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# Effects of chronic oral administration of a high dose of nicorandil on in vitro contractility of rat arterial smooth muscle

Kamolchai Trongvanichnam, Minori Mitsui-Saito, Hiroshi Ozaki *, Hideaki Karaki

*Department of Veterinary Pharmacology, Graduate School of Agriculture and Life Sciences, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan*

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**Abstract**

Nicorandil, which is structurally a nitrate and also a nicotinamide, has a vasodilator action by stimulating guanylate cyclase and ATP-sensitive $K^+$ channel. The aim of present study was to examine the effects of chronic oral administration of a high dose of nicorandil on in vitro vascular reactivity. Nicorandil (30 mg/kg), at a dose 6–10-times higher than to decrease blood pressure in rat, was orally administered 2-times daily for 2–4 weeks to the rats. At the end of the administration period, thoracic aorta was isolated for in vitro study. Treatment with nicorandil for 4 weeks markedly reduced the relaxant effect of nicorandil itself and other vasodilators including sodium nitroprusside, nitric oxide, endothelium-derived relaxing factor released by carbachol, 8-Br-cyclic guanosine 3′,5′-monophosphate (cGMP), a $K^+$ channel opener, levcromakalim, and forskolin. Increase in cGMP content induced by nicorandil and sodium nitroprusside was less in the aorta from nicorandil-treated rat than in the vehicle-control rat. Chronic administration of nicorandil altered neither the contractile responses to norepinephrine nor the vasodilator effect of verapamil. On the other hand, a 4-week treatment with a dose of nicorandil (2 mg/kg) sufficient to decrease blood pressure in rat showed no change in aortic response. These results suggest that in vivo chronic treatment with a high dose of nicorandil inactivates not only the guanylate cyclase activity but also the mechanism mediated by cGMP; it also attenuates the sensitivity of $K^+$ channels to levcromakalim. Prolonged activation of the specific site may desensitize its site of action.

*Keywords:* Nicorandil; Aorta, rat; Relaxation; cGMP; Tolerance; Levcromakalim

## 1. Introduction

Nicorandil [*N*-(2-hydroxyethyl)-nicotinamide nitrate], a combined chemical structure of an organic nitrate and a nicotinamide, is an orally efficacious antianginal drug with potent coronary vasodilating, vasospasmolytic and cardioprotective activities (Shibata, 1987). This drug relaxes vascular smooth muscle by stimulating soluble guanylate cyclase leading to increased cGMP levels (Endoh and Taira, 1983; Holzmann, 1983; Meisheri et al., 1991) and also by opening of ATP-sensitive $K^+$ channels to hyperpolarize membrane (Furukawa et al., 1981; Taira, 1987, 1989; Kukovetz et al., 1991, 1992; Holzmann et al., 1992).

Development of tolerance to nitrovasodilator, particularly to nitroglycerin, is a phenomenon occurring in vivo and in vitro after a prolonged treatment (Flaherty, 1989). Tolerance towards nitroglycerin was observed in isolated vascular smooth muscle of rat aorta (Needleman, 1970; Keith et al., 1982), bovine coronary artery (Kukovetz and Holzmann, 1985) and human peripheral vein (Ahlner et al., 1986). At present, however, there is no general agreement as to the specific mechanisms of this tolerance. According to Needleman and Johnson (1973), tolerance to organic nitrate involves the oxidation of a critical sulfhydryl group of nitrate receptor and the decreased vascular biotransformation of nitrates. Recent reports demonstrated that in vitro treatment with organic nitrates decreases the activity of soluble guanylate cyclase (Keith et al., 1982; Axelsson and Anderson, 1983; Axelsson and Karlson, 1984; Romanin and Kukovetz, 1989) or elevates the activity of phosphodiesterase (Ahlner et al., 1986; Axelsson and Ahlner, 1987). In contrast to nitroglycerin, nicorandil causes less tolerance (Sakai and Kuromaru, 1987; Henry et al., 1990) or less cross-tolerance toward isosorbide dinitrate or isosorbide-5-mononitrate (Kukovetz and Holzmann, 1990) although higher concentrations induce tolerance (Nabata et al., 1981). However, no information is available about whether overt tolerance or cross-tolerance

* Corresponding author. Tel.: (81-3) 3812-2111, ext. 5393; Fax: (81-3) 5802-2959; e-mail: aozaki@hongo.ecc.u-tokyo.ac.jp


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to other vasodilators develops in the vasorelaxant effect of nicorandil particularly at high doses. The study was designed to investigate; (1) if the long term in vivo treatment with a high dose of nicorandil changes the relaxant effect of nicorandil and other nitric oxide-releasing compounds, (2) if the nicorandil treatment changes the ability to produce cGMP, and (3) if the nicorandil treatment changes the relaxant effect of vasodilators other than nitric oxide-releasing compounds.

