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# ACTIONS OF NITROGLYCERINE ON THE MEMBRANE AND MECHANICAL PROPERTIES OF SMOOTH MUSCLES OF THE CORONARY ARTERY OF THE PIG

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**1** Effects of nitroglycerine (NG) on the membrane and contractile properties of the smooth muscle cell of the isolated coronary artery of the pig were observed.

**2** NG, up to a concentration of $10^{-5}$ M, modified neither the membrane potential nor the membrane resistance. Increased concentrations of NG ($>2.8 \times 10^{-5}$ M) hyperpolarized the membrane, reduced the membrane resistance and enhanced the rectifying property of the membrane measured by depolarization pulses. These phenomena observed with a high concentration of NG are the result of an increase in the K-conductance of the membrane.

**3** NG ($2.8 \times 10^{-5}$ M) did not modify the membrane potential displaced by various concentrations of excess $[K]_o$. In low $[K]_o$, NG ($2.8 \times 10^{-5}$ M) hyperpolarized the membrane to a greater extent than that observed in Krebs solution. The effects of NG ($10^{-6}$ to $2.8 \times 10^{-5}$ M) on the membrane potential were not modified by simultaneous application of $2 \times 10^{-6}$ M acetylcholine (ACh).

**4** NG ($2.8 \times 10^{-6}$ M) consistently raised the mechanical threshold required for tension development and suppressed the amplitude of the contraction evoked by excess $[K]_o$, ACh or electrical depolarization of the membrane. The dose-response curve shifted to the right in the presence of NG noncompetitively in all the conditions employed to develop the tension.

**5** When the tissue was immersed in Ca-free (EGTA) solution, ACh ($5 \times 10^{-6}$ M) evoked a contraction even after the tissue had lost the ability to contract to repetitive applications of 118 mM $[K]_o$ in Ca-free (EGTA) solution. However, the tissue finally failed to contract to repetitively applied ACh. At this stage, 2.5 mM $[Ca]_o$ evoked a small contraction, after which the response was briefly restored to $5 \times 10^{-6}$ M ACh. This transient response to ACh was reduced by NG ($5.6 \times 10^{-6}$ M) when NG was added either simultaneously with ACh or with the previous Ca application. However, the inhibition was greater in the former than the latter case.

**6** Cysteine (1 to 2 mM), without modifying the membrane potential or membrane resistance, partly restored the contraction evoked by excess $[K]_o$ or ACh which had been reduced by NG.

**7** The mechanism of action of NG on the smooth muscle cell of the coronary artery of the pig is postulated to be due to a nonselective suppression of the Ca-mobilization from the store site with no noticeable change in the membrane properties.

## Introduction

The effectiveness of nitrates in the treatment of angina pectoris has been evident for over a century since the classic reports of Brunton (1867) and Murrell (1879). Despite the wide-spread, daily administration of these compounds to patients with coronary heart disease, as well as extensive studies using experimental animals, the mechanisms involved in their action remain poorly understood.

Brunton (1867) initially investigated the effects of amyl nitrate in the relief of angina pectoris as related to its hypotensive action (Voegtlin & Macht, 1913; Bogaert, 1972) and it is widely believed that the effects of nitrates are derived primarily from their peripheral actions and not necessarily due to direct effects on the coronary circulation. Recently, in the light of data demonstrating that nitrates affect the regional distribution of coronary flow, the coronary bed has again been advocated as an important site for the action of nitroglycerine (NG) (review of Vatner & Heyndricks, 1975).

Winbury, Howe & Hefner (1969) indicated that the resistance of the large coronary vessels normally constitutes about 5% of the total resistance of the coronary arterial system. This means that the total coron-

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ary resistance is localized almost entirely in the small vessels and that the resistance of the large vessels is not a limiting factor in the blood supply. However, these authors found that NG produced a prolonged dilatation of the large coronary arteries, and only a transient dilatation of the small coronary arteries; and Schnaar & Sparks (1972) reported that NG produced a more prolonged relaxation in strips excised from the large arteries than in those from small ones. Therefore, to investigate the vasodilator action of NG on coronary arteries, the epicardial coronary arteries seem to be a suitable preparation.

The present study was an attempt to clarify the vasodilator mechanism of NG on the single smooth muscle of the pig coronary artery. This particular preparation was chosen because Ito, Kitamura & Kuriyama (1979) have studied in detail its passive properties and the effects both of acetylcholine (ACh) and catecholamines on its electrical and mechanical properties.

## Methods

Adult pigs of either sex were killed in a local slaughter house; the hearts were removed into oxygenated Krebs solution at 15 to 18°C and then brought to our laboratory at which time they were still beating.

