

Influence of Nicorandil on Aortic Strip's Contractility and Blood Pressure of the Rat

Dong-Yoon LIM^{1*}, Yong-Jik KIM¹, and Soon-Pyo HONG²

¹Department of Pharmacology, College of Medicine, Chosun University, Kwang Ju 501-759, KOREA

²Department of Internal Medicine (Cardiology), Chosun University, Kwang Ju 501-759, KOREA

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Abstract – The present study was conducted to investigate the effects of nicorandil on arterial blood pressure and vascular contractile responses in the normotensive anesthetized rats and to establish the mechanism of action. Nicorandil (30–300 µg/kg) given into a femoral vein of the normotensive anesthetized rat produced a dose-dependent depressor response. These nicorandil-induced hypotensive responses were not affected by pretreatment with atropine (3.0 mg/kg, i.v.) or propranolol (2.0 mg/kg, i.v.), while markedly inhibited in the presence of chlorisondamine (1.0 mg/kg, i.v.) or phentolamine (2.0 mg/kg, i.v.). Furthermore, after the pretreatment with 4-aminopyridine (1.0 mg/kg/30 min, i.v.) or glibenclamide (50.0 µg/kg/30 min, i.v.), nicorandil-induced hypotensive response was greatly reduced. Interestingly, the infusion of nicorandil (30 µg/kg/30min) into a femoral vein made a significant reduction in pressor responses induced by intravenous norepinephrine. In the isolated rat aortic strips, both phenylephrine (10⁻⁵ M)- and high potassium (5.6 x 10⁻² M)-induced contractile responses were dose-dependently depressed in the presence of nicorandil (25–100 µM). Collectively, these experimental results demonstrate that intravenous nicorandil causes a dose-dependent depressor action in the anesthetized rat at least partly through the blockade of vascular adrenergic α₁-receptors, in addition to the well-known mechanism of potassium channel opening-induced vasorelaxation.

Keywords □ Nicorandil, Vasorelaxation, K⁺ Channel Opener, Adrenergic α₁-Receptors

The vasodilator nicorandil, *N*-(2-hydroxyethyl)-nicotinamide nitrate ester, has a combined chemical structure of an organic nitrate and a nicotinamide and is clinically an efficacious drug for treatment of angina pectoris (Frampton *et al.*, 1992; Goldschmidt *et al.*, 1996). Nicorandil has at least two mechanisms of action; 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 opening of ATP-sensitive K⁺ (K_{ATP}) channels to hyperpolarize the plasma membrane (Furukawa *et al.*, 1981; Kukovetz *et al.*, 1991; Holzmann *et al.*, 1992). The contribution of these two pathways to vasorelaxation appears to vary according to the tissue under study and the concentration of nicorandil used, the relative importance of the K⁺ channel opening mechanism being greater in small vessels and at lower concentrations of nicorandil (Holzmann *et al.*, 1992; Kukovetz *et al.*, 1991; Akai *et al.*, 1995).

Nicorandil is found to dose-dependently inhibit halothane-epinephrine arrhythmias in rats through mitochondrial ATP-sensitive K⁺ channels, and nitric oxide is required for the anti-arrhythmic effect of nicorandil (Kawai *et al.*, 2002). It has also been reported that the potency of nicorandil to cause coronary vasorelaxation is increased under conditions of metabolic inhibition. This effect appears to result from the K⁺ channel opening action of the drug, and may have significant consequences for its therapeutic effectiveness (Davie *et al.*, 1998).

It has been found that nicorandil reduces sympathetic coronary vasoconstriction by decreasing the reactivity of the vasculature to sympathetic neurotransmitters and by suppressing neuropeptide Y (NPY) overflow during cardiac sympathetic nerve stimulation (Chujo *et al.*, 1994). The norepinephrine (NE)-induced contraction was suppressed by 2-nicotinamidoethyl nitrate (2-NN) with hyperpolarization of the membrane in the porcine mesenteric artery, but with no change in the membrane potential in the guinea-pig mesenteric artery, presumably due to suppression of the Ca²⁺-mobilization in the cell (Furukawa *et al.*, 1981). Nicorandil had a greater relaxing effect on the maximum contractile response to norepinephrine

*Corresponding author

Tel: +82-62-230-6335, Fax: +82-62-227-4693

E-mail: dylim@chosun.ac.kr

(NE) than on the potassium (K^+) response on all vascular smooth muscles of rabbit aorta, cat coronary arteries and rabbit basilar arteries used (Shibata *et al.*, 1984).

In view of the current pharmacological actions of nicorandil, the present study was therefore designed to investigate whether it can produce the hypotensive action in the normotensive anesthetized rats and the vasorelaxation on phenylephrine-induced contractile response of the isolated rat aortic strips, and to establish its mechanism of action, in addition to the well-known K_{ATP} -channel opening.

MATERIALS AND METHODS

Experimental Procedure

Mature male Sprague-Dawley rats, weighing 150 to 350 g, were used in the experiment. The animals were housed individually in separate cages, and food (Cheil Animal Chow) and tap water were allowed *ad libitum* for at least a week to adapt to experimental circumstances. On the day of experiment, a rat was anesthetized with thiopental sodium (50 mg/kg) intraperitoneally, and tied in supine position on fixing panel.

A) Isolation of Aortic Strips: The thorax was opened by a midline incision, and placing three-hook retractor exposed the heart and surrounding area. The heart and portion of the lung were not removed, but pushed over to the right side and covered by saline-soaked gauge pads in order to obtain enough working space for isolating aortic vessel. The aorta was isolated from the proximal part of the heart to the vicinity of liver and immediately immersed in cold Krebs solution. The blood within the aorta was rapidly removed. The aorta was cut into the ring of 4-5 mm length.