## 2. Materials and methods

### 2.1. Experimental protocols

The male Wistar rats (8 weeks old) were divided into vehicle control group (5% gum arabic solution) and nicorandil-treated group. Nicorandil (30 mg/kg) was given orally twice a day (60 mg/kg as a daily dose) at 9:30 a.m. and 18:30 p.m. every day (except on Sunday) for 2 and 4 weeks. Approximately 16 h after the last administration, rats were killed by stunning at neck and bleeding. The thoracic aorta was then quickly removed and used for measurement of muscle tension and of cGMP contents. In some experiments, age-matched, untreated rats were used.

### 2.2. Measurement of muscle tension

The isolated aorta was cut into rings 2–3 mm wide and placed in a normal physiological salt solution which contained (mM): NaCl 136.9, KCl 5.4, $CaCl_2$ 1.5, $MgCl_2$ 1.0, $NaHCO_3$ 23.8, ethylenediamine tetraacetic acid 0.01 and glucose 5.5. The endothelium was removed by gently rubbing the intimal surface with a finger moistened with physiological salt solution except otherwise stated. High $K^+$ solution was prepared by replacing NaCl with equimolar KCl. These solutions were equilibrated with a mixture containing 95% $O_2$ and 5% $CO_2$ at 37°C to maintain the pH at 7.4. Muscle tension was recorded isometrically with a force displacement transducer. Each muscle strip was attached to a holder under a resting tension of 10 mN. All the preparations were allowed to equilibrate for 60–90 min at 37°C until the contractile response to high $K^+$ (65.4 mM) solution became stable.

At the end of the experiments, muscle rings were blotted with filter paper and weighed on an analytical balance. The contractions were expressed as mN tension developed/mg wet tissue weight.

### 2.3. Measurement of cGMP levels

Thoracic aorta was longitudinally cut open and further cut transversely into 3 segments. Each segment weighed about 10 mg. After equilibration in physiological salt solution at 37°C for 1 h, the strips were exposed to test agents for 5 min. Some strips were used as untreated controls. Tissue cGMP content was measured with a competitive radioimmunoassay. Subsequent to an incubation, muscle strips were frozen in liquid nitrogen and homogenized in 6% trichloroacetic acid solution. After centrifugation at $1400 \times g$ for two times, trichloroacetic acid in the supernatant was removed by washing with water-saturated ether, and the succinylated cGMP was assayed (Yamasa Shoyu, Tokyo, Japan). Tissue cGMP levels were expressed as pmol/g wet weight.

### 2.4. Chemicals

Chemicals used were nicorandil (Chugai Pharmaceutical, Tokyo, Japan), norepinephrine bitartrate, sodium nitroprusside (Wako Pure Chemicals, Osaka, Japan), carbachol hydrochloride, 8-Br-cGMP, methylene blue, glibenclamide and 3-isobutyl-1-methylxanthine (IBMX) (Sigma, St. Louis, MO, USA). Nicorandil was dissolved in 50% ethanol at a concentration of 100 μM and diluted to the desired concentration in distilled water. Glibenclamide was dissolved in dimethyl sulfoxide (Wako, Tokyo, Japan). Levcromakalim was kindly donated by Smith Kline Beecham (Surrey, UK) and dissolved in 70% ethanol. Other drugs were dissolved in distilled water. Stock solution of nitric oxide was prepared as described by Thornbury et al. (1991).

### 2.5. Statistics

The results of the experiments are expressed as means ± S.E.M. Student's *t*-test or analysis of variance (ANOVA, when comparison involved more than two groups) was used for the statistical analysis of the data. A $P$ value less than 0.05 was considered to be statistically significant.

