had no effect on the mechanical response.

When the stimulus frequency was increased to 5.0 Hz (1 ms pulse duration, 10 s stimulus duration), the mechanical response was still enhanced by treatment with 1×10^{-6} M phentolamine (1.21 ± 0.06 times the control, $n = 5$), inhibited by treatment with 3×10^{-6} M K-351 (0.6 ± 0.08 times the control, $n = 5$) but was not affected by application of 4.8×10^{-6} M prazosin (1.0 ± 0.1 times the control, $n = 5$). When 1×10^{-6} M phentolamine with 3×10^{-6} M K-351 were applied simultaneously, the mechanical response was not affected (1.01 ± 0.04 times the control, $n = 5$). The mechanical response

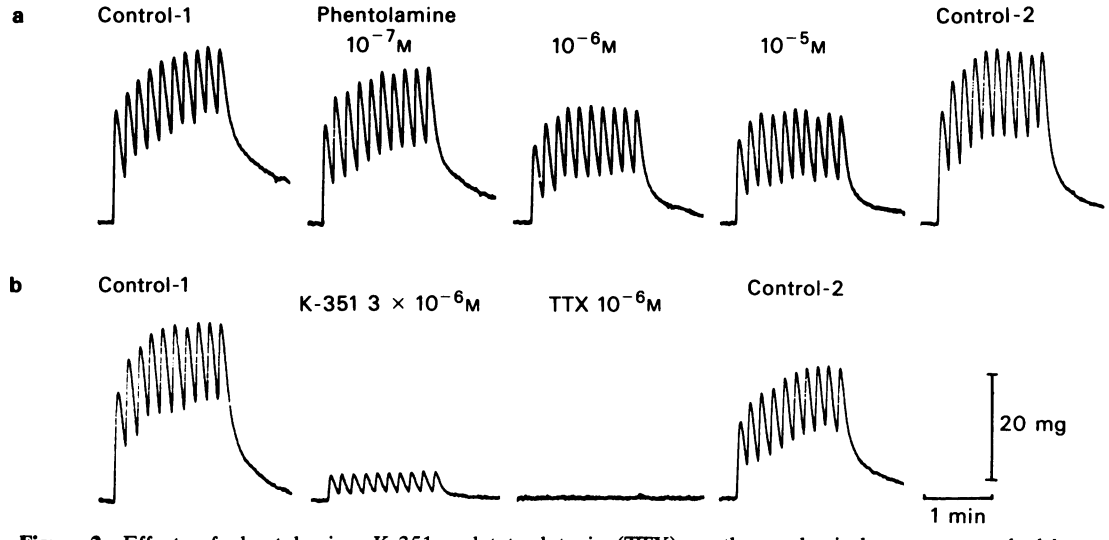

Figure 2 Effects of phentolamine, K-351 and tetrodotoxin (TTX) on the mechanical responses evoked by perivascular nerve stimulation (1 ms pulse duration, 100 V intensity, 0.1 Hz and 10 pulses). (a) Effects of various concentrations of phentolamine on the mechanical response. (b) Effects of K-351 and TTX on the mechanical response.

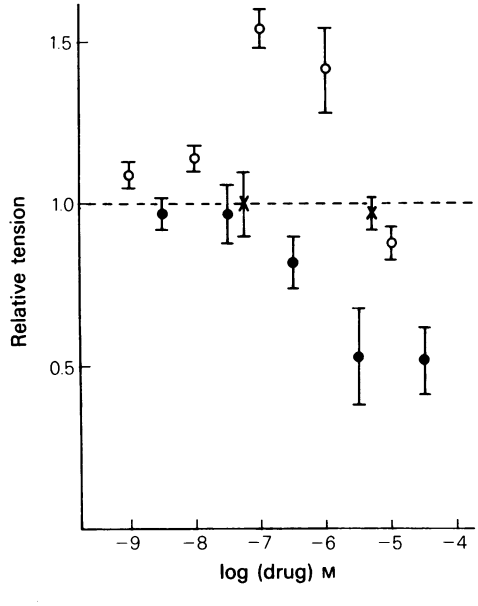

Figure 3 Effects of phentolamine (○), K-351 (●) and prazosin (×) on the mechanical response evoked by perivascular nerve stimulation (1 ms pulse duration, 1 Hz stimulus frequency, 100 V stimulus intensity and 10 s stimulation). Vertical axis shows amplitude of contraction as relative value to that obtained before application of drugs. Dotted line indicates control amplitude of the mechanical response evoked in the absence of an α-adrenoceptor blocking agent. Each value shows mean of 6–12 observations; vertical lines indicate s.d.

evoked by the above conditions was abolished by treatment with 3×10^{-7} M TTX.

