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European Journal of Pharmacology 273 (1995) 153–159

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The inhibitory mechanisms of nicorandil in isolated rat urinary bladder and femoral artery

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Received 21 July 1994; revised MS received 12 October 1994; accepted 4 November 1994

Abstract

Nicorandil or cromakalim inhibited contractile responses to acetylcholine and KCl in detrusor muscles of rat urinary bladder, whereas nitroglycerin inhibited only the responses to acetylcholine. In the detrusor muscles contracted by electrical stimulations, relaxations caused by nicorandil and cromakalim were inhibited by glyburide, but not by nitroglycerin or apamin. Methylene blue slightly potentiated the nicorandil-relaxation without affecting the cromakalim-relaxation. N^G-Monomethyl-L-arginine also did not affect the relaxation induced by nicorandil. The level of cGMP was increased by both nicorandil and nitroglycerin. In rat femoral arteries contracted by phenylephrine, the relaxation induced by nicorandil was inhibited by methylene blue, glyburide and apamin. The relaxation induced by cromakalim was inhibited by glyburide, but not by apamin or methylene blue. These results suggest that the effect of nicorandil is due to activation of K_{ATP} channels in rat detrusor muscles and is due to the activation of guanylate cyclase, K_{ATP} and K_{Ca} channels in rat femoral arteries. The effect of cromakalim is due to the activation of K_{ATP} channels in both smooth muscles.

Keywords: Nicorandil; Cromakalim; cGMP; Detrusor muscle, rat; Femoral artery, rat; K^+ channel

1. Introduction

Nicorandil, an anti-anginal agent with a potent vasodilating action (Kato et al., 1987; Kishida and Murao, 1987; Yokota and Shibata, 1990), is known to hyperpolarize the cell membrane in vascular smooth muscle through the activation of ATP-dependent potassium (K_{ATP}) channels (Takata and Kuriyama, 1980; Karashima et al., 1982). It was also shown that the nitro group in its structure enables nicorandil to activate soluble guanylate cyclase, thereby increasing the cGMP level in the smooth muscle (Endoh and Taira, 1983; Inoue et al., 1984; Schmidt et al., 1985; Murakami et al., 1987; Meisheri et al., 1991; Holtzmann et al., 1992) and also leading to relaxation. These reports, thus, indicate that nicorandil relaxes vascular smooth muscle by two independently operating mechanisms, hyperpolarization through opening K_{ATP} channels and stimulation of guanyl cyclase, leading to increased cGMP levels, depending on the experimental system as to which of the two predominates. It was recently found that nicorandil at a low concentration (50 nM) causes vasorelaxation predominantly by hyperpolarization, whereas the effect on cGMP becomes predominant at higher concentrations (Kukovetz et al., 1991).

It has been reported that cromakalim, a K-channel opener, increased the potassium permeability and hyperpolarized the cell membrane in the detrusor muscle of human, pig and guinea-pig urinary bladder (Foster et al., 1989; Fujii et al., 1990), consistent with its repolarized action in other smooth muscles. The action of cromakalim in the bladder was inhibited by glyburide, a specific blocker of K_{ATP} channels, suggesting that the relaxing effect of cromakalim is due to hyperpolarization through opening K_{ATP} channels (Fujii et al., 1990). In addition, studies in isolated human detrusor muscle (Foster et al., 1989) and bladder tissues from several animal species have shown that K^+ channel openers reduce not only spontaneous contractions, but also contractions induced by electrical stimulation, carbachol, and K^+ at low concentrations (Foster et al., 1989; Edwards et al., 1991). Recently, clinical applications of the K^+ channel openers have been proposed

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Elsevier Science B.V.
SSDI 0014-2999(94)00685-7

in the treatment of urinary bladder instability which is secondary to bladder outflow obstruction (Andersson et al., 1988; Quast, 1992; Andersson, 1992; Malmgren et al., 1990).

The characterization of the pharmacological action of nicorandil is not clarified yet in the smooth muscle of urinary bladder. We have recently found that nicorandil inhibited the contractions induced by electrical stimulation and acetylcholine in the smooth muscle of rat bladder. Therefore, the present experiment was undertaken to determine which type of inhibitory mechanisms is involved in the relaxing effect of nicorandil, such as hyperpolarization through the opening of K^+ channels or the stimulation of guanylate cyclase leading to an increased cellular level of cGMP. Cromakalim was used as a standard K_{ATP}-channel opener. The effects of nicorandil on the rat urinary bladder and femoral arteries were also compared.

