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The effects of 2-nicotinamidoethyl nitrate on smooth muscle cells of the dog mesenteric artery and trachea

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1 The effects of 2-nicotinamidoethyl nitrate (nicorandil; 2-NN), a synthesized coronary vasodilator, on smooth muscle cells of dog mesenteric artery or trachea were investigated using microelectrode, double sucrose gap and isometric tension recording methods.

2 Nicorandil hyperpolarized the smooth muscle membrane of the mesenteric artery (5×10^{-6} M) and the trachea (5×10^{-5} M).

3 In both these smooth muscle cells, the nicorandil-induced hyperpolarization remained constant in various concentrations of $[Cl^-]_o$, but changed with various concentrations of $[K^+]_o$. This hyperpolarization was partly inhibited following pretreatment with tetraethylammonium (TEA > 1 mM) and was completely inhibited following pretreatment with procaine (1 mM or 5 mM), indicating that the nicorandyl-induced hyperpolarization is due to increase in the K-conductance, in both these membranes.

4 Following pretreatment with TEA (5 mM), outward current pulses evoked an action potential in tracheal smooth muscle cells. Nicorandil inhibited the generation of action potential due to the hyperpolarization of the membrane but not due to inhibition of the spike generating mechanism.

5 Nicorandil (10^{-6} M) inhibited the contracture evoked by excess $[K]_o$, in both tissues. The contracture evoked by noradrenaline or repetitive field stimulation with short duration (50 μs) pulses was also inhibited in the mesenteric artery, while a higher dose of nicorandil (10^{-5} M) was required to inhibit the contracture evoked by acetylcholine or repetitive field stimulation in the trachea.

6 Excitatory junction potentials (e.j.ps) evoked by field stimulation in the mesenteric artery due to the release of noradrenaline, or in the trachea due to release of acetylcholine, were suppressed by 10^{-5} M or 5×10^{-5} M nicorandil, respectively. The reduction in the amplitude of e.j.p. was mainly due to the hyperpolarization of the membrane with increase in the membrane conductance.

7 In the mesenteric artery, following pretreatment with TEA (1 mM) an action potential was generated on the e.j.p.. Nicorandil suppressed the generation of the action potential by reduction in the amplitude of the e.j.p., below the threshold required for generation of the action potential.

8 These results indicate that nicorandil hyperpolarizes the membrane by increasing K-conductance and inhibits the generation of contraction, in both tissues. Higher concentrations of nicorandil are required to suppress the mechanical response in the trachea than in the mesenteric artery. Depression of the mechanical responses in both tissues is partly due to suppression of Ca-mobilization inside the muscle cells and partly to hyperpolarization of the membrane.

Introduction

2-Nicotinamidoethyl nitrate (nicorandil; 2-NN), a synthesized coronary vasodilator, reportedly hyperpolarized the muscle membrane and suppressed the mechanical response of the coronary artery induced by electrical depolarization, acetylcholine, excess $[K^+]_o$ or repetitive short pulse stimulation. Relatively high concentrations of nicorandil were required to suppress the excess $[K^+]_o$-induced contraction without causing hyperpolarization of the membrane. The marked vasodilator action of nicorandil on the porcine and guinea-pig coronary arteries is mainly due to hyperpolarization of the membrane and partly due to suppression of the Ca-mobilization inside the smooth muscle cell (Furukawa, Itoh, Kajiwara, Kitamura, Suzuki, Ito & Kuiyama, 1981).

Nicorandil had similar effects on the mesenteric artery, and mesenteric and portal veins excised from the pig or guinea-pig (Itoh, Furukawa, Kajiwara,

Kitamura, Suzuki, Ito & Kuriyama, 1981; Karashima, Itoh & Kuriyama, 1982), suggesting that in porcine tissues, reduction in the mechanical responses of these vascular smooth muscle may be mainly due to the hyperpolarization of the membrane, and in the guinea-pig to suppression of Ca-mobilization in the muscle cells. Therefore this vasodilator possesses potent anti-anginal and/or antihypertensive effects, as postulated from *in vivo* experiments (Taira, Satoh, Yanagisawa, Imai & Hiwatari, 1979; Karashima et al., 1982).

To determine whether or not nicorandyl exerts the same effects on the other visceral smooth muscle cells, the effects of nicorandyl on the muscle membrane, excitation-contraction coupling and neuroeffector transmission in the dog trachea were investigated and the dog mesenteric artery was used to assess the action on vascular tissues. These particular preparations were used because the electrical membrane properties of the smooth muscle and neuroeffector transmission have already been described in detail (Suzuki, Morita & Kuriyama, 1976; Ito & Tajima, 1981 a & b; Kou, Kuriyama & Suzuki, 1982).

Methods

Materials

Mongrel dogs of either sex, weighing 10–15 kg were anaesthetized with an intravenous injection of pentobarbitone (30 mg kg^{-1}). Segments of cervical trachea were excised, and a dorsal strip of transversely running smooth muscle fibre was separated from the cartilage. The mucosa and adventitial areolar tissue were carefully removed. The tracheal smooth muscle was cut at a width of 2.0–2.5 mm and a length of about 20 mm for use in the double sucrose gap method, and 15–20 mm in length, 4–5 mm in width and 0.3–0.4 mm thick for microelectrode-related experiments. The mesenteric artery running in parallel with veins and lymph ducts was dissected from the mesentery of the jejunal region. The artery (diameter 0.5–1.0 mm) was used for the microelectrode and double sucrose gap methods. The microelectrode was inserted from the outer surface of the artery. For the double sucrose gap method, helically cut preparations (2–2.5 mm wide and about 20 mm in length) were used.

Experimental procedures

To measure the membrane resistance and membrane potential of the smooth muscle, the tissue was mounted in a 2 ml organ bath and superfused at a rate of 2 ml min^{-1} at 30–31°C. A conventional microelectrode filled with 3M KCl (resistance of the electrode was 50–80 megohms) was used to record the membrane potential. For measurements of membrane resistance, the partition stimulating method described by Abe & Tomita (1968) was used.

For simultaneous observation of the membrane property and tension development, the double sucrose gap method was used. The apparatus used has been described elsewhere (Ito & Tajima, 1981a).

To measure the isometric contraction, two strips prepared from the mesenteric artery (5–6 mm in length and 1 mm in diameter, helical preparation) or from trachea were mounted simultaneously in a 1 ml organ bath; one end of each strip was fixed at the bottom of the bath and the other end was connected by a hook to a tension recorder (Nihon Kohden Ltd., FD pick up; TB-612T). The flow rate of the superfusate was 3 ml min^{-1} at a temperature of 30–31°C. To evoke the mechanical response by electrical stimulation, two silver-silver chloride ring electrodes were placed in the organ bath at the two ends of the tissue. The resting tension of the tissue was kept at about 0.1 g for both tissues. Results (membrane potential and amplitude of contraction) are expressed as mean ± s.d..

Solution

Krebs solution was of the following ionic concentration (mM); Na^+ 137.4, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134.0, H_2PO_4 1.2, HCO_3, 15.5, and glucose 11.5. The solution was bubbled with 97% O_2 : 3% CO_2 the pH was maintained at 7.2 to 7.3.

Excess $[K^+]_o$ solution was prepared by replacing NaCl with an equivalent amount of KCl, up to 118 mM, isotonically.

The following drugs were used; nicorandil; 2-NN (mol.wt. 211.18, $C_8H_9N_3O_4$, SG 75; Chugai), indomethacin (Sigma), (−)-propranolol (Sumitomo), noradrenaline (Sankyo), phentolamine (CIBA-Geigy), acetylcholine (Nikken Chemical), tetraethylammonium chloride and procaine (Daiichi).