The mechanical response could also be evoked by application of noradrenaline. Figure 4 shows the effects of K-351, phentolamine and prazosin on the noradrenaline-induced contraction following application of 5×10^{-7} M. The amplitude of contraction evoked by 5×10^{-7} M was normalized as 1.0. When the ID_{50} was compared, prazosin proved to be a more effective inhibitor for the noradrenaline-induced contraction, as compared to phentolamine or K-351. The ID_{50} values as the logarithmic concentration were prazosin:phentolamine:K-351 = 8.1:7.5:7.1. These results indicate that potencies required to inhibit the activation of extra-junctional adrenoceptors are of the following order: prazosin > phentolamine > K-351, while the inhibition of the mechanical response evoked by perivascular nerve stimulation by the above agents was in the order: K-351 > phentolamine > prazosin.

Effects of K-351 and other α-adrenoceptor blocking agents on neuromuscular transmission

The resting membrane potential of smooth muscle cells of the canine mesenteric artery was -68.4 ± 1.6 mV ($n = 20$), and the cells were electrically quiescent. With application of 3×10^{-5} M K-351, the membrane potential was not modified by superfusion for over 40 min. Furthermore, the membrane resistance measured from amplitudes of electrotonic potentials produced by the constant intensity of inward current pulse (2 s) in the presence or ab-

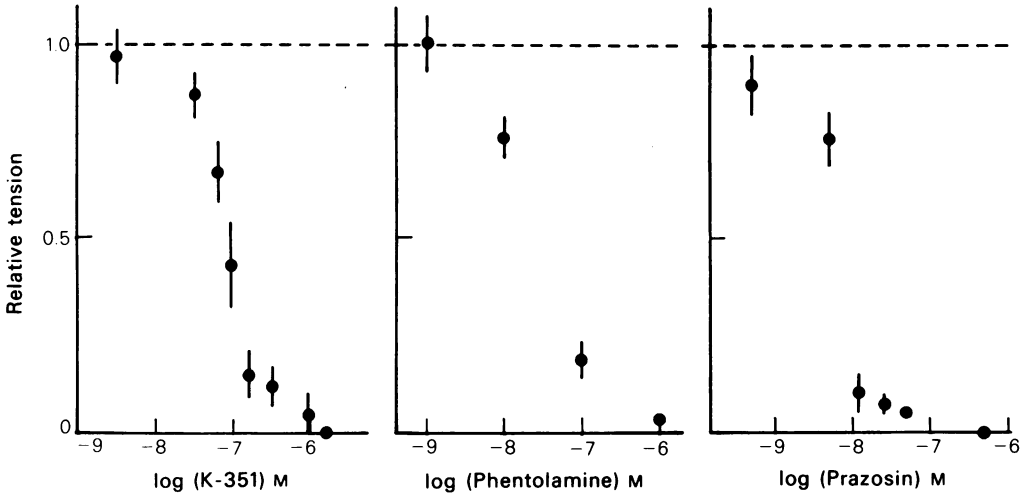

Figure 4 Effects of K-351, phentolamine and prazosin on the noradrenaline-induced contraction (5×10^{-7} M). The amplitude of contraction evoked in the absence of agent was registered as a relative tension of 1.0. Vertical bars indicate 2 × s.d. ($n = 6-9$). Dotted line indicates the control amplitude of contraction registered as a relative value of 1.0.

sence of K-351 (3×10^{-5} M) was not changed.

In the guinea-pig mesenteric artery, repetitive perivascular nerve stimulation produces e.j.ps whose amplitude gradually increases to a certain level if stimulus frequency is greater than 0.25 Hz (Kuriyama & Suzuki, 1981). Such was observed in the canine mesenteric artery, but infrequently. Repetitive perivascular nerve stimulation with low frequencies (0.25–1 Hz) often reduced the amplitude of e.j.ps again after several stimulations (depression phenomenon), or produced e.j.ps of irregular amplitude.