2. Materials and methods

2.1. Mechanical response

Adult male Wistar rats weighing 200–300 g were killed by cervical dislocation under ether anesthesia. Rat urinary bladders and femoral arteries were isolated, and excess fat and connective tissue were removed. The urinary bladder above the ureteral orifices was isolated and horizontal detrusor strips of about 2 mm wide and 5 mm long were obtained from the middle region of the bladder body. The femoral artery was cut into helical strips (about 1 mm wide and 15 mm long). Endothelium was removed by rubbing the helical strips on a filter paper moistened with Krebs solution. Preparations were mounted in organ baths containing 20 ml of Krebs solution of the following compositions (mM): NaCl, 120.3; KCl, 4.8; $CaCl_2$, 2.2; $MgSO_4 \cdot 7H_2O$, 1.3; KH_2PO_4, 1.2; $NaHCO_3$, 24.2; glucose 5.8 at pH 7.4. $CaCl_2$ was omitted from this solution to make Ca^{2+}-free medium. The tissue bath solution was maintained at 37°C and bubbled with a 95% O_2 and 5% CO_2 mixture. Ligatures were placed around both ends of the muscle strips, one attaching the muscle to a metal holder and the other to a transducer. In some experiments, the detrusor muscle strip was placed between two platinum wires and stimulated at 1 ms duration, one-seventh Hz, and supramaximal voltage. The absence of endothelium was determined by the absence of acetylcholine (3×10^{-6} M) relaxation on the artery contracted by phenylephrine (3×10^{-5} M). The strips were preloaded with a tension of 1 g for the detrusor muscles and 0.5 g for the femoral arteries. Isometric tension changes were recorded through force-displacement transducers (Grass FT-03) connected to a 6-channel Grass polygraph.

2.2. Measurement of cyclic GMP levels

The detrusor strips of rat bladder were equilibrated in Krebs solution bubbled with 95% O_2 and 5% CO_2 for 1 h. Some tissues were, then, incubated with nicorandil (3×10^{-3} M) or nitroglycerin (3×10^{-5} M) for 5 min and quickly frozen in liquid nitrogen. Frozen tissues were homogenized in 6% of ice-cold trichloroacetic acid and centrifuged at $1600 \times g$ for 20 min at 4°C. The supernatant was extracted with 3 volumes of water-saturated ether and pH was adjusted to 7.5. The level of cyclic GMP was measured in duplicate by a radioimmunoassay kit (Amersham Corporation, Arlington Heights, IL, USA) and radioactivity was counted in a scintillation counter (Beckman, LS 6000SE).

2.3. Chemicals

The following drugs were used: acetylcholine (Sigma Chemical Co., St. Louis, MO, USA), apamin (Sigma), cromakalim (Beecham Pharmaceuticals, UK), glyburide (Upjohn Co., Kalamazoo, MI, USA), methylene blue (Fisher Scientific Co., Fair Lawn, NJ, USA), nicorandil (Chugai Pharmaceutical Co., Japan), N^G-monomethyl-L-arginine (Calbiochem Corp., La Jolla, CA, USA), nitroglycerin (Warner-Lambert, Morris Plain, NJ, USA), phenylephrine (Sigma).

2.4. Statistical analysis

The data were presented as the means ± S.E.M. and were analyzed statistically by using Student's *t*-test. ED_{50} values (concentrations which cause 50% of the maximal response) are obtained directly from the graph.

3. Results

3.1. Rat detrusor muscles

In rat detrusor muscles, KCl (5–100 mM) and acetylcholine (10^{-8} to 10^{-3} M) induced contractile responses in a concentration-dependent manner (Fig. 1A and B). Pretreatment with nicorandil (10^{-4} M) or cromakalim (10^{-6} M), but not nitroglycerin (10^{-5} M), partially inhibited the contractile responses to KCl (5–100 mM) (Fig. 1A). Specifically, cromakalim (10^{-6} M) significantly inhibited the responses to KCl at 20 mM and 40 mM without affecting those to higher concentrations (70 mM and 100 mM) of KCl. Nicorandil (10^{-4} M), however, inhibited the KCl responses at all the concentrations used (Fig. 1A). ED_{50} value for KCl was also significantly increased by cromakalim (10^{-6} M) (control: 32.2 ± 2.20 mM, cromakalim: 45.0 ± 1.91 mM), but not by nicorandil (10^{-4} M) (39.8 ± 4.26 mM) or nitroglycerin (10^{-5} M) (38.7 ± 4.10 mM).