## 3. Results

### 3.1. Effects on norepinephrine-induced contraction

Cumulative addition of norepinephrine (1 nM to 10 μM) induced contractions in a concentration-dependent manner in either preparations from vehicle-treated or nicorandil-treated rats. Chronic treatment with nicorandil for 4 weeks did not change the responses to norepinephrine [$EC_{50}$ ($-\log M$): 7.9 ± 0.2 for vehicle control, 8.2 ± 0.1 for nicorandil treatment, $n = 4$ each]. The maximum contraction induced by norepinephrine was not affected, either (9.5 ± 1.2 mN/mg wet weight for vehicle-control; 7.9 ± 1.0 mN/mg wet weight for nicorandil treatment, $n = 4$ each).

### 3.2. Attenuation of nicorandil-induced relaxation

Fig. 1A shows the effect of cumulative addition of nicorandil (1–30 μM) on norepinephrine (100 nM)-in-

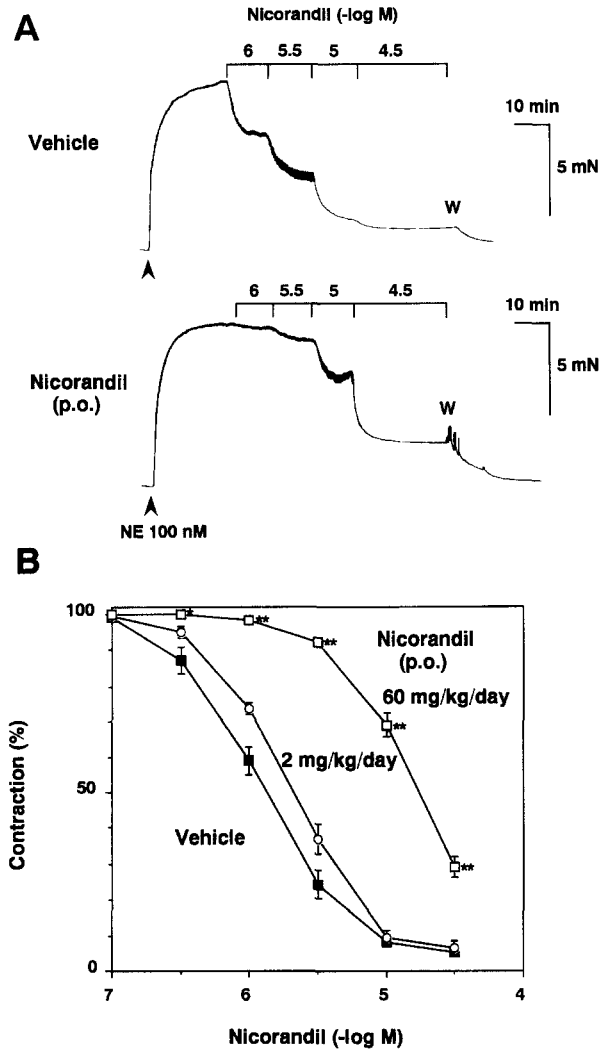


Fig. 1. Effects of chronic treatment with nicorandil (60 mg/kg per day for 4 weeks and 2 mg/kg per day for 4 weeks) on nicorandil-induced relaxation in vitro. Panel A shows a profile of relaxation of norepinephrine (100 nM)-induced contraction by nicorandil (1–30 μM) (upper trace, vehicle administration; lower trace, nicorandil administration). Panel B shows the concentration-response relationship for the inhibitory effect of nicorandil on norepinephrine-induced contraction in the aortas from vehicle- and nicorandil-treated (2 and 60 mg/kg per day) rats (○, 2 mg/kg; □, 60 mg/kg). Results are expressed as means ± S.E. of 8 experiments. *,** Significantly different from control (■) with $P < 0.05$ and $P < 0.01$, respectively (ANOVA).

duced contraction in the aorta with or without nicorandil administration. After a 4-week administration with nicorandil (60 mg/kg per day), relaxation induced by nicorandil was greatly reduced. The decreased responsiveness to nicorandil was detected after a 2-week nicorandil (60 mg/kg per day) treatment (data not shown). Fig. 1B shows that the concentration-response curve for nicorandil was shifted to the right after a 4-week administration with nicorandil (60 mg/kg per day); $IC_{50}$ ($-\log M$) was $5.9 \pm 0.1$ ($n = 8$) for vehicle-control and $4.7 \pm 0.03$ ($n = 8$) for aorta from nicorandil-treated rats ($P < 0.01$). Fig. 1B also shows the effect of treatment with a lower dose of nicorandil (2 mg/kg per day) on the nicorandil-induced relaxation in vitro, showing that this treatment did not develop tolerance.

