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ACTIONS OF NITROGLYCERINE ON SMOOTH MUSCLES OF THE GUINEA-PIG AND RAT PORTAL VEINS

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1 Effects of nitroglycerine (NG) on the electrical and mechanical activities of smooth muscle cells of the rat and guinea-pig portal veins were studied by a microelectrode technique and an isometric tension recording method.

2 The membrane potentials of smooth muscle cells in the rat and guinea-pig were −44.4 mV and −47.6 mV, respectively. In both species the smooth muscle cells generated spikes as burst discharges.

3 In the guinea-pig portal vein, NG (2.8×10^{-8} M) produced biphasic potential changes, an initial transient hyperpolarization followed by depolarization. The hyperpolarization suppressed and depolarization enhanced spike activities.

4 From the changes in the membrane potential produced by NG in various concentrations of $[K]_o$, $[Na]_o$ or $[Ca]_o$, it is postulated that the hyperpolarization is due to an increase in the K-permeability and the depolarization is due to an increase in the Na-permeability of the membrane.

5 NG (2.8×10^{-5} M) had no effect on the membrane activity of the rat portal vein.

6 NG consistently suppressed mechanical activities generated in both tissues. The minimum concentration required to suppress the mechanical activity was lower in the guinea-pig than in the rat portal vein.

7 NG suppressed the contraction due to noradrenaline more than that evoked by excess $[K]_o$ in both species.

8 From these experiments, it is concluded that NG relaxes the muscular bed of portal veins of both species. In the rat portal vein, suppression of mechanical activity had no causal relation to the membrane activity. In contrast, in the guinea-pig portal vein, suppression of mechanical activity was slightly modified by changes in the membrane activity, i.e. hyperpolarization additively contributes to the relaxation and depolarization slightly suppresses the relaxation.

Introduction

It is a century since Brunton (1867) and Murrell (1879) suggested that nitroglycerine (NG) would be effective in the treatment of angina pectoris. Recent clinical studies have tended to confirm that the main effect is on peripheral capacitance vessels (Parker, 1972; Parratt, 1974; 1975).

In isolated vessels (dog femoral artery, saphenous vein and rat portal vein), both NG and sodium nitrate preferentially relax venous smooth muscle, especially when this tissue is contracted with noradrenaline (Mackenzie & Parratt, 1977). These authors also suggested that the selective dilator effects of NG on venous smooth muscle might explain the action in alleviating the pain of angina pectoris.

Keatinge (1966) found that amyl nitrate and sodium nitrate relaxed and repolarized sheep carotid arterial strips which had been previously partially contracted and depolarized by either noradrenaline or histamine. Nitrates did not alter the membrane potential of unstimulated arteries. However, these agents to some extent produced relaxation, without electrical change, of arteries which had been completely depolarized by high $[K]_o$ solution. Recently, Harder, Berlardinelli, Sperelakis, Rubio & Berne (1979) demonstrated that NG completely blocked the Ca-current which had been induced by addition of tetraethylammonium on electrical stimulation in dog coronary artery, and that 10^{-7} to 10^{-5} M NG had no significant effect on resting potential or input resistance. Meanwhile, Ito, Kitamura & Kuriyama (1980) showed that in the porcine coronary artery NG, in a low dose (2.8×10^{-10} to 2.8×10^{-8} M), did not modify the membrane properties, although the mechanical response was suppressed. Increased concentration of NG (2.8×10^{-5} M) hyperpolarized the membrane, reduced the membrane resistance and

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markedly suppressed the mechanical response. They concluded that NG acted as a nonselective suppressor of the Ca-mobilization from the storage sites in the cell with no noticeable change in the membrane properties.

Häusler & Thoren (1976), using microelectrodes, suggested that a concentration-dependent hyperpolarization of pulmonary smooth muscle may be of significance for the vasodilator action of sodium nitroprusside. In fact, this agent hyperpolarized the membrane, and suppressed spike generation and contraction of the rabbit portal vein (Ito, Suzuki & Kuriyama, 1978). These results may indicate that the actions of nitrate compounds differ between spontaneously active and electrically quiescent vascular smooth muscle tissues.

The effects of NG on the electrical and mechanical properties of venous smooth muscle have not been investigated. It seemed of interest to clarify the vasodilator mechanism of NG on smooth muscle cells of the portal veins as these tissues possess spontaneous electrical and mechanical activities. Since marked species differences in drug actions exist, the effects of NG were observed on both rat and guinea-pig portal veins.

Method

Guinea-pigs, weighing 250 to 300 g and Wistar strain rats, 180 to 250 g were stunned and bled. The portal vein was excised and the connective tissue was carefully removed in Krebs solution at room temperature under a binocular microscope. The preparation and experimental procedures were the same as those described by Ito & Kuriyama (1971). The tissue was mounted in an organ bath with a volume of 2 ml and perfusion was carried out at a rate of 3 ml/min at a temperature 35 to 36°C using a thermo-unit with perfusion pump.

