Br. J. Pharmac. (1983), **79**, 285–295

The effects of 3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran (K-351) and its denitrated derivative on smooth muscle cells of the dog coronary artery

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1 Effects of 3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran (K-351) and its derivative, 3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-hydroxy-2H-1-benzopyran (K-351 (N−)) on the electrical and mechanical properties of smooth muscles of the dog coronary and mesenteric arteries were investigated, and the findings were compared with data obtained with nitroglycerine.

2 In both proximal and distal regions of the coronary arteries, K-351 and nitroglycerine reduced the resting tone and suppressed the contractions produced by high-potassium solution or by current passage to the same extent, with no remarkable change in the electrical properties of the smooth muscle membrane.

3 In the proximal regions of the descending coronary artery, low and high concentrations of noradrenaline (NA) produced relaxation and contraction of the muscle, respectively. In the distal region, NA consistently relaxed the muscle with concentrations up to 10^{-5}M. In both regions, the contraction or relaxation was suppressed by phentolamine or propranolol, respectively.

4 K-351 suppressed the NA-induced contraction. K-351(N−) potentiated the NA-induced contraction and suppressed the relaxation, but these actions were weaker than those of propranolol. Nitroglycerine suppressed the NA-induced contraction and the potency was weaker than that of K-351.

5 In the mesenteric artery, K-351 depressed excitatory junction potentials, spikes and contractions evoked by perivascular nerve stimulation, while K-351(N−) potentiated or depressed mechanical responses, with no change in the electrical responses. Nitroglycerine also depressed the mechanical responses evoked by perivascular nerve stimulation with no change in the electrical responses.

6 These results suggest that K-351 has a blocking action on postjunctional adrenoceptors, and also dilator actions similar to the actions of nitroglycerine on the dog coronary artery, while K-351 (N−) possesses a weak β-adrenoceptor blocking action.

Introduction

The α-adrenoceptors in the vascular system are classified pharmacologically into two subtypes, α_1 in the postjunctional smooth muscle membrane, α_2 on the prejunctional nerve terminal (Langer, 1977) and also in the postjunctional smooth muscle membrane (Vanhoutte, Verbeuren & Webb, 1981). In addition to these two types of receptor, a new type of receptor which responds to α-agonists (γ-receptor) is proposed in the postjunctional membrane (Hirst & Neild, 1980). The two subtypes of receptor are blocked by drugs which are generally classified as α_1- or α_2-antagonists; however the γ-receptor is resistant to these blocking agents.

K-351 has been found to suppress electrical and mechanical responses evoked by perivascular nerve stimulation in the mesenteric artery of the guinea-pig (Asada, Nanjo, Itoh, Suzuki & Kuriyama, 1982) and dog (Kou, Kuriyama & Suzuki, 1982). The compound reduced the amplitude of excitatory junction potentials (e.j.ps) generated by perivascular nerve stimulation with no change in the facilitation of e.j.ps, electrical threshold of perivascular nerves or input resistance of the postjunctional membrane. Electrical and mechanical responses of these vascular smooth muscles to exogenous noradrenaline(NA) were also suppressed by K-351. Thus, K-351 blocked

both α_1- and γ-adrenoceptors, its action differing from that of other α_1- and α_2-adrenoceptor blocking agents (e.g., phentolamine interacts with both α_1- and α_2-adrenoceptors). Thus it enhanced the amplitude of the e.j.p. due to interference with the negative feedback regulation of transmitter release and inhibited the contraction evoked by exogenous NA due to blockade of α_1-adrenoceptors (Kuriyama & Makita, 1983).

Uchida, Nakamura, Shimizu, Shirasawa & Fujii (1983) found that K-351 blocked β-adrenoceptors *in vivo* and produced hypotension in rats. Smooth muscle cells of the dog coronary artery possess α- and β-adrenoceptors, and high concentrations of exogenously applied NA produced biphasic mechanical responses (Ito, Kitamura & Kuriyama, 1980a; Toda, 1981). If K-351 does possess α- and β-adrenoceptor blocking actions, the responses of the coronary artery to NA should be suppressed by K-351.

K-351 contains an organic nitrate and such compounds are known to produce vasodilatation (see Needleman 1975; Kreye & Gross, 1977). A denitrated derivative of K-351 was recently synthesized by Kowa Pharmac. Co. (Tokyo). We investigated the effects of K-351 and denitrated K-351 (K-351 (N−)) on the dog coronary artery to determine whether vasodilatation induced by K-351 is due solely to an adrenoceptor blocking action or is related to the properties of organic nitrates. The effects of triglycerol nitrate, nitroglycerine, were also investigated for comparison. Some experiments were carried out to investigate the effects of these agents on the dog mesenteric artery.

Methods

Mongrel dogs of either sex, weighing 10–15 kg, were given sodium pentobarbitone (40 mg kg i.v.) and exsanguinated from the femoral artery. The main trunk of the descending coronary artery at the left ventricle, and the second branch of the mesenteric artery leading to the ileum were excised and kept in Krebs solution at room temperature. Connective tissues surrounding the vessels were removed and helical strips (0.7–1 mm width) were cut.

