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Influence of Nicorandil on Arterial Blood Pressure of the Anesthetized Rabbits

조선대학교 대학원

의학과

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이 논문을 의학석사 학위신청논문으로 제출함.

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마취 토끼의 동맥혈압에 미치는 Nicorandil 의 영향

민 선 영

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니코란딜은 적어도 다음과 같은 두가지 작용기전에 의해서 현재 혈관이완제로서 임상적으로 협심증 치료에 사용되고 있는 니코틴아마이드 유도체이다 (Frampton 등, 1992; Goldschmidt 등, 1996). 즉, 니코란딜은 칼륨통로 개방제로써 작용과 또한 니트로혈관이완작용을 가지고 있어서 guanylyl cyclase 를 활성화시켜 guanosine 3', 5'-cyclic monophosphate (cGMP)를 증가시키는 것으로 알려져 있다 (Holzmann, 1983; Holzmann 등, 1992). 또한, 최근 임등(2005)은 니코란딜이 마취흰쥐에서 아드레날린 α 수용체 차단에 의한 혈관이완반응을 일으킨다고 보고하였다. 따라서 니코란딜이 정상혈압 마취토끼의 동맥혈압에 미치는 영향을 검색하고 작용기전을 규명코자 본 연구를 시행하여 다음과 같은 연구결과를 얻었다.

니코란딜(30-300 μg/kg)은 정상혈압 토끼의 일측 대퇴동맥 내로 투여 시 용

량의존성의 혈압하강반응을 나타내었다. 이러한 니코란딜의 혈압하강반응은 atropine (3.0 mg/kg, i.v.) 이나 propranolol (2.0 mg/kg, i.v.)의 전처치로 영향을 받지 않았으나 chlorisondamine (1.0 mg/kg, i.v.)이나 phentolamine (2.0 mg/kg, i.v.)의 존재 하에서 유의하게 억제되었다. 또한 4-aminopyridine (1.0 mg/kg/30min)로 전처치 한 후에는 니코란딜의 혈압하강 반응이 크게 감약되었다. 더욱이 니코란딜 (30.0 μg/kg/30 min)을 대퇴정맥 내로 주입한 후에 노르에피네프린의 승압반응을 뚜렷이 억제되었다.

이와 같은 연구결과를 종합하여 보면, 마취토끼에서 정맥 내로 투여한 니코란딜은 용량의존적으로 혈압하강반응을 일으켰으며, 이러한 니코란딜의 혈압하강작용은 기존의 칼륨통로개방작용에 의한 혈관이완작용 외에 적어도 혈관평활근의 아드레날린 α_1 수용체 차단작용에 기인되는 것으로 사료된다.

I. INTRODUCTION

The vasodilator nicorandil, *N*-(2-hydroxyethyl)-nicotinamide nitrate ester, 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 antiarrhythmic 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 Ca^{2+}-mobilization in the cell (Furukawa et al., 1981). Nicorandil had a greater relaxing effect on the maximum contractile response to norepinephrine (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). Recently, it has been shown that intravenous nicorandil causes depressor action in the anesthetized rat at least partly through the blockade of vascular adrenergic α_1-receptors, in addition to the known mechanism of potassium channel opening-induced vasorelaxation (Lim et al., 2005).

Based on these pharmacological actions of nicorandil, the present study was designed to investigate whether it can produce the hypotensive action in the normotensive anesthetized, and to compare this action with that in rats, in addition to the well-known K_{ATP}-channel opening.

II. MATERIALS AND METHODS

Experimental Procedure

Mature male New Zealand rabbits, weighing 2.0 to 3.0 kg, were used in this 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 rabbit was anesthetized with urethane (1.0 g/kg) subcutaneously, and tied in supine position on fixing panel.

A) 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^{o}C by a thermostatically controlling blanket and heating lamp throughout the course of the experiment.

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.): 1.0 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.: 0.5 mm) was made into a femoral vein for the administration of drugs and supplemental anesthetic agents as needed to maintain light surgical anesthesia. Each rabbit 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 intervals of 60 minutes.

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 computer program described by Tallarida and Murray (1987).

Drugs and Their Sources

The following drugs were used: nicorandil, heparin sodium (Daehan Choongwae Pharm. Co., Korea), norepinephrine bitartrate, and 4-aminopyridine (Sigma Chemical Co., U. S. A.), atropine sulfate, urethane (Aldrich Chemical Co., U.S.A.), chlorisondamine chloride (CIBA Co., U.S.A.), phentolamine mesylate (CIBA Co., U.S.A.). Drugs were dissolved in distilled water (stock) and added to the normal saline solution as required. However, nicorandil was dissolved in dimethyl

sulfoxide. 4-Aminopyridine was 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. Concentrations of all drugs used are expressed in terms of molar base and gram.