### 3.3. Cross-tolerance to other nitric oxide-related compounds

Treatment with nicorandil (60 mg/kg per day) for 4 weeks markedly shifted the concentration-response curve for sodium nitroprusside (0.1 nM to 1 μM) to the right (Fig. 2A); $IC_{50}$ ($-\log M$) was $8.7 \pm 0.1$ ($n = 8$) for vehicle-control and $7.7 \pm 0.1$ ($n = 8$) for nicorandil treatment ($P < 0.01$). The relaxant effect of nitric oxide (0.15, 1.5 and 15 μM) on norepinephrine (100 nM)-induced contraction was also markedly reduced after the chronic treatment with nicorandil for 4 weeks (Fig. 2B). Fig. 2C shows the effects of the chronic treatment with nicorandil on the endothelium-dependent, carbachol-induced relaxation in norepinephrine-precontracted aorta. The concentration-response curve for carbachol was shifted to the right by the nicorandil treatment; $IC_{50}$ ($-\log M$) was $7.1 \pm 0.2$ ($n = 4$) for vehicle-control and $6.3 \pm 0.1$ ($n = 4$) for nicorandil treatment ($P < 0.05$).

### 3.4. Effect on cGMP formation

The ability of nicorandil and sodium nitroprusside to generate cGMP was compared between preparations from vehicle- and nicorandil-treated (60 mg/kg per day for 4 weeks) rats (Table 1). In vehicle-control muscles, cGMP levels significantly increased after incubation with 30 μM nicorandil or 100 nM sodium nitroprusside for 5 min. Phosphodiesterase inhibitor, IBMX (1 mM), also increased cGMP levels. The effects of nicorandil and sodium nitroprusside were greatly enhanced in the presence of IBMX. In the presence of IBMX, the increase in cGMP levels due to nicorandil (30 μM) or sodium nitroprusside (0.1 μM) were significantly lower in aorta from nicorandil (60 mg/kg per day)-treated rats than those in vehicle-control.

Table 1
Effects of 4-week administration with nicorandil (60 mg/kg per day) on the production of cGMP by rat aorta

| Treatment | Concentration | cGMP (pmol/g wet weight) | |
|---|---|---|---|
| | | Vehicle | Nicorandil |
| Control | – | 6.2 ± 2.5 | 3.3 ± 2.2 |
| Nicorandil | 30 μM | 44.6 ± 16.5 [a] | 28.0 ± 10.2 [a] |
| Nitroprusside | 0.1 μM | 56.5 ± 21.7 [a] | 42.4 ± 9.5 [a] |
| IBMX | 1 mM | 71.1 ± 12.8 [a] | 47.1 ± 13.6 [a] |
| IBMX | 1 mM | 258.9 ± 39.4 [b] | 132.5 ± 16.9 [b,c] |
| + nicorandil | 30 μM | | |
| IBMX | 1 mM | 457.6 ± 47.3 [b] | 213.8 ± 48.3 [b,c] |
| + nitroprusside | 0.1 μM | | |

[a] Significantly different from control with $P < 0.05$. [b] Significantly different from IBMX with $P < 0.01$. [c] Siginificantly different from respective vehicle-control (with $P < 0.05$ for IBMX + nicorandil and $P < 0.01$ for IBMX + sodium nitroprusside).