Results

Effects of nicorandil on the membrane potential of the dog trachea and mesenteric artery

Figure 1a shows the effects of various concentrations of nicorandil on the membrane potential of smooth muscle cells of the dog mesenteric artery. Application of nicorandil in concentrations over 5×10^{-6} M hyperpolarized the membrane of the smooth muscle of mesenteric arteries and the nicorandil-induced hyperpolarization was dose-dependent. The maximum hyperpolarization was observed with 10^{-4} M nicorandil (from -63.1 ± 1.8 to -86.2 ± 2.1 mV,

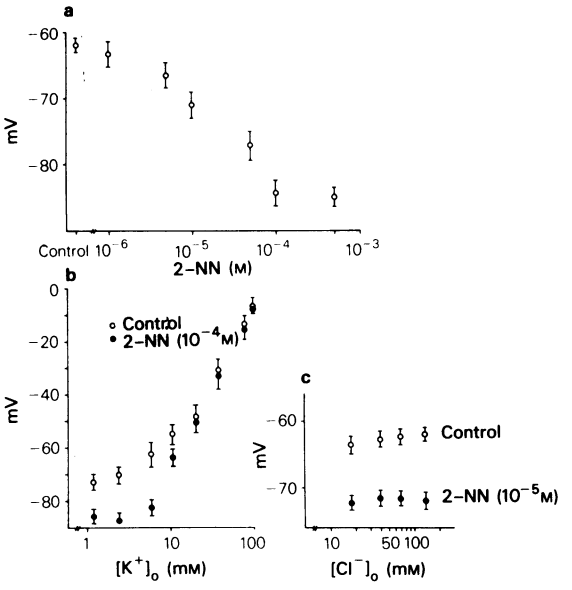

Figure 1 Effects of nicorandil (2-NN) on the membrane potential of smooth muscle cells of the dog mesenteric artery in control Krebs solution (a), various $[K^+]_o$ (b) and in various $[Cl^-]_o$ (c).

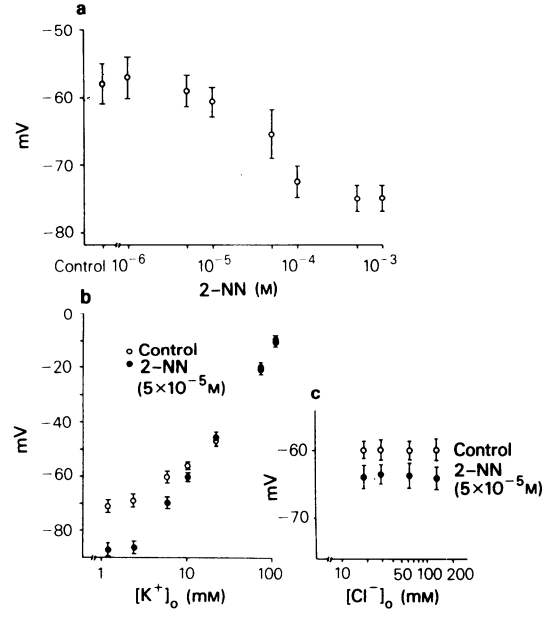

Figure 2 Effects of nicorandil (2-NN) on the membrane potential of smooth muscle cells of the dog trachea in control Krebs solution (a), various $[K^+]_o$ (b) and in various $[Cl^-]_o$ (c).

$n = 30$, $P < 0.01$). Further increase in the concentration of nicorandil caused no further hyperpolarization of the membrane. Figure 1b shows the effects of nicorandil on the membrane potential of muscle cells of the mesenteric artery, in the presence of various concentrations of $[K^+]_o$. The membrane depolarization calculated from the maximum slope produced by a 10 fold increase in $[K^+]_o$ ranging between 10.7 mM and 118 mM $[K]_o$ was 60 mV and was not affected by nicorandil (10^{-4} M). At concentrations of $[K^+]_o$ below 10.7 mM, 10^{-4} M nicorandil hyperpolarized the membrane, to various degrees. The maximum hyperpolarization was observed in 2.4 mM $[K^+]_o$ (from -65.8 ± 1.9 mV to -84.3 ± 2.1 mV, $n = 20$). However, in the presence of $[K^+]_o$ over 20.2 mM, nicorandil did not alter the membrane potential. Figure 1c shows effects of nicorandil on the membrane potential of muscle cells of the mesenteric artery, in various concentrations of $[Cl^-]_o$. A reduction in $[Cl^-]_o$ from 134 to 18 mM slightly hyperpolarized the membrane in the control experiments (from -61.6 ± 1.3 mV to -63.6 ± 1.6 mV, $n = 20$, $P < 0.1$), whereas nicorandil (10^{-4} M) hyperpolarized the membrane to the same level at all concentrations of $[Cl^-]_o$ used. These observations indicate that the hyperpolarization induced by nicorandil was not related to the Cl-permeability of the muscle membrane.

Effects of nicorandil on smooth muscle cells of dog trachea were observed (Figure 2). This agent (10^{-5} M) had no effect on the membrane potential (-58.2 ± 2.4 mV, $n = 20$ in the control solution; -60.3 ± 1.8 mV, $n = 20$ in nicorandil, $P > 0.1$), but increased concentrations of nicorandil hyperpolarized the membrane (in 5×10^{-5} M; -65.5 ± 3.1 mV, ($n < 0.01$), and in 5×10^{-4} M; -75.2 ± 1.4 mV, ($n = 30$)). Increased or reduced concentrations of $[K^+]_o$ modified the membrane potential. The effects of nicorandil on the membrane potential in various concentrations of $[K^+]_o$ were investigated. At concentrations $[K^+]_o$ below 10.7 mM, nicorandil (5×10^{-5} M) hyperpolarized the membrane. The maximum hyperpolarization was observed in 2.4 mM $[K^+]_o$ (from -64.7 ± 1.8 mV to -87.1 ± 2.1 mV). In concentrations of $[K^+]_o$ ranging between 20.2 mM – 118 mM, nicorandil did not alter the membrane potential or the membrane depolarization calculated from the maximum slope produced by a 10 fold increase in $[K^+]_o$ plotted on a log scale (45 mV in control condition and 46 mV in the presence of 5×10^{-5} M nicorandil). Figure 2c shows the effects of 5×10^{-5} M nicorandil on the membrane potential as a function of $[Cl^-]_o$. The membrane potential was not significantly altered by the reduction in $[Cl^-]_o$ (-58.6 ± 1.8 mV, $n = 20$ in 134 mM $[Cl^-]_o$; -58.6 ± 1.9 mV, $n = 20$ in 18 mM $[Cl^-]_o$). When 5×10^{-5} M nicorandil was applied, the membrane