B) Preparation of Arterial Cannulation: The animal was tied in supine position on fixing panel to insert a T- formed cannula into the trachea for securing free air passage. The rectal temperature was maintained at 37-38°C by a thermostatically controlling blanket and heating lamp throughout the course of the experiment.

Recording of Mechanical Activity

The ring segment of aorta was mounted in a muscle bath by sliding the ring over two parallel stainless-steel hooks (0.15 mm in diameter). The lower hook was fixed on bottom of the bath and the upper was connected to isometric transducer (Grass FT. 03). The signal from the transducer was displayed on a polygraph (Grass Instruments Model 79). The volume of bath was 25 ml and the bath solution was saturated with 95%

O_2 and 5% CO_2 at 37°C (Fig. 1). The composition (mM) of Krebs was: NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgCl_2$, 1.18; $NaHCO_3$, 25; KH_2PO_4 , 1.2; glucose, 11.7. The final pH of the solution was maintained at 7.4 - 7.5. During equilibration period of 2 hours, the resting tension was adjusted to 0.5 g. After the equilibration period, the ring was challenged with 35 mM KCl two times, and if it responded with contraction, the proper experiment was started. Vasoconstrictors were administered into the bath in order to obtain dose-response curves. In the subsequent experiments, under the presence of nicorandil, some vasoconstrictors were administered, respectively. The data were expressed as % of the control tension.

Measurement of Blood Pressure

In order to observe the change of arterial pressure, one of the common carotid arteries or of the femoral arteries was catheterized with polyethylene tubing [outside diameter (o.d.): 0.5 mm]. The tubing was connected to a pressure transducer (Gould Co., U.S.A.) and pulse of mean arterial blood pressure was recorded on a biological polygraph (Grass Co., U.S.A.) continuously. The chart speed was adjusted to 2 cm per minute. The artery tubing was filled with heparin solution (400 I.U.) to prevent the blood coagulation during the experiment. Another cannulation with polyethylene tubing (o.d.: 0.3 mm) was made into a femoral vein for the administration of drugs and supplemental anesthetic agents as needed to maintain light surgical anesthesia. Each rat was left undisturbed for at least 30 minutes after completion of the operative procedures to permit cardiovascular parameters to be stabilized and drugs under investigation were administered at 60 minutes intervals.

Statistical Analysis

The statistical significance between groups was determined by the Student's *t*- and ANOVA- tests. A P-value of less than 0.05 was considered to represent statistically significant changes unless specifically noted in the text. Values given in the text refer to means and the standard errors of the mean (S.E.M.). The statistical analysis of the experimental results was made by computerized program described by Tallarida and Murray (1987).

Drugs and Their Sources

The following drugs were used: nicorandil (Daehan Choongwae Pharm. Co., Korea), phenylephrine hydrochloride, potassium chloride, norepinephrine bitartrate, glibenclamide and 4-aminopyridine (Sigma Chemical Co., U. S. A.), atropine sulfate

(Aldrich Chemical Co., U.S.A.), chlorisondamine chloride (CIBA Co., U.S.A.), phentolamine mesylate (CIBA Co., U.S.A.), thiopental sodium and heparin sodium (Daehan Choongwae Pharm. Co., Korea). Drugs were dissolved in distilled water (stock) and added to the normal saline solution as required. However, nicorandil was dissolved in dimethyl sulfoxide. Glibenclamide and 4-aminopyridine were dissolved in ethanol. The concentration of dimethyl sulfoxide or ethanol in the aortic bath and in injecting solution was less than 1%, which had no effect on the vascular contractility and blood pressure under the conditions employed in this study.

RESULTS

Effects of intravenous nicorandil on arterial blood pressure in the anesthetized rats

When cardiovascular parameters were stabilized for 30 min before the experimental protocols were initiated, the administration of physiological saline solution in a volume of 0.2 ml into a femoral vein did not cause any changes in both arterial blood pressure. Then, nicorandil injected intravenously to the normotensive thiopental-anesthetized rat produced a dose-dependent decrease in arterial blood pressure.

In 8 rats, as shown in Fig. 1, intravenous 30 $\mu\text{g}/\text{kg}$ of nicorandil produced a fall in arterial blood pressure to -6 ± 0.8 mmHg ($P < 0.01$) from the original baseline of 122 ± 11 mmHg. Increasing intravenous doses of nicorandil to 100 and 300 $\mu\text{g}/\text{kg}$ caused the dose-related reduction in arterial pressure responses to -27 ± 4.4 mmHg ($P < 0.01$) and -57 ± 2.6 mmHg ($P < 0.01$) from the original baseline, respectively. Furthermore, these present experimental results were similar to those effects obtained from previous studies (Holzmann *et al.*, 1992; Kukovetz *et al.*, 1991; Akai *et al.*, 1995).