For recording the membrane potential, a conventional glass microelectrode filled with 3 M KCl was inserted from the serosal side and its tip resistance was 70 MΩ to 100 MΩ. The preamplifier was MZ-3B (Nihon Kohden). Application of electrical stimulation was by the partition stimulating method described by Abe & Tomita (1968).

To measure isometric contractions, a strip of the tissue was mounted in a vertical tubular organ bath with a volume of 2 ml. One end of the tissue was fixed at the bottom of the bath and the other end was connected to an isometric tension recorder by a fine silk thread. The flow rate of the solution was 3 ml/min and the temperature was maintained at 32°C.

Modified Krebs solution (Bülbring, 1955) served as the control solution, and had the following composition (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 and 3% CO_2, and the pH was maintained at 7.2 to 7.3.

Excess $[K]_o$ solution was prepared by replacing NaCl with equivalent amounts of KCl. In the Na-deficient solution, NaCl was replaced with an equimolar concentration of Tris-Cl (Tris(hydroxymethyl-animomethan)-Cl) or choline chloride Cl (atropine 1 μg/ml was added), and the pH of the solution was adjusted to 7.1 to 7.2 with 1 N HCl. In the Ca-deficient solution, $CaCl_2$ was replaced with the same concentration of $MgCl_2$ to restore the membrane potential (see results). To prepare the excess $[Ca]_o$ solution, solid $CaCl_2$ was added to Krebs solution.

The following drugs were used at concentrations (molar) described in the results; nitroglycerine (0.637 mg/ml distilled water, prepared by Nippon Kayaku), (±)-noradrenaline (Sankyo), phentolamine mesylate (CIBA-Geigy) and propranolol (Sumitomo).

Results

Effects of nitroglycerine on the membrane potential

The mean membrane potentials of smooth muscle cells of the rat and guinea-pig portal veins were -44.4 ± 1.6 mV s.d. ($n = 30$) and -47.6 ± 1.8 mV s.d ($n = 50$), respectively. Both membranes were spontaneously active. NG (2.8×10^{-5} M) did not affect the membrane potential of the rat portal vein, but in the guinea-pig portal vein the membrane was hyperpolarized transiently (10 to 15 s) by the application of 2.8×10^{-8} M NG. High concentrations of NG ($> 2.8 \times 10^{-8}$ M) had biphasic effects on the membrane potential, an initial hyperpolarization which was followed by depolarization. Therefore, the membrane potential in the presence of NG was measured at the maximum hyperpolarized level. In 2.8×10^{-6} M NG, the membrane was hyperpolarized to -51.9 ± 1.8 mV ($n = 15$). With a further increase in concentration of NG (2.8×10^{-5} M), the amplitude of hyperpolarization remained the same (-52.4 ± 1.9 mV $n = 15$) but the degree of depolarization was increased.

To investigate the nature of hyperpolarization produced by NG, the action of NG was observed in various ionic environments. In Figure 1a, effects of NG (2.8×10^{-5} M) on the membrane potential of the guinea-pig portal vein were observed in various $[K]_o$. Increased $[K]_o$ depolarized the membrane and the maximum slope of the membrane depolarization produced by a 10 fold increase in $[K]_o$ plotted on a log scale was -44 mV. In 5.9 mM $[K]_o$, the membrane was hyperpolarized by treatment with 2.8×10^{-5} M NG from -47.6 ± 1.8 mV ($n = 50$) to -51.8 ± 1.4 mV ($n = 15$, $P < 0.05$). In 1.2 mM $[K]_o$,