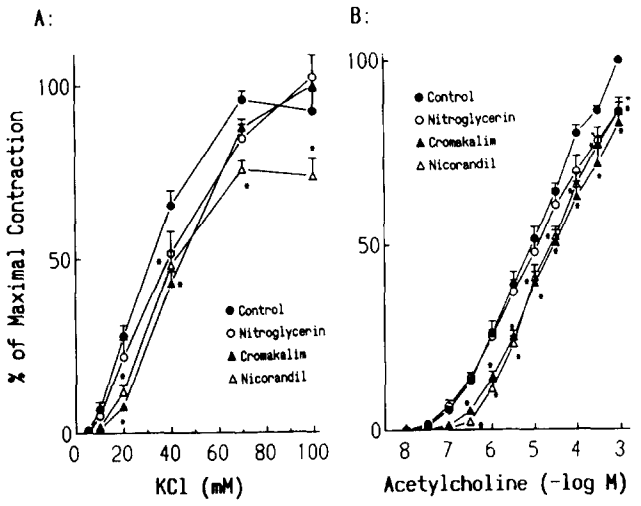

Fig. 1. Inhibitory effects of nicorandil, cromakalim and nitroglycerin on contractile responses to KCl and acetylcholine in rat detrusor muscles. Rat detrusor muscles were contracted by (A) KCl (5–100 mM) ($n = 7$) or (B) acetylcholine (10^{-8} to 10^{-3} M) ($n = 7$). In some experiments, nitroglycerin (10^{-5} M) (A: $n = 6$, B: $n = 7$), cromakalim (10^{-6} M) (A: $n = 6$, B: $n = 7$) or nicorandil (10^{-4} M) (A: $n = 5$, B: $n = 7$) was added to the bath 20 min before the addition of KCl or acetylcholine. The maximal contractions induced by KCl (100 mM: 4.9 ± 0.40 g) or acetylcholine (10^{-3} M: 7.3 ± 0.62 g) in the absence of inhibitors were taken as 100%. Data are expressed as the means ± S.E.M. * Significantly different from respective controls ($P < 0.05$).

Pretreatment with nicorandil (10^{-4} M), cromakalim (10^{-6} M) or nitroglycerin (10^{-5} M) also partially inhibited the contractile responses to acetylcholine (10^{-8} to 10^{-3} M) (Fig. 1B). Specifically, nicorandil (10^{-4} M) and cromakalim (10^{-6} M) shifted the concentration-response curve for acetylcholine to the right (control ED_{50}: $0.93 \pm 0.24 \times 10^{-5}$ M, nicorandil: $2.76 \pm 0.64 \times 10^{-5}$ M, cromakalim: $4.34 \pm 0.21 \times 10^{-5}$ M). Nitroglycerin (10^{-5} M), however, only inhibited the responses to acetylcholine at 3×10^{-4} M and 10^{-3} M without significantly affecting the ED_{50} value ($2.25 \pm 0.93 \times 10^{-5}$ M) (Fig. 1B).

In rat detrusor muscles, electrical stimulations (1 ms, one- seventh Hz at supramaximal voltage) induced contractile responses. Nicorandil (3×10^{-6} to 3×10^{-3} M) relaxed the responses to the electrical stimulations in a concentration-dependent manner (Fig. 2, Fig. 3A and B). Pretreatment of the tissues with glyburide (10^{-6} M) inhibited the relaxing responses to nicorandil (control ED_{50}: $3.81 \pm 0.64 \times 10^{-4}$ M, glyburide: $1.75 \pm 0.50 \times 10^{-3}$ M) (3×10^{-6} to 3×10^{-3} M) (Fig. 3A), while pretreatment with methylene blue (10^{-6} M) slightly potentiated the relaxation (control ED_{50}: $4.38 \pm 0.77 \times 10^{-4}$ M, methylene blue: $1.61 \pm 0.47 \times 10^{-4}$ M) (Fig. 3B). The relaxing responses to nicorandil, however, were not significantly affected by pretreatment with apamin (10^{-6} M) (Fig. 3A), nitroglycerin (3×10^{-5} M), or N^G-monomethyl-L-arginine (NMMA: 10^{-4} M) (Fig. 3B).