Figure 5 shows the effects of K-351 (3×10^{-6} M), phentolamine (1×10^{-6} M) and prazosin (1×10^{-6} M) on the e.j.p. evoked by perivascular nerve stimulation (pulse duration, 0.05 ms; intensity, 100 V) at a frequency of 0.5 Hz. In these three cells, K-351 reduced the amplitude of the first e.j.p. and also the amplitude of the e.j.p. after completion of the facilitation process. Phentolamine reduced the amplitude of the first e.j.p., but enhanced the amplitude of the e.j.p. after the facilitation was completed. Prazosin had no effect on the amplitude of the first e.j.p. or the e.j.p. after the completion of the facilitation process.

To investigate in detail the effects of K-351, phentolamine and prazosin on the facilitation process, two

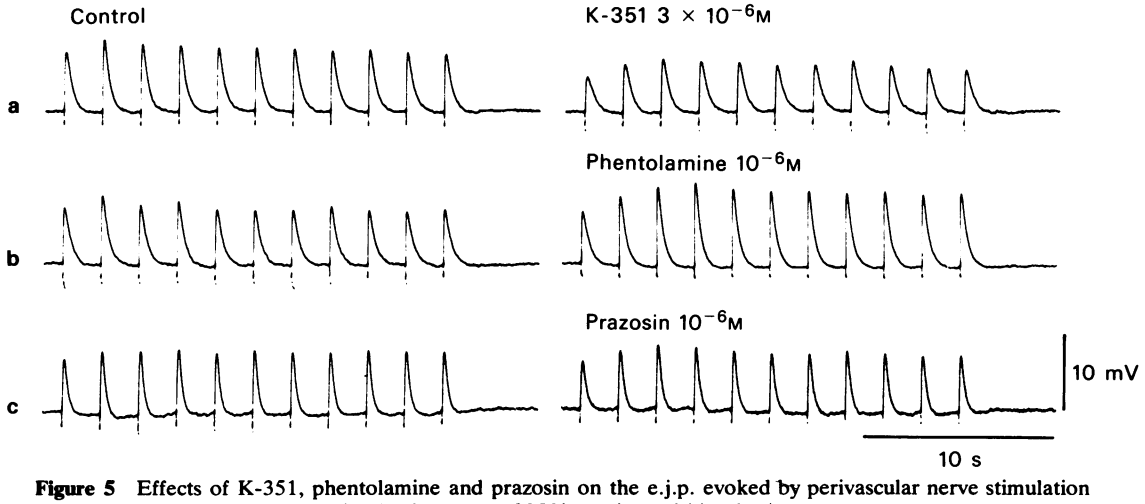

Figure 5 Effects of K-351, phentolamine and prazosin on the e.j.p. evoked by perivascular nerve stimulation (0.05 ms pulse duration, 0.5 Hz stimulus frequency, 30 V intensity and 11 pulses).