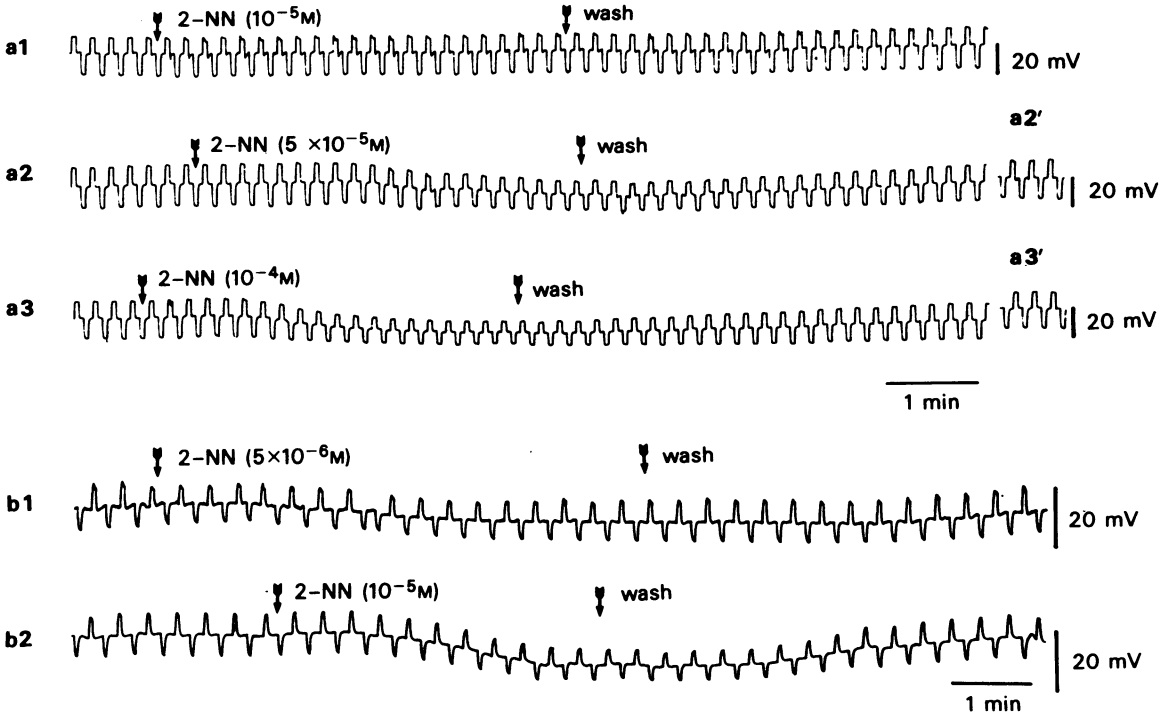

Figure 3 Effects of nicorandil (2-NN) on the membrane potential and membrane resistance observed in the smooth muscle cells of trachea (a_1-a_3) and mesenteric artery (b_1 and b_2). (a_1-a_3) Effects of 2-NN (10^{-5} M, 5×10^{-5} M or 10^{-4} M) on the membrane resistance measured from the amplitude of electrotonic potentials evoked by alternately applied inward and outward current pulses with constant amplitude (4 s in duration). (b_1-b_2). Effects of nicorandil (5×10^{-6} M or 10^{-5} M) on the membrane resistance of the mesenteric artery. Arrows indicate application and removal of nicorandil.

was hyperpolarized to the same level in all concentrations of $[Cl^-]_o$ in 5.9 mM $[K^+]_o$ ($-64.2 + 2.4$ mV, $n = 20$ in 134 mM $[Cl^-]_o$; -62.1 ± 1.4 mV, $n = 20$ in 18 mM $[Cl^-]_o$).

Figure 3 shows the effect of nicorandil on the amplitude of electrotonic potential evoked by alternately applied inward and outward current pulses from the cell at the 50 μm distance from the stimulating electrode. In the mesenteric artery, application of nicorandil ($> 5\times10^{-6}$ M) hyperpolarized the membrane and reduced the amplitude of electrotonic potential, in a concentration-dependent manner. The input membrane resistance, measured from the change in the amplitude of electrotonic potential, was reduced to 69% in 5×10^{-6} M or 41% in 10^{-5} M nicorandil of the control value. On the other hand, in the dog trachea, treatment with nicorandil (10^{-5} M) changed neither the membrane potential nor the amplitude of the electrotonic potential. Increased concentrations of nicorandil ($> 5\times10^{-5}$ M), hyperpolarized the membrane and reduced the amplitude of the electrotonic potential, i.e. the input membrane resistance was reduced to 70% of the control value in 5×10^{-5} M or 31% in 10^{-4} M nicorandil.

The effects of nicorandil were also confirmed from the current-voltage relationship. As shown in Figure 4, the current-voltage relationship was observed before and during application of nicorandil. In the presence of nicorandil (10^{-4} M in the trachea and 10^{-5} M in mesenteric artery), the relationship became less steep compared with that observed in Krebs solution, in both tissues. To eliminate factors influencing the hyperpolarization of the membrane induced by nicorandil, the membrane potential in the presence of nicorandil was displaced to the control level by applications of outward d.c. currents. Under these conditions, nicorandil also increased the ionic conductance of the membrane.

These results indicate that nicorandil increases the K-conductance of the muscle membrane, in both tissues.

Since tetraethylammonium (TEA) or procaine suppressed the K-conductance of membranes in various visceral smooth muscle cells (see for example

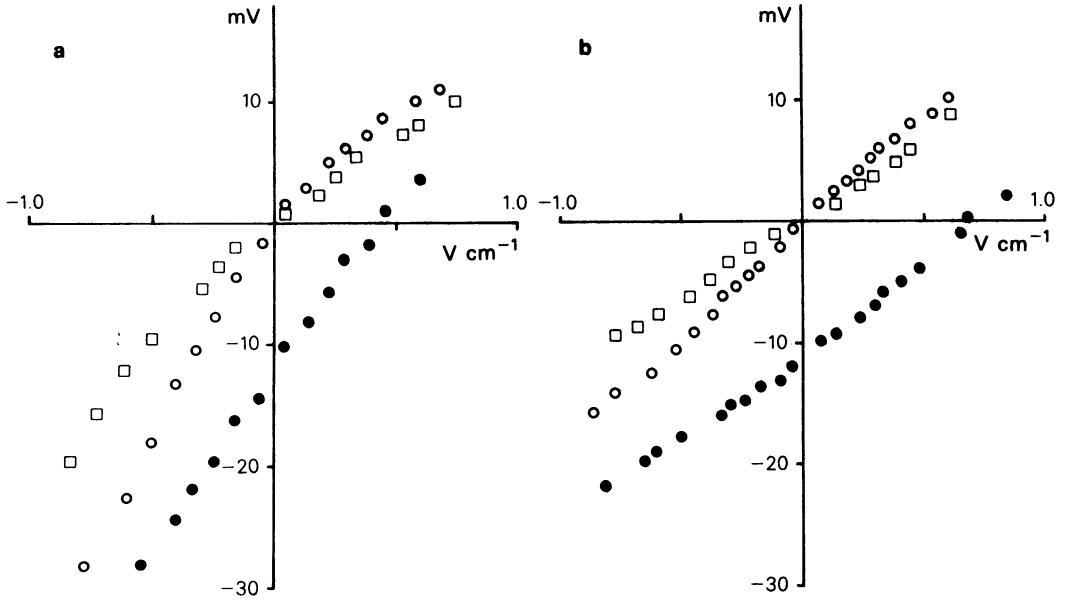

Figure 4 Effects of nicorandil (2-NN) on the current-voltage relationships observed in the trachea (a) or mesenteric artery (b). (○) Control; (●) in the presence of nicorandil (10^{-4} M in the trachea and 10^{-5} M in the mesenteric artery respectively); (□) the membrane potential was displaced to the control level after the membrane had been hyperpolarized by nicorandil.