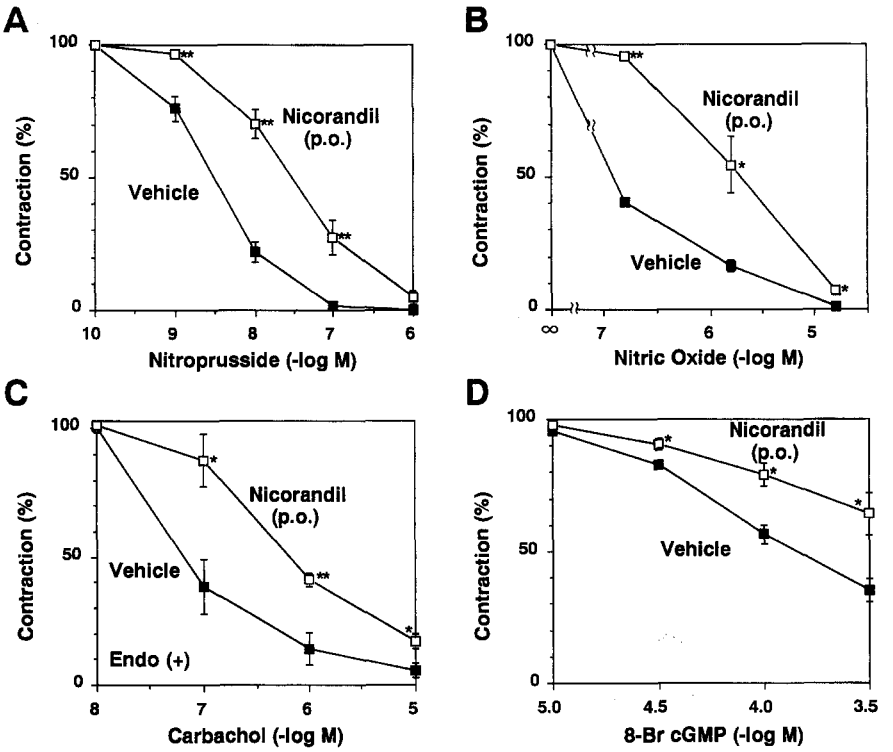


Fig. 2. Effects of chronic treatment with nicorandil (60 mg/kg per day for 4 weeks) on sodium nitroprusside-, nitric oxide-, carbachol (with endothelium)- and 8-Br-cGMP-induced relaxation in vitro. After the norepinephrine-induced contraction reached a steady level, sodium nitroprusside (A, 0.1 nM–1 μM), nitric oxide (B, 0.15, 1.5 and 15 μM), carbachol (C, 10 nM to 10 μM) and 8-Br-cGMP (D, 10–300 μM) were cumulatively added. (■) Aorta from vehicle-administered rats; (□) aorta from nicorandil-administered rats. Each point represents mean ± S.E. of 4–8 experiments. *,** Significantly different from control (■) with $P < 0.05$ and $P < 0.01$, respectively (Student's $t$-test).

### 3.5. Effects on 8-Br-cGMP-induced relaxation

As shown in Fig. 2D, the membrane permeable analogue of cGMP, 8-Br-cGMP, produced a concentration-dependent relaxation of the norepinephrine-induced contraction. The effect of 8-Br-cGMP was significantly reduced by the nicorandil (60 mg/kg per day) treatment.

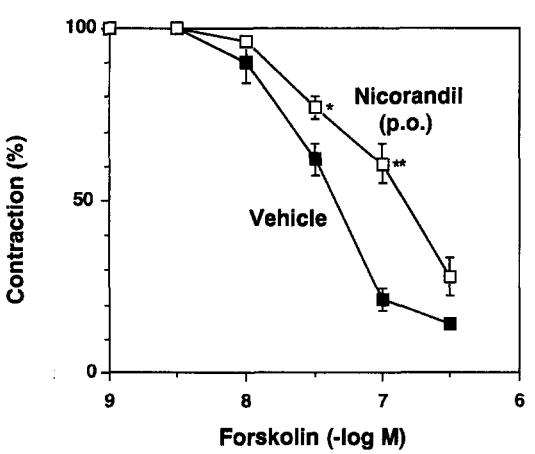


Fig. 3. Effects of chronic treatment with nicorandil (60 mg/kg per day for 4 weeks) on forskolin-induced relaxation in vitro. Aortic rings were contracted by 100 nM norepinephrine and then forskolin (1 nM to 1 μM) was cumulatively added. (■) Aorta from vehicle-administered rats; (□) aorta from nicorandil-administered rats. Results are expressed as means ± S.E. of 4–12 experiments. *,** Significantly different from control (■) with $P < 0.05$ and $P < 0.01$, respectively (Student's $t$-test).

### 3.6. Effects on forskolin-induced relaxation

Forskolin (1–300 nM), an activator of adenylate cyclase, also produced a concentration-dependent relaxation of the norepinephrine-induced contraction. The effects of forskolin was significantly reduced by the nicorandil (60 mg/kg per day) treatment; $IC_{50}$ ($-\log M$) was $7.4 \pm 0.1$ ($n = 4$) for vehicle-control and $6.9 \pm 0.1$ ($n = 12$) for nicorandil treatment ($P < 0.01$) (Fig. 3).

### 3.7. Effects on verapamil-induced relaxation

As shown in Fig. 4, the relaxant effect of $Ca^{2+}$-channel blocker, verapamil (10 nM to 10 μM), was unaffected by the nicorandil treatment. Concentration which inhibits the contraction by 50% ($-\log M$) was $6.0 \pm 0.4$ ($n = 4$) for vehicle-control and $6.7 \pm 0.2$ for nicorandil treatment).