Electrical responses of the smooth muscle were recorded by means of glass capillary microelectrodes (Hilgenberg, No. 1103207) filled with 3 M KCl and of tip resistance 40–100 MΩ. The recording chamber of Lucite had a volume of about 2 ml. The tissue was mounted on a silicon-rubber plate (Shinetsu Kagaku) fixed to the bottom of the chamber, and Krebs solution (35.5°C) was superfused at a rate of 3 ml/min. Electrotonic potentials were evoked using the partition stimulating method (Abe & Tomita, 1968). Field stimulation was applied to the tissue for perivascular nerve stimulation (Suzuki & Fujiwara, 1982), with a current pulse of 0.05–0.1 ms duration and 10–100 V intensity. Electrical responses of the smooth muscle membrane were displayed on a pen-writing recorder (Nihonkohden Recticorder, RJG 4202).

Mechanical responses of the muscle were recorded isometrically from the helical strip (1 mm width, 1.5 cm length). The tissue was mounted vertically, the bottom end being fixed and the other end connected to a mechanotransducer (Nihonkohden FD-pick-up, TB-612T). The recording chamber had a volume of 1.5 ml, and Krebs solution (35.5°C) was superfused at a rate of 3 ml/min. Field stimulation was applied through a pair of silver plates fixed at the sides of the chamber.

The composition of Krebs solution was as follows (mM): Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0 HCO_3^- 15.1, $H_2PO_4^-$ 1.2 and glucose, 11.5. High-potassium solution was prepared by replacing NaCl with KCl, isotonically. The solution was aerated with 97% O_2 and 3% CO_2 and the pH was maintained at 7.2–7.4.

Drugs used in the experiment were (−)-noradrenaline HCl, tetrodotoxin (Sigma), propranolol HCl (Sumitomo Kagaku), phentolamine mesylate (CIBA Geigy), nitroglycerine (Nihonkayaku), 3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy)-3-nitroxy-2H-1-benzopyran (K-351, Kowa) and its denitrated derivative 3,4-dihydro-8-(2-hydroxy-3-isopropylaminopropoxy) -3-hydroxy- 2H-1- benzopyran (K-351 (N−), Kowa). To prepare the appropriate concentrations of K-351 or K-351 (N−), each of these compounds was dissolved in 0.1 N HCl at a concentration of 10^{-2}M, and added to the Krebs solution to obtain the desired concentration.

Appropriate concentrations required for our experiments did not modify the pH of the Krebs solution.

Electrical or mechanical responses were expressed as means ± s.d., and statistical significance was determined by Student's *t* test, $P < 0.05$ being taken as significant.

Results

Effect of K-351 on electrical properties of the smooth muscle membrane

The smooth muscle cells in the dog coronary artery (main trunk) were electrically quiescent. The resting membrane potential was -49.5 ± 1.8 mV ($n = 30$), this value being much the same as that obtained by Ito *et al.* (1980a).

Application of K-351, K-351 (N−) or nitroglycerine at a concentration of 10^{-5}M did not modify

Table 1 Effects of K-351, K-351 (N−) and nitroglycerine on the membrane potential of smooth muscle in dog coronary artery in different potassium solutions

$[K]_o$ (mM)	*Control*	*K-351* (10^{-5}M)	*K-351 (N−)* (10^{-5}M)	*Nitroglycerine* (10^{-5}M)
5.9	−49.8±2.3(26)	−48.2±2.4(9)	−49.8±2.8(11)	−49.7±1.7(11)
39.2	−25.8±2.4(21)	−27.9±1.7(11)	−24.6±2.0(14)	−26.6±1.6(11)

Mean ±s.d. is shown. Number of observations is given in parentheses. Control = membrane potential (mV) observed before application of drug.

the membrane potential of smooth muscle cells from the proximal region of the left anterior descending branch (Table 1). The membrane potential (−49.8±2.3 mV) was much the same as that measured from the main trunk. These drugs did not modify the membrane resistance measured from the amplitude of electrotonic potentials evoked by application of constant intensity inward or outward current or measured from the current-voltage relationship using various intensities of inward and outward currents (2 s pulse duration). These agents had no effect on the membrane potential and membrane resistance measured from small branches of the coronary artery (2–3 cm distal to the branching point of the anterior descending artery, where the artery was 100–200 μm in outer diameter).