Bolton, 1979), the effects of nicorandil following treatment with these agents were observed. Figure 5 shows effects of nicorandil on the membrane potential of the mesenteric artery (Figure 5a, b) and trachea (Figure 5c, d) in the presence of various concentrations of TEA or porcaine. In the mesenteric artery, TEA (> 1 mM) significantly depolarized the membrane (from -63.8 ± 1.8 mV, $n = 20$ to -55.9 ± 2.2 mV, $n = 25$ in 1 mM or to -53.4 ± 1.4 mV, $n = 30$ in 5 mM TEA). There was no further depolarization with increases in the concentrations of TEA. In the presence of TEA (> 0.1 mV), nicorandil hyperpolarized the membrane, dose-dependently, however the hyperpolarization was less than that in the controls. In the mesenteric artery, procaine (> 0.1 mM) significantly depolarized the membrane (from -63.7 ± 2.1 mV to -57.6 ± 1.2 mV in 0.1 mM, -52.9 ± 1.4 mV in 1 mM, -46.6 ± 2.0 mV in 5 mM, -45.2 ± 1.2 mV in 10 mM procaine; $n = 30 - 40$). In the presence of 0.1 or 1 mM procaine, nicorandil hyperpolarized the membrane, dose-dependently; however, the relationship between membrane potential and concentration of nicorandil shifted to a more depolarized level. In the presence of 5 or 10 mM procaine, nicorandyl ($< 5 \times 10^{-5}$ M) did not alter the membrane potential.

In the tracheal smooth muscle cells, TEA (5 or 10 mM) depolarized the membrane from -59.0 ± 1.5 mV ($n = 30$) to -53.7 ± 1.1 mV ($n = 25$) or -51.1 ± 1.2 mV ($n = 20$) respectively, and nicorandil hyperpolarized the membrane dose-dependently, although the maximum hyperpolarization was smaller than that observed in the control. Procaine (> 1 mM) also depolarized the membrane as shown in Figure 5d, while following pretreatment with procaine (> 1 mM), nicorandil evoked no hyperpolarization of the membrane.

In the dog trachea, TEA suppressed the rectifying property of the membrane and evoked the action potential, in response to outward current pulse, as previously reported (Suzuki, Morita & Kuriyama, 1976; Ito & Tajima, 1982). Figure 6a shows effects of nicorandil on the spike evoked by the outward current pulse (2 s duration), in the presence of 5 mM TEA. Nicorandil (5×10^{-5} M) hyperpolarized the membrane, reduced the membrane resistance and inhibited the spike generation. However, this inhibition of spike generation was restored by applications of strong intensities of the outward current pulse.

In the mesenteric artery, TEA (> 5 mM) evoked spontaneous and transient depolarization of the membrane (1–8 mV in amplitude and 10–20 min^{-1}) with or without generation of the action potential. In the presence of 10 mM TEA, nicorandil (5×10^{-5} M) hyperpolarized the membrane from −54 mV to −67 mV, and reduced the

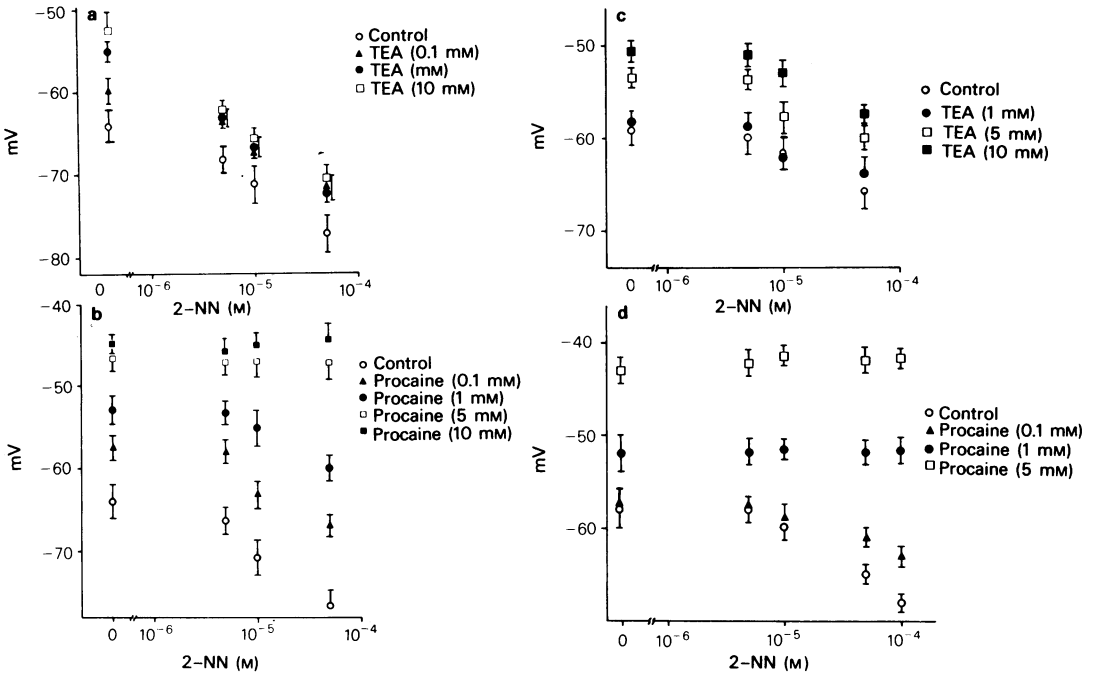

Figure 5 Effects of nicorandil (2-NN) on the membrane potential of the mesenteric artery (a and b) or trachea (c and d) in the presence or absence of various concentrations of tetraethylammonium (TEA) (a and c) or procaine (b and d). Open circles in (a) to (d), show the relationship between the concentration of nicorandil and membrane potential in normal Krebs solution. (a) Effects of pretreatment of the mesenteric artery with TEA (▲ 0.1 mM; ● 1 nM; □ 10 mM) on the nicorandil-induced membrane hyperpolarization. (b) Effects of the pretreatment of the mesenteric artery with procaine (▲ 0.1 mM; ● 1 mM; □ 5 mM; ■ 10 mM) on the nicorandil-induced membrane hyperpolarization in the mesenteric artery. (c) Effects of TEA on the nicorandil-induced membrane hyperpolarization (● 1 mM; □ 5 mM; □ 10 mM) observed in the dog trachea. (d) Effects of procaine (▲ 0.1 mM; ● 1 mM; □ 5 mM) on the nicorandil-induced membrane hyperpolarization observed in the dog trachea.

frequency of amplitude of spontaneous membrane fluctuation to roughly one-third the control, but did not completely abolish the generation of the spontaneous membrane fluctuation (Figure 6b). These potential changes ceased following treatment with tetrodotoxin (10^{-7} M). Figure 6c shows the effect of nicorandil on the membrane potential and membrane resistance of the muscle membrane in the presence of 1 mM TEA. With application of nicorandil (10^{-5} M) following pretreatment with 1 mM TEA, the depolarized membrane (from -64 to -60 mV) was repolarized and the increased amplitude of the electrotonic potential was reduced to 38% of the value in 1 mM TEA.

Effects of nicorandil on the mechanical properties of smooth muscle of the mesenteric artery and trachea

Electrical field stimulation (20 Hz and 0.5 ms pulse duration at a constant intensity) to both muscle strips produced rapidly developing phasic contractions, and the amplitudes were dependent on the number of stimuli. Tetrodotoxin (10^{-7} M) abolished these mechanical responses.

Figure 7 shows the effects of nicorandil on the relationship between the contraction and the number of field stimuli. The amplitude of the phasic contraction evoked by 90 pulses was registered as a relative amplitude of 1.0 in the trachea, and in the mesenteric artery 30 pulses at 20 Hz, since this was the minimum condition to reproduce the maximum and constant amplitudes of phasic contractions with repetitive trials. The minimum concentrations of nicorandil required to inhibit the nerve mediated mechanical response was 10^{-5} M in the trachea and 10^{-6} M in the mesenteric artery, and the stimulus-response relationship shifted vertically toward a low level, irrespective of the number of stimuli applied.

