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Does potassium induce the release of nitric oxide in the rabbit corpus cavernosum?

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Abstract We investigated the effects of increases in the extracellular potassium concentration on the function of the rabbit corpus cavernosum. The resting tissue tension increased as the potassium concentration was increased from 4.7 mM to 20 mM or 30 mM. The maximum contraction induced by 200 μM phenylephrine was significantly decreased in the presence of 30 mM potassium compared with 4.7 mM potassium. After precontraction was induced with 200 μM phenylephrine, the magnitude of field-stimulated relaxation increased significantly as the potassium concentration was increased from 4.7 mM to 10 or 20 mM, but was almost completely abolished at 30 mM potassium. There was no difference in the suppressive effect of L-NAME on field-stimulated relaxation between specimens treated with 4.7 mM or 20 mM potassium. ATP- and bethanechol-induced relaxation was not affected by increases in the extracellular potassium concentration. A high-dose potassium solution (124 mM) induced contraction of the corpus cavernosum. In tissue precontracted with phenylephrine, a high-dose potassium solution that contained phenylephrine induced relaxation of corpus cavernosum; this relaxation was completely suppressed by L-NAME. These findings suggest that small increases in the extracellular potassium concentration increase field-stimulated relaxation of the corpus cavernosum and that this relaxation is not related to the effects of nitric oxide. Relaxation induced by high-dose potassium in tissue precontracted with phenylephrine is probably the result of release of nitric oxide.

Key words Rabbit corpus cavernosum · Calcium · Potassium · Impotence · Nitric oxide

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Introduction

Erectile function is mediated by the interaction of several types of neurotransmission, including the local production and release of nitric oxide [3, 11, 14, 15, 18, 19]. Contraction, which results in the detumescence of the corpus cavernosum, is primarily mediated by alpha-adrenergic stimulation [3, 14, 19]. Erection is initiated and sustained by relaxation of the corpus cavernosum, which is mediated primarily by nitric oxide [11, 15, 18]. Nitric oxide is released directly from nerve terminals and from cholinergic-activated endothelial cells.

Calcium channel blockers and potassium channel openers, which modulate the function of ion channels on the smooth muscle cell membrane, can alter the function of the corpus cavernosum [1]. Potassium channel openers reduce the tissue tension or contractile force in response to stimulation of the corpus cavernosum [1]. Injection of a potassium channel opener hyperpolarizes the cell membrane, inducing tumescence or erection [7]. An increase in the extracellular potassium concentration induces depolarization. An increase in the extracellular potassium concentration can also shift the ionic equilibrium in the plasma membrane and the membrane of sarcoplasmic reticulum [2, 4, 16]. Recently, it has been reported that the maxi-K channel regulated by both cell membrane potential and intracellular calcium concentration is an important modulator of corporal smooth muscle tone [6, 22, 23].

We investigated the effects of increases in the extracellular potassium concentration on the function of the corpus cavernosum in rabbits.

Materials and methods

Male Japanese white rabbits ($n = 14$) weighing 3 kg obtained from Chubu Kagaku Co (Aichi Japan) were sedated with intramuscular injections of ketamine/xylazine (25 mg ketamine/kg, 6 mg xylazine/kg). Anesthesia was maintained by intravenous injections of sodium pentobarbital (25 mg/kg). The penis was

removed at the point at which the corporal body attaches to the ischium, and the grossly dissected organ preparation placed in Krebs' solution at room temperature. Rabbits were killed by intravenous air injection. Most of the overlying striated muscle was then removed, taking care not to damage the underlying tunica albuginea. Once the tunica was fully exposed, a slit was made in the proximal end and extended distally. The corpus cavernosal tissue was bilaterally dissected free of the tunica. Two tissue strips were obtained from each rabbit. This study followed "standards relating to the arc and management, etc. of experimental animals (notification No. 6 March 27, 1980 of the Prime Minister's office)" and "Law concerning the protection and control of animals (Law No. 105 October 1, 1973)".