The large branches of the left and right coronary arteries were carefully dissected under a binocular microscope. The vessel diameter was about 3 to 5 mm at the Valsalva sinus and 1 mm at 50 to 60 mm distal to the sinus; tissue was taken mainly from the regions where the diameter was between 1.5 and 2 mm. After an initial longitudinal incision of the artery, the circular muscle tissue (2 mm in width and 6 to 7 mm in length) was mounted in a 2 ml organ bath and superfused at a rate of 3 ml/min with fluid at 35 to 36°C by means of a thermo-unit with perfusion pump (Taiyo Co. Ltd. type C-550).

For recording the membrane potential, a conventional glass microelectrode filled with 3 M KCl was inserted from the serosal side (adventitial side). Electrical stimulation was carried out by the partition stimulating method described by Abe & Tomita (1968).

For simultaneous recording of the electrical and mechanical activities, the double sucrose gap method was used (Ito, Suzuki & Kuriyama, 1977) on circular muscle strips 0.5 mm in width and 6 to 7 mm in length.

To measure isometric contractions, two circular muscle preparations (1.0 to 1.5 mm in width and 6 mm in length) were mounted in parallel in a 1 ml organ bath; one end of each muscle was fixed at the bottom of the bath and other end connected by a hook to the tension recorder (Nihon Kohden Ltd.). The flow rate of the solution was 3 ml/min at a temperature of 32°C.

Modified Krebs solution (Bülbring, 1955) was of the following composition (mM); $Na^+$ 137.4; $K^+$ 5.9, $Mg^{2+}$ 1.2, $Ca^{2+}$ 2.5, $Cl^-$ 134.0, $K_2PO_4^-$ 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 up to 118 mM isotonically. To prepare 136 mM $[K]_o$ solution, $NaHCO_3$ was replaced with $KHCO_3$. Na-deficient solution was prepared by substituting NaCl with an appropriate amount of Tris-Cl (Tris(hydroxymethyl)aminomethane-Cl).

The following drugs were used; nitroglycerine (stock solution of 0.637 mg/ml in distilled water prepared by Nippon-Kayaku Co); acetylcholine chloride (Daiichi Pharm. Co); cysteine (Merck); and glycoletherdiamine tetraacetic acid (GEDTA, EGTA, Dojin Chem-Pharm. Instit.).

## Results

### *Effects of nitroglycerine on the muscle membrane*

The mean (±s.d.) membrane potential of the circular muscle cells measured by glass microelectrodes was $-51.4 \pm 1.8$ mV ($n = 50$) and the membrane was electrically quiescent.

Figure 1 shows the effects of NG on the membrane potential in Krebs solution, in the absence or presence of ACh ($2 \times 10^{-6}$ M) or in various concentrations of $[K]_o$. Increasing the concentration of NG up to $1 \times 10^{-5}$ M did not modify the membrane potential but with a concentration of $2.8 \times 10^{-5}$ M the membrane was hyperpolarized from $-51.4$ mV to $-54.7 \pm 1.9$ mV ($n = 20$; $P < 0.05$ by Student's $t$ test) shown in Figure 1a. These changes in the membrane potential produced by $2.8 \times 10^{-5}$ M NG were not modified by pretreatment with $2 \times 10^{-6}$ M ACh which alone in Krebs solution gave a value of $-49.8 \pm 3.1$ mV ($n = 15$) and, together with NG, a value of $-53.5 \pm 2.2$ mV ($n = 18$; $P < 0.05$). The hyperpolarization of the membrane induced by NG ($2.8 \times 10^{-5}$ M) was also observed in Ca-deficient (0.5 mM) or Na-deficient (15.6 mM) solutions.

Increased $[K]_o$ depolarized the membrane and the maximum slope of a membrane depolarization produced by a tenfold increase in $[K]_o$ plotted on a log scale was 50 mV. In 59 mM $[K]_o$, the membrane potential was $-22.4 \pm 1.8$ mV ($n = 10$) and with added NG ($2.8 \times 10^{-5}$ M) was $-22.6 \pm 1.5$ mV ($n = 15$). However, in 5.9 mM $[K]_o$, the membrane was hyperpolarized by pretreatment with NG from $-50.7 \pm 2.4$ mV ($n = 18$) to $-54.2 \pm 2.1$ mV ($n = 20$, $P < 0.05$), and in 3 mM $[K]_o$, NG hyperpo-