III. RESULTS

Influence of intravenous nicorandil on arterial blood pressure in urethane-anesthetized rabbits

When cardiovascular parameters were stabilized for 30 min before the experimental protocols were initiated, the administration of physiological saline solution in a volume of 0.5 ml into a femoral vein did not cause any changes in both arterial blood pressure. Then, nicorandil injected intravenously to the normotensive urethane-anesthetized rabbit produced a dose-dependent decrease in arterial blood pressure.

In 14 rabbits, as shown in table 1, Fig. 1 and 2, intravenous 30 μg/kg of nicorandil produced a fall in arterial blood pressure to –6.2±1.4 mmHg ($P< 0.01$) from the original baseline of 118 ± 12 mmHg. Increasing intravenous doses of nicorandil to 100 and 300 μg/kg caused the dose-related reduction in arterial pressure responses to –16.8±2.2 mmHg ($P< 0.01$) and –28.8±1.2 mmHg ($P< 0.01$) from the original baseline, respectively. Furthermore, these present experimental results were identical to those obtained from previous studies (Holzmann et al., 1992; Kukovetz et al., 1991; Akai et al., 1995; Lim et al., 2005).

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Influence of atropine and chlorisondamine on nicorandil-induced depressor responses in the anesthetized rabbits

In 5 experimental animals, the effect of atropine on the cardiovascular reponses 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, nicorandil given intravenously at all doses (30, 100 and 300 μg/kg) elicited nonsignificant changes in arterial blood pressure of –3.0±0.6 mmHg (ns), -12.0±1.8 mmHg (ns) and –28.0±1.2 mmHg (ns), respectively by comparing with their corresponding control, as shown in Fig. 3 and Table 2.

Chlorisondamine (1.0 mg/kg), an autonomic ganglionic blocking agent was given intravenously into a femoral vein of the rabbit to examine the cardiovascular effects of nicorandil. Following the administration of chlorisondamine, the baseline of blood pressure was reduced from 120 ± 12 mmHg to 76 ± 8 mmHg. In 5 rabbits, responses of arterial blood pressure by nicorandil, when given intravenously at 30, 100 and 300 μg/kg, were –5.0±0.4 mmHg, -18.0±1.4 mmHg and –28.0±1.2 mmHg from the pre-injection level, respectively. However, after pretreatment with chlorisondamine they were markedly inhibited by –2.0±0.1 mmHg ($P<0.05$), -5.6±0.6 mmHg ($P< 0.01$) and –10.6±0.6 mmHg ($P< 0.01$) from the original baseline, respectively as shown in Fig. 4 and Table 2.

Influence of phentolamine and propranolol on intravenous nicorandil-induced depressor responses in the anesthetized rabbits

In order to investigate whether nicorandil-induced hypotensive response is mediated by the blockade of adrenergic α-receptors or of autonomic ganglia, it

was of interest to test the influence of phentolamine, an antagonist of adrenergic α-receptors, on nicorandil-evoked hypotensive responses. In 4 rabbits, in order to examine the relationship 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 responses induced by intravenous nicorandil at doses of 30, 100 and 300 μg/kg were inhibited to –6.0±0.1 mmHg ($P< 0.01$), -14.0±0.1 mmHg ($P< 0.01$) and –18.0±1.2 mmHg ($P< 0.01$), respectively, in comparison with their control responses of –12.0±1.2 mmHg, -21.0±1.8 mmHg and –30.0±2.0 mmHg, as shown in Fig. 5 and Table 3.

In order to examine the relationship between nicorandil-induced cardiovascular effects and adrenergic β-receptors, propranolol (2.0 mg/kg) was administered intravenously. In 6 rabbits, prior to administration of propranolol, nicorandil-induced hypotensive responses at doses of 30, 100 and 300 μg/kg were –5.2±1.8 mmHg, -13.8±1.6 mmHg and –26.0±3.0 mmHg from the preinjection level, respectively. However, following pretreatment with propranolol nicorandil-induced depressor responses (which were 4.4±1.4 mmHg, -12.0±1.4 mmHg and –23.6±2.6 mmHg) were not altered as compared with their corresponding control responses, as shown in Fig. 6 and Table 3.