In rat detrusor muscles, cromakalim (3×10^{-8} to 3×10^{-5} M) also induced relaxation of the responses to the electrical stimulation in a concentration-dependent manner (Fig. 2, Fig. 4A and B). The relaxing responses were inhibited by pretreatment of the tissues with glyburide (10^{-6} M), but not with apamin (10^{-6} M) (Fig. 4A). Nitroglycerin (3×10^{-5} M) or methylene blue (10^{-6} M) also had no significant effect on the relaxation (Fig. 4B). In addition, glyburide (10^{-6} M)

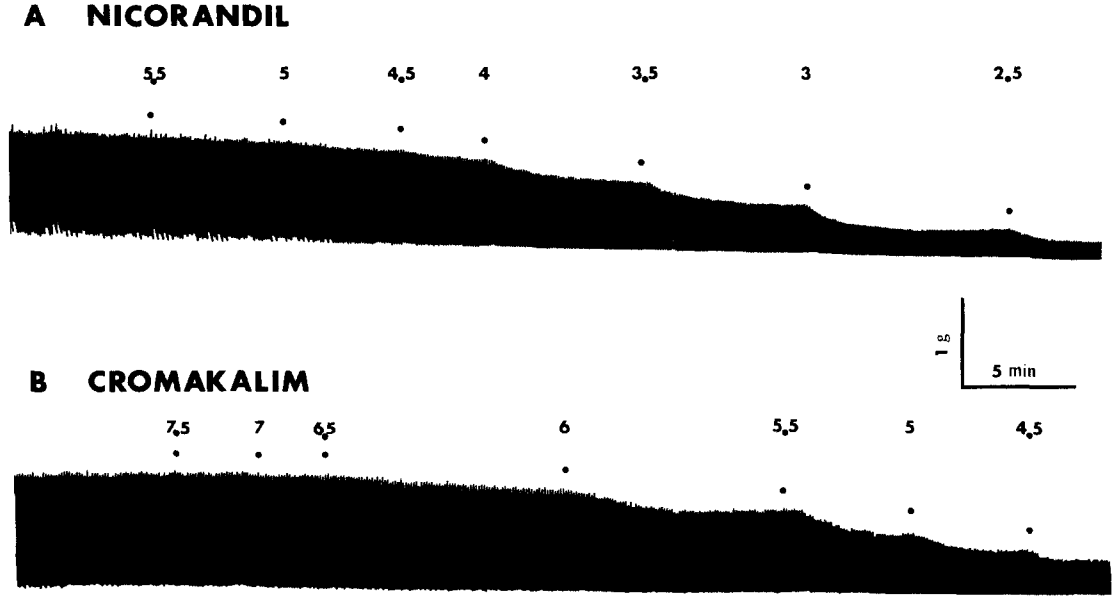

Fig. 2. Typical recordings showing (A) nicorandil and (B) cromakalim relaxations in rat bladder detrusor muscles. Rat detrusor muscles were repeatedly contracted by electrical stimulations (1 ms duration, one-seventh Hz at supramaximal voltage). Concentrations (M) are expressed in negative logarithms.