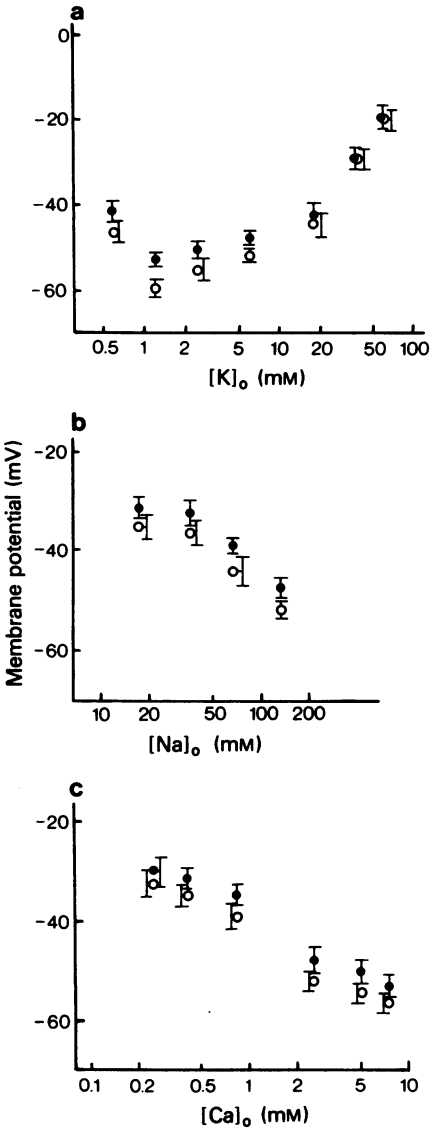

Figure 1 Effects of 2.8×10^{-5} M nitroglycerine (NG) on the membrane potential of the guinea-pig portal vein in various concentrations of $[K]_o$, $[Na]_o$ or $[Ca]_o$: (a) effects of NG in various concentrations of $[K]_o$; (b) effects of NG in various concentrations of $[Na]_o$; (c) effects of NG in various concentrations of $[Ca]_o$. In (a), (b) and (c) (●) control; (○) in the presence of NG. Vertical bars indicate 2 × s.d. ($n = 15 - 25$).

NG-induced hyperpolarization (2.8×10^{-5} M) showed the maximum value, i.e. the membrane was hyperpolarized from -53.1 ± 0.9 mV ($n = 15$) to -59.4 ± 1.0 mV ($n = 15$). However, a reduction of $[K]_o$ to 0.6 mM, decreased the amplitude of hyperpolarization from -41.5 ± 2.5 mV ($n = 15$) to -46.2 ± 2.4 mV ($n = 15$). When $[K]_o$ was increased more than 34.4 mM, NG did not hyperpolarize the membrane.

Figure 1b and c shows the effects of NG on the membrane potential of the guinea-pig portal vein in various $[Na]_o$ and $[Ca]_o$. When the tissue was superfused with the Na-deficient solution, the membrane was depolarized (-39.1 ± 1.5 mV, $n = 18$ in 68.7 mM $[Na]_o$; -32.5 ± 1.8 mV, $n = 16$ in 30.8 mM $[Na]_o$; -31.6 ± 2.1 mV. $n = 15$ in 15.4 mM $[Na]_o$). After application of 2.8×10^{-5}M NG, the membrane was consistently hyperpolarized in Na-deficient solutions to the same extent as observed in Krebs solution (-44.2 ± 2.6 mV, $n = 15$ in 68.7 mM $[Na]_o$; -36.3 ± 2.3 mV, $n = 16$ in 30.8 mM $[Na]_o$; -35.2 ± 2.6 mV, $n = 17$ in 15.4 mM $[Na]_o$). In Na-deficient solution (< 68.7 mM), the depolarization induced by NG was greatly reduced and in 15.4 mM $[Na]_o$, it completely ceased. Similar effects were also observed in 15.4 mM $[Na]_o$ substituted by choline chloride.

As shown in Figure 1c when $[Ca]_o$ was reduced or increased, the membrane was depolarized or hyperpolarized, respectively. To prevent marked depolarization of the membrane in low $[Ca]_o$, an equimolar concentration of $MgCl_2$ was added. In Krebs solution, 2.5 mM $[Mg]_o$ had no effect on the membrane potential and spike activities, yet in low $[Ca]_o$ solution, the membrane potential was slightly restored. By treatment with NG (2.8×10^{-5} M), the membrane was hyperpolarized at any given concentration of $[Ca]_o$, but the amplitude of hyperpolarization was small in low $[Ca]_o$. In 0.8 mM $[Ca]_o$, the membrane was hyperpolarized from -29.9 ± 2.9 mV ($n = 18$) to -32.3 ± 2.3 mV ($n = 15$) and in 7.5 mM $[Ca]_o$ from -52.7 ± 1.8 mV ($n = 15$) to -56.0 ± 2.0 mV ($n = 15$).

Figure 2 shows the effects of 2.8×10^{-5} M NG on the membrane resistance measured by alternate applications of inward and outward current pulses (1.5 s in duration). The microelectrode was inserted in the same cell throughout the experiment. In Krebs solution, applications of inward current pulses suppressed and outward current pulses generated the spike. NG hyperpolarized the membrane, decreased the spike frequency and reduced the amplitude of the electrotonic potential evoked by inward and outward current pulses. The number of spikes evoked by outward current pulses was also reduced by treatment with NG. To observe the change of amplitude of tonic potential in detail, fast speed recording was used. In (c) and (d), a decrease in resistance is clearly seen. These results suggest that the NG-induced hyperpolarization is due to an increase in the ionic conductance of the membrane, presumably an increase in the K-conductance. Furthermore, the depolarization is presumably due to an increase in the Na-conductance of the membrane.