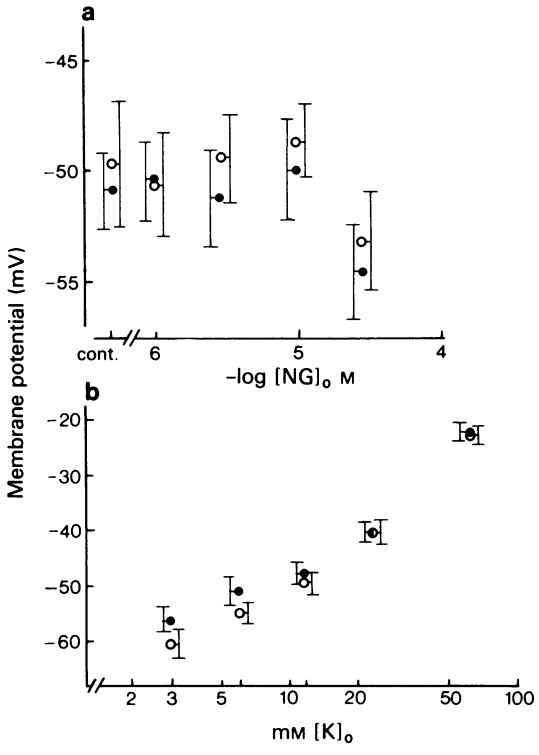


**Figure 1** (a) Effects of various concentrations of nitroglycerine (NG) in the presence or absence of $2 \times 10^{-6}$ M acetylcholine (ACh): (●) NG alone; (○) NG with ACh. (b) Effects of $2.8 \times 10^{-5}$ M NG on the membrane potential in various concentrations of $[K]_o$: (●) various concentrations of $[K]_o$ without NG; (○) various concentrations of $[K]_o$ with NG.

larized the membrane from $-55.8 \pm 2.2$ mV ($n = 12$) to $-60.2 \pm 2.5$ mV ($n = 20$, $P < 0.05$). Thus, in Krebs solution, NG in a concentration of below $10^{-5}$ M did not modify the membrane potential but membrane hyperpolarization occurred with $2.8 \times 10^{-5}$ M NG provided that $[K]_o$ did not exceed 5.9 mM.

Figure 2 shows the effects of NG on the membrane potential and membrane resistance. NG ($10^{-5}$ M) modified neither the membrane potential nor the membrane resistance, as measured from current-voltage relationships (Figure 2a and c). A higher concentration of NG ($2.8 \times 10^{-5}$ M) hyperpolarized the membrane, reduced the membrane resistance (as measured from the slope of the current-voltage relationship) and enhanced the rectifying property of the membrane, measured from the depolarization produced by various intensities of the outward current pulse (Figure 2b). When the membrane potential was displaced by the inward current in Krebs solution to the level where NG hyperpolarized, or when the membrane was depolarized to the control level by application of the outward current in the presence of NG, the current-voltage relationship was consistently less steep in the presence of NG.

Figure 3 shows the effects of NG on the membrane potential and membrane resistance, measured in the various concentrations of $[K]_o$. When the effects of NG ($2.8 \times 10^{-5}$ M) were observed in 59 mM or 23.6 mM $[K]_o$, it modified neither the membrane potential nor the membrane resistance. However, changes in these parameters were observed with NG in the presence of 5.9 mM and 2.9 mM $[K]_o$.

These results indicate that a high concentration of NG increases the K-conductance of the membrane, thus causing hyperpolarization, reducing the membrane resistance and enhancing its rectifying property.

*Effects of nitroglycerine on evoked contractions*

Depolarization-contraction relationships in the pig coronary artery were observed by the double sucrose gap method. The resting membrane potential was $-42 \pm 2.5$ mV ($n = 6$), compared with $-51.4 \pm 1.8$ mV ($n = 50$) measured by the microelectrode method. Outward current pulse (2 s) produced a graded response and when a depolarization exceeded 6 mV a contraction was evoked, the amplitude of which was increased with stronger pulses. Application of NG ($2.8 \times 10^{-6}$ M) suppressed the amplitude of contraction and raised the mechanical threshold. Figure 4 (a and b) shows examples of the effects of NG ($2.8 \times 10^{-6}$ M) on the electrically induced contraction and Figure 4c shows the relationship between the depolarization and contraction in the presence or absence of NG ($2.8 \times 10^{-6}$ M and $8.4 \times 10^{-6}$ M). The amplitude of the contraction evoked by 30 mV depolarization was registered as 1.0. In the presence of $2.8 \times 10^{-6}$ M NG, the membrane potential remained the same but the minimum depolarization required for the tension development increased from 7 mV to 12 mV ($n = 5$) and in $8.4 \times 10^{-6}$ M NG increased from 7 mV to 17 mV ($n = 5$). The amplitude of the contraction at 30 mV depolarization was reduced to 52% and to 26% the control in the presence of $2.8 \times 10^{-6}$M and $8.4 \times 10^{-6}$ M NG ($n = 4$), respectively.