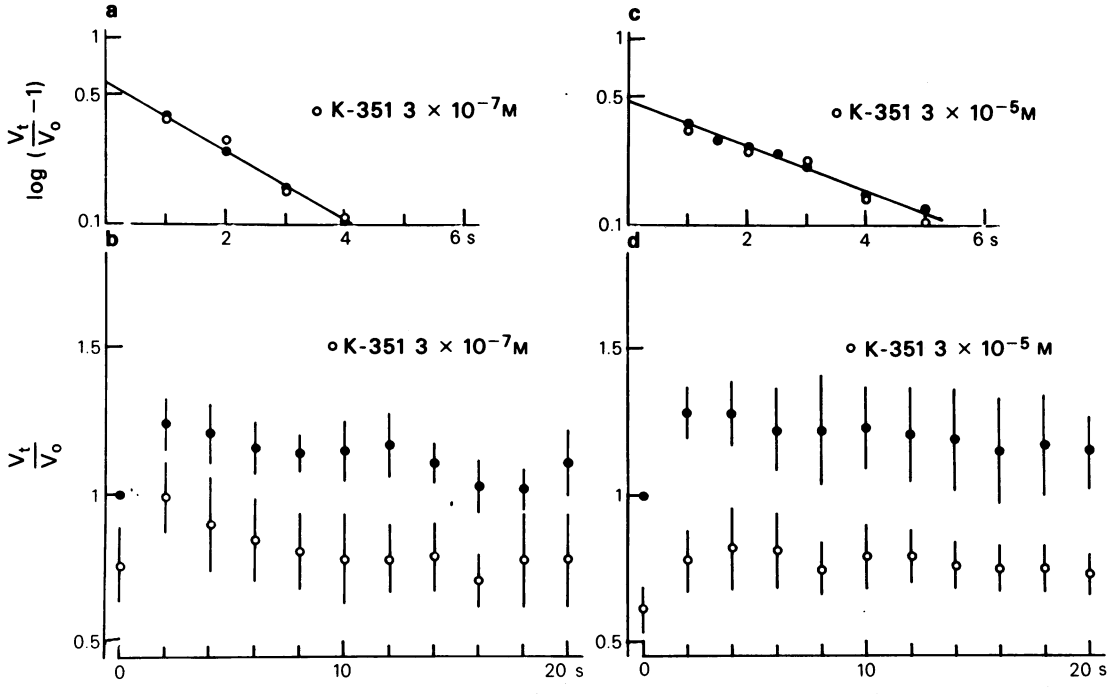

Figure 6 Effects of K-351 (a and b, 3×10^{-7} M; c and d, 3×10^{-5} M) on the facilitation process of the e.j.p. evoked by perivascular nerve stimulation (0.05 ms pulse duration, 30 V intensity). (a) and (c) The relationship between the amplitude of the second e.j.p. relative to that of the first e.j.p. and the interval between the paired pulses. Vertical axis indicates log $(V_t/V_0 - 1)$, and horizontal axis the interval between paired pulses. More details in the text. (b) and (d) The relationship between the amplitude of e.j.p. and number of stimuli (0.5 Hz stimulus frequency, 30 V intensity and 0.05 ms pulse duration). Amplitude of e.j.ps were recorded relative to that of the first e.j.p. of a train of stimuli generated in the control condition. Each point indicates mean ± s.d. ($n = 6$–12). (●) Control; (○) presence of K-351. The effects of drugs were observed after application for 10–20 min.

separate experiments were done. First, pairs of stimuli separated by different intervals were applied, and the size of the e.j.p. elicited by the second was compared with that following the first, and second, the growth of e.j.p. amplitude during a train of stimuli at 0.5 Hz was measured, in the presence or absence of α-adrenoceptor blocking agents.

Figure 6(a) shows the effects of K-351 (3×10^{-7} M and 3×10^{-5} M) on the amplitudes of e.j.p. produced by application of the paired pulses. The amplitude of e.j.ps evoked by the pair of stimuli was analysed according to the method described by Mallart & Martin (1967); briefly, a value of $\log (V_t/V_0 - 1)$ was plotted against the interval of two pulses, where V_0 and V_t were the amplitudes of the first and the second e.j.ps respectively. These pairs of stimuli were applied with an interval of over 1 min, and the results were obtained from single cells. When the amplitude of the e.j.p. was expressed by the above equation against the interval of two pulses, a linear decay was observed in the presence or absence of K-351, on the same line. This result indicates that the facilitation process is not affected by application of K-351 (3×10^{-7} M and 3×10^{-5} M) even when the amplitude of the first e.j.p. is reduced. Furthermore, the growth of the amplitude of e.j.p. produced by application of a train of stimuli at 0.5 Hz was compared before and during application of K-351 (3×10^{-7} M and 3×10^{-5} M). The amplitude of the first e.j.p. in Krebs solution was registered as a relative amplitude of 1.0. As shown in Figure 6(b), the amplitude of the first e.j.p. was reduced but the growth of e.j.ps produced by repetitive stimulation was not affected by K-351.