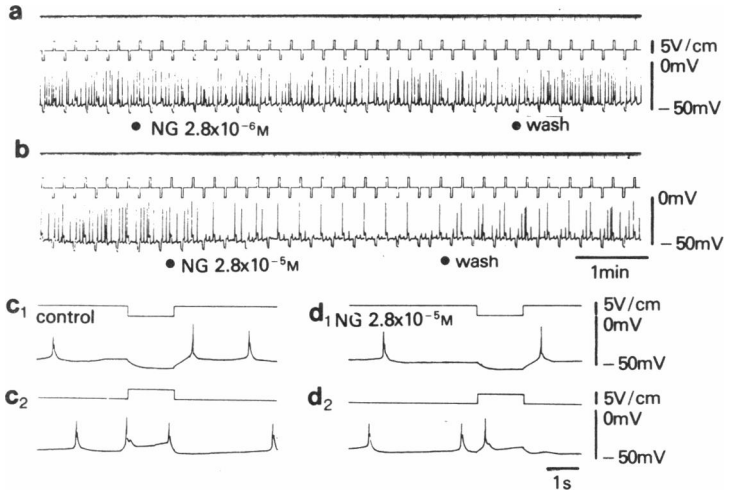

Figure 2 Effects of nitroglycerine (NG, 2.8×10^{-6} M and 2.8×10^{-5} M) on the membrane potential, spike activity and electrotonic potential evoked by application of inward and outward current pulses (1.5 s in pulse duration) of the guinea-pig portal vein: (a) effects of 2.8×10^{-6} M NG; (b) effects of 2.8×10^{-5} M NG. Vertical bar in (b) indicates the absolute potential calibration (0 to −15 mV). (c) and (d) are recorded at fast speed; (c) control, (d) in the presence of NG. (c_1) and (d_1); application of inward current pulse (1.5 s); (c_2) and (d_2) outward current pulse (1.5 s).

Effects of nitroglycerine on the membrane activity

The effects of NG on the spontaneous activity of the muscle membrane of the rat and guinea-pig portal veins were observed (Figure 3). The spike mainly appeared as a burst discharge on the sustained depolarization between the silent periods in both tissues. NG (2.8×10^{-6} M) had no effect on the frequency and amplitude of spike generation in the rat portal vein. On the other hand, as shown in Figure 3a, NG affected the membrane activities of the guinea-pig portal vein even in a low dose (2.8×10^{-8} M). At a concentration of 2.8×10^{-6} M (c) or 2.8×10^{-5} M NG, the membrane was transiently hyperpolarized and this was followed by depolarization of the membrane. When the membrane was hyperpolarized the spike generation was suppressed and when it was depolarized train discharges were generated, i.e. the spike generation was voltage-dependent. After washing out NG, the membrane was gradually repolarized, and the burst pattern was restored from the train discharge. When NG was reapplied within a short interval (less than 5 min), hyperpolarization and the following depolarization were less marked. Figure 3 (e, f) also shows the effects of NG (2.8×10^{-6} M) on the membrane and mechanical activities recorded from muscle cells of the rat portal vein. NG had no effect on the membrane activity but suppressed the amplitude of the mechanical activity.

The effects of NG (2.8×10^{-5} M) on the membrane activity in various $[K]_o$ is shown in Figure 4. When $[K]_o$ was decreased to 1.9 mM (b) the membrane was hyperpolarized and the frequency of spontaneous burst discharge was decreased. With further reduction in $[K]_o$ to 0.6 mM (a), the membrane was depolarized and the spike generation became sparse. By application of NG (2.8×10^{-5} M) in various $[K]_o$ from 0.6 mM to 17.7 mM, the spike generation was inhibited by hyperpolarization of the membrane. Increasing the $[K]_o$ to 17.7 mM depolarized the membrane and changed the burst pattern to train discharges. NG slightly hyperpolarized the membrane and suppressed the spike generation (e). With a further increase in $[K]_o$ to 59 mM (f), a depolarization block of the spike generation occurred, and NG neither changed the membrane potential nor reproduced the spike generation.