Figure 5 shows the effects of NG ($8.4 \times 10^{-6}$ M) on contractions induced by ACh or excess $[K]_o$. As has been described by Ito *et al.* (1979), ACh generated a contraction with no change in either the membrane potential or the membrane resistance. The minimum effective concentration of ACh was $5 \times 10^{-9}$ M; and that of $[K]_o$ was 23.6 mM (at which the membrane was depolarized from $-51.6 \pm 2.4$ mV ($n = 20$), to $-40.3 \pm 2.3$ mV ($n = 15$)). The amplitudes of the contraction evoked by 136 mM $[K]_o$ and that by $5 \times 10^{-5}$ M ACh were each registered as a relative

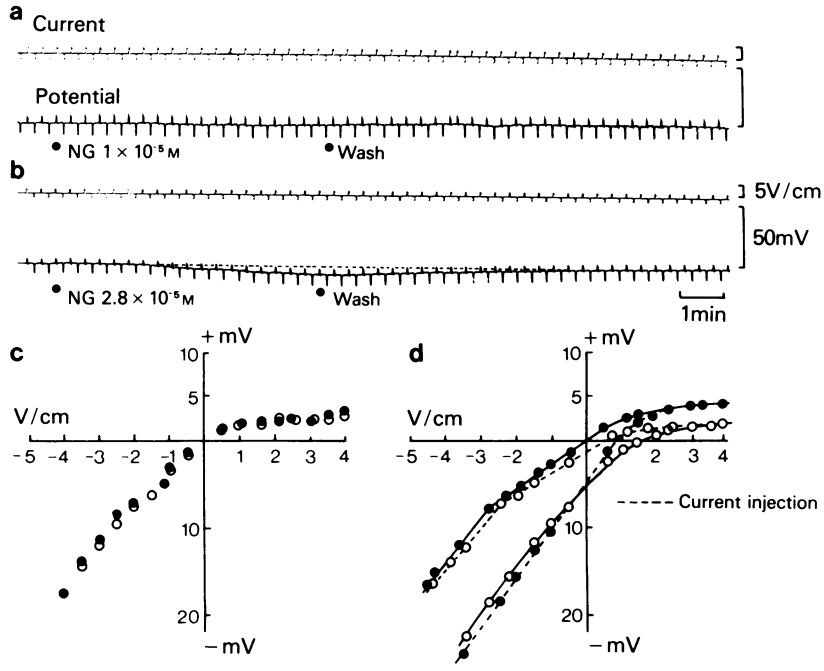


**Figure 2** Effects of nitroglycerine (NG, $10^{-5}$ M and $2.8 \times 10^{-5}$ M) on the membrane potential and membrane resistance. In (a) and (b), inward and outward current pulses (1.5 s pulse duration) were applied alternately. Dotted lines in (b) indicate the resting membrane potential level. (c and d) Current-voltage relationships observed in the presence or absence of NG ($10^{-5}$ M and $2.8 \times 10^{-5}$ M, respectively). In (c) and (d), (○) is the control and (●) is in the presence of NG. Dotted lines indicate electrical displacement of the membrane potential to the control potential level following treatment with NG (---○---) and also electrical displacement of the membrane potential to the NG-induced hyperpolarization in Krebs solution (---●---). Pulse duration was 1.5 s and the recording electrode was placed at 0.5 mm distance from the stimulating electrode (a–d).