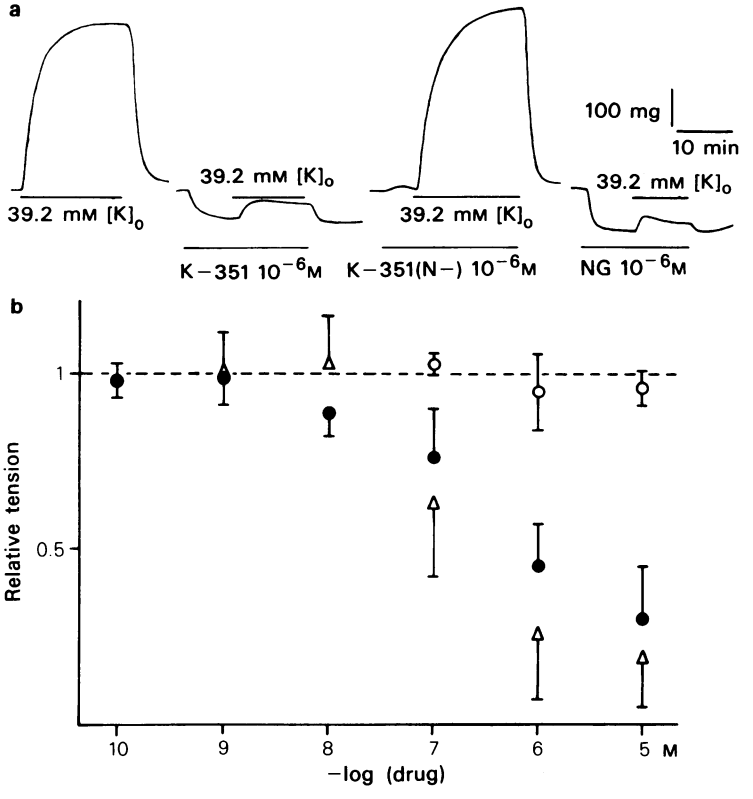

Figure 1 Effects of K-351, K-351 (N−) and nitroglycerine (NG) on 39.2 mM $[K]_o$-induced contraction. (a) Bars indicate application of 39.2 mM $[K]_o$ or chemical agents. (b) Effects of various concentrations of agents on the relative tension of the K-induced contraction: (●) K-351; (○) K-351 (N−); (Δ) nitroglycerine. The 39.2 mM $[K]_o$-induced contraction in the absence of drug was expressed as 1.0 (dotted line), and mean (n = 4–8) is shown; s.d. shown by vertical lines.

Effect of K-351 on mechanical responses induced by high $[K]_o$ or electrical depolarization

The resting tone of the muscle tissue was kept at 0.1–0.2 g by light stretching. With applications of K-351 or nitroglycerine in a concentration over 10^{-8}M, the tissue relaxed dose-dependently. However, application of 10^{-6}M K-351 (N−) either had no effect on the resting tone, or in some preparations, elevated the resting tone (Figure 1). To prevent the effects of transmitter release by excess concentrations of $[K]_o$, guanethidine (10^{-6}M) and tetrodotoxin (TTX, 3×10^{-7}M) were applied throughout the following experiments. With application of 39.2 mM $[K]_o$, the amplitude of contraction was consistently reduced under treatment with K-351. Figure 1 shows the effects of 10^{-6}M K-351, K-351 (N−) and nitroglycerine on the K-induced contraction. Inhibitory actions of K-351 and nitroglycerine were observed at concentrations of over 10^{-7}M (Figure 1b). The ID_{50} for the inhibitory action of K-351 and nitroglycerine was 8×10^{-7}M and 5×10^{-7}M, respectively. These values were not significantly different. K-351 (N−) had no effect on the K-induced contraction in doses up to 10^{-5}M.

Application of 39.2 mM $[K]_o$ solution depolarized the muscle membrane to −26 mV and this depolarization was not affected by application of K-351, K-351 (N−), or nitroglycerine at a concentration of 10^{-5}M (Table 1).

Effects of K-351, K-351 (N−) and nitroglycerine on the mechanical responses produced by depolarizing current were observed. Tensions were expressed relative to the contraction evoked in Krebs solution which was taken as 1.0, and 10^{-6}M guanethidine and 3×10^{-7}M TTX were applied throughout. K-351 and nitroglycerine reduced the contraction to the same extent in concentrations over 10^{-6}M, while K-351 (N−) had no effect. At a concentration of 10^{-5}M, K-351 or nitroglycerine reduced the depolarization-induced contraction to 0.49 ± 0.11 ($n = 16$) or 0.46 ± 0.07 ($n = 14$) of the control (= 1.0), respectively.

The effects of K-351, K-351 (N−) or nitroglycerine on noradrenaline-induced contractions

In the dog coronary artery, NA in a low concentration relaxed the tissue and in a high concentration contracted it with no change in the membrane potential (Ito *et al.*, 1980a). There were regional differences in the mechanical responses, e.g., in the proxi-