In 68.7 mM, 30.8 mM, or 15.4 mM $[Na]_o$, the muscle membrane of the guinea-pig portal vein was transiently hyperpolarized and then depolarized (Figure 5a, c, d). The membrane activities were suppressed during the hyperpolarization and frequency of spike generation was still reduced during the depolarization period (a, c). Spike generation completely ceased in 15.4 mM $[Na]_o$ (e). When the spontaneous membrane activity had ceased in low $[Na]_o$, field stimulation (1 ms duration) of the tissue evoked the spike, i.e. Na-deficient solution suppressed spontaneous membrane activity but not spike generation. In the above three different Na-deficient solutions, the maximum membrane potential changes were observed in 15.4 mM $[Na]_o$. NG (2.8×10^{-5} M) hyper-

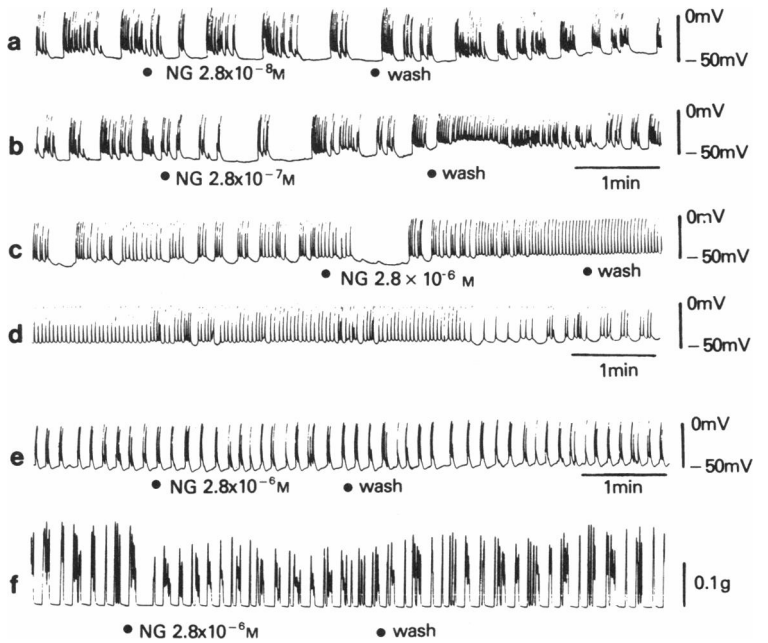

Figure 3 Effects of various concentrations of nitroglycerine (NG, 2.8×10^{-8} to 2.8×10^{-6} M) on the spontaneously generated membrane activity of the guinea-pig and rat portal veins; (c) and (d) are a continuous record. (●) indicates application and removal of NG. (a–d) Guinea-pig portal vein. (e–f) Rat portal vein; (e) electrical activity; (f) mechanical activity. (c–f) Application of 2.8×10^{-6} M NG. Vertical bars in (a–e) indicate the absolute potential calibration (0 to −50 mV).

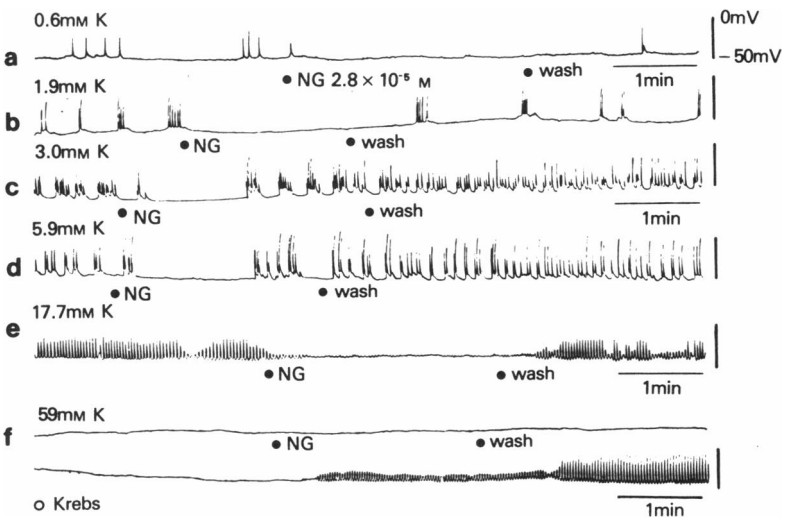

Figure 4 Effects of nitroglycerine (NG, 2.8×10^{-5} M) on the membrane activity recorded from the guinea-pig portal vein in various concentrations of $[K]_o$: (●) application and removal of NG; (○) washed with Krebs solution. Vertical bars indicate the absolute potential calibration (0 to −50 mV). Effects of NG on the membrane activity were observed after the tissue was superfused with various concentrations of $[K]_o$ (10 to 15 min).