Longitudinal sections of the rabbit corpus cavernosum with an unstretched length of about 8 mm were placed in organ baths containing 10 ml of Krebs' solution (NaCl 119 mM, KCl 4.7 mM, $NaHCO_3$ 25 mM, $MgSO_4$ 1.2 mM, NH_2PO_4 1.2 mM, $CaCl_2$ 2.5 mM, and glucose 11 mM) at 37°C. Tissues were equilibrated with a mixture of 95% oxygen and 5% carbon dioxide. One end of each strip was connected to a force displacement transducer, and changes in muscle tension were measured and recorded on a Rectigraph 8K (San-ei, Tokyo, Japan)

Following 1 h of equilibration, the tissue tension was adjusted to approximately 2 g. We measured the increases in tension in response to a low (2 μM), intermediate (20 μM) or high (200 μM) dose of phenylephrine. We examined the relaxation effects of field stimulation, ATP, and bethanechol in tissues precontracted with 200 μM of phenylephrine. Field stimulation (2–60 Hz) and maximal doses of ATP (2 mM) and bethanechol (600 μM) were administered after phenylephrine-induced contraction reached a plateau.

Field stimulation was delivered via platinum electrodes applied to both sides of muscle strips suspended in organ baths. Transmural nerve stimulation was performed with a DPS-160 field stimulator (Dia Medical System, Tokyo, Japan) that delivered 50-V biphasic square-wave pulses of 0.5 ms duration. The polarity of each pulse was changed using a polarity changing unit. Stimulation was delivered at 2-min intervals. A preliminary study showed that the maximum relaxation induced by field stimulation was completely blocked by 10^{-6} M tetrodotoxin.

The incubation medium was then replaced with 10 mM of potassium-containing Krebs' solution. To maintain the same osmolality, this solution was prepared by replacing the sodium with an equimolar amount of potassium. After tissues had been incubated for 30 min in this solution, we measured the contraction induced by three doses of phenylephrine and the relaxation induced by field stimulation, ATP, and bethanechol. The procedure was repeated in the presence of 20 and 30 mM potassium.

To investigate the mechanism of the effect of increases in potassium on the relaxation induced by field stimulation, we evaluated the suppressive effect of L-NAME NG-monometyl L-arginine (10^{-4} M) on relaxation in the presence of 4.7 mM and 20 mM potassium. We measured relaxation of the corpus cavernosum in response to field stimulation before and after 30 min exposure to 10^{-4} M L-NAME.

To avoid the effect of tissue fatigue, we examined the effects of different concentrations of potassium (4.7 mM, 10 mM, 20 mM, and 30 mM) in a random order. The basal tissue tension was equal to the resting tension at the end of the 30-min incubation in each solution.

We also examined the effect of a high concentration of potassium (124 mM) on the corpus cavernosum. After tissue specimens had been incubated in normal Krebs' solution containing 4.7 mM potassium without precontraction, the incubation medium was replaced with a high potassium solution prepared by replacing the sodium in the Krebs' solution with 119 mM potassium. The same procedure was then performed with tissues precontracted with 200 μM phenylephrine.

Drugs and data analysis

ATP, phenylephrine, L-NAME, and bethanechol were purchased from Sigma (St. Louis, Mo.). The resting tension and increases in tension in response to phenylephrine are expressed in absolute grams. Relaxation is expressed as the percent relaxation of the total tonic tension (basal tissue tension plus increased tension in response to 200 μM phenylephrine). Data were assessed by analysis of variance with Fisher's protected least significant difference. A P value < 0.05 was accepted as statistically significant.

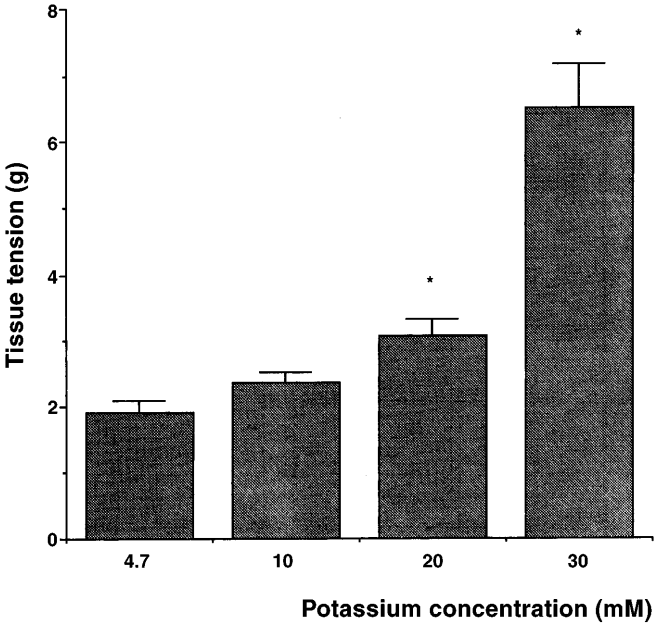

Fig. 1 Effect of increases in the potassium concentration on the basal tension of the isolated corpus cavernosum. *Bars* represent the mean ± SEM of six duplicate observations. * Significantly different from the response at normal potassium level, $P < 0.05$