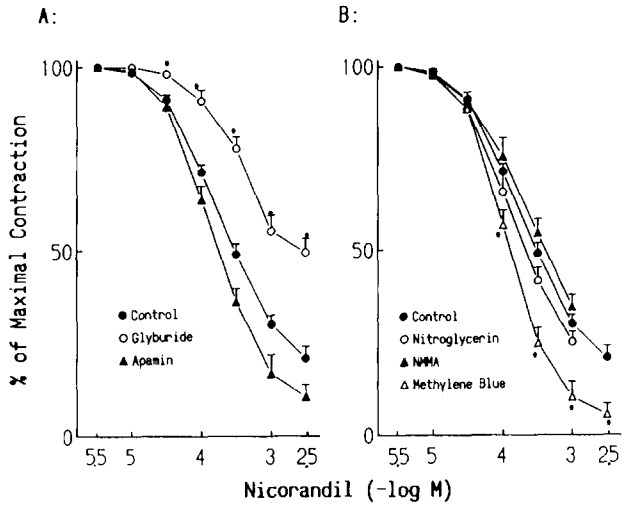

Fig. 3. Inhibitory effects of various agents on the relaxations induced by nicorandil in rat detrusor muscles. Rat detrusor muscles were repeatedly contracted by the electrical stimulations (1 ms, one-seventh Hz, 90 V). Nicorandil (3×10^{-6} to 3×10^{-3} M) (A: $n=18$, B: $n=14$) was added cumulatively to induce relaxations. In some experiments, (A) glyburide (10^{-6} M) ($n=10$), apamin (10^{-6} M) ($n=5$), (B) nitroglycerin (3×10^{-5} M) ($n=6$), NMMA (10^{-4} M) ($n=8$) or methylene blue (10^{-6} M) ($n=5$) was added to the bath 20 min before the addition of nicorandil. Maximal contractions induced by the stimulation before the addition of nicorandil were expressed as 100% (A: 1.0 ± 0.06 g, B: 0.9 ± 0.05 g). Data are expressed as the means ± S.E.M. * Significantly different from respective controls ($P<0.05$).

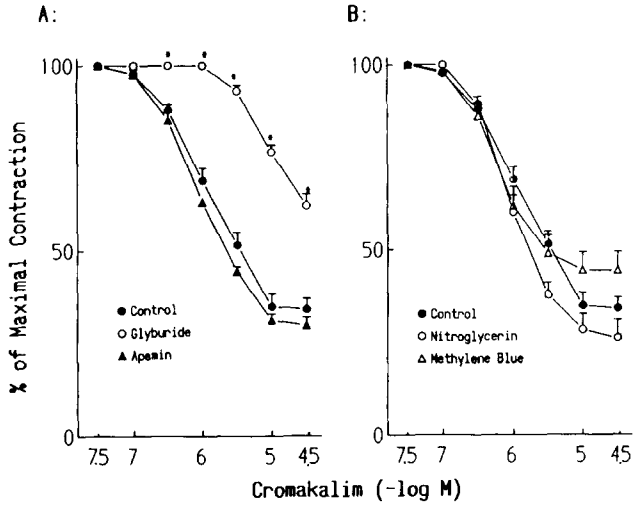

Fig. 4. Inhibitory effects of various agents on the relaxations induced by cromakalim in rat detrusor muscles. Rat detrusor muscles were repeatedly contracted by the electrical stimulations (1 ms, one-seventh Hz, 90 V). Cromakalim (3×10^{-8} to 3×10^{-5} M) (A: $n=9$, B: $n=4$) was added cumulatively to induce relaxations. In some experiments, (A) glyburide (10^{-6} M) ($n=4$), apamin (10^{-6} M) ($n=5$), (B) nitroglycerin (3×10^{-5} M) ($n=6$), or methylene blue (10^{-6} M) ($n=4$) was added to the bath 20 min before the addition of cromakalim. Maximal contractions induced by the stimulation before the addition of nicorandil were expressed as 100% (A: 1.0 ± 0.08 g, B: 1.2 ± 0.09 g). Data are expressed as the means ± S.E.M. * Significantly different from respective controls ($P<0.05$).

and methylene blue (10^{-6} M) by themselves had no effect on the responses to electrical stimulations. However, apamin (10^{-6} M) ($117.4\pm2.93\%$ of the control without the inhibitor, $n=10$) increased and nitroglycerin (3×10^{-5} M) ($93.0\pm0.93\%$, $n=12$) decreased the magnitude of the responses to electrical stimulations.

The effects of nicorandil and nitroglycerin on the level of cGMP in rat detrusor muscles were also examined. Both nicorandil (3×10^{-3} M) and nitroglycerin (3×10^{-5} M) significantly increased the cGMP levels (0.52 ± 0.020 pmol/mg wet tissue, $n=4$ and 0.59 ± 0.060 pmol/mg wet tissue, $n=6$, respectively) as compared to the control (0.35 ± 0.048 pmol/mg wet tissue, $n=10$).