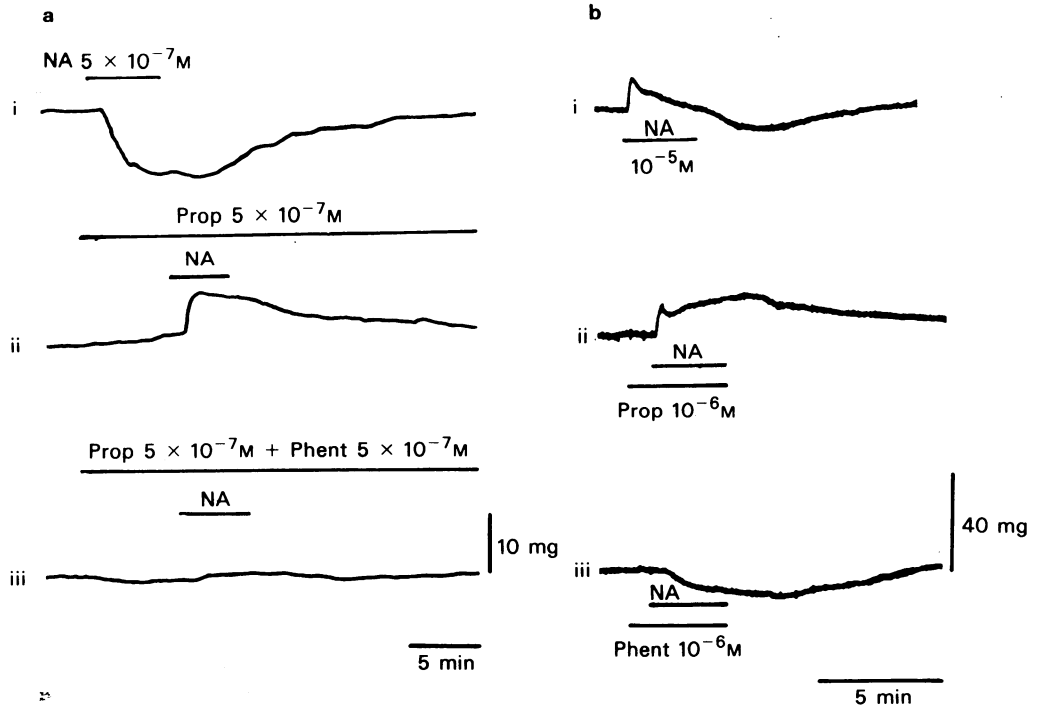

Figure 2 Noradrenaline (NA)-induced mechanical responses from the proximal region of the descending coronary artery. (a) noradrenaline, 5×10^{-7}M. (b) Noradrenaline 10^{-5}M. Effects of pretreatment with propranolol (Prop) or propranolol with phentolamine (Phent) are shown. (a) and (b) were obtained with different tissues.

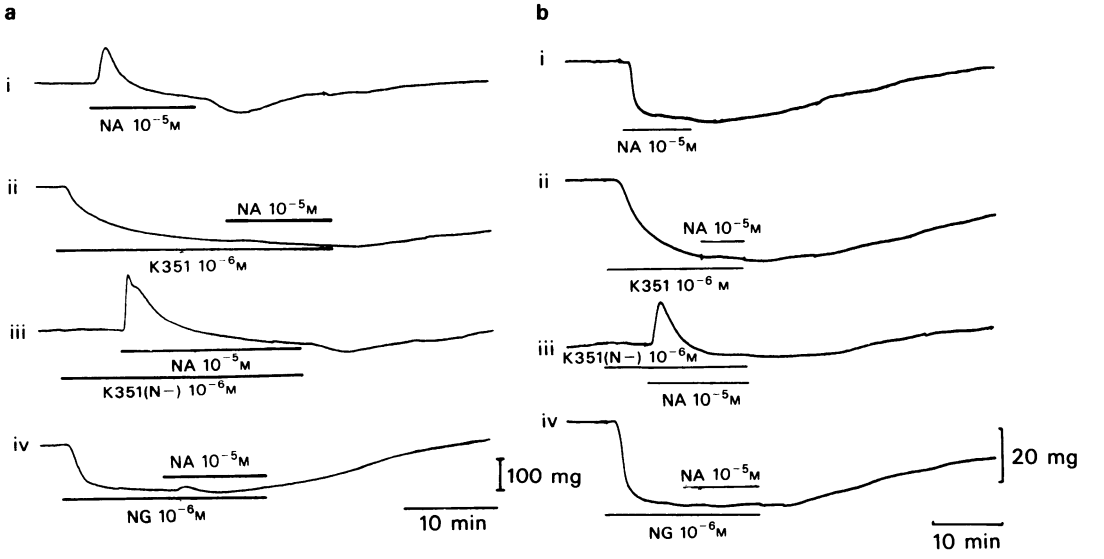

Figure 3 Effects of K-351, K-351 (N−) and nitroglycerine (NG) on the noradrenaline (NA 10^{-5}M)-induced contractions. (a) Proximal region of the descending coronary artery; (b) distal region. Drugs were applied during the period indicated by the bar in each record.

mal region of the descending artery relaxation occurred with low concentrations and contraction with high concentrations (up to 10^{-5}M), while the distal region (2–3 cm distal to the branching point of the descending artery) consistently relaxed on application of concentrations up to 10^{-5}M.

Figure 2 shows the effects of two concentrations of NA on the mechanical responses obtained from the proximal region. Relaxation of the muscle on application of 5×10^{-7}M NA was changed to contraction in the presence of 5×10^{-7}M propranolol (Figure 2a(ii)). This contraction ceased with additional application of 5×10^{-7}M phentolamine (Figure 2a(ii)). Application of 10^{-5}M NA produced a contraction with a delayed relaxation (Figure 2b). Pretreatment with propranolol (10^{-6}M) potentiated the tonic phase of the contraction and suppressed the delayed relaxation (Figure 2b(ii)). Pretreatment with 10^{-6}M phentolamine led to a relaxation of the tissue when 10^{-5}M NA was applied (Figure 2b(iii)). Thus, smooth muscles of the proximal region of dog coronary artery possess both α- and β-adrenoceptors.

The effects of K-351, K-351 (N−) and nitroglycerine on contractions induced by 10^{-5}M NA in the proximal region were studied. K-351 10^{-6}M was found to reduce the resting tone and to suppress the NA-induced contraction (Figure 3a(ii)), while 10^{-6}M K-351 (N−) potentiated the NA-induced contraction (1.50 ± 0.14 times; $n = 3$; $P < 0.05$) (Figure 3a(iii)). During the nitroglycerine (10^{-6}M-induced relaxation of the tissue, 10^{-5}M NA produced a small contraction as shown in Figure 3a(iv) (0.19 ± 0.10 times the control, $n = 3$).

Effects of K-351, K-351 (N−) and nitroglycerine on NA-induced mechanical responses were also studied in tissues from the distal region. The tissue relaxed in the presence of 10^{-5}M NA, and during the muscle relaxation produced by K-351 (10^{-6}M) or nitroglycerine (10^{-6}M), NA produced no mechanical responses (Figure 3b). In the presence of 10^{-6}M K-351 (N−), 10^{-5}M NA produced a biphasic mechanical response, an initial contraction and then a relaxation (Figure 3b(iii)).