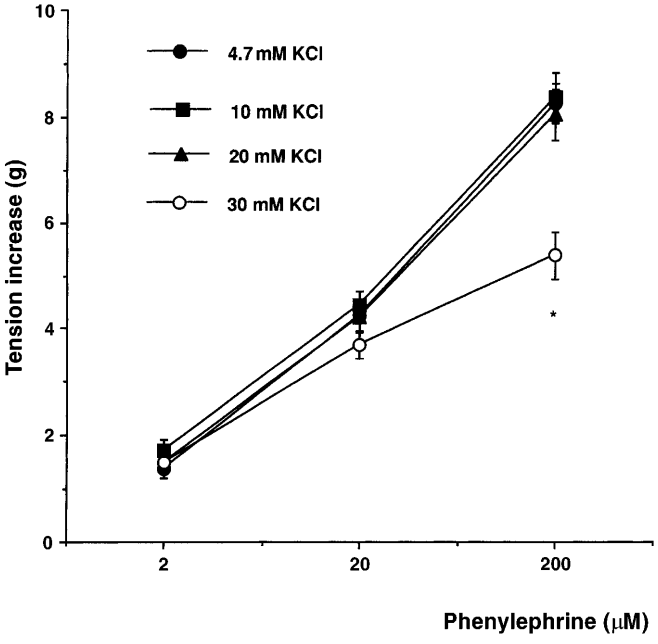

Fig. 2 Effect of alterations in the potassium concentration on the response to low-, intermediate-, and high-dose phenylephrine. *Points* represent the mean ± SEM of six duplicate observations. * Significantly different from the response at normal potassium level, $P < 0.05$

Results

The basal tissue tension increased significantly as the potassium concentration was increased from 4.7 mM to 20 mM or 30 mM (Fig. 1). Increasing the potassium concentration from 4.7 mM to 10 mM or 20 mM had no significant effect on the contractile response to phenylephrine. The maximum contraction induced by 200 μM phenylephrine in the presence of 30 mM potassium was significantly smaller than the contraction induced in the presence of 4.7 mM potassium (Fig. 2). There was no difference in the total tonic tension (basal tissue tension plus the tension increase induced by 200 μM phenylephrine) according to the potassium concentrations: 4.7 mM KCl, 10.1 ± 0.45 g; 10 mM KCl, 10.7 ± 0.52 g, 20 mM KCl, 11.1 ± 0.54 g,

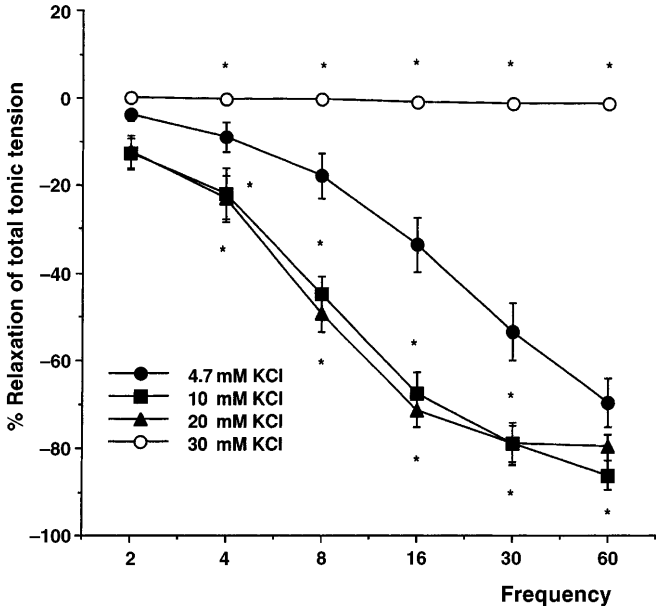

Fig. 3 Effect of alterations in the potassium concentration on the response to field stimulation. *Points* represent the mean ± SEM of six duplicate observations. * Significantly different from the response at normal potassium level, $P < 0.05$