Influence of atropine and chlorisondamine on nicorandil-induced hypotensive responses in the anesthetized rats

In 6 experimental animals, the effect of atropine on the cardiovascular responses to intravenous injection of nicorandil was studied. Atropine (3.0 mg/kg, i.v.) was given to block cholinergic muscarinic receptors after obtaining the control responses of nicorandil. Preliminary studies revealed that this dose of atropine blocked vasodepressor effect of muscarine. In the presence of atropine, the arterial blood pressure responses induced by nicorandil given intravenously at doses of 30, 100 and 300 $\mu\text{g}/\text{kg}$ were -7 ± 1.4 mmHg (ns), -18 ± 3.0 mmHg (ns) and -36 ± 1.4 mmHg (ns) from the original baseline, respec-

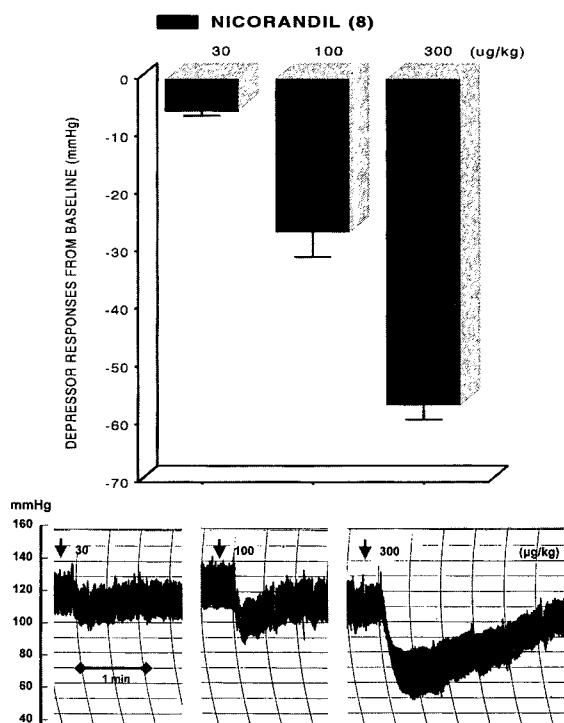


Fig. 1. Upper: Influence of intravenous nicorandil on arterial blood pressure in the thiopental-anesthetized rats. Ordinate: changes of arterial blood pressure in mmHg from 8 rats. Abscissa: intravenous doses of nicorandil in $\mu\text{g}/\text{kg}$. Vertical bar on each column indicates standard error of mean (S.E.M.). There was statistically significant difference in changes of arterial pressure responses induced by nicorandil from pre-injection level. Numeral in the parenthesis denotes number of animals used in the experiment. **Lower:** The representative tracing of the intravenous nicorandil-induced depressor response in the thiopental-anesthetized rat. At arrow mark, the indicated doses (30, 100 and 300 $\mu\text{g}/\text{kg}$) of nicorandil were administered into a femoral vein at 20-30 min interval. The chart speed was 20 mm/min.

tively, which are not significant as compared with their corresponding control responses (Fig. 2-upper). Chlorisondamine (1.0 mg/kg), an autonomic ganglionic blocking agent was given intravenously into a femoral vein of the rat to examine whether nicorandil acts on autonomic ganglia. Following the administration of chlorisondamine, the baseline of blood pressure was reduced from 122 ± 10 mmHg to 75 ± 6 mmHg. In 5 rats, responses of arterial blood pressure by intravenous nicorandil at 30, 100 and 300 $\mu\text{g}/\text{kg}$ before pretreatment with chlorisondamine were -9 ± 1.4 mmHg, -19 ± 1.2 mmHg and -40 ± 6.4 mmHg from the pre-injection level, respectively. However, after pretreatment with chlorisondamine they were markedly inhibited by 55-53% of the corresponding control responses, respectively as shown in Fig. 2 (lower).

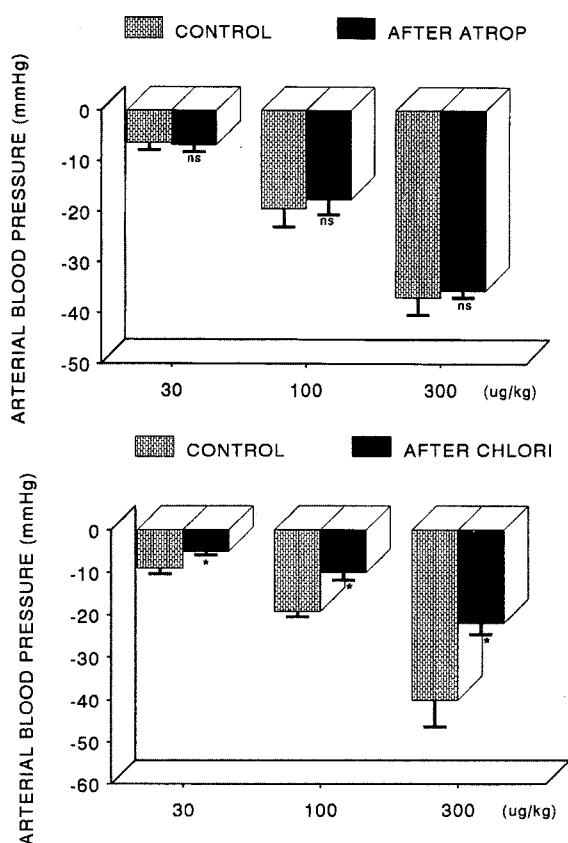


Fig. 2. Upper: Influence of atropine (ATROP) on intravenous nicorandil-evoked hypotensive responses. Atropine (3.0 mg/kg) was given intravenously after completion of the corresponding control responses of nicorandil. **Lower:** Influence of chlorisondamine (CHLORI) on intravenous nicorandil-evoked hypotensive responses. Chlorisondamine (1.0 mg/kg) was given intravenously after completion of the corresponding control responses of nicorandil. "CONTROL" and "AFTER" represent changes of arterial pressure induced by nicorandil before (CONTROL) and after pretreatment with atropine or chlorisondamine. Statistical significance was obtained by comparing the changes between groups of "CONTROL" and "AFTER". Other legends are the same as in Fig.1. *: $P < 0.05$. ns: Statistically not significant.