Influence of 4-aminopyridine on intravenous nicorandil-induced depressor responses in the anesthetized rabbits

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 reticulim (SR) (Fink and Stephenson, 1987). By inhibiting SR K^+ channels, 4-aminopyridine can inhibit SR calcium sequestration (Fink and Stephenson, 1987). Therefore, 4-aminopyridine, presently employed as a potassium channel blocking agents was injected intravenously in order to examine the interrelationship between nicorandil-induced hypotensive responses and potassium channel. After pretreatment with 4-aminopyridine (2.0 mg/kg, i.v.), depressor responses of intravenous nicorandil at all doses of 30, 100 and 300 μg/kg were greatly inhibited by –2.6±0.4 mmHg ($P< 0.01$), -7.2±1.4 mmHg ($P< 0.05$) and –14.0±0.6 mmHg ($P< 0.01$), respectively, as compared with control responses of –7.0±1.2 mmHg, -11.6±1.2 mmHg and –22.0±1.8 mmHg from 8 rabbits, as shown in Fig. 7 and Table 3.

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

Since pretreatment with both chlorisoindamine and phentolamine greatly inhibited nicorandil-induced hypotensive responses as shown in Fig. 3~4. 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 4 rabbits, norepinephrine injected intravenously at doses of 1, 3 and 10 μg/kg caused dose-dependent pressor responses of 8.0±0.9 mmHg, 18.0±1.0 mmHg

and 44.0±1.7 mmHg from the original baseline (120±12 mmHg), respectively. However, after infusion of nicorandil with a rate of 30 μg/kg/30min, they were significantly depressed to 5.0± 0.6 mmHg ($P< 0.05$), 13.0±0.6 mmHg ($P< 0.01$) and 24.0±1.2 mmHg ($P< 0.01$) at the same doses, respectively (Table 4 and Fig. 8). Fig. 9 shows that norepinephrine-evoked pressor responses are attenuated after pretreatment with intravenous nicorandil in an anesthetized rabbit.

IV. DISCUSSION

Based on these experimental results, it is suggested that nicorandil given intravenously causes a dose-dependent depressor action in the normotensive anesthetized rabbit. It also looks likely that this nicorandil-induced depressor action is exerted through the blockade of vascular adrenergic α_1-receptors, in addition to the known mechanisms of stimulating soluble guanylate cyclase and potassium channel opening-induced vasorelaxation.

In support of this idea, it has been shown that this hypotensive action of nicorandil is very similar with that in the anesthetized rats (Lim et al., 2005). Also, 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 that 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 sympathetic nervous system hyper-response to exercise. 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 mechanisms of potassium channel-opening and stimulating soluble guanylate cyclase. 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. Moreover, it has been reported that nicorandil also inhibited the contractile responses evoked by phenylephrine in the isolated rat aortic strips (Lim et al., 2005). This 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.

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 (Aschroft, 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 (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 may produce 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 Ca^{2+}-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 stimulayion of cholinergic muscarinic receptors and the blockade of adrenergic β–receptors.

Taken together, these results obtained from the present study demonstrate that nicorandil given intravenously causes the hypotensive action in a dose-dependent fashion at least partly through the blockade of vascular adrenergic α_1-receptors in the rabbit anesthetized with urethane, in addition to the well-known mechanisms of stimulating soluble guanylate cyclase and potassium channel opening-induced vasorelaxation.

V. SUMMARY

The vasodilator nicorandil is a nicotinamide derivative used clinically for the treatment of angina (Frampton et al., 1992; Goldschmidt et al., 1996). Nicorandil has at least two mechanisms of action; it acts as a K^+ channel opener and also has a nitrovasodilator action, activating guanylyl cyclase and so increasing guanosine 3':5'-cyclic monophosphate (Holzmann, 1983; Holzmann et al., 1992). Recently, it has been shown that nicorandil administered intravenously causes depressor action at least partly through the blockade of vascular adrenergic α_1-receptors in the anesthetized rats (Lim et al., 2005).

Therefore, the present study was conducted to investigate the effects of nicorandil on arterial blood pressure in the normotensive rabbits anesthetized with urethane and to establish the mechanism of action. Nicorandil (30 ~ 300 μg/kg) given into a femoral vein of the normotensive rabbit 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.). Also, after the pretreatment with 4-aminopyridine (2.0 mg/kg/30 min, i.v.), nicorandil-induced hypotensive response was greatly reduced. Interestingly, the infusion of nicorandil (1.0 mg/kg/30min) into a femoral vein made a significant reduction in hypertensive responses induced by intravenous norepinephrine.

Collectively, these results obtained from the present study demonstrate that intravenous nicorandil causes a dose-dependent depressor action at least partly

through the blockade of vascular adrenergic α_1-receptors in the anesthetized rabbits, in addition to the known mechanisms of stimulating soluble guanylate cyclase and potassium channel opening-induced vasorelaxation.

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