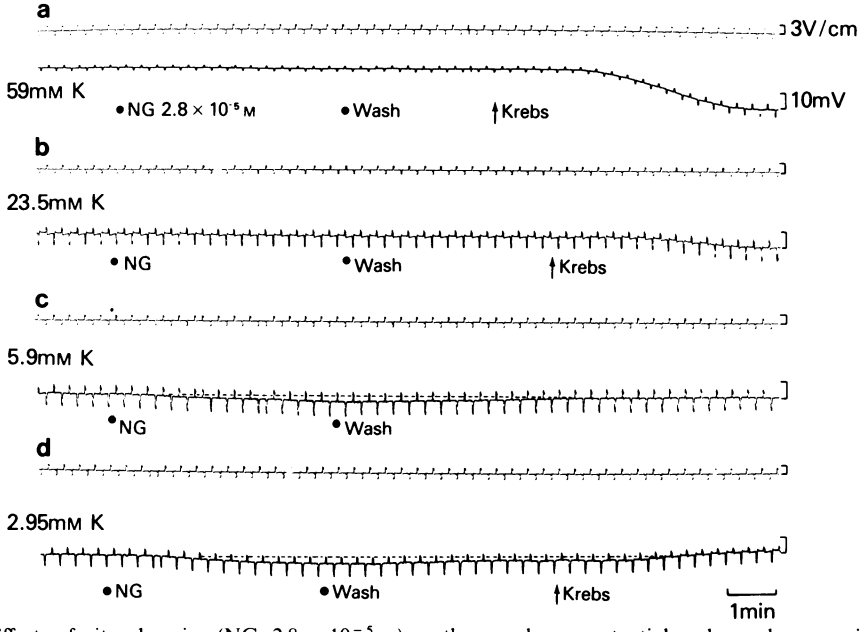


**Figure 3** Effects of nitroglycerine (NG, $2.8 \times 10^{-5}$ M) on the membrane potential and membrane resistance in various concentrations of $[K]_o$. Inward and outward current pulses (1.5 s in pulse duration) were applied alternately. Dots in the figure indicate application and removal of NG. Arrows indicate removal of various concentrations of $[K]_o$. Dotted lines in (c) and (d) indicate the resting membrane potential level.

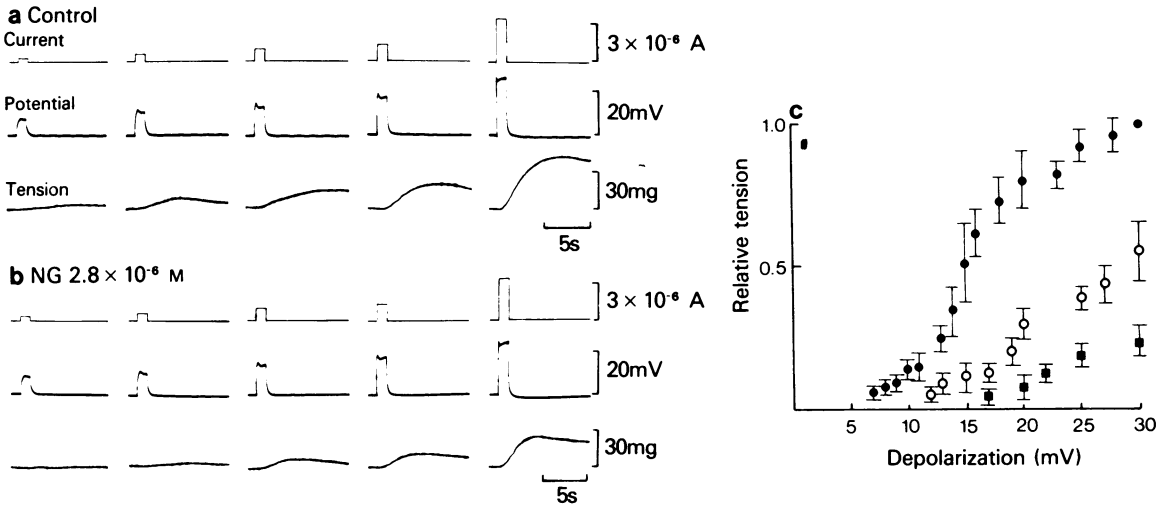


**Figure 4** Depolarization-contraction relationship observed in the presence or absence of nitroglycerine (NG): (a) and (b) show actual traces of depolarization-contraction relationship observed in Krebs solution and in the presence of NG ($2.8 \times 10^{-6}$ M). (c) Depolarization-contraction relationships observed in the presence of NG, $2.8 \times 10^{-6}$ M (○) or $8.4 \pm 10^{-6}$ M (■) and in Krebs solution (●). The double sucrose gap method was used. The resting membrane potential levels were varied from −41 mV to −44 mV. The relative amplitude of contraction recorded by 30 mV depolarization was taken as 1.0. Horizontal bars indicate 2 × s.d., $n = 5$ to 10.

tension of 1.0. A sigmoidal relationship existed between the amplitude of contraction and the concentration of excess $[K]_o$ or ACh.

Application of NG ($5.6 \times 10^{-6}$ M) suppressed more noticeably the amplitude of contractions induced by low concentrations ($4 \times 10^{-8}$ M and $10^{-7}$ M) of ACh and by 29.8 mM $[K]_o$ than those by high concentrations (Fig. 5c). However, the depression of the maximum response suggested that the inhibition is of a non-competitive type. Furthermore, despite the fact that the mechanisms of contraction differ, NG suppressed equally well the contraction induced by both agents.