Effects of phentolamine and propranolol on intravenous nicorandil-induced depressor responses in the anesthetized rats

In order to investigate whether nicorandil-induced hypotensive response is mediated by the blockade of adrenergic α -receptors, it was of interest to test the influence of phentolamine, an antagonist of adrenergic α -receptors, on nicorandil-evoked hypotensive responses. In 6 rats, in order to examine the interaction between adrenergic α -receptors and nicorandil-induced depressor action, phentolamine (2.0 mg/kg) was given intravenously after obtaining the control responses of intravenous nicorandil. In the presence of phentolamine, depressor

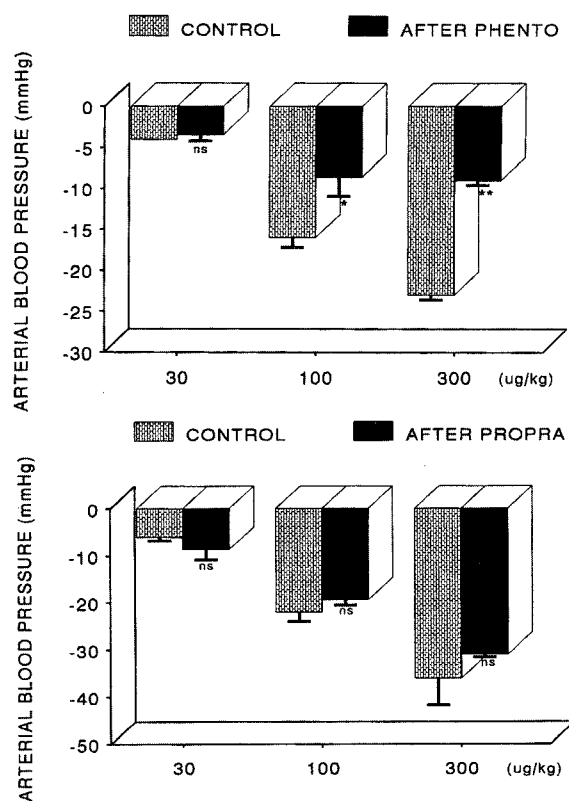


Fig. 3. Upper: Influence of phentolamine (PHENTO) on intravenous nicorandil-evoked hypotensive responses. Phentolamine (2.0 mg/kg) was given into a femoral vein after obtaining the corresponding control responses of intravenous nicorandil. **Lower:** Influence of propranolol (PROPRA) on intravenous nicorandil-evoked hypotensive responses. Propranolol (2.0 mg/kg) was given into a femoral vein after obtaining the corresponding control responses of intravenous nicorandil. Other legends are the same as in Fig. 1 and 2. *: $P < 0.05$, **: $P < 0.01$. ns: Statistically not significant.

responses induced by intravenous nicorandil at doses of 30, 100 and 300 $\mu\text{g}/\text{kg}$ were inhibited to 85–39% of the control responses, as shown in Fig. 3 (upper), which were statistically significant.

In order to examine the relationship between nicorandil-induced cardiovascular effects and adrenergic β -receptors, propranolol (2.0 mg/kg) was administered intravenously. Prior to administration of propranolol, nicorandil-induced hypotensive responses at doses of 30, 100 and 300 $\mu\text{g}/\text{kg}$ were -6 ± 0.8 mmHg, -22 ± 2.0 mmHg and -36 ± 0.6 mmHg from the preinjection level, respectively. However, following pretreatment with propranolol, nicorandil-induced depressor responses were not altered as compared with their corresponding control responses, as shown in Fig. 3 (lower).

Effects of 4-aminopyridine and glibenclamide on intravenous nicorandil-induced depressor responses in the anesthetized rats

It has been found that 4-aminopyridine is capable of inhibiting various types of K^+ channels in both the outer membrane of the cell (Nelson and Quayle, 1995; Brayden, 1996) and the intracellular membrane associated with the sarcoplasmic reticulum (SR) (Fink and Stephenson, 1987). By inhibiting SR K^+ channels, 4-aminopyridine can inhibit SR calcium sequestration (Fink and Stephenson, 1987). Therefore, 4-aminopyridine (1.0 mg/kg/30 min), presently employed as a potassium channel blocking agent was injected intravenously in order to observe the interrelationship between nicorandil-induced hypotensive responses and potassium channel. After pretreatment with 4-aminopyridine (1.0 mg/kg, i.v.) in 6 rats, depressor responses of intravenous nicorandil at all doses of 30, 100 and 300 $\mu\text{g}/\text{kg}$ were greatly inhibited by 71~54% of the corresponding control responses, respectively as compared with control responses of -7 ± 0.4 mmHg, -25 ± 2.0 mmHg and -57 ± 2.6 mmHg, as shown in Fig. 4 (upper).