The actions of phentolamine or prazosin on the facilitation process were also observed for comparison with the effects of K-351 (Figure 7). When the amplitude of e.j.ps evoked by the paired pulses in the presence of phentolamine (1×10^{-6} M) was compared with that in Krebs solution, the amplitude of the second e.j.p. was consistently enlarged, compared to that observed in Krebs solution (log $(V_t/V_0 - 1)$ vs interval). Furthermore, the facilitation persisted with a longer duration of the interval compared to that observed in the control solution, even

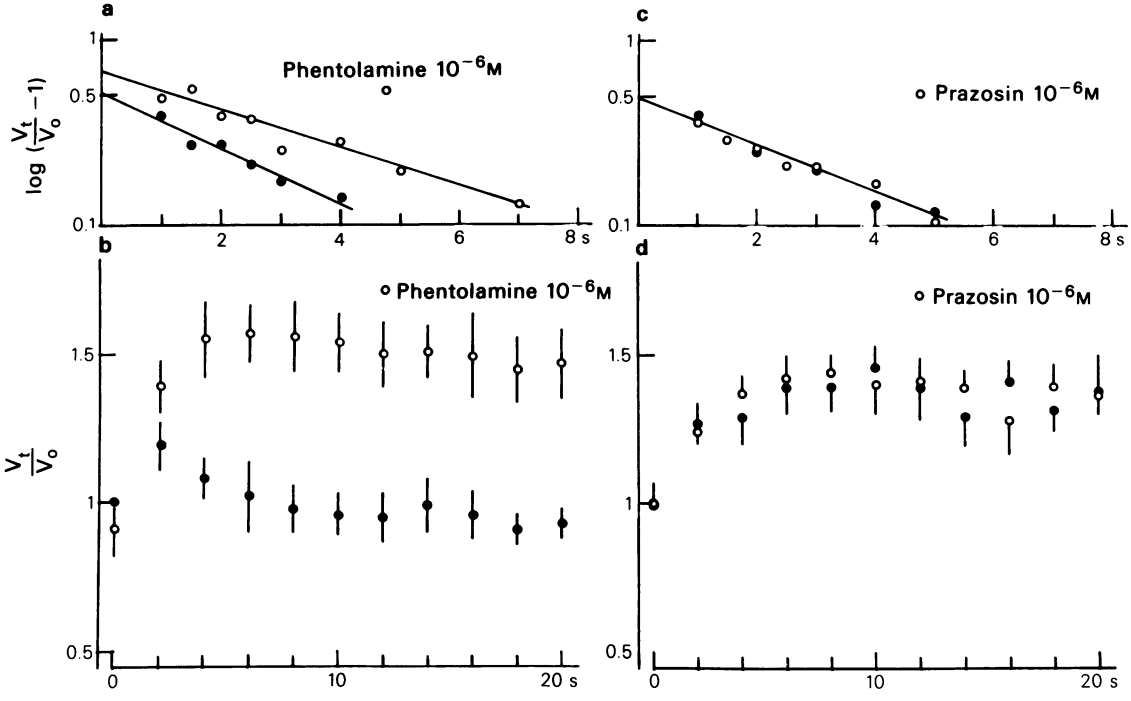

Figure 7 Effects of phentolamine and prazosin on the facilitation process of the e.j.p. evoked by perivascular nerve stimulation. The stimulus conditions were the same as those described in Figure 6 (a) and (c). The relationship of the amplitude of the second e.j.p. relative to the first and the interval between the paired pulses, before and during application of 1×10^{-6}M phentolamine (a) and 1×10^{-6}M prazosin (c). Each point is a mean value of 5–8 observations. (b) and (d), The relationship between the amplitude of the e.j.p. and the number of stimuli; 1×10^{-6}M phentolamine (b) and 1×10^{-6}M prazosin (d) were used. The experimental procedures were the same as those described in Figure 6. (●) Control; (○) presence of phentolamine or prazosin. Each point shows mean ± s.d. of 6 observations.

when the amplitude of the first e.j.p. was reduced. The relationship in the presence of phentolamine also gave a straight line. The growth of the e.j.ps after repetitive stimulation with 0.5 Hz stimulus frequency was faster and larger than that observed in Krebs solution. In these particular cells ($n = 6$), the first few e.j.ps showed a facilitation phenomenon and then the depression phenomenon of the growth of e.j.ps followed.

Prazosin (1×10^{-6}M) had no effect on the amplitudes of e.j.ps evoked by application of the paired pulses or on growth of the amplitude of the e.j.ps produced by repetitive stimulation at the stimulus frequency of 0.5 Hz ($n = 6$).