3.2. Rat femoral arteries

In rat femoral arteries, nicorandil (10^{-7} to 10^{-4} M) and cromakalim (10^{-8} to 3×10^{-6} M) induced relaxations of the tissues precontracted by phenylephrine (3×10^{-5} M) (Fig. 5A, B and 6A, B). Pretreatment of the tissues with apamin (10^{-6} M) slightly inhibited the relaxations caused by low concentrations (3×10^{-7} to 10^{-5} M) of nicorandil and increased the ED_{50} value for nicorandil (control: $3.62\pm0.36\times10^{-6}$ M, apamin: $6.64\pm0.96\times10^{-6}$ M) (Fig. 5A), whereas glyburide (10^{-6} M) inhibited the relaxations caused by high concentrations (3×10^{-5} M and 10^{-4} M) of nicorandil without significantly affecting the ED_{50} value ($4.94\pm1.16\times10^{-6}$ M) (Fig. 5A). Methylene blue (10^{-6} M) inhibited the relaxations induced by nicorandil at all concentrations (control ED_{50}: $1.94\pm0.31\times10^{-6}$ M, methylene blue: $1.17\pm0.38\times10^{-5}$ M) (Fig. 5B). Glyburide (10^{-6} M), but not apamin (10^{-6} M), also significantly inhibited the relaxing responses to cromakalim (10^{-7} to 3×10^{-5} M) (Fig. 6A). The ED_{50} value for cromakalim was significantly increased by glyburide (10^{-6} M) but not by apamin (10^{-6} M) (control: $2.62\pm0.18\times10^{-7}$ M, glyburide: $6.75\pm0.90\times10^{-6}$ M, apamin: $2.70\pm0.31\times10^{-7}$ M). Methylene blue (10^{-6} M) (Fig. 6B) had no significant effect on the relaxation induced by cromakalim (10^{-8} to 3×10^{-6} M) (control ED_{50}: $1.91\pm0.69\times10^{-7}$ M, methylene blue: $3.55\pm0.51\times10^{-7}$ M).

4. Discussion

In the present study, both nicorandil and cromakalim inhibited contractile responses to acetylcholine and KCl in rat detrusor muscles. Since cromakalim inhibited the contractions induced by low concentrations (< 40 mM) of KCl, but not by high concentrations (> 70 mM) of KCl, the inhibitory effect of cromakalim may be solely due to K^+-channel open-

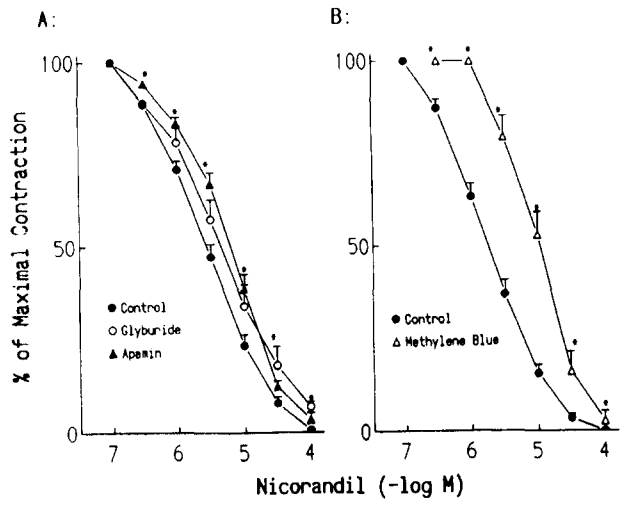

Fig. 5. The effects of various inhibitors on the relaxations induced by nicorandil in rat femoral arteries. Rat femoral arteries were contracted by phenylephrine (3×10^{-5} M). Nicorandil (10^{-7} to 10^{-4} M) (A: $n = 8$, B; $n = 6$) was added cumulatively to induce relaxations. In some experiments, (A) glyburide (10^{-6} M) ($n = 4$), apamin (10^{-6} M) ($n = 4$) or (B) methylene blue (10^{-6} M) ($n = 5$) was added to the bath 20 min before the addition of nicorandil. Maximal contractions induced by phenylephrine (3×10^{-5} M) before the addition of nicorandil were expressed as 100% (A: 0.16 ± 0.02 g, B: 0.19 ± 0.03 g). Data are expressed as the means ± S.E.M. * Significantly different from respective controls ($P < 0.05$).