Experiments were carried out in the presence of cysteine, which itself did not modify the membrane potential (control; $-51.7 \pm 3.5$ mV, $n = 13$ and in 2 mM cysteine; $-51.6 \pm 1.5$ mV, $n = 11$). NG ($2.8 \times 10^{-5}$ M) hyperpolarized the membrane in the presence of 2 mM cysteine ($-53.5 \pm 1.7$ mV, $n = 12$) but the value was much the same as that observed in Krebs solution alone. This means that cysteine does not suppress the hyperpolarization induced by NG. However, the inhibitory effects of NG on evoked contractions were partly reversed by 2 mM cysteine; inhibition of 59 mM $[K]_o$ induced contractions ranged from 0.72 to 0.84 of control values and of ACh-induced contractions ($2 \times 10^{-7}$ M) ranged from 0.68 to 0.75 of control values ($n = 4$) when cysteine was present. Cysteine itself had no effect on the mechanical response induced by either ACh or excess $[K]_o$ in the absence of NG.

To investigate in detail the effects of NG on the mechanical response only, NG was applied to tissue superfused with Ca-free EGTA (0.5 mM) solution.

Repetitively applied 118 mM $[K]_o$ produced contractions that were gradually lowered in amplitude in Ca-free (EGTA) Krebs solution, so that after 40 min, their size was reduced to below 0.5% of the control in normal Krebs solution. Initially $5 \times 10^{-6}$ M ACh produced a relatively large contraction in Ca-free Krebs solution after the tissue had lost the ability to respond to 118 mM $[K]_o$, although when ACh was applied repetitively the contraction became smaller and eventually disappeared. Under these circumstances, the Ca in the cell is probably insufficient to generate a contraction (Ito *et al.*, 1979).

At a time when tissues bathed with Ca-free solution had become refractory to ACh, exposure to 2.5 mM Ca (normal Krebs solution for 2 min) evoked a slight contraction. A short-lived recovery of the ACh-induced response was seen if ACh was added within a few minutes of the brief exposure to Ca, the response being larger, but irregular if ACh was added within 5 min and smaller, but regular if the interval was extended up to 10 min. In the present experiments a time interval was chosen of 7 min to provide measurable contractions of similar size. Some deterioration was seen in tissues bathed for long periods in Ca-free solution in that after 60, 120 and 180 min, the contractions induced by ACh as above were 55%, 50% and 35% of the initial values, respectively. It was reported previously (Ito *et al.*, 1979) that, like ACh,