Figure 4 shows dose-response relationships of the effect of K-351 (N−) on the NA-induced mechanical response from the distal region. Relative amplitudes of relaxation or contraction produced by 10^{-5}M NA in the presence of different concentrations (10^{-8}–10^{-5}M) of K-351 (N−) are shown. The effect of propranolol (10^{-6}M) is shown for a comparison. K-351 (N−) or propranolol was applied 10 min before application of NA. K-351 (N−) reduced the NA-induced relaxation in a dose-dependent manner and, at 10^{-5}M, completely suppressed it. In the presence of K-351 (N−) at concentrations of 10^{-7}–10^{-6}M, NA produced biphasic responses, i.e., initial contraction followed by relaxation. The NA-induced contraction in the presence of K-351 (N−) increased dose-dependently at concentrations over 10^{-7}M. Propranolol (10^{-6}M) completely suppressed the NA-induced relaxation and NA produced a contraction with much the same amplitude as that seen in

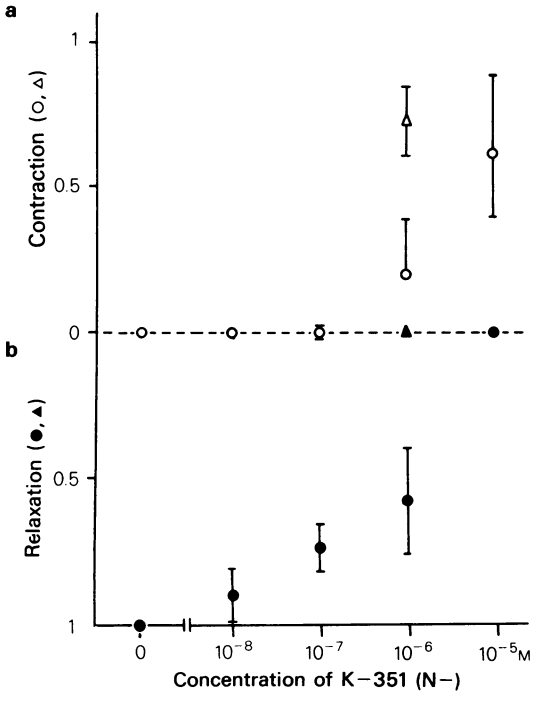

Figure 4 Dose-response relationship of the effect of K-351 (N−) on 10^{-5}M noradrenaline (NA)-induced mechanical responses. Distal region of dog coronary artery. Maximum amplitude of 10^{-5}M NA-induced relaxation was taken as 1, and the relative amplitudes of NA-induced relaxation (b) or contraction (a) are plotted. Each point is the mean of 4–8 observations; s.d. shown by vertical lines. Responses to NA in the presence of 10^{-6}M propranolol (▲, △) are also shown. K-351 (N−) or propranolol was applied 10 min before application of NA. Line at zero indicates resting tension.

the presence of 10^{-5}M K-351 (N−). Thus K-351 (N−) blocked β-adrenoceptors in the dog coronary artery but the potency was about 10 times weaker than that of propranolol.

To rule out the possible involvement of β-adrenoceptors in the K-351-induced relaxation, its effects were compared in the presence or absence of propranolol (Figure 5). In dog coronary artery, relaxation produced by application of 5×10^{-7}M NA was reversed to contraction by pretreatment with 5×10^{-7}M propranolol (Figure 5b). After pretreatment with propranolol, the K-351-induced relaxation was not affected and during the relaxation, NA produced no response (Figure 5d). Similar experiments were done on tissues from the distal region of the coronary artery and similar results obtained. Thus, K-351-induced relaxation is not due to stimulation of β-adrenoceptors.

Effects of K-351, K-351 (N−) and nitroglycerine on neuroeffector transmission

Figure 6 shows effects of K-351, K-351 (N−) and nitroglycerine on the e.j.p. evoked by perivascular nerve stimulation in the dog mesenteric artery. Increase in amplitude of e.j.p. was intensity-dependent and when the amplitude of depolarization exceeded the electrical threshold, an action potential was generated. Repetitive stimulation (> 0.2 Hz) produced a depression of e.j.p. amplitude. Therefore, to produce the same amplitude of response (action potential plus e.j.p.), stimulation was applied every 20 s. When 10^{-5}M K-351 was applied, e.j.p. amplitude was reduced and the action potential was not generated at high intensity stimulation (Figure 6b). K-351 (N−) 10^{-5} and nitroglycerine 10^{-5}M did not modify the amplitudes of e.j.p. and action potential (Figure 6c and d).

Repetitive stimulation of perivascular nerves (0.1 Hz, 100 V) produced twitch contractions in the dog mesenteric artery. Figure 7 shows effects of K-351, K-351 (N−) and nitroglycerine on the twitch contractions. The contraction ceased in the presence of TTX 3×10^{-7}M. The amplitude of contraction decreased with application of 10^{-6}M K-351 or 10^{-6}M nitroglycerine. However in the presence of K-351 (N−) the nerve-induced contractions were either potentiated or suppressed. In three of six preparations there was an enhancement, and in three others there was a suppression with no relation to differences in sex and body weight. The electrical responses produced by perivascular nerve stimulation in these two groups were not detectably different, i.e., the e.j.p. and action potential were not modified by application of 10^{-6}M K-351 (N−).