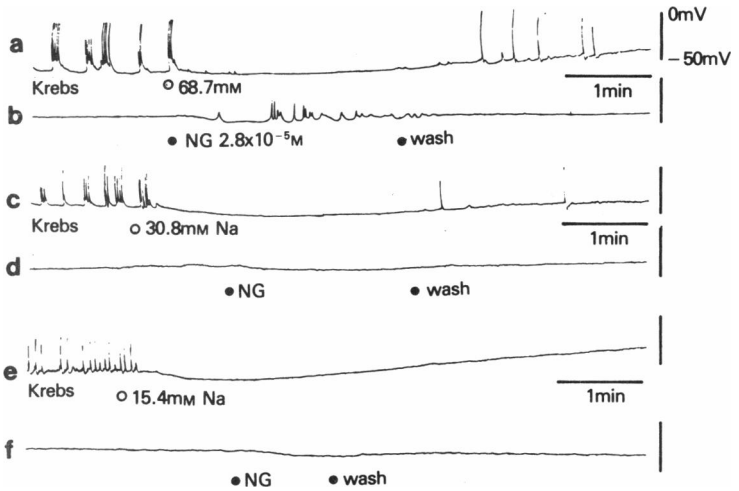

Figure 5 Effects of nitroglycerine (NG, 2.8×10^{-5} M) on the membrane activity recorded from the guinea-pig portal vein in various concentrations of $[Na]_o$. Symbols in the figure are the same as those described in Figure 4. (a, c and e) Effects of low $[Na]_o$ on the membrane activity; (b, d and f) effects of NG in low $[Na]_o$ solution. The effects of NG were observed after about 20 min in low $[Na]_o$ solution. Vertical bars indicate the absolute membrane calibration (0 to −50 mV).

polarized the membrane to the same extent in all the above Na-deficient solutions, and in 68.7 mM $[Na]_o$, spikes reappeared with hyperpolarization (b). In 30.8 mM $[Na]_o$ or 15.4 mM $[Na]_o$, spikes did not appear on treatment with NG (d, f). Depolarization which appeared after the hyperpolarization in Krebs solution induced by NG was not observed in Na-deficient solution. When $[Na]_o$ was reduced to 15.4 mM by choline chloride, the membrane was hyperpolarized then depolarized and spike generation ceased. NG (2.8×10^{-5} M) also hyperpolarized the membrane. The response of smooth muscle membrane to NG in low $[Na]_o$ substituted with either Tris–Cl or choline chloride was the same.

In 7.5 mM $[Ca]_o$ the membrane was hyperpolarized as previously stated and the appearance of burst discharge was reduced but the number of spikes in a train discharge was increased. By application of 2.8×10^{-5} M NG, the membrane was further hyperpolarized, and depolarization after transient hyperpolarization in the presence of NG was suppressed. In 5.0 mM $[Ca]_o$, NG (2.8×10^{-5} M) hyperpolarized the membrane to the same extent as in Krebs solution, but the depolarization following the hyperpolarization was small and the spikes appeared as the burst discharge. In 0.25 mM $[Ca]_o$, the membrane was depolarized and spike activity was suppressed. By treatment with NG (2.8×10^{-5} M) in low $[Ca]_o$, both the amplitude of the hyperpolarization and the following depolarization were smaller than those observed in Krebs solution.

Effects of nitroglycerine on the mechanical responses

Figure 6 shows the effects of NG on the electrical and mechanical activities of the guinea-pig portal vein. In the portal vein, it was difficult to obtain a synchronized record of electrical and mechanical activities because of the segmental arrangement of the muscle structures (Kitamura, Suzuki & Kuriyama, 1976). NG (2.8×10^{-5} M) hyperpolarized the membrane transiently, and markedly suppressed the amplitude of the contraction during the hyperpolarization. During the depolarization phase after hyperpolarization, mechanical responses were restored to some extent, but not to the control value. When NG was washed out, the amplitude of contractions was gradually but completely restored. Figure 6f shows clear suppression of contractions which consisted of an initial marked suppression and a following sustained phase in 2.8×10^{-5} M NG.

On the other hand, in the rat portal vein, NG (2.8×10^{-7} to 2.8×10^{-5} M) did not affect the membrane activities but did suppress the amplitude of contractions.

When the effects of NG on the spontaneous contraction of the guinea-pig portal vein in various ionic environments were observed, NG decreased the amplitude of phasic contractions in 0.8 mM $[Ca]_o$, in 7.5 mM $[Ca]_o$ and in 1.2 mM $[K]_o$ as it did in Krebs solution. In both 15.4 mM $[Na]_o$ and 17.7 mM $[K]_o$, phasic contractions appeared on the tonic contraction. Application of NG also suppressed