### 3.8. Effects of activator and inhibitor of ATP-sensitive $K^+$ channel

In vehicle-control muscles, levcromakalim, an activator of ATP-sensitive $K^+$ channel, inhibited norepinephrine

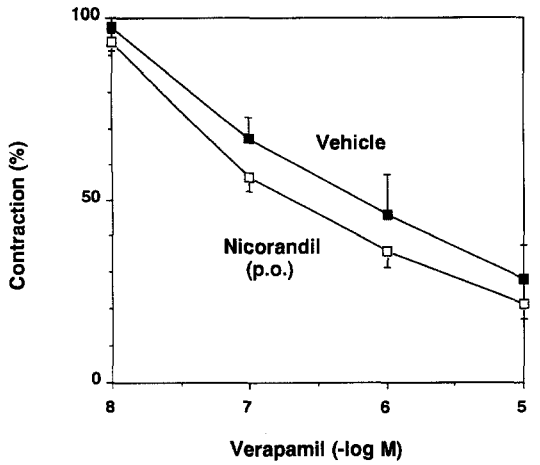


Fig. 4. Effects of chronic treatment with nicorandil (60 mg/kg per day for 4 weeks) on verapamil-induced relaxation in vitro. Aortic rings were contracted by 100 nM norepinephrine and then verapamil (10 nM to 10 μM) was cumulatively added. ■: aorta from vehicle-administered rats. □: aorta from nicorandil-administered rats. Results are expressed as means ± S.E. of 4 experiments.

(100 nM)-induced contraction in a concentration-dependent manner. The maximum effects of levcromakalim was obtained at 1 μM reducing the contraction to 32.0 ± 3.7% ($n = 4$). In the aorta from nicorandil (60 mg/kg per day)-treated rats, the concentration-response curve for levcromakalim was shifted to the right; concentration which inhibits the contraction by 50% ($-\log M$) was 6.9 ± 0.2 ($n = 4$) for vehicle-control and 6.1 ± 0.02 for nicorandil treatment ($P < 0.01$) (Fig. 5). The maximum relaxation was also reduced to 44.2 ± 0.8% ($n = 4$, $P < 0.05$).

We further characterized the nicorandil-induced relaxation using glibenclamide, a selective inhibitor of ATP-

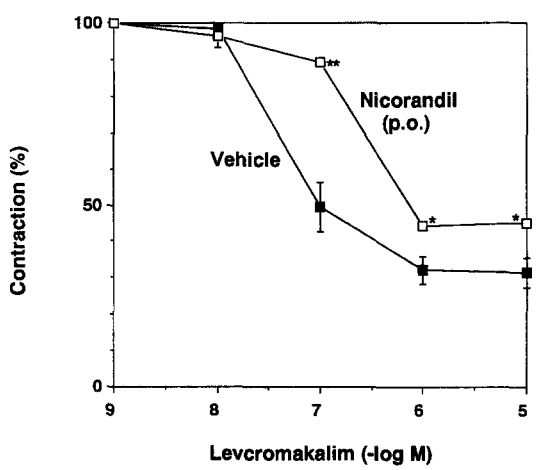


Fig. 5. Effects of chronic treatment with nicorandil (60 mg/kg per day for 4 weeks) on levcromakalim-induced relaxation in vitro. Aortic rings were contracted by 100 nM norepinephrine and then levcromakalim (1 nM to 10 μM) was cumulatively added. ■: aorta from vehicle-administered rats. □: aorta from nicorandil-administered rats. Results are expressed as means ± S.E. of 4 experiments. *,** Significantly different from control (■) with $P < 0.05$ and $P < 0.01$, respectively (Student's $t$-test).