Since it is known that glibenclamide inhibits the opening of the K_{ATP} channel on pancreatic β -cells, which results in the increased release of insulin, employing presently in treating type 2 diabetes mellitus (Edwards and Weston, 1993), and it is a nonselective inhibitor of all subtypes of the K_{ATP} channel (Liu *et al.*, 2001), it is likely of particular interest to investigate the effect of glibenclamide on nicorandil-induced hypotensive effects. Intravenous nicorandil-induced hypotensive responses at 30, 100 and 300 $\mu\text{g}/\text{kg}$ before treatment with glibenclamide were -19 ± 0.8 mmHg, -26 ± 1.2 mmHg and -40 ± 1.2 mmHg from the original arterial pressure baseline, respectively, while following pretreatment with intravenous glibenclamide (30.0 $\mu\text{g}/\text{kg}$, i.v.) they were greatly inhibited to 60~47% of the corresponding control responses (Fig. 4-lower).

Effect of nicorandil on norepinephrine-induced hypertensive responses in the anesthetized rats

Since pretreatment with both chlorisondamine and phentolamine greatly inhibited nicorandil-induced hypotensive responses, as shown in Fig. 2 (lower) and 3 (upper). It suggests that nicorandil can cause hypotension through the blockade of peripheral adrenergic α -receptors. Therefore, it is of interest to examine the effect of intravenous nicorandil on norepinephrine-evoked pressor responses. In 8 rats, norepinephrine injected intravenously at doses of 0.3, 1 and 3 $\mu\text{g}/\text{kg}$ caused dose-dependent pressor responses of 18 ± 1 mmHg, 31 ± 2 mmHg

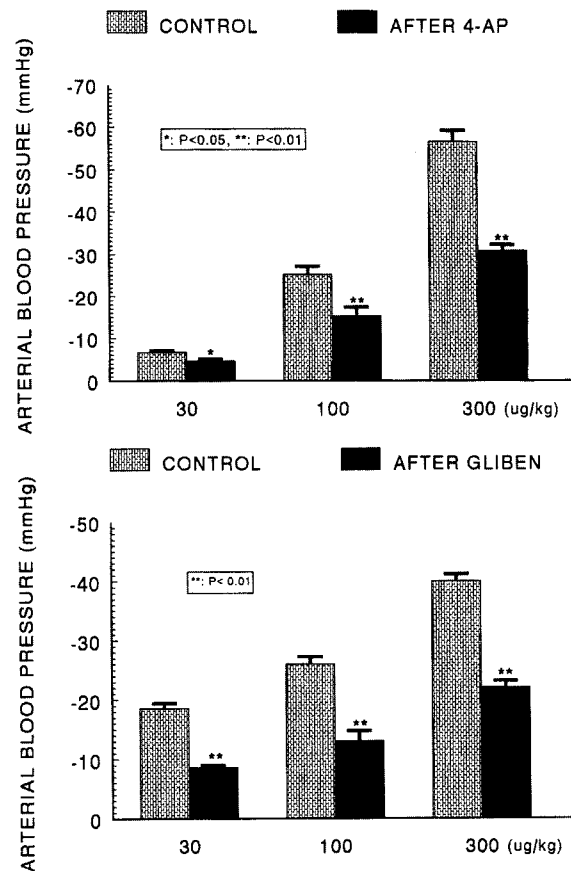


Fig. 4. Upper: Influence of 4-aminopyridine (4-AP) on intravenous nicorandil-evoked hypotensive responses. 4-Aminopyridine (1.0 mg/kg/30 min) was given into a femoral vein after obtaining the corresponding control responses of intravenous nicorandil. **Lower:** Influence of glibenclamide (GLIBEN) on intravenous nicorandil-evoked hypotensive responses. Glibenclamide (50.0 $\mu\text{g}/\text{kg}/30$ min) was given into a femoral vein after obtaining the corresponding control responses of intravenous nicorandil. Other legends are the same as in Fig. 1 and 2. . *: $P < 0.05$, **: $P < 0.01$.

and 58 ± 3 mmHg from the original baseline (121 ± 13 mmHg), respectively. However, after infusion of nicorandil with a rate of 30 $\mu\text{g}/\text{kg}/30$ min, they were significantly depressed to 57~41% of the corresponding control responses, respectively (Fig. 5). Fig. 5 (lower) shows that norepinephrine-evoked pressor responses are greatly attenuated after pretreatment with intravenous nicorandil.

Effects of nicorandil on contractile responses induced by phenylephrine and high K^+ in the rat aortic strips

Since it has been found that nicorandil causes depressor responses by the blockade of adrenergic α -receptors in the anesthetized rats as shown in Fig. 3 (upper) and 5, it was of