Figure 8 shows dose-response relationships of the effect of K-351, K-351 (N−) or nitroglycerine on the mechanical responses produced by a single stimulus. The response ceased on application of TTX 3×10^{-7}. This response was evoked every 90 s, and the amplitudes of nerve-mediated contraction evoked during the application of different concentrations of K-351, K-351 (N−) or nitroglycerine were measured relative to that seen in Krebs solution (1.0). Since K-351 (N−) produced either an enhancement or a depression, experiments in which depression occurred were treated separately and are pooled in the graph of Figure 8b. K-351 and nitroglycerine consistently and dose-dependently reduced the twitch contraction. K-351 (N−)-induced inhibition was dose-dependent while K-351 (N−)-induced enhancement was not related to dose.

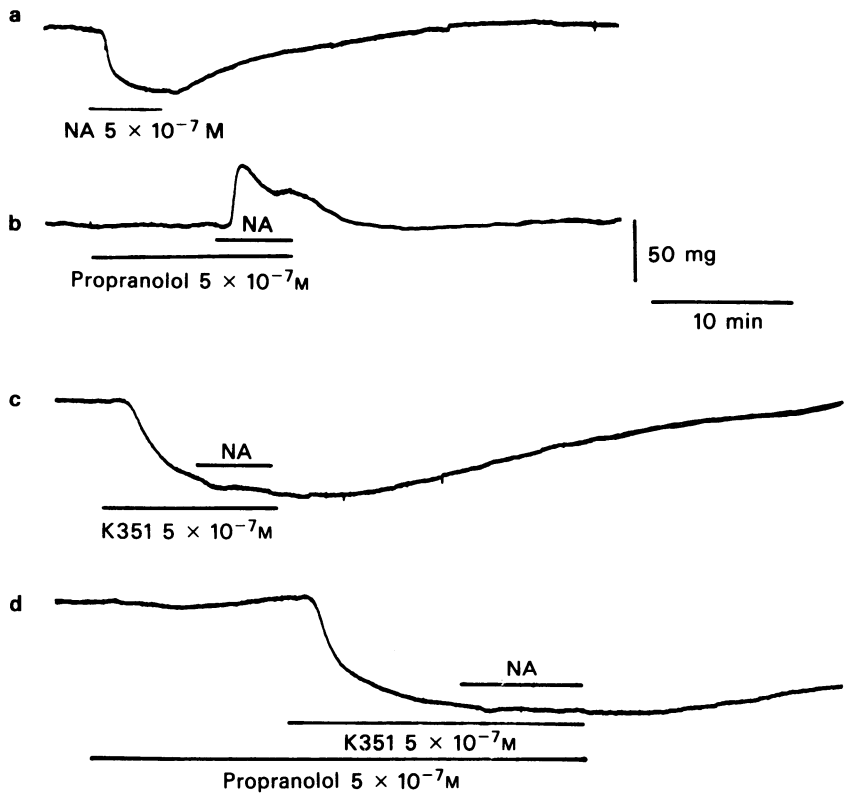

Figure 5 Effect of propranolol on the K-351-induced relaxation of dog coronary artery (proximal region). Noradrenaline (NA) at a concentration of 5×10^{-7}M was applied during the period indicated by the bar. All responses obtained from the same tissue.

Discussion

In the dog coronary artery, K-351 but not K-351 (N−) relaxed the smooth muscle with no detectable changes in membrane potential and membrane resistance. K-351 but not K-351 (N−) inhibited contractions produced by high-potassium solution and depolarizing current pulses. These observations suggest that the presence of the organic nitrate in the structure of K-351 is essential for appearance of the inhibitory action in the coronary artery. The relaxation induced by K-351 cannot be attributed to activation of β-adrenoceptors, since propranolol did not prevent the K-351-induced relaxation.

Nitroglycerine showed much the same potency as K-351 in producing relaxation of the resting tissue and also when stimulated by high $[K]_o$ or electrical depolarization. Electrophysiological experiments suggest that the inhibitory effect of nitroglycerine in the canine and porcine coronary arteries is due to suppression of Ca mobilization from storage sites. There is no marked effect on the myoplasmic membrane of smooth muscle cells (Ito *et al.*, 1980 a, b). However, the efflux of intracellularly accumulated ^{45}Ca is not affected by application of nitroglycerine (Nakazawa & Imai, 1981). Comparing the effect of K-351 with that of nitroglycerine, the NA-induced contraction in the proximal region of dog coronary artery was completely suppressed by K-351, yet this contraction was only partly suppressed by nitroglycerine. Clear differences in action between K-351 and nitroglycerine could also be seen in electrical responses evoked by perivascular nerve stimulation from the canine mesenteric artery, e.g., K-351 reduced e.j.p. emplitude and nerve-induced contraction (see also Asada *et al.*, 1982; Kou *et al.*, 1982).