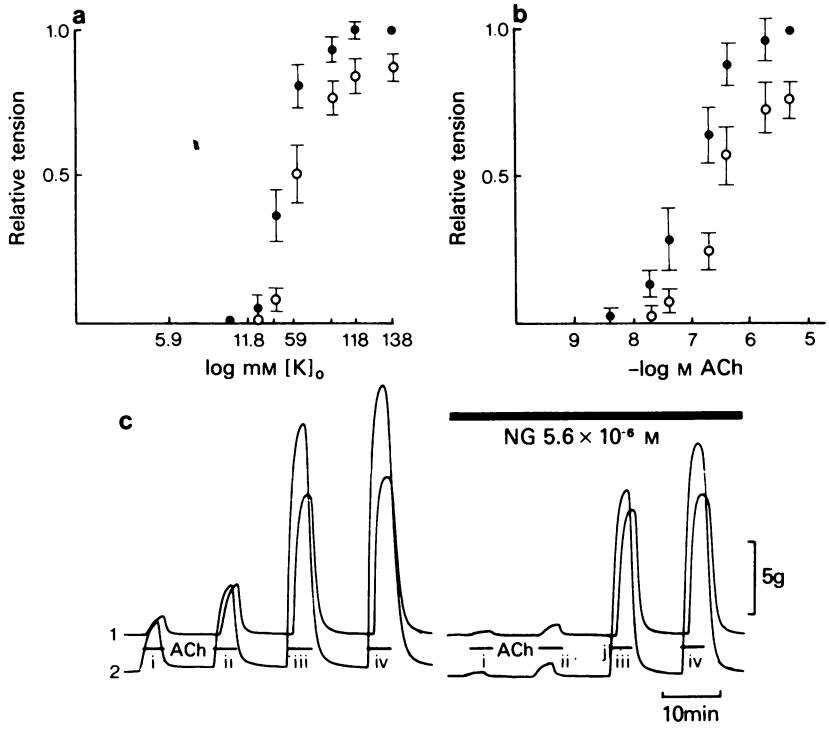


**Figure 5** Effects of nitroglycerine (NG) on the contraction evoked by excess $[K]_o$ or acetylcholine (ACh). (a) The relationship between $[K]_o$ and contraction in the presence (○) or absence (●) of NG ($8.4 \times 10^{-6}$ M). The amplitude of contraction evoked by 138 mM $[K]_o$ was taken as 1.0. $n = 4$ for both control and NG. Horizontal bars = 2 × s.d. (b) The relationship between various concentrations of ACh and contraction in the presence (○) or absence (●) of NG ($8.4 \times 10^{-6}$ M). The contraction evoked by $5 \times 10^{-6}$ M was taken as 1.0. $n = 4$ for both control and NG. Horizontal bars = 2 × s.d.. (c) Effects of ACh on the mechanical response in the presence or absence of NG ($5.6 \times 10^{-6}$ M). Numbers 1 and 2 indicate two different preparations; i, ii, iii and iv indicate $4 \times 10^{-8}$ M, $10^{-7}$ M, $4 \times 10^{-7}$ M and $10^{-6}$ M ACh, respectively.

excess $[K]_o$ could also induce cnntractions in Ca-free solution if it was added after a brief exposure of the tissue to Ca.

Figure 6 shows some of the findings in Ca-free solution. The decreased effectiveness of ACh and of excess $[K]_o$ is illustrated, as is the small contraction on exposing the Ca-deprived tissue to 2.5 mM Ca for 2 min. Furthermore, the recovery of the response to ACh when it was added 7 min after Ca is also depicted. In the presence of NG, a reduction in amplitude occurred of the contractions induced (a) by ACh (preceded by exposure to Ca) and (b) by Ca itself. The reduction of ACh-induced responses by NG was always greater when the NG was present simultaneously with ACh than when it was present only during the preceding exposure to Ca (6 experiments).

These findings suggest that NG more effectively suppressed the mobilization of Ca from tissue stores than it suppressed the Ca-influx such as would occur when tissues were briefly exposed to Ca.

## Discussion

Ito *et al.* (1979) showed that the smooth muscle of the coronary artery of the pig possesses a cable-like property (length constant, 0.67 mm) and that $\beta$-adrenoceptors are distributed such as to modify the ionic permeability of the membrane. Since catecholamines modified the membrane potential only in very high concentrations and ACh had no influence on it, their respective receptors probably do not play a physiological role in altering the ionic permeability of the membrane. They further showed that mechanical responses could be evoked by application of ACh, excess $[K]_o$ or electrical depolarization, seemingly by different mechanisms. For example, excess $[K]_o$ depolarized the membrane, increased Ca-influx and Ca-mobilization from the store site, while ACh mainly increased Ca-mobilization without affecting the membrane property. Furthermore, after excess $[K]_o$ failed to generate the contraction in Ca-free (EGTA solu-

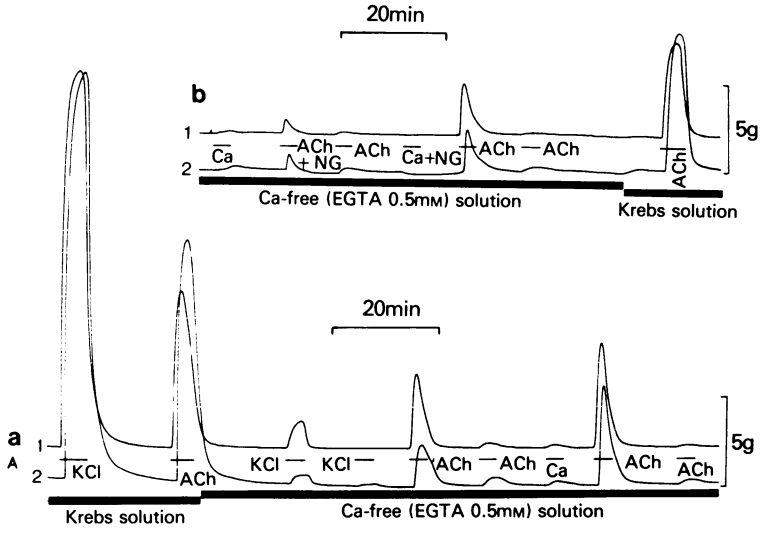


**Figure 6** Effects of Ca (2.5 mM), acetylcholine (ACh, $5 \times 10^{-6}$ M), 118 mM $[K]_o$ and nitroglycerine (NG, $5.8 \times 10^{-6}$ M) on the mechanical response in Ca-free (EGTA 0.5 mM) Krebs solution: KCl = application of 118 mM$[K]_o$; ACh = application of $5 \times 10^{-6}$ M acetylcholine; Ca = application of Krebs solution (2.5 mM Ca); NG = application of $5.6 \times 10^{-6}$ M NG with Ca or ACh. (a) and (b) are successive records and 1 and 2 show two different preparations. Bars indicate applications of agents.