To determine if the reduction in the amplitude of e.j.p. produced by application of perivascular nerve stimulation during treatment with K-351 was due to inhibition of nerve activities, the e.j.ps were evoked by different intensities of perivascular nerve stimulation (0.05 ms pulse duration). Figure 8 shows the effects of 3×10^{-5}M K-351 on the amplitude of e.j.ps evoked by various intensities (10–100 V) of stimulation. When intensities of stimulation were gradually reduced from 100 V to 10 V, the amplitude of e.j.ps was reduced stepwise in Krebs solution. This stepwise reduction in the amplitudes of e.j.ps is postulated to be due to a reduction in the number of nerve fibres contributing to the generation of e.j.ps (Kuriyama & Suzuki, 1981). In the presence of K-351, the amplitude of e.j.p. was reduced but the number of steps was not affected. This suggests that the reduction in the amplitude of e.j.p. is not due to a diminution of the number of nerve fibres contributing the generation of the e.j.p. As also shown in Figure 8, when the stimulus intensity was over 60 V, a spike was superimposed on the e.j.p.

Figure 9 shows the effects of K-351, phentolamine and TTX on the compound action potential evoked from peripheral nerve bundles distributed on the mesenteric artery. Phentolamine (1×10^{-5}M) and K-351 (3×10^{-5}M) had no effect on the amplitude and shape of the compound action potential, but with TTX (1×10^{-6}M) these potential changes ceased completely.