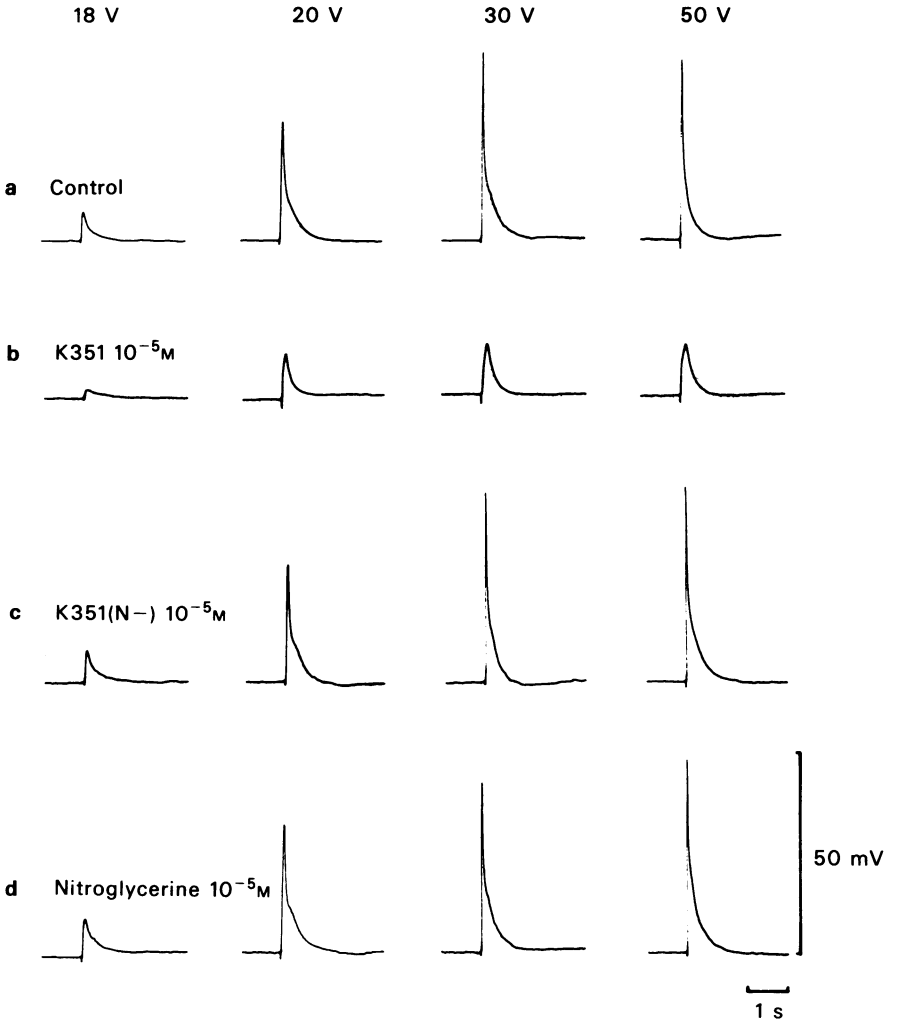

Figure 6 Effects of K-351, K-351 (N−) and nitroglycerine on electrical responses in the mesenteric artery generated by perivascular nerve stimulation. Pulses at 0.05 ms at four different intensites. All responses obtained from the same tissue during application of drugs for 10–15 min.

Nitroglycerine suppressed only the mechanical response with no change in the e.j.p. and superimposed spike, as has been reported for the mesenteric artery of guinea-pig and pig (Itoh, Furukawa, Kajiwara, Kitamura, Suzuki, Ito & Kuriyama, 1981). Thus the inhibitory action of K-351 and nitroglycerine differs.

Uchida *et al.* (1983) reported that K-351 blocks β-adrenoceptors in the circulatory system, thus leading to hypotension in SHR and DOCA rats. Our present and previous results suggest that K-351 blocks α-adrenoceptors in the muscle of mesenteric and coronary arteries (Asada *et al.*, 1982; Kou *et al.*, 1982). In the presence of K-351 (N−), the NA-induced relaxation was suppressed and contraction developed. Thus, K-351 (N−) possesses β-adrenoceptor blocking properties, although this action is weaker than that of propranolol. Whether or not K-351 possesses β-receptor blocking actions is not clear.

There were regional differences in responses to NA in the dog coronary artery, i.e., a high concentration (10^{-5}M) produced contraction in the proximal