Figure 8 shows the effects of nicorandil on the contracture evoked by excess-$[K^+]_o$ or noradrenaline in the mesenteric artery. To prevent the release of noradrenaline from nerve terminals in the presence

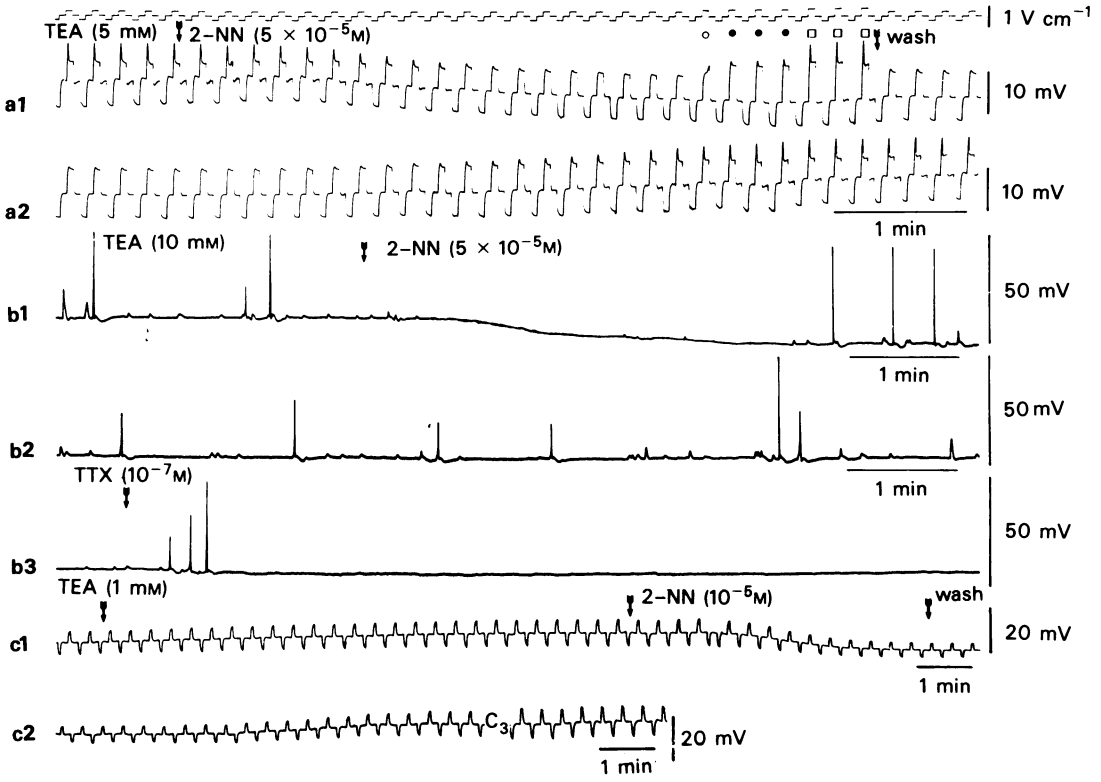

Figure 6 (a_1 and a_2) Effects of nicorandil (2-NN) on the action potential evoked from the muscle cells of the dog trachea by outward current pulses in the presence of tetraethylammonium (TEA 5 mM). Inward and outward current pulses (4 s in pulse duration) were alternately applied. Application of nicorandil hyperpolarized the membrane, reduced the input membrane resistance and suppressed the generation of action potential. However, increase in the outward current pulses in a step-wise manner (○, ● or □) restored the generation of action potential. (b_1 and b_2) Effects of nicorandil (5×10^{-5} M) or tetrodotoxin (TTX 10^{-7} M) on the spontaneous membrane depolarization with or without action potential recorded from the mesenteric artery in the presence of TEA (10 mM). Nicorandil (5×10^{-5} M) hyperpolarized the membrane and reduced the amplitude and frequency of the spontaneous membrane depolarization evoked by TEA, (b_1 and b_2). Tetrodotoxin (10^{-7} M (b_3) completely suppressed these potential changes. (c_1 and c_2) Effects of nicorandil (10^{-5} M) on the input membrane resistance and membrane potential of the mesenteric artery in the presence of 1 mM TEA. TEA (1 mM) depolarized the membrane and increased the membrane resistance, and application of nicorandil (10^{-5} M) hyperpolarized the membrane and reduced the input membrane resistance measured from the amplitude of electrotonic potentials evoked by constant inward current pulses in the presence of TEA (1 mM). Arrows indicate application or removal of nicorandil.

of excess-$[K^+]_o$, guanethidine (10^{-6} M) was present throughout the K-induced contraction experiments. The amplitude of the K-induced contraction (> 20 mM) was proportionally increased up to 57.6 mM $[K^+]_o$. Application of nicorandil (> 10^{-6} M) reduced the amplitude of contraction consistently in the concentration range between 20.2 and 57.6 mM of $[K^+]_o$. As described above, nicorandil (10^{-4} M) did not hyperpolarize the membrane when concentrations of $[K^+]_o$ exceeded 20.2 mM, thus inhibition of the K-induced contraction by nicorandil is not due to hyperpolarization of the membrane. As shown in Figure 8b, nicorandil (> 10^{-6} M) also consistently reduced the amplitude of the noradrenaline-induced contraction (10^{-7}–10^{-5} M) in the mesenteric artery.

In the dog trachea, the minimum concentration of $[K^+]_o$ required to evoke the contraction was 10.7 mM in the presence of 10^{-6} M atropine (Figure 9a). Amplitudes of the K-induced contraction were increased, in a dose-dependent manner. In Figure 9a the relative amplitude of 57.6 mM $[K^+]_o$-induced contraction was registered as 1.0. Application of nicorandil (10^{-6} M) consistently reduced amplitudes of the K-induced contraction, at any given concentration of $[K^+]_o$. The minimal concentration of ACh required to evoke the contraction was 10^{-8} M. Nicorandil

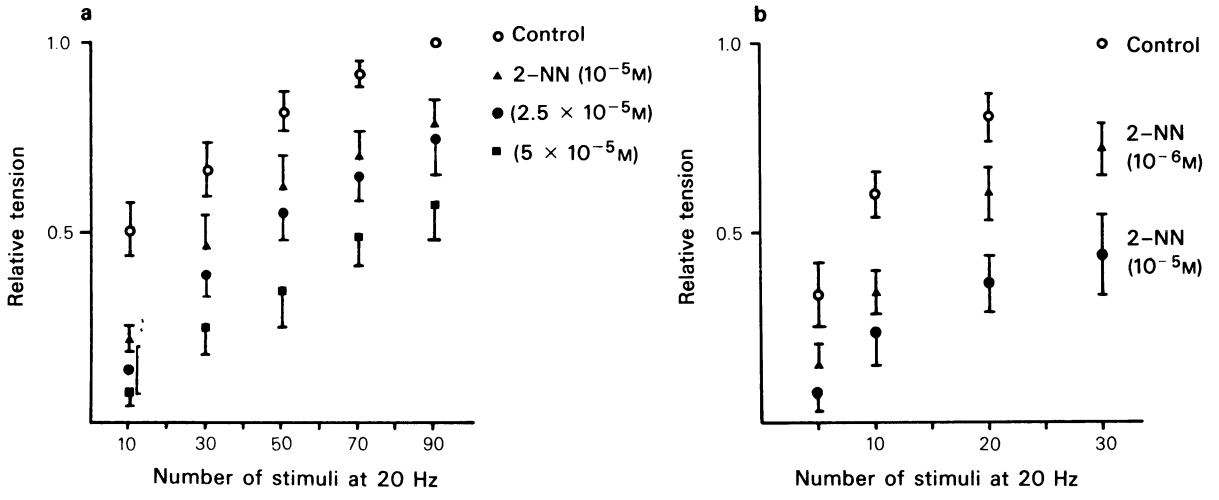

Figure 7 Effects of nicorandil (2-NN) on the relationship between the amplitude of tension development and number of indirect muscle stimuli (50 μs in duration) at a constant intensity and frequency (20 Hz), where the relative amplitude of tension development evoked by 90 stimuli or 30 stimuli were registered as a relative amplitude of 1.0 in the trachea (a) or mesenteric artery (b). Vertical bars indicate s.d. or 2 × s.d.