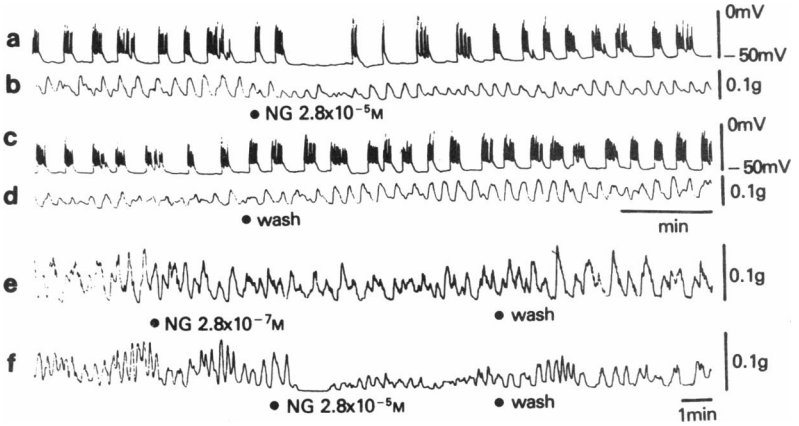

Figure 6 Effects of nitroglycerine (NG, 2.8×10^{-7} and 2.8×10^{-5} M) on electrical and mechanical activities of the guinea-pig portal vein: (a, b) and (c, d) are a simultaneous record of electrical and mechanical activities from the same segment. (a) and (c), and (b) and (d) are continuous records; (e) and (f) show the effects of NG on the mechanical properties of the guinea-pig portal vein.

both contractions. This means that NG consistently suppresses the mechanical response in any moderate change in the ionic environment.

Effects of nitroglycerine on the membrane and contractile responses evoked by excess $[K]_o$ or noradrenaline

Figure 7 shows the effects of NG (2.8×10^{-5} M) on the contracture of the guinea-pig and rat portal vein evoked by 59 mM $[K]_o$ or 4.9×10^{-7} M noradrenaline. NG suppressed the contracture evoked by both procedures. However, NG suppressed more extensively the contractions induced by both procedures in the guinea-pig tissue than those in the rat tissue, and it suppressed more extensively the noradrenaline induced contracture than that induced by excess $[K]_o$ in both species.

Figure 7(e, f) shows the effects of NG (2.8×10^{-5} M) on the membrane activity during treatment with 4.9×10^{-7} M or 4.9×10^{-6} M noradrenaline. Treatment with NG (2.8×10^{-5} M) of the membrane already depolarized by pretreatment with noradrenaline (4.9×10^{-7} M) caused slight repolarization and decrease in spike frequency. NG slightly repolarized the membrane during treatment with noradrenaline (4.9×10^{-6} M); the suppressed spike frequency caused by application of NG was instantly restored to the previous state, after washing out of NG with Krebs solution containing noradrenaline.

The effects of NG on the membrane activity observed in Krebs solution were not altered by pretreatment with either phentolamine (3.9×10^{-7} M) or propranolol (3.6×10^{-7} M).

Discussion

In the guinea-pig portal vein, high concentrations of NG transiently hyperpolarized and then depolarized the membrane. The spike generation was modified in a manner dependent upon the membrane potential. The hyperpolarization of the membrane of the guinea-pig portal vein by treatment with NG is presumably due to an increase in the K-conductance of the membrane, because the amplitude of hyperpolarization was changed by various low $[K]_o$ but it was less in various $[Na]_o$ or $[Ca]_o$, and because hyperpolarization is accompanied by a reduction in the membrane resistance. Furthermore, the depolarization which followed hyperpolarization in the presence of NG ceased in Na-deficient solution which suggests an increase in the Na-permeability of the membrane. This means that NG produces a rapid increase in the K-permeability and a delayed increase in the Na-permeability of the membrane. However, these responses of the membrane were not observed in the rat portal vein.

Ito *et al.* (1980) studied the effects of NG on electrically quiescent muscle of the porcine coronary artery and observed that NG ($\leqq 10^{-5}$ M) neither modified the membrane potential nor the membrane resistance, while at 2.8×10^{-8} M, NG markedly suppressed the contraction induced by excess $[K]_o$ or acetylcholine. Differences in the mechanical response to NG in high $[K]_o$ or acetylcholine in Ca-free solution (EGTA) were apparent, therefore they concluded that NG might suppress the Ca releasing mechanism from the storage site rather than suppress Ca influx.

On the other hand, in the dog coronary artery, Harder *et al.* (1979) observed that the cell was electri-