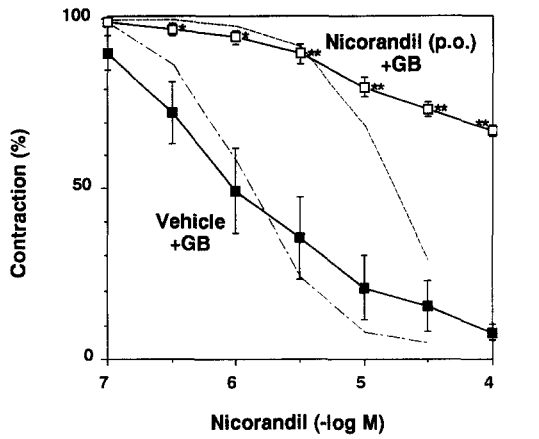


Fig. 6. Effects of glibenclamide on nicorandil-induced relaxation in vitro after the chronic treatment with vehicle (■) or nicorandil (□, 60 mg/kg per day for 4 weeks). The aortic rings were pretreated with glibenclamide (GB, 1 μM) for 20 min in the presence of norepinephrine (100 nM) before addition of nicorandil (0.1–100 μM). Dotted lines indicate the concentration-response relationships in the absence of or glibenclamide ( — - —, vehicle-control; – – –, nicorandil treatment) (data are adapted from Fig. 1B). Values are expressed as means ± S.E. of 4 experiments. *,** Significantly different from vehicle-control (■) with $P < 0.05$ and $P < 0.01$, respectively (Student's $t$-test).

sensitive $K^+$ channels. In the aorta from nicorandil (60 mg/kg per day)-treated rats, the inhibitory effects of nicorandil (0.1–100 μM) on norepinephrine-induced contraction was decreased by approximately 10-fold, as shown in Fig. 1A,B. The nicorandil-induced relaxation was more greatly attenuated (more than 100-fold) in the presence of glibenclamide (1 μM) by which the ability of nicorandil to activate ATP-sensitive $K^+$ channel would be greatly attenuated (Fig. 6). Furthermore, glibenclamide attenuated the nicorandil-induced relaxation at a nicorandil concentration of > 3 μM.

## 4. Discussion

The present study revealed that in vivo administration of nicorandil for 2–4 weeks markedly attenuated the in vitro vasodilator actions of nicorandil. It is possible that the attenuated responsiveness to nicorandil may be due to nicorandil remaining in the tissue even 16 h after the final administration and the subsequent washout in vitro (60–90 min). If this is the case, the contractile activity would be reduced in the preparations from nicorandil-treated rats. This possibility was ruled out because we did not observe any difference in the responses to norepinephrine between vehicle- and nicorandil-treated groups.

The present results also demonstrated that prolonged nicorandil administration in vivo induced cross-tolerance to sodium nitroprusside, carbachol and nitric oxide (Fig. 2A,B,C). The effects of nitrovasodilators, such as nitroglycerin, sodium nitroprusside and nicorandil, and the effects of endothelium-dependent vasodilators, such as car-

bachol, are mediated by the activation of guanylate cyclase and the resulting cGMP accumulation (Galvas and Disalvo, 1983; Lincoln, 1989; Ignarro and Kadowitz, 1985; Ahlner et al., 1991). cGMP activates cGMP-dependent protein kinase which leads to muscle relaxation by decreasing cytosolic $Ca^{2+}$ levels and/or $Ca^{2+}$ sensitivity of contractile apparatus (Rapoport and Murad, 1983; Lincoln and Johnson, 1984; Karaki et al., 1988). Since tolerance to nitroglycerin has been suggested to be due to decrease in cGMP formation, we measured cGMP contents. Results showed that desensitization to nicorandil and sodium nitroprusside was associated with a reduction in the formation of cGMP measured in the presence of a phosphodiesterase inhibitor, IBMX. These results suggest that activity of guanylate cyclase was decreased after the nicorandil treatment and this mechanism may at least partly be responsible for the nicorandil-induced tolerance. Phosphodiesterase activity may not be impaired after the nicorandil treatment since there was no significant difference between the two groups in the absence of IBMX.

It has been reported that the relaxant effect of 8-Br-cGMP, a lipophilic derivative which activates cGMP-dependent kinases (Schultz et al., 1979), did not change the in vitro contractility of vascular tissue after the establishment of nitroglycerin tolerance in vitro (Keith et al., 1982). Furthermore, after the administration of nitroglycerin in vivo for 4–8 days, the relaxation of rat aorta produced by 8-Br-cGMP was not changed (Molina et al., 1987). The authors of these reports have suggested that once cGMP is formed, its subsequent action is not adversely affected. Consistent with the observations by Keith et al. (1982) and Molina et al. (1987), we have recently shown that the in vivo chronic treatment with a high dose of isosorbide dinitrate (ISDN) reduces the relaxant effect of ISDN itself but had little effect on the 8-Br-cGMP (Kamolchai et al., 1996a). These results suggest that nitroglycerin and ISDN desensitize the nitric oxide-generating step rather than the downstream pathways after the cGMP formation. In the present study, in contrast, administration of nicorandil for 4 weeks significantly decreased the relaxation produced by 8-Br-cGMP (Fig. 2D). This result suggests that nicorandil-induced tolerance occurs not only at guanylate cyclase, but also at the mechanisms after generation of cGMP.