(> 10^{-5} M) inhibited the ACh-induced contraction (10^{-8}–10^{-6} M), but nicorandil (10^{-5} M) did not reduce the amplitude of ACh-induced contraction evoked by concentrations of over 10^{-5} M.

The relationship between the membrane depolarization and contraction was observed in the presence or absence of nicorandil, using the double sucrose gap method. In the dog trachea, outward current pulses (2 s in duration) did not produce an action potential, however, when a depolarization exceeded 7 mV, a contraction was evoked as previously reported (Ito & Tajima, 1982). As shown in Figure 10 a–c, nicorandil (10^{-6} M) reduced the amplitude of contraction, at any given depolarization of the membrane, and raised the threshold membrane depolarization required to produce the contraction. Figure 10d shows the relationship between the membrane depolarization and contraction of the dog trachea, in the presence or absence of nicorandil. The amplitude of the contraction evoked by 30 mV depolarization

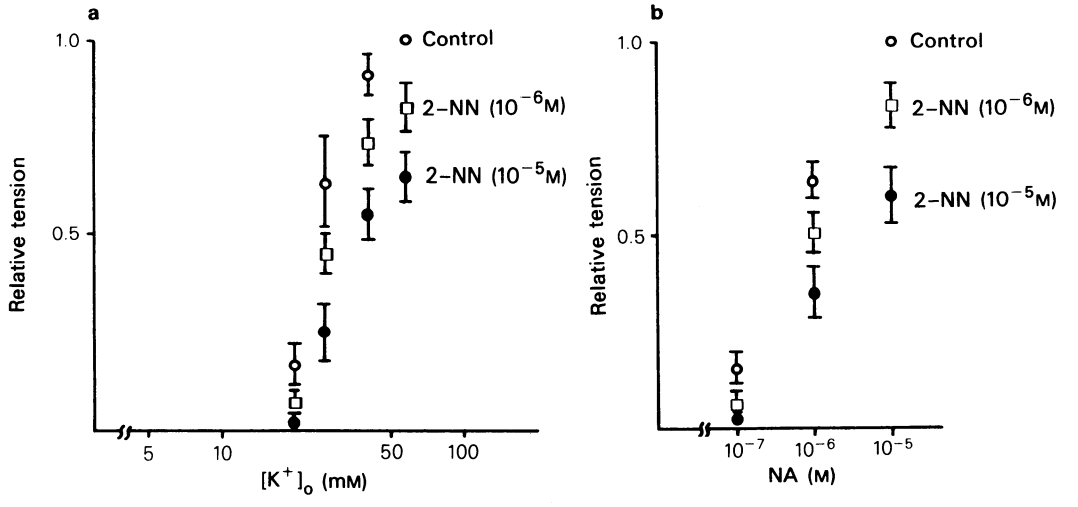

Figure 8 Effects of nicorandil (2-NN) on the contracture evoked by excess-$[K^+]_o$ or noradrenaline (NA) in the mesenteric artery, where the amplitudes of contracture evoked by 57.6 mM $[K^+]_o$ or 10^{-5} M noradrenaline were registered as a relative amplitude of 1.0. Vertical bars indicate s.d. or 2 × s.d..

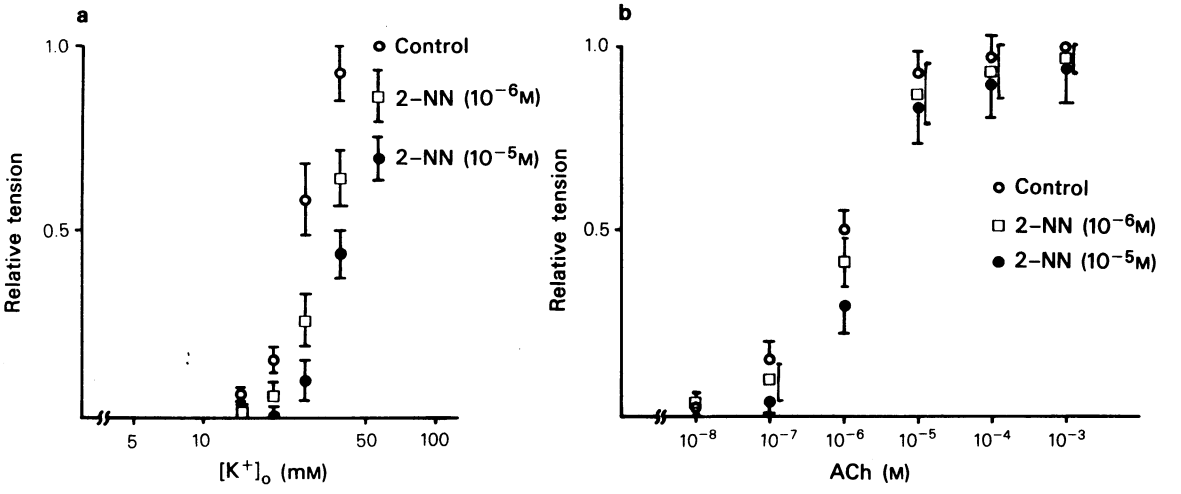

Figure 9 Effects of nicorandil (2-NN) on the contracture evoked by excess-$[K^+]_o$ or acetylcholine (ACh) in the dog trachea, where the amplitudes of contracture evoked by 57.6 mM $[K^+]_o$ or 10^{-3} M acetylcholine were registered as a relative amplitude of 1.0. Vertical bars indicate s.d. or 2× s.d..

was registered as a relative contraction of 1.0. In the presence of nicorandil (10^{-6} M), the membrane potential remained unaffected but the minimum depolarization required to produce tension development was increased from 7 mV to 10 mV. The amplitude of contraction evoked by 30 mV depolarization was reduced to 70% in 10^{-6} M or 48% in 10^{-5} M nicorandil.

Effects of nicorandil on the neuro-effector transmission in the mesenteric artery and trachea

In the dog trachea, after pretreatment with propranolol (10^{-6} M) and indomethacin (10^{-5} M), single pulse field stimulation (50 μs duration) evoked an excitatory junction potential (e.j.p.) with constant amplitude followed by twitch contraction, as already reported (Ito & Tajima, 1981 a, b). Figure 11 shows the effects of nicorandil on the e.j.p.. As shown in Figure 11 (d, e) nicorandil ($< 10^{-5}$ M had no effect on the amplitudes of e.j.p. or on the electrotonic potential, yet an increased concentration of nicorandil (5×10^{-5} M) hyperpolarized the membrane by about 10 mV and reduced the amplitude of the electrotonic potential (Figure 11a). The mean amplitudes of e.j.p. and of electrotonic potential during the application of nicorandil (5×10^{-5} M) were reduced to 46 ± 9% (± s.d., $n = 4$) or 45.5 ± 10.6% (± s.d., $n = 5$) the control.