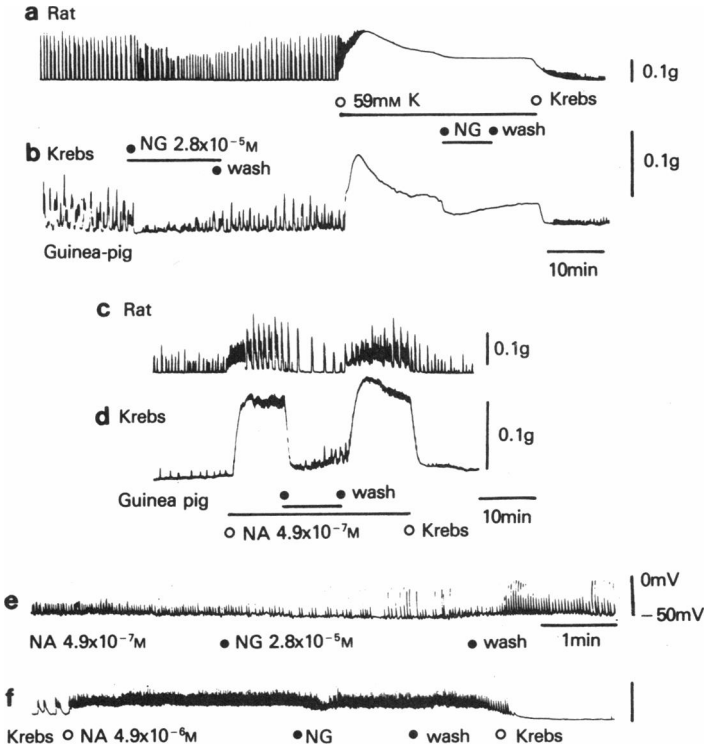

Figure 7 Effects of nitroglycerine (NG, 2.8×10^{-5} M) on the contraction of the rat and guinea-pig portal veins induced by 59 mM $[K]_o$ or noradrenaline (NA, 4.9×10^{-7} M) and on the membrane activity of the guinea-pig portal vein pretreated with 4.9×10^{-6} M or 4.9×10^{-7} M noradrenaline. (a and c) Mechanical activity of the rat portal vein; (b and d) mechanical activity of the guinea-pig portal vein; (e and f) membrane activities recorded from muscle cells of the guinea-pig. Vertical bar in (f) indicates the absolute potential calibration (0 to −50 mV). (●) Application and removal of NG; (○) application and removal of noradrenaline or excess $[K]_o$.

cally quiescent but NG blocked the spike which was induced by electrical stimulation in the presence of tetraethylammonium in large coronary arteries, while adenosine blocked the spike preferentially in small coronary arteries. Verapamil blocked these Ca-dependent action potentials in both large and small arteries. In physiological solution, NG (10^{-7} to 10^{-5} M) had no significant effect on the resting potential or the input resistance of the membrane, as measured by the Wheatstone bridge method.

In the dog and rat portal veins, Mackenzie & Parratt (1977) studied the effects of NG and concluded that it inhibited the inherent spontaneous myogenic activities, and that this phenomenon might be related to the vasodilator action *in vivo*. These conclusions confirmed the view of Häusler & Thorens (1976). In human saphenous vein, Mikkelsen, Andersson & Bengtsson (1978) studied the effects of verapamil and NG on the contractile responses to $[K]_o$ or noradrenaline, and suggested that prevention of the Ca-influx from the extracellular medium was not of primary importance for the relaxant effect of NG.

The portal veins of both rat and guinea-pig are spontaneously active and their phasic contraction might have a causal relation to the Ca-spike (Ito & Kuriyama, 1971; Kuriyama & Suzuki, 1978). If the vasodilator action of NG is indeed caused by suppression of Ca-current, the spontaneous or evoked membrane activity observed in the portal vein should be suppressed. Such was the case in the guinea-pig but not the rat portal vein. Even in the guinea-pig portal vein, membrane activity was consistently increased and mechanical activity was suppressed during the depolarization phase which followed a transient hyperpolarization in the presence of NG. Therefore, the relaxant action of NG on the mechanical activity may not depend solely on the inhibition of Ca-influx in spontaneously active veins of either of these species.

The initial marked suppression of the mechanical

activity in the guinea-pig portal vein might be due to hyperpolarization of the membrane which suppressed the activation of the voltage-dependent Ca-channel of the membrane, and thereby abolished or decreased spike activity. Korth (1975) suggested that in guinea-pig heart, the NG-induced increase in contractile force was induced by the liberation of noradrenaline and an inhibitory effect on the monoamine oxidase of sympathetic nerve endings might be involved. However, in these experiments phentolamine and propranolol did not modify the effects of NG on the membrane and mechanical activities of the guinea-pig portal vein.

From the present experiments, it is postulated that NG possesses two different actions on the guinea-pig portal vein, i.e. it acts on the membrane as a modulator of the ionic permeability; an initial increase in the K-permeability is followed by a slowly developed late increase in Na-permeability, causing hyperpolarization and then depolarization respectively. It also acts to suppress the mechanical responses. The suppression of spike generation by transient hyperpolarization of the muscle cells of the guinea-pig portal vein may contribute additively to the vasodilator mechanism. In contrast, in the rat portal vein, NG only inhibits the mechanical activity presumably by suppressing the excitation-contraction coupling mechanism.

This means that the response of the muscle membrane in the rat portal vein to NG is not an essential factor for vasodilatation, and responses of the muscle cells to NG differ between animal species even in the same vascular beds.

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