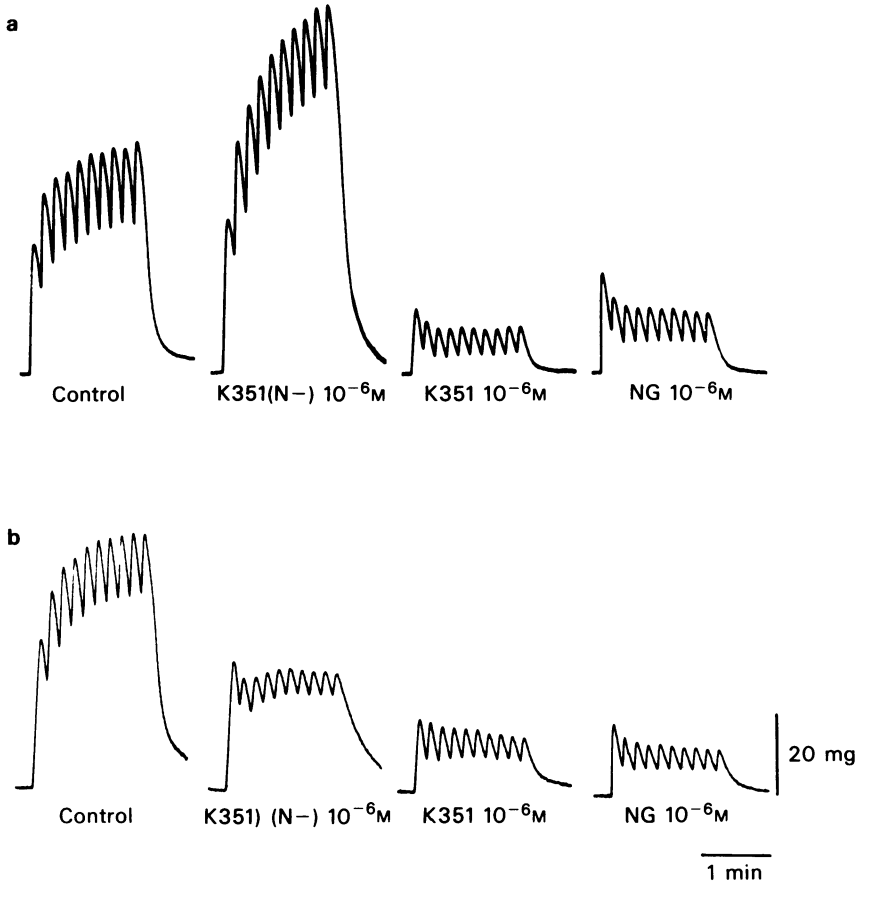

Figure 7 Effects of K-351 (N−), K-351 and nitroglycerine (NG) on the mechanical responses of the mesenteric artery produced by perivascular nerve stimulation. A train of 10 pulses (1 ms, 100V) was applied at frequency of 0.1 Hz. Responses were obtained 5–10 min after application of these drugs. (a) and (b) were obtained with tissues from different dogs.

region yet relaxation occurred in the distal region. These regional differences in response to NA are presumably due to different densities of α- and β-adrenoceptors.

Harder, Belardinelli, Sperelakis, Rubio & Berne (1979) reported that in the dog coronary artery the effects of nitroglycerine differ between large and small coronary arteries, i.e. in large but not small arteries, evoked action potentials in the presence of tetraethylammonium chloride were suppressed by nitroglycerine. In the present experiments, nitroglycerine produced no detectable change in membrane potential, either in large or small coronary arteries, and consistently relaxed the tissue in both regions. These observations suggest that nitroglycerine action may depend on whether resting or active conditions pertain in the smooth muscle membrane.

In conclusion, K-351 reduces resting tone and mechanical responses produced by high-potassium solution, depolarizing current and NA in the dog coronary artery with no change in the electrical properties of the smooth muscle membrane. K-351 (N−) does not inhibit these contractions, but blocks β-adrenoceptors. The effects of K-351 are much the same as those of nitroglycerine. However, in the mesenteric artery, nitroglycerine suppresses mechanical responses evoked by perivascular nerve stimulation with no change in the electrical response, while K-351 suppresses both electrical and mechanical responses. These results suggest that K-351 has a blocking action at α-adrenoceptors and that the

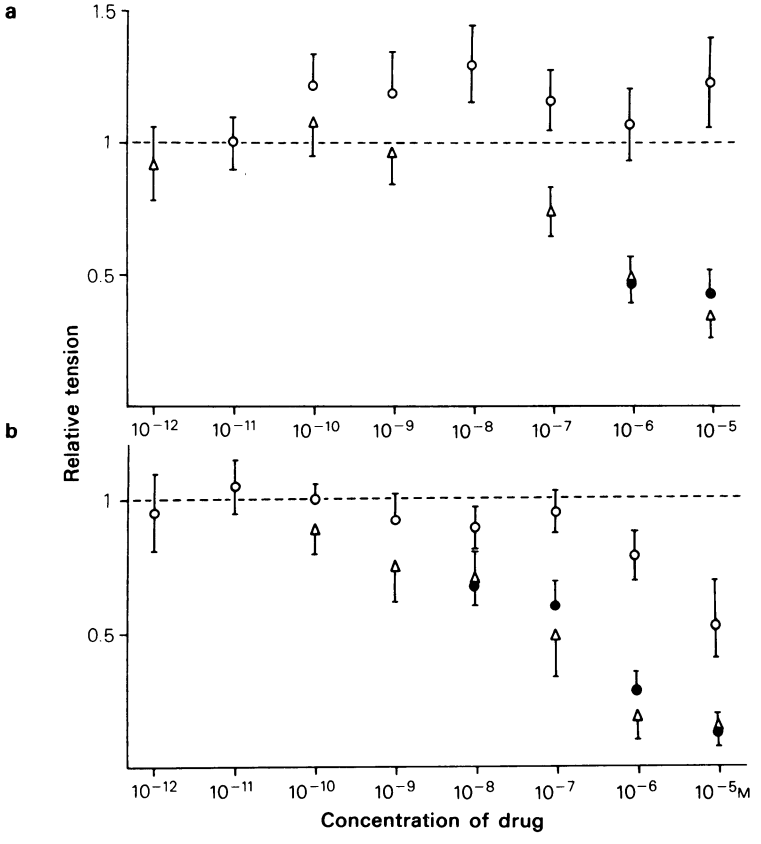

Figure 8 Dose-response relationship of the mechanical responses of the dog mesenteric artery produced by nerve stimulation in the presence of K-351 (●), K-351 (N−) (○) or nitroglycerine (△). Perivascular nerves were stimulated with a single pulse (1 ms, 100V) at intervals of 90 s. Tension in the absence of drug is expressed as 1, and relative tension (mean) is shown; s.d. shown by vertical lines. Each point represents 8–16 observations from 3 different tissues. (a) and (b) obtained from different animals.

nitroglycerine-like action is mainly due to the presence of an organic nitrate. Whether any β-adrenoceptor blocking action of K-351 is masked by the relaxant actions or whether this action is dominant only after K-351 is denitrated remains to be determined.

We thank Professor H. Kuriyama for pertinent discussions and M. Ohara for reading the manuscript. K-351 was a gift from Kowa Pharmac. Co. (Tokyo).

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(Received November 22, 1982.
Revised January 6, 1983.)