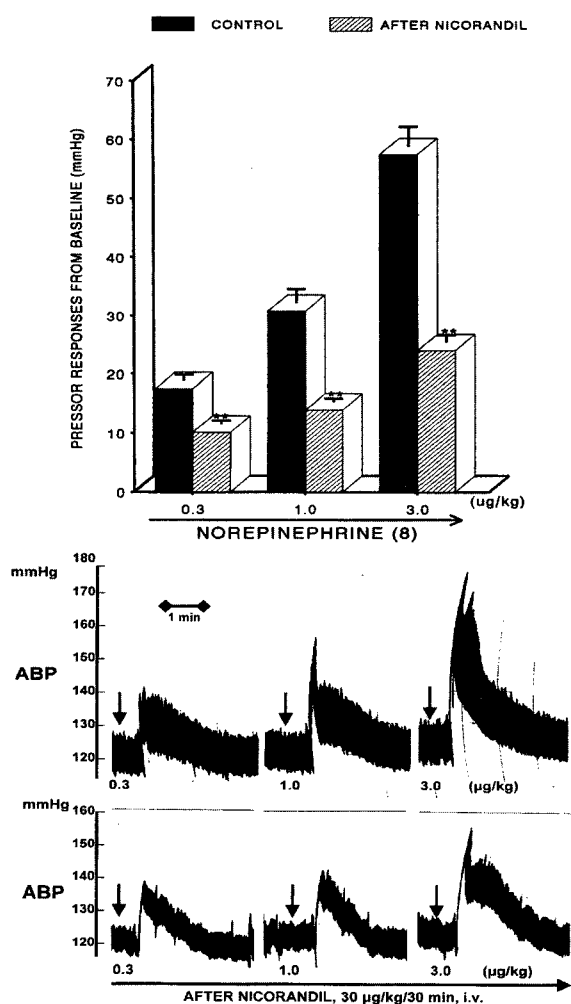


Fig. 5. Upper: Influence of intravenous nicorandil on norepinephrine-evoked pressor responses. Ordinate: Changes of blood pressure from baseline level in mmHg. Abscissa: Intravenous doses of norepinephrine in µg/kg. Vertical bar on the top of each column indicates standard error of mean. There was statistically significant difference in changes of norepinephrine-evoked pressor responses between before and after pretreatment with nicorandil. Nicorandil was infused into a femoral vein with a rate of 30.0 µg/kg/30 min after obtaining the corresponding control responses of intravenous norepinephrine. The original base-line of arterial blood pressure was 121± 13 mmHg. **: P< 0.01. **Lower:** The representative tracing of nicorandil effect on intravenous norepinephrine (NE)-induced pressor responses in the anesthetized rat. At arrow marks, the indicated doses (0.3, 1.0 and 3.0 µg/kg) of NE were administered into a femoral vein. Upper panel: NE-induced hypertensive responses in a non-treated rat. Lower panel: NE-induced hypertensive responses in a nicorandil-pretreated rat. ABP: Arterial blood pressure in mmHg. The chart speed was 20 mm/min.

interest to test the influence of nicorandil phentolamine on the isolated rat aortic contractile responses evoked by phenylephrine, a selective adrenergic α₁-receptor agonist. The resting

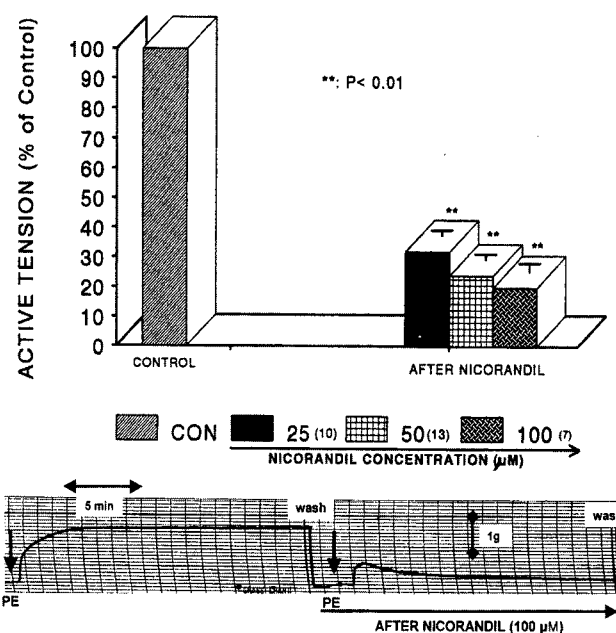


Fig. 6. Upper: Influence of nicorandil on phenylephrine (PE)-induced contractile response in the isolated rat aortic strips. The contractile response was induced by adding 10 µM of PE after adaptation with normal Krebs solution for two hours prior to initiation of the experimental protocol. "CONTROL" denotes active tension induced evoked by PE before adding nicorandil (100%). Ordinate: the active tension (% of control). Abscissa: concentration of PE (µM). Statistical difference was obtained by comparing the control with the nicorandil-pretreated groups (25, 50 and 100 µM). **Lower:** The typical tracing showing the effect of nicorandil on phenylephrine (PE)-induced contractile responses in the rat aortic strip. Left panel: PE-induced contractile response (Control). Right panel: PE-induced contractile response in the presence of 100 µM nicorandil. At arrow mark, the indicated dose (10 µM) of PE was added to the bath. The chart speed was 5 mm/min.

(basal) tension from the isolated rat aortic strips reaches a steady state after the perfusion with oxygenated Krebs-bicarbonate solution for 90 min before the experimental protocol is initiated. The resting tension was adjusted to 0.5 g. The effect of nicorandil on phenylephrine- as well as high potassium chloride-mediated contractile responses in the isolated rat aorta was examined. In the present study, nicorandil itself did not produce any effect on the resting tension in the aortic strips isolated from the rat (data not shown).

When 10⁻⁵ M concentration of phenylephrine was administered into the aortic bath, its active tension amounted to 1.8 ± 0.2 g from the resting tension level. In the presence of nicorandil at concentrations of 25~100 µM, 10⁻⁵ M-phenylephrine-induced contractile responses were dose-dependently inhibited to 32~23% of the control responses (Fig. 6). High