In the mesenteric artery, field stimulation (50 μs duration) evoked e.j.p., but there was no evidence of contraction. The amplitude of the e.j.p. increased in proportion to the number of stimuli, at constant stimulus intensity and frequency. Application of nicorandil (10^{-5} M) hyperpolarized the membrane by about 5 mV, and reduced the amplitude of e.j.p. The mean size of the e.j.p. evoked by a single stimulus during application of nicorandil was reduced to 74.8 ± 14.7% (± s.d., $n = 5$) of the control. After pretreatment with TEA (1 mM), single field stimulation of the same intensity as used in Krebs solution (Figure 12A) evoked e.j.p. followed by an action potential (Figure 12c). Nicorandil (10^{-5} M) hyperpolarized the membrane to a lesser extent in the presence of TEA than that in the control, and reduced the amplitude of e.j.p. to below the threshold depolarization required for generation of the action potential. To produce the action potential on e.j.ps, increased stimulus number (3 stimuli at 20 Hz) was required (Figure 12e).

Discussion

Nicorandil hyperpolarized the smooth muscle membrane of both the dog mesenteric artery and trachea; however, to produce the same amplitude of potential change, higher concentrations of nicorandil were required in the trachea (5×10^{-6} M in the dog mesenteric artery and 5×10^{-5} M trachea). The hyperpolarization of membranes induced by nicorandil in both tissues was due to increase in the K-conductance of the membrane, because the hyperpolarization was closely related with the K-equilibrium potential (E_K) but not E_{Cl}. These findings confirmed the previous observations that nicorandil increases the K-conductance of the smooth muscle membrane, as observed in coronary and mesenteric arteries of the

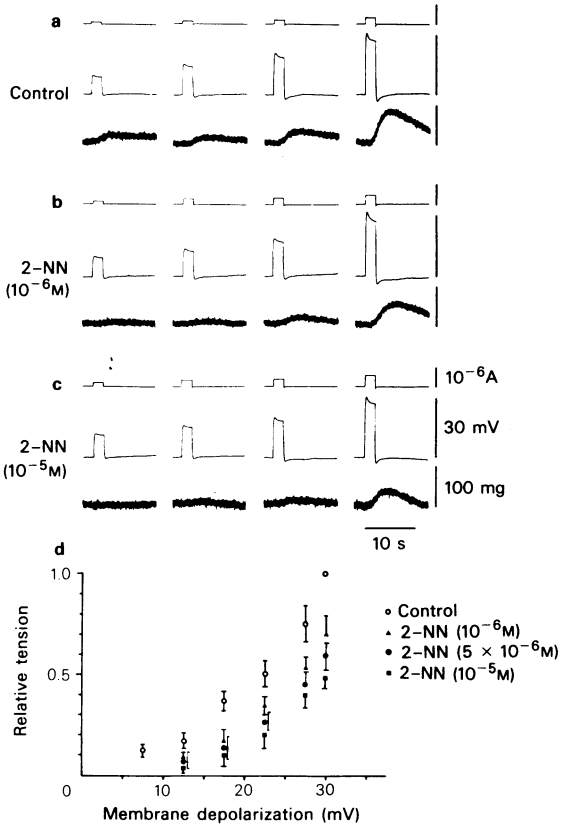

Figure 10 Effects of nicorandil (2-NN) on the depolarization-contraction coupling observed from the dog trachea, using the double sucrose gap method. (a–c) Actual records of depolarization-induced contraction produced by various intensities of outward current pulse (2 s in pulse duration) in the presence or absence of 10^{-6} M or 10^{-5} M nicorandil. (d) Depolarization-induced contractions observed in the presence of 10^{-6} M or 10^{-5} M nicorandil, where the relative amplitude of tension evoked by 30 mV depolarization was registered as 1.0. Vertical bars indicate s.d. or 2 × s.d..

pig, and coronary and mesenteric arteries or mesenteric veins of the guinea-pig (Furukawa *et al.*, 1981; Itoh *et al.*, 1981a; Karashima *et al.*, 1982). TEA suppressed the nicorandil-induced hyperpolarization in the smooth muscle cell of the mesenteric artery (> 0.1 mM) or trachea (> 5 mM). Furthermore, procaine completely inhibited the nicorandil-induced hyperpolarization in the trachea (1 mM) or mesenteric artery (5 mM).

In the guinea-pig basilar artery, TEA and procaine depolarized the membrane dose-dependently, but nicorandil (10^{-4} M) or 4-aminopyridine (10^{-3} M) did not modify the membrane potential or the membrane resistance, indicating the lack of a K-channel sensitive to nicorandil or 4-aminopyridine (Fujiwara & Kuriyama, 1983). On the other hand, Takata & Kuriyama (1980) found two types of K-channel, one being a $[Ca^{2+}]_o$ sensitive channel and the other being less sensitive to $[Ca^{2+}]_o$ in the guinea-pig coronary artery, and that membrane hyperpolarization induced by nicorandil is mainly due to activation of the latter K-channel (Karashima *et al.*, 1982). The present finding that the effects of nicorandil on the membrane potential in the presence of TEA or procaine differs qualitatively in the mesenteric artery and trachea, indicates that in both these tissues, two types of K-channel may be present, or that the affinity of nicorandil, procaine or TEA for the K-channel differs in the following order of the potency; procaine > nicorandil > TEA.

Nicorandil (> 10^{-6} M) suppressed the mechanical responses evoked by excess-$[K^+]_o$ in the mesenteric artery or trachea following pretreatment with guanethidine or atropine, respectively. In the mechanical response evoked by repetitive field stimulation, 10^{-5} M nicorandil in the trachea (mediated by ACh), but 10^{-6} M in the mesenteric artery (mediated by noradrenaline) was required to inhibit this response. The mechanical responses of the trachea evoked by the electrical membrane depolarization in the presence of atropine were inhibited by nicorandil (10^{-6} M), i.e. nicorandil inhibited the mechanical response evoked by indirect muscle or chemical stimulation to a greater extent in the mesenteric artery than in the trachea.

The minimal concentration of nicorandil required to evoke the membrane hyperpolarization was 5×10^{-6} M or 5×10^{-5} M in the mesenteric artery or trachea, yet it suppressed the K-induced contractions, or contraction evoked by electrical membrane depolarization, in a concentration of 10^{-6} M. Therefore, in both tissues, inhibitions of mechanical responses are not solely due to the hyperpolarization of membrane. This agent may suppress the response mainly due to the immobilization of Ca^{2+} within the muscle cell, since nicorandil did not abolish the generation of an action potential in the presence of TEA, in both tissues. The voltage-dependent Ca^{2+}-influx may not be suppressed by nicorandil. Furukawa *et al.*, (1981) found that in the pig coronary and mesenteric arteries, nicorandil suppressed the contraction, only when the membrane was hyperpolarized. On the other hand, in the guinea-pig coronary and mesenteric arteries or in the mesenteric veins, nicorandyl inhibited the contraction without any marked change in the membrane potential, in the presence of $[K]_o$ (> 20 mM] or noradrenaline (> 10^{-6} M). Therefore, the inhibition of Ca^{2+}-mobilization in the cell may play an important role in producing the relaxation of tissues (Itoh *et al.*, 1981b; Karashima *et al.*, 1982). Nitrate is included in