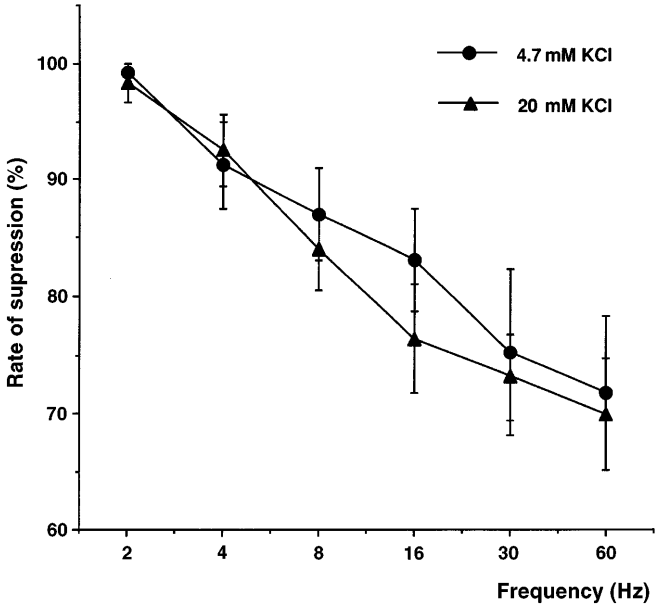

Fig. 4 Effect of 10^{-4} M L-NAME on field-stimulated relaxation in the presence of 4.7 mM and 20 mM potassium. *Points* represent the mean ± SEM of six individual observations

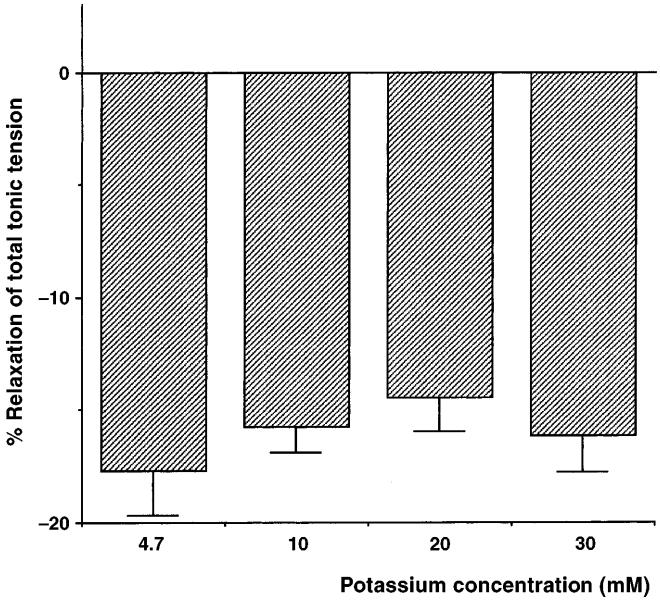

Fig. 5 Effect of alterations in the potassium concentration on ATP-induced relaxation. *Bars* represent the mean ± SEM of six individual observations. * Significantly different from the response at normal potassium level, $P < 0.05$

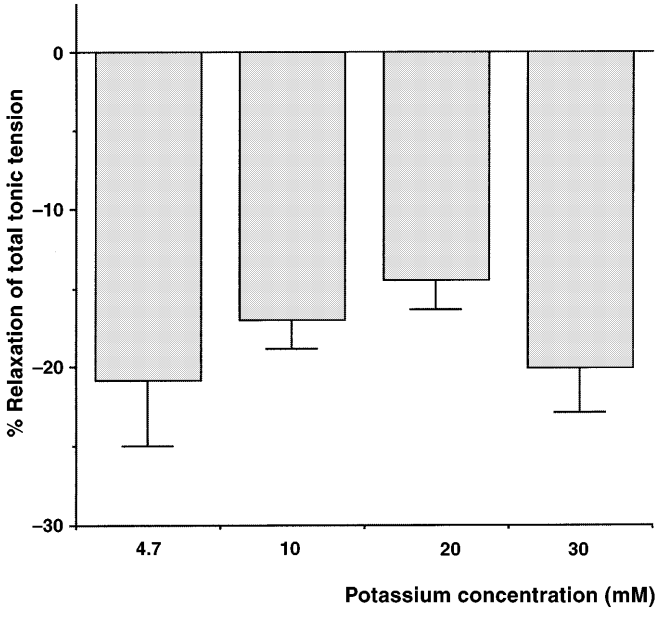

Fig. 6 Effect of alterations in the potassium concentration on bethanechol-induced relaxation. *Bars* represent the mean SEM of six individual observations. * Significantly different from the response at normal potassium level, $P < 0.05$