In the present study, we also observed that the chronic nicorandil treatment decreased the relaxant effect of forskolin. This result suggests that nicorandil desensitizes the cAMP-dependent vasorelaxation pathway. It has been reported that, in vascular smooth muscle cells, cAMP is able to activate cGMP-dependent kinase (Lincoln et al., 1990; Jiang et al., 1992). Therefore, the decreased activity of forskolin after the nicorandil treatment may indicate that part of the forskolin-induced relaxation is mediated by the activation of cGMP-dependent kinase.

To further characterize the mechanism of nicorandil-induced tolerance, we examined the effects of an inhibitor of ATP-sensitive $K^+$ channels, glibenclamide. In rat aorta, 1 μM glibenclamide almost completely suppresses the relaxation induced by levcromakalim (unpublished observation), suggesting that this concentration of levcromakalim may greatly attenuate the activity of ATP-sensitive $K^+$ channels. It was found that glibenclamide did not change the relaxant effect of nicorandil in the vehicle-control, suggesting that the major effect of nicorandil is to increase the cGMP level but not to activate the $K^+$ channel. In the aorta from nicorandil-treated rats, the relaxation induced by nicorandil was obtained at relatively higher concentrations ($> 3$ μM) than in vehicle-control ($> 0.1$ μM). Glibenclamide inhibited the relaxant effect of nicorandil in nicorandil-treated aorta. This result suggests that although lower concentrations of nicorandil ($< 3$ μM) activate cGMP-related mechanism, higher concentrations ($> 3$ μM) also activate ATP-sensitive $K^+$ channel. Thus, nicorandil treatment desensitized mainly the cGMP-related mechanism and partly the opening of $K^+$ channel and this may be reason for inhibition of levcromakalim-induced relaxation in the aorta from nicorandil-treated rats. It has been reported that the spasmolytic effect of nicorandil on canine conductive coronary vessels is not mediated by $K^+$-channel opening but by a nitroglycerin-like action and that the dilatation of resistive coronary vessels induced by nicorandil may be largely due to its action as a $K^+$-channel opener (Imagawa et al., 1992).

The effects of levcromakalim to relax norepinephrine-induced contraction was inhibited after the nicorandil treatment. We have recently observed that the chronic treatment of the rats with levcromakalim attenuates the activity of ATP-sensitive $K^+$ channels to relax vascular smooth muscle (Kamolchai et al., 1996b). These results are consistent with the suggestion that nicorandil activates ATP-sensitive $K^+$ channels at high concentrations and therefore chronic nicorandil treatment desensitized $K^+$ channels.

The chronic treatment with nicorandil at a moderate dosage (9 mg/kg per day) develops only a slight tolerance to nicorandil itself (Nabata et al., 1981). In vivo studies (Sakai and Kuromaru, 1987) also suggested that, unlike nitroglycerin or isosorbide dinitrate, nicorandil does not cause acute tolerance or cross tolerance to other nitrovasodilators. We confirmed such advantage of this drug by showing that treatment with a low dose (2 mg/kg per day) of nicorandil for 4 weeks induced almost no changes (see Fig. 1B). Although nicorandil bears such favorable feature compared to other nitrovasodilators, nicorandil at very high doses is capable of producing tolerance and cross-tolerance to other nitrovasodilators. It has been suggested that nitroglycerin and ISDN desensitize the NO-generating step because this step is more tachyphylatic than the downstream mechanisms (see, Abrams, 1986; Henry et al., 1988). In contrast, nicorandil did not desensitize the NO-generating step possibly because nicorandil releases NO by a pathway different from that for nitroglycerin or ISDN.

In summary, the results of the present study showed

that the aortic strips isolated from the rats administered with a high dose of nicorandil for 2–4 weeks exhibited a desensitization towards not only the cGMP-mediated pathway but also the $K^+$ channel opening pathway. Long term administration of a high drug dose may desensitize its specific site of action. Further studies are necessary to elucidate the molecular mechanism of nicorandil-induced changes in vascular smooth muscle.

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