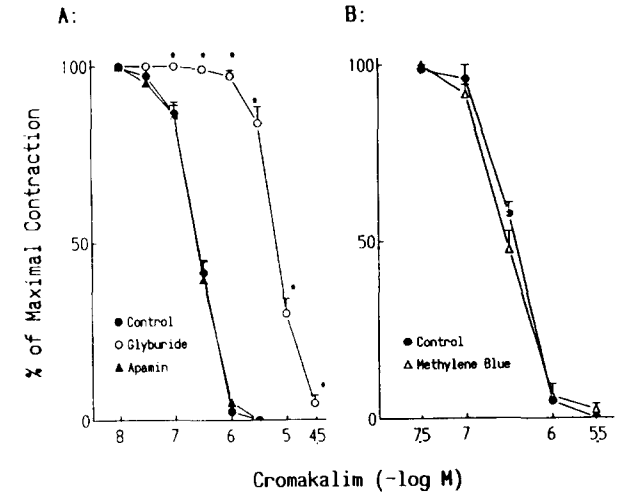

Fig. 6. The effects of various inhibitors on the relaxations induced by cromakalim in rat femoral arteries. Rat femoral arteries were contracted by phenylephrine (3×10^{-5} M). Cromakalim (10^{-8} to 3×10^{-5} M) (A: $n = 5$, B: $n = 5$) was added cumulatively to induce relaxations. In some experiments, (A) glyburide (10^{-6} M) ($n = 5$), apamin (10^{-6} M) ($n = 8$), or (B) methylene blue (10^{-6} M) ($n = 9$) was added to the bath 20 min before the addition of cromakalim. Maximal contractions induced by phenylephrine (3×10^{-5} M) before the addition of cromakalim were expressed as 100% (A: 0.16 ± 0.02 g, B: 0.14 ± 0.03 g). Data are expressed as the means ± S.E.M. * Significantly different from respective controls ($P < 0.05$).

ing. This is in agreement with previous reports that cromakalim produces the vasoinhibitory effects by activating K_{ATP} channels (Cavero et al., 1989; Beech and Bolton, 1989; Fujii et al., 1990).

It is known that nicorandil has dual mechanisms of vasoinhibition: hyperpolarization of cell membranes through activation of K_{ATP} channels and increase in cGMP levels due to activation of guanylate cyclase (Kukovetz et al., 1991). The results in the present study indicated that nitroglycerin, which increases the cGMP levels, only slightly inhibited the responses to high concentrations of acetylcholine. This suggests that the activation of guanylate cyclase may not be the major reason for the inhibitory effect of nicorandil in rat detrusor muscle. This is in agreement with the previous reports that drugs activating guanylate cyclase have little effect on rat detrusor muscles (Morita et al., 1992; Persson et al., 1992). In addition, the inhibitory effect of nicorandil on acetylcholine response is similar to that of cromakalim. It is, therefore, possible that the activation of K^+ channels may be involved in the inhibitory effect of nicorandil in rat detrusor muscles. Therefore, further experiments were carried out in order to clarify the mechanisms involved in the inhibitory action of nicorandil in rat detrusor muscles and femoral arteries.

It has been reported that there are several types of K^+ channels which mediate hyperpolarization. One is the K_{ATP} channel (Cavero et al., 1989; Quast and Cook, 1989; Standen et al., 1989), which is sensitive to sulfonylurea blockers, like glyburide (Nelson et al., 1990; Andersson, 1992; Holzmann et al., 1992; Richardson et al., 1992). Another is the Ca^{2+} activated K^+ channel (K_{Ca}) (Nakao et al., 1988; Gelband et al., 1989), which is inhibited by apamin (Nelson et al., 1990; Foster et al., 1989). The results of the present study indicate that the relaxations induced by both nicorandil and cromakalim in rat detrusor muscles are inhibited by glyburide. These results suggest that nicorandil, like cromakalim, activates K_{ATP} channels in rat detrusor muscles. K_{Ca} channels may not be involved in the inhibitory effects of nicorandil and cromakalim in the detrusor muscles, because apamin failed to affect the relaxations induced by nicorandil and cromakalim. This is in agreement with a previous report that cromakalim only activates K_{ATP} channels in guinea pig urinary bladder (Bonev and Nelson, 1993).