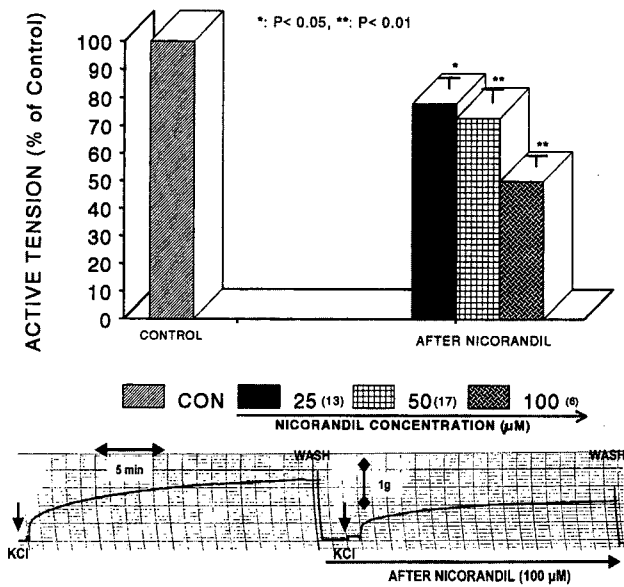


Fig. 7. Upper: Influence of nicorandil on high potassium-induced contractile responses in the isolated rat aorta. High potassium (56 mM) was added into the bath before and after pretreatment with 25, 50 and 100 μ M of nicorandil. **Lower:** The typical tracing showing the effect of nicorandil on high potassium (KCl)-induced contractile responses in the rat aortic strip. Left panel: KCl-induced contractile response (Control). Right panel: KCl-induced contractile response in the presence of 100 μ M nicorandil. At arrow mark, the indicated dose of KCl (56 mM) was added to the bath. The chart speed was 5 mm/min. Other legends are the same as in Fig. 6.

potassium exerts two distinct effects on cells: (1) depolarization of cell membrane, and (2) depolarization-induced influx of calcium via voltage-dependent calcium channels (Wada *et al.*, 1985). When added through the bath, high potassium at the concentration of 5.6×10^{-2} M, which is a membrane-depolarizing agent, caused an increased aortic contraction (1.5 ± 0.1 g). As shown in Fig. 7, high potassium (5.6×10^{-2} M)-induced contractile responses after pre-loading with 25–100 μ M of nicorandil were inhibited by 78–50% of their corresponding control responses in a dose-dependent fashion, respectively.

DISCUSSION

The present experimental results demonstrate that intravenous nicorandil causes a dose-dependent depressor action in the normotensive anesthetized rat. It seems that this hypotensive action of nicorandil is exerted through the vascular blockade of adrenergic α_1 -receptors, in addition to the well-known mechanism of potassium channel opening-induced vasorelaxation.

In support of this idea, it has been reported that nicorandil

reduces sympathetic coronary vasoconstriction by decreasing the reactivity of the vasculature to sympathetic neurotransmitters and by suppressing NPY overflow during cardiac sympathetic nerve stimulation (Chujo *et al.*, 1994). Itoh and his co-workers (1981) also showed that the NE-induced contraction was suppressed by 2-nicotinamidoethyl nitrate (2-NN) with hyperpolarization of the membrane in the porcine mesenteric artery, but with no change in the membrane potential in the guinea-pig mesenteric artery, presumably due to suppression of the Ca^{2+} -mobilization in the cell. Moreover, it is known that, in a Ca^{2+} -free medium, the residual NE-induced contraction was inhibited by nicorandil or nitroglycerin but not by nifedipine in rabbit aorta, cat coronary arteries and rabbit basilar arteries. The combined treatment with nicorandil and nitroglycerin caused a stronger suppression of residual NE response than that of a single treatment with either agent suggesting the different site of action for the two agents (Shibata *et al.*, 1984). In 21 patients with coronary artery disease, Ogino and his colleagues (1992) showed that plasma norepinephrine levels after exercise were suppressed with nicorandil. Also, the percent change in norepinephrine with nicorandil was significantly decreased during exercise and recovery. Therefore, nicorandil suppressed the exercise-induced hyper-response of the sympathetic nervous system. These results suggest that the mode of vasorelaxing action of nicorandil may be due to the alteration (inhibition) of Ca^{2+} kinetics in the cell.

In terms of these findings, the results obtained from the present study seem likely that nicorandil can cause the depressor effect at least through the blockade of sympathetic adrenergic α -receptors, in addition to the pre-existing mechanism of potassium channel-opening. Moreover, the present results that nicorandil-induced hypotensive responses were greatly inhibited by the pretreatment with chlorisondamine, an autonomic ganglionic blocking agent, or phentolamine, an adrenergic α -receptor blocking agent support that nicorandil has adrenergic α -receptor blocking activity. In the present study, nicorandil also inhibited the pressor responses evoked by NE in anesthetized rats as well as the contractile responses evoked by phenylephrine in the isolated rat aortic strips. This result suggests that nicorandil possesses the effective property inhibiting the activity of the sympathetic nervous system, in addition to the known mechanism of action. This inhibition seems likely to contribute to the relaxing effect of vascular smooth muscle by nicorandil.

In general, among drugs that interfere with peripheral sympathetic function, adrenergic α -receptor blocking agents alone

cause reversal of the epinephrine pressor response (Constantine *et al.*, 1973). When epinephrine is administered to untreated animals, its α -agonist properties predominate, resulting in a rise in mean arterial pressure. However, in the presence of adrenergic α -receptor blockade, the peripheral β_2 -agonist properties of epinephrine predominate and a fall in arterial pressure or reversal of the pressor response is observed. In contrast, the pressor responses to norepinephrine are impaired by adrenergic α -receptor blockade, but are not reversed (Freis *et al.*, 1951) as this agent processes little β_2 -agonist activity (Ablad *et al.*, 1975). In terms of the fact that nicorandil greatly depresses phenylephrine-evoked contractile response as well as NE-induced hypertensive responses, and nicorandil-induced hypotensive responses are reduced by phentolamine, it is thought that nicorandil has vascular relaxing activity through the adrenergic α -receptor blockade. In view of these reports, in the present work, the finding that nicorandil attenuated the NE-induced pressor responses demonstrates that nicorandil possesses the antagonistic activity of adrenergic α_1 -receptors.