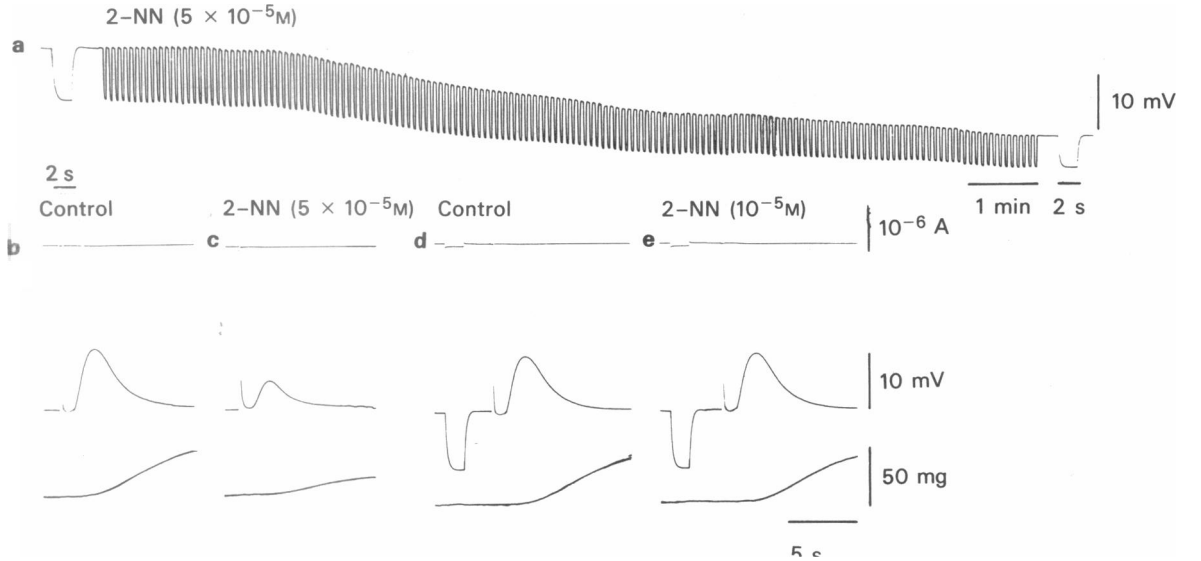

Figure 11 Effects of nicorandil (2-NN) on the membrane potential, membrane resistance or e.j.p. observed in the dog trachea, using the double sucrose gap method. (a) Nicorandil (5×10^{-5} M) hyperpolarized the membrane by about 10 mV, reduced the amplitude of electrotonic potential evoked by constant inward current pulses; (b) e.j.p. evoked by single stimulus (50 μs in duration) in the control solution; (c) e.j.p. observed during application of nicorandil (5×10^{-5} M); (d and e) electrotonic potentials or e.j.p. before (d) and during application of nicorandil (10^{-5} M).

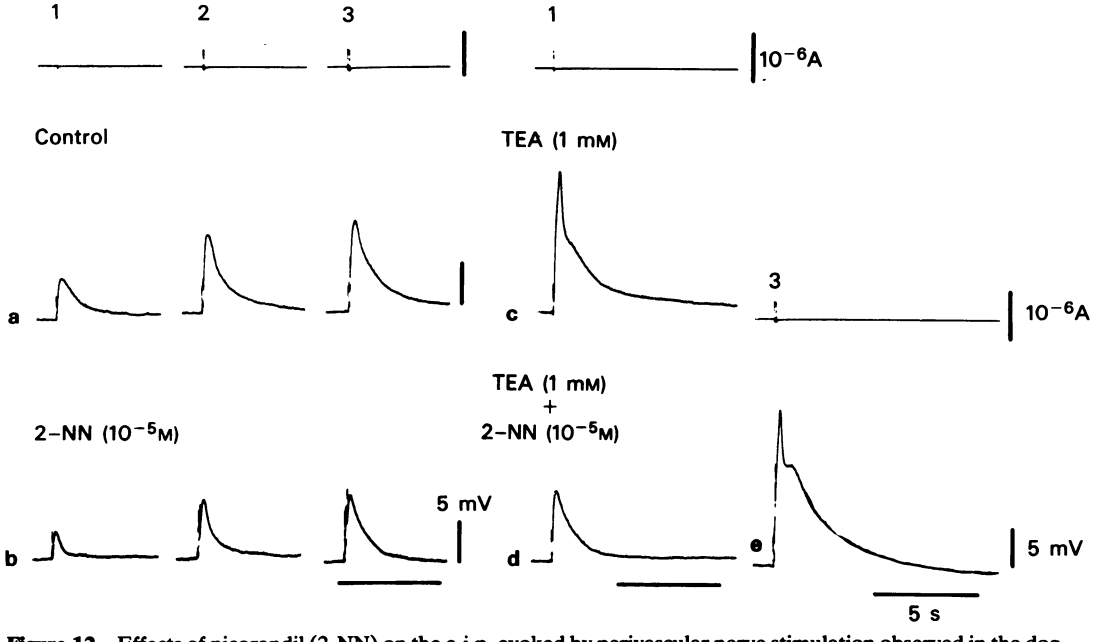

Figure 12 Effects of nicorandil (2-NN) on the e.j.p. evoked by perivascular nerve stimulation observed in the dog mesenteric artery. (a) The amplitude of e.j.p. increased in proportion to the number of stimuli given (1, 2, or 3 stimuli at constant intensity and frequency (20 Hz)); (b), nicorandil (10^{-5} M) hyperpolarized the membrane by about 5 mV and consistently reduced the amplitude of e.j.p. at any given stimulus condition; (c) after the pretreatment of the tissue with tetraethylammonium (TEA 1 mM), single field stimulation in the same intensity and duration used in (a) evoked e.j.p. followed by an action potential; (d) nicorandil (10^{-5} M) hyperpolarized the membrane, reduced the amplitude of e.j.p. and suppressed the generation of action potential in the presence of 1 mM TEA; (e) repetitive field stimulation (3 stimuli at 20 Hz) restored e.j.p. followed by an action potential.

molecular structure of nicorandil, therefore, actions of this vasodilator, as related to muscle relaxation resemble the findings for nitroglycerine or nitroprusside for the K-induced contraction (Ito, Kitamura & Kuriyama, 1980; Itoh, Kajiwara, Kitamura & Kuriyama, 1981b).

In the dog mesenteric artery or trachea, the e.j.p. generated by electrical field stimulation was attributed to release of noradrenaline or ACh from the nerve terminal, since tetrodotoxin suppressed the generation of e.j.p. in both tissues, and guanethidine or atropine suppressed the generation of e.j.p. (Kuriyama & Makita, 1983; Ito & Tajima, 1981a). Nicorandil reduced the amplitude of e.j.p. and input membrane resistance in the mesenteric artery or trachea, thus the reduction in the amplitude of e.j.p. would be mainly due to the increase in the ionic conductance and membrane potential (Katz & Thesleff, 1957), and not to inhibition of release of chemical transmitter from the nerve terminal.

In conclusion, nicorandil hyperpolarizes the membrane and inhibits the contraction evoked by various factors, in both the mesenteric artery and the trachea. The inhibition of contraction is partly related to the immobilization of Ca^{2+} in the cell and also to hyperpolarization of the membrane. As the potency of drug action was much weaker in smooth muscle cells of the trachea than in those of the mesenteric artery, nicorandil will probably be a more effective antihypertensive agent than an anti-asthmatic agent.

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