The inhibitory effects of nicorandil have partially been attributed to the formation of cGMP due to stimulation of guanylate cyclase (Kukovetz et al., 1991; Meisheri et al., 1991). However, there was no interaction between nicorandil and pretreatment by a high concentration of nitroglycerin in rat detrusor muscles. If cGMP is involved in the effect of nicorandil, the increase in cGMP levels by pretreatment with nitroglycerin should have, at least partially, inhibited the

effect of a low concentration of nicorandil, since a large amount of cGMP should have already been produced by nitroglycerin. In addition, methylene blue, a guanylate cyclase inhibitor (Gruetter et al., 1981) failed to block the inhibitory effect of nicorandil, but it instead potentiated it. This clearly suggests that cGMP does not play a major role in the effect of nicorandil in rat detrusor muscle. The potentiating effect of methylene blue appears to be, at least, specific to the action of nicorandil, since it does not affect the inhibitory effect of cromakalim. However, the reason for the potentiation of the relaxing response by methylene blue cannot be ascertained from the present study. Further, NMMA, an inhibitor of nitric oxide synthase (Hibbs et al., 1987; Palmer et al., 1988) which catalyzes the formation of nitric oxide from arginine, failed to affect the relaxation induced by nicorandil. This also suggests that the citrulline-arginine system which generates nitric oxide may not be involved in the effect of nicorandil in the detrusor muscle. Direct measurement of cGMP confirms that nicorandil increases the tissue level of cGMP. Since cGMP does not have marked effects on the detrusor muscle, which is evidenced by a negligible effect of nitroglycerin, it is likely that the formation of cGMP by nicorandil in the detrusor muscle may not play a major role in the pharmacological action of nicorandil. The present study also indicates that the inhibitory effect of cromakalim is not related to cGMP, since nitroglycerin, methylene blue and NMMA have no effect at all on the relaxation induced by cromakalim. Therefore, these results suggest that, in rat detrusor muscles, the activation of K_{ATP} channels play a major role in the inhibitory effects of nicorandil and cromakalim.

The present study also indicates that, in rat femoral arteries, the effect of nicorandil can be markedly inhibited by methylene blue, suggesting the major involvement of the cGMP formation in the effect of nicorandil. This is in contrast to the effect of nicorandil in rat detrusor muscles in which the formation of cGMP has no marked effect. The formation of cGMP is also not involved in the effect of cromakalim, since methylene blue has no significant effect at all on the relaxation induced by cromakalim. It has been reported that, in the bovine coronary arteries, nicorandil at concentrations lower than 48 μM causes relaxation predominantly by hyperpolarization due to activation of K_{ATP} channels, whereas the effect due to cGMP becomes predominant at higher concentrations of nicorandil (Kukovetz et al., 1991; Holtzmann et al., 1992). In rat aorta, however, the effect of nicorandil at concentrations higher than 3.2 μM was associated with the activation of K_{ATP} channels (Borg et al., 1991). In addition, the effect of nicorandil was not affected by glyburide in canine large coronary arteries (Satoh et al., 1991). More recently, it was reported that, in rabbit blood vessels, the involvement of K channels in the effect of nicorandil can vary with the anatomic location of the conductance vessels (Magnon et al., 1994). In the present study, in rat femoral arteries, the activation of K_{ATP} channels may be partly involved in the response to higher concentrations of nicorandil, since the responses to higher concentrations of nicorandil were slightly inhibited by glyburide. Further, in rat femoral arteries, the effects of nicorandil at lower concentrations may be partly due to activation of K_{Ca} channels, since they were also slightly inhibited by apamin. This is in agreement with a recent report (Robertson et al., 1993) that, in rabbit cerebral arteries, K_{Ca} channels can be activated by cGMP-dependent protein kinase, which may be activated by nicorandil through the formation of cGMP. The inhibitory effect of cromakalim seems to be dependent mostly on the activation of K_{ATP} channels in the rat femoral arteries, since the effect of cromakalim is only inhibited by glyburide but not by apamin.

The present study suggests that the inhibitory effect of nicorandil in rat femoral arteries is mostly due to the formation of cGMP through the activation of guanylate cyclase whereas in rat detrusor muscles the activation of K_{ATP} channels appears to be the major mechanism for the effect of nicorandil. In addition, in rat femoral arteries, the activation of K_{ATP} channels and K_{Ca} channels may, at least in part, be involved in the inhibitory effect of nicorandil at higher and lower concentrations, respectively. The inhibitory effect of cromakalim may be due to the activation of K_{ATP} channels in both rat detrusor muscles and femoral arteries.

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