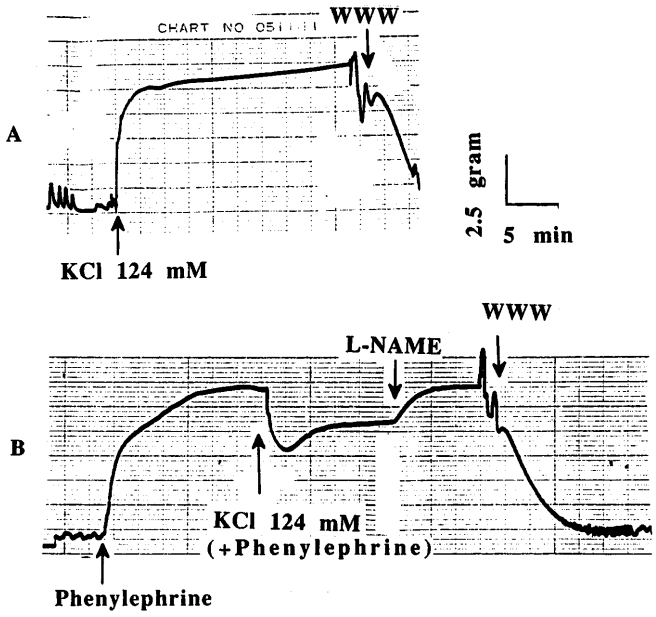

Fig. 7 Representative tracings of the tension changes induced by a high-dose potassium solution. **A** Without precontraction: the incubation medium was changed from a normal Krebs' solution to a high-dose potassium solution (124 mM). **B** With precontraction induced by 200 μM phenylephrine: the incubation medium was replaced with a high-dose potassium solution containing 200 μM phenylephrine followed by administration of 10^{-4} M L-NAME. WWW: three time wash with a normal Krebs' solution.

30 mM KCl, 11.2 ± 0.50 g. Following precontraction with 200 μM phenylephrine, the magnitude of field-stimulated relaxation increased as the potassium concentration was increased from 4.7 mM to 10 mM or 20 mM, but was almost completely abolished in the presence of 30 mM potassium (Fig 3). There was no difference in the suppressive effect of L-NAME on field-simulated relaxation in the presence of 4.7 mM and 20 mM potassium (Fig. 4). ATP- and bethanechol-induced relaxation were not altered by increases in the potassium concentration (Figs. 5, 6). A high-dose potassium solution (124 mM) alone induced contraction of the corpus cavernosum (Fig. 7A). However, in tissue precontracted with 200 μM phenylephrine, the relaxation of the corpus cavernosum was induced by a high-dose potassium solution containing 200 μM phenylephrine. The relaxation induced by the high-dose potassium solution was completely suppressed by L-NAME (Fig. 7B).

Discussion

Multiple biologic systems are involved in the maintenance of the ionic gradient and the transport of ion across intracellular and extracellular spaces for normal cellular function [2, 4, 9, 10, 16, 20, 22]. Among these systems are ion channels, which open in response to stimulation, and ion pumps, which maintain the ionic gradient in the resting condition. The distribution of these channels and pumps differs among different types of tissue. The neuronal and smooth muscle cells involved in contraction and relaxation of the corpus cavernosum are also controlled by these systems. Drugs acting on ion channels, such as potassium channel openers and calcium channel blockers, have favorable effects on erectile function [1]. The intracavernous injection of potassium channel openers induces tumescence or erection in monkeys and cats [7, 8]. Christ et al. [5] reported that papaverine and nifedipine had synergic effects on isolated human erectile tissue.