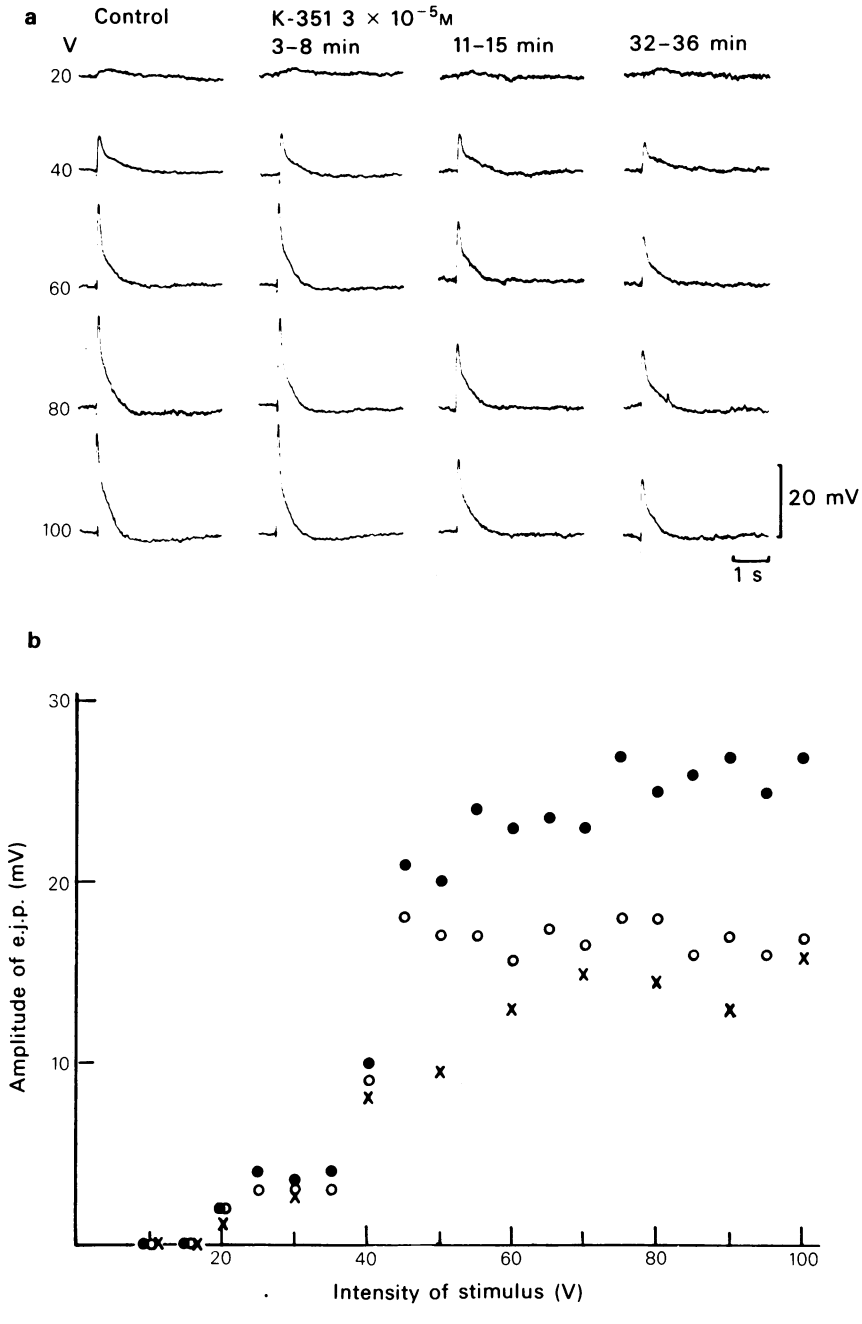

Figure 8 Effects of K-351 (3×10^{-5} M) on the e.j.p. evoked by various intensities of stimulation (1 ms pulse duration, single pulse). The intensity of stimulation was reduced from 100 V to 10 V. (a) The effects of K-351 on the e.j.p.. Five different intensities of stimulus were applied to the tissue and e.j.ps were recorded at 3–8 min, 11–15 min and 32–36 min after application of K-351. (b) The relationship between the amplitude of e.j.ps recorded by applications of various intensities of stimulation in the absence (control, (●)) or presence of 3×10^{-5} M K-351 (after 10–17 min (○) and 32–36 min (×)). Records were taken from the same cell.

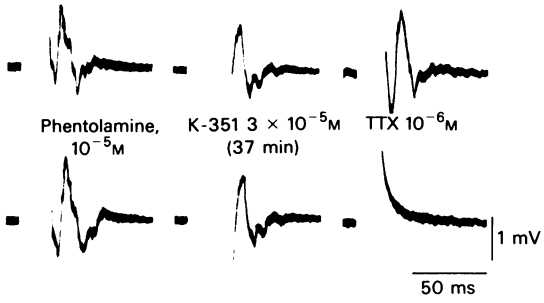

Figure 9 Effects of K-351, phentolamine and tetrodotoxin (TTX) on the compound action potential recorded from small nerve bundles distributed on the mesenteric artery. Electrical stimulation (1 ms duration, 100 V intensity) was applied by a pair of silver wire electrodes and electrical activity was recorded using the suction electrode. Upper traces: control; lower traces: after application of chemical agents. A large upward deflection is a stimulus artifact. Records were taken 10–20 min after application of chemical agents. The compound action potentials evoked by 1.0 Hz stimulus frequency in 30 traces were superimposed.

Discussion

The e.j.p. is generated by perivascular nerve stimulation in many vascular smooth muscles. A single stimulus could generate an e.j.p. in the mesenteric (Hirst, 1977; Suzuki & Kuriyama, 1980), ear (Kajiwara, Kitamura & Kuriyama, 1981) and uterine arteries (Bell, 1969) of guinea-pig, saphenous artery of rabbit (Holman & Surprenant, 1979) or caudal artery of rat (Surprenant, 1980). On the other hand, in the guinea-pig mesenteric vein, repetitive, but not a single stimulation generates slow depolarization of the membrane (Suzuki, 1981). These e.j.ps are believed to be generated by activation of α-adrenoceptors with noradrenaline released from the nerves (Westfall, 1977). However, they could be classified into two groups from the pharmacological point of view, i.e., phentolamine-sensitive and phentolamine-resistant e.j.ps. The e.j.p. observed in the mesenteric, ear or saphenous artery cannot be suppressed by application of phentolamine (Holman & Surprenant, 1980; Kajiwara *et al.*, 1981; Kuriyama & Makita, 1982), or requires a very high concentration of phentolamine (3×10^{-4} M) to be suppressed (Suzuki & Kuriyama, 1980), while the e.j.p. observed in the guinea-pig mesenteric vein could be suppressed by phentolamine (Suzuki, 1981). In all these tissues, the electrical and mechanical responses induced by exogenously applied noradrenaline are inhibited by application of phentolamine or prazosin (Holman & Surprenant, 1980; Suzuki, 1981; Kuriyama & Makita, 1982; Asada, *et al.*, 1982). Phentolamine possesses more complicated actions on vascular tissues. For example, in the mesenteric artery of guinea-pig, the amplitude of e.j.p. is increased by application of this agent (Kuriyama & Makita, 1982). This effect of phentolamine is explained by assuming the presence of negative feedback regulation of transmitter release from the nerve terminal through α-adrenoceptors in the prejunctional membrane (Westfall, 1977; Vanhoutte, *et al.*, 1981). Therefore. the muscle contractions induced by exogenous noradrenaline, but not by endogenous noradrenaline, are suppressed by phentolamine or prazosin (Holman & Surprenant, 1980; Asada *et al.*, 1982; Kuriyama & Makita, 1982).