tion, ACh still did so, thus suggesting a different mechanism of Ca-mobilization between chemical stimulation and depolarization (Ito *et al.*, 1979). These earlier findings helped the analysis of the action of NG in the present experiments.

NG did not modify the membrane potential or the membrane resistance but did raise the mechanical threshold. Experiments in Ca-free solution indicated that NG suppressed the mobilization of Ca from the store site, providing an explanation for the reduction in the amplitude of contraction produced by excess $[K]_o$, by ACh or by electrical depolarization. These inhibitory effects of NG were considered to be non-competitive as suggested by the nature of displacement of the stimulus-response curves. Therefore, it is concluded that NG produces a non-selective suppression of the Ca-mobilization from the store site which is induced by these different stimuli.

Needleman & Johnson (1975) discussed the action of glyceryl-trinitrate in relation to the SH group distributed on the muscle membrane, and stated that this agent as well as isoprenaline and sodium nitroprusside, inhibited the noradrenaline-induced contraction of rabbit aortic strips at a very low concentration. Therefore, these suppressions are probably not due to nonspecific sulphydryl reactivity on the surface membrane. Needleman & Johnson (1973, 1975) concluded that the vasodilatation induced by glyceryl-trinitrate is caused by a direct interaction with a specific receptor site and as such, does not necessarily require SH-oxidation as a primary event. In the present experiments, cysteine partly restored the NG-induced inhibition of the mechanical response of the pig coronary artery evoked by excess $[K]_o$, ACh or electrical depolarization. Since the mechanisms of generating the contraction by the above procedures differed, and NG inhibited the contraction non-selectively, the most probable site of the action of cysteine is considered to be the final step, the Ca-releasing site, rather than the surface membrane.

The action of vasodilators has been attributed either to the activation of adenyl cyclase or to the inhibition of phosphodiesterase. However, sodium nitrate did not alter phosphodiesterace activity in coronary vessels (Kukovitz, Poch & Juan, 1969). Furthermore, Triner, Nahas, Vulliemox, Overweg, Verosky, Habif & Ngal (1971) found that very high concentrations of glyceryl-trinitrate and sodium nitroprusside produced only a slight increase in cyclic adenosine 3',5'-monophosphate (cyclic AMP) levels in aortic strips. Thus, there is little or no compelling evidence to indicate that the vasodilatation caused by glyceryl-trinitrate is mediated by alterations in the levels of cyclic AMP.

When a sufficiently large dose of NG is administered to humans *in vivo*, a striking coronary vasodilatation occurs. Vatner & Heyndricks (1975) stated that this mechanism involves a direct dilating action on the coronary vascular smooth muscle and results in primary vasodilatation. In addition, the major com-

ponent of the coronary vasodilatation is secondary to the increased myocardial metabolic demand. Winbury *et al.* (1969) showed that NG produces a prolonged vasodilatation of the large coronary arteries yet only a slight transient dilatation of the small coronary arteries; and that with the nitrates, there is a redistribution of the coronary blood supply to areas that are ischaemic. The present experiments, demonstrated that NG nonselectively reduced the elevated muscle tone of the epicardial coronary artery produced by various procedures *in vitro*. The mechanisms causing vasoconstriction of the pig coronary artery in physiological and pathological situations are uncertain, as noradrenaline, a common vasoconstrictor, produced solely $\beta$-adrenoceptor activation (Ito *et al.*, 1979), thus resulting in relaxation of this smooth muscle.

NG reduces the tone in many smooth muscles; for example, it slightly hyperpolarized the membrane of the guinea-pig portal vein and suppressed the spike generation, thus causing relaxation of the tissue (T. Karashima, unpublished observations) and these findings are in agreement with the observations made on the guinea-pig taenia coli (Imai & Takada, 1968). Therefore, the effects of NG may depend on differences between vascular smooth muscles and regional functions; in the pig large coronary artery, NG suppresses the mechanical response with no evident change in the membrane property.

It is concluded from the present experiments that NG may act by suppressing the Ca-releasing mechanism from the store site, regardless of how it is released, rather than the Ca-influx.

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