Potassium channel openers induce hyperpolarization of the smooth muscle cell membrane. Potassium primarily increases the tonus of smooth muscle by depolarizing the muscle cell membrane, resulting in an increase in the intracellular free calcium concentration. In the present study, tissue tension increased as extracellular potassium concentration was increased, which is consistent with the results of a previous study showing that a potassium channel opener reduced corporal tension [1].

Under precontraction with phenylephrine an increase in the extracellular potassium concentration from 4.7 mM to 10 mM or 20 mM significantly increased field-stimulated relaxation in the present study. The maxi-K channel regulated by both cell membrane potential and concentration of intracellular calcium, has recently been reported to be an important modulator of vascular smooth muscle tone [6, 22, 23]. Evidence in the literature supports the idea that nitric oxide (NO) may relax smooth muscle through the maxi-K channel [6, 22, 23]. An increase in extracellular potassium concentration induces depolarization of the cell membrane. Since the open probability for the maxi-K channel increases as cell membrane depolarizes, in the presence of 10 mM or 20 mM potassium NO released at field stimulation may relax corporal smooth muscle to a greater degree through an enhanced maxi-K channel. However, in this study there was no difference in the effect of L-NAME on field-stimulated relaxation in the presence of 4.7 mM or 20 mM of potassium. One can speculate that although NO plays a role in increasing relaxation of the corpus cavernosum in the presence of high potassium, L-NAME might equalize it.

In the presence of 30 mM potassium, the response to field stimulation was almost completely abolished. This finding is consistent with the results of a study using rat bladders, in which 24.7 mM potassium impaired nerve-mediated detrusor contraction [20, 21]. Since the relaxation induced by direct receptor activation with ATP or bethanechol was not altered by 30 mM potassium, the decreased field-stimulated relaxation in the presence of 30 mM potassium was probably induced by a disturbance in nerve function. When a nerve fires, its action potential is induced via the depolarization initiated by the opening of a sodium channel, and subsequently by a calcium channel, a potassium channel, and then chloride

channel [9]. Since tetrodotoxin, a potent sodium channel blocker, completely inhibits nerve-mediated responses, sodium channels are clearly indispensable to nerve function [9]. Patients with familial hyperkalemic periodic paralysis exhibit increases in the extracellular potassium concentration. An increase in the serum concentration of potassium disturbs the sodium channels in the skeletal muscle in these patients [13], leading to a loss of muscle tonus [12]. Local anesthetics, which are also sodium channel blockers, act by depolarizing the membrane potential [17]. These findings suggest that excessive extracellular potassium can deactivate sodium channels on the nerve.

A high-dose potassium solution relaxed the corpus cavernosum in tissues precontracted with 200 μM phenylephrine in the present study. High-dose potassium alone induced contraction of the corpus cavernosum by depolarizing the tissue membrane. This paradoxical phenomenon is very interesting. The potassium-induced relaxation was blocked by L-NAME, suggesting that NO release was involved. One hypothesis, which involves interplay of voltage-dependent calcium channels and maxi-K channels, would explain the potassium-induced tissue relaxation. A depolarization of cell membrane induced by a high dose of potassium led to opening of both voltage-dependent calcium channels and maxi-K channels. If it is assumed that there is predominance of opening of voltage-dependent calcium channels over maxi-K channels, this would lead to tissue contraction (Fig. 7A). In tissue precontracted with phenylephrine, intracellular calcium is high. In this case depolarization induced by a high dose of potassium led to predominance of opening of maxi-K channels over voltage-dependent calcium channels, which may lead to tissue relaxation (Fig. 7B). NO is released from nerve terminals and the endothelium [11, 15, 18]. At erection, NO from both these sources contributes to relaxation of the corpus cavernosum. Because nerve function was disturbed by excessive potassium (>30 mM), NO was probably released from the endothelium during relaxation induced by high-dose potassium.

In conclusion, a small increase in the extracellular potassium concentration (up to 20 mM) enhanced nerve-mediated relaxation of the corpus cavernosum. However, 30 mM potassium disturbed neuronal function, resulting in a decrease in field-stimulated relaxation. Although a high-dose potassium solution (124 mM) alone induced contraction of the corpus cavernosum, it induced relaxation when tissues were precontracted with an alpha stimulator. This high-dose potassium-induced relaxation appeared to have been mediated by release of endothelial nitric oxide.

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