In the canine mesenteric artery, prazosin was more potent than phentolamine or K-351 in inhibiting the noradrenaline-induced contraction. However, prazosin had no effect on the e.j.p. evoked by single or repetitive perivascular nerve stimulation. As observed in the guinea-pig mesenteric artery, phentolamine inhibited the noradrenaline-induced contraction (inhibition of the extra-junctional adrenoceptor), slightly inhibited the intra-junctional adrenoceptor and also inhibited the presynaptic adrenoceptor. Therefore, the first e.j.p. was inhibited but the facilitation process evoked by perivascular stimulation at frequencies greater than 1.0 Hz was markedly enhanced. This action of phentolamine on the intra-junctional adrenoceptor differed from findings in the case of the guinea-pig mesenteric artery, in which phentolamine enhances the amplitude of the first e.j.p. and also the facilitation process of the e.j.ps elicited by a train of stimuli (Kuriyama & Makita, 1982).

In the canine mesenteric artery, K-351 had no effect on the compound action potential evoked by activation of small nerve bundles. If K-351 acts as an α_2-adrenoceptor blocking agent, the amplitude of the e.j.p. after the completion of the facilitation process should increase; however, the result did not support this postulate. If this agent acts as a β-adrenoceptor blocker, the amplitude of e.j.p. after completion of the facilitation process would be reduced due to inhibition of positive feedback mechanisms promoted by activation of β-adrenoceptors distributed on the presynaptic nerve terminals for noradrenaline release (Vanhoutte *et al.*, 1981). In fact, K-351 inhibited the amplitude of e.j.ps. However, K-351 did not modify the facilitation process of e.j.ps in Krebs solution. Furthermore, propranolol did not modify the facilitation process of e.j.ps produced by repetitive perivascular nerve stimulation (unpublished observations). Therefore, K-351 inhibited mainly the intra-junctional adrenoceptors and reduced the amplitude of e.j.ps. K-351 also inhibited the extra-junctional adrenoceptor as prazosin and phentolamine did.

The intra-junctional adrenoceptor has been

termed the γ-adrenoceptor by Hirst (1981), and this region possesses different properties from the extra-junctional adrenoceptor. The region contributing to the neuromuscular transmission in the guinea-pig and canine mesenteric arteries might possess a similar property (Kuriyama & Suzuki, 1981; Makita & Kuriyama, 1982; Asada *et al.*, 1982). However, these intra-junctional adrenoceptors are not distributed in all vascular beds but only in the region where the e.j.p. is generated by perivascular nerve stimulation, namely many small arteries, but not the venous systems. Therefore, α-adrenoceptor blocking agents other than K-351 may also modify the muscle tone of vascular beds through inhibition of activation of adrenergic nervous systems.

Prazosin was more potent than K-351 in inhibiting the extra-junctional adrenoceptors; therefore, application of this agent may induce a hypotension, whereas K-351 possessed a weaker inhibitory action on the extra-junctional adrenoceptor but did inhibit the intra-junctional adrenoceptor. Therefore, the concomitant hypotensive actions induced by K-351 may differ from the action of prazosin. Furthermore, K-351 reportedly possesses the action of a β-adrenoceptor blocker in *in vivo* experiments (Uchida *et al.*, 1982). Both α- and β-adrenoceptor blocking agents tend to induce hypotension.

Our results suggest that because K-351 is a drug which inhibits the intra-junctional adrenoceptors, evidence on the distribution and nature of α-adrenoceptors located on the vascular smooth muscles can be obtained by application of this drug in pertinent studies.

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