Generally, it well known that potassium chloride (KCl) opens voltage-dependent calcium channels by depolarizing the cell membrane of vascular smooth muscle, resulting in increased influx of extracellular Ca^{2+} (Bolton, 1979; Schwartz & Taira, 1983; Dube *et al.*, 1985; 1988). Kim and his colleagues (1989) have shown that the contractile responses of vascular smooth muscle induced by CaCl_2 and KCl may result most likely from increased influx of extracellular Ca^{2+} through the voltage-dependent calcium channels. In terms of these results, the present findings that nicorandil inhibited the contraction of rat aortic smooth muscle evoked by not only phenylephrine (an α_1 -adrenergic receptor agonist) but also by KCl (a membrane depolarizer) indicate that the vascular relaxation of nicorandil is mediated by the blockade of α_1 -adrenergic receptors, in addition to the known mechanism of potassium channel opening action.

In previous studies, three cellular mechanisms have been proposed to explain relaxant response of vascular smooth muscle: (i) blockade of extracellular Ca^{2+} entry into cells (Fleckenstein, 1977; Schwartz & Triggle, 1984), (ii) increase in binding or sequestration of intracellular Ca^{2+} (Watkins & Davidson, 1980; Imai & Kitagawa, 1981), and (iii) inhibiting the release of intracellular stored Ca^{2+} (Imai & Kitagawa, 1981; Ito *et al.*, 1980a; 1980b). In contrast, the contractions of vascular smooth muscles induced by neurohumoral agents have been composed of two components: Phasic contraction induced by the Ca^{2+} released from inside the cell and tonic tension related to the

Ca^{2+} influx (Bevan, 1982; Dube *et al.*, 1988), both leading to increased intracellular calcium. In the light of these findings, it could not be ruled out that nicorandil can dilate the contractile responses of vascular smooth muscle evoked by phenylephrine through the blockade of extracellular Ca^{2+} entry into the muscle cells.

In this study, nicorandil-induced hypotensive responses were greatly suppressed by both glibenclamide and 4-aminopyridine, ATP-dependent potassium channel blockers. The present results demonstrate that nicorandil-induced hypotensive action could be mediated through the opening of vascular K_{ATP} -channels. Glibenclamide is a nonselective inhibitor of all subtypes of the K_{ATP} channel (Liu *et al.*, 2001). The K_{ATP} channels are specifically blocked by sulfonylurea derivatives such as tolbutamide and glibenclamide (Ascroft, 1990; Hamada *et al.*, 1990) and are activated by a number of ATP-dependent K^+ channel openers (Sanguinetti *et al.*, 1988; Hiraoka and Fan, 1989; Arena and Kass, 1989; Thuringer *et al.*, 1995; Kwak *et al.*, 1995). The opening of ATP-sensitive K^+ channels results in hyperpolarization of the plasma membrane (Longman and Hamilton, 1992) and hence, in vasodilatation of vascular smooth muscle. It has been found that 4-aminopyridine is capable of inhibiting various types of K^+ channels in both the outer membrane of the cell (Nelson and Quayle, 1995; Brayden, 1996) and the intracellular membrane associated with the SR (sarcoplasmic reticulum) (Fink and Stephenson, 1987). By inhibiting K^+ channels of SR, 4-aminopyridine can inhibit SR calcium sequestration (Fink and Stephenson, 1987). Based on these findings, the present results that nicorandil-induced hypotensive responses were greatly suppressed by both glibenclamide and 4-aminopyridine indicate that nicorandil produces depressor responses through the activation of vascular ATP-dependent inhibiting K^+ channels.

However, it is well-known that the ATP-sensitive K^+ channel openers consist of compounds with diverse chemical structures (Cook, 1988; Edwards and Weston, 1990). Activation of ATP-sensitive K^+ channels by these compounds depends on the intracellular concentrations of ATP, dinucleotide diphosphates, Mg^{2+} and H^+ ions (Findlay, 1987; Horie *et al.*, 1987; Arena and Kass, 1989; Shen *et al.*, 1991; Forestier *et al.*, 1996). Altered signalling pathways have been demonstrated in cultured vascular smooth muscle cells from SHR (Tuttle *et al.*, 1995) as well as altered properties of ATP-sensitive K^+ channels in mesenteric artery cells (Ohya *et al.*, 1996) and in ventricular myocytes from diabetic rats (Smith and Wahler, 1996). It has been reported that nicorandil, an ATP-sensitive K^+ channel opener,

produces smooth muscle relaxation by multiple mechanisms. In addition to the well-known effects on the opening of ATP-sensitive K⁺ channels, nicorandil-induced relaxation also involves the activation of guanylate cyclase as well as the opening of Ca²⁺-activated K⁺ channels (Zhou *et al.*, 1995).

On the other hand, in the present work, nicorandil-induced depressor responses were not affected by atropine or propranolol. This result suggests that nicorandil-induced depressor action is not mediated via stimulation of cholinergic muscarinic receptors and the blockade of adrenergic β-receptors.

Taken together, these results obtained from the present study demonstrate that intravenous nicorandil causes a dose-dependent depressor action in the anesthetized rat at least partly through the blockade of vascular adrenergic α₁-receptors, in addition to the well-known mechanism of potassium channel opening-induced vasorelaxation.

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