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Interrelationship of Cardiovascular Effects, Plasma Levels of Nicorandil, and Vascular cGMP Formation in Conscious Rats

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Abstract

The relationship between the dual activity of nicorandil (K_{ATP} channel-opening activity and nitrate-like action), plasma levels, and changes in vascular cGMP levels and cardiovascular parameters was investigated in conscious rats.

Nicorandil (3 mg kg^{-1}, p.o.) was rapidly absorbed and caused a significant reduction in blood pressure, lasting for at least 1 h, increases in heart rate and femoral blood flow, and decreases in femoral vascular resistance. These were entirely abolished by intravenous glibenclamide (20 mg kg^{-1}). The plasma concentration of nicorandil reached a maximum 30 min after dosing. After administration of nicorandil, a correlation was observed between blood pressure and plasma nicorandil level or femoral vascular resistance. A significant increase ($P < 0.05$) in the cGMP content of the thoracic aorta occurred 15 min after administration of nicorandil, and persisted for at least 2 h.

These results imply that nicorandil induces vasodilatation by opening K_{ATP} channels in peripheral resistance vessels, leading to overt reduction of blood pressure, but acts on conductance vessels mainly through nitrate-like activity.

Nicorandil (*N*-(2-hydroxyethyl)nicotinamide nitrate ester) has the hybrid biological properties of a K_{ATP}-channel opener and a nitrate (Taira 1989) and is an orally efficacious coronary vasodilator which is currently being utilized in the therapy of angina pectoris (Sakai 1989; Kinoshita & Sakai 1990; Krumenacker & Roland 1992). It is well known that K_{ATP}-channel openers, such as cromakalim and minoxidil, are potent vasodilating drugs which act directly on the vascular smooth muscle cell (Kreye et al 1993), whereas nitrates elevate cGMP levels in vascular tissues by stimulating soluble guanylate cyclase, leading to vasorelaxation (Kukovetz & Holzmann 1987). Although several experiments have demonstrated a dual mechanism of nicorandil, the relationships between this mechanism, plasma levels of nicorandil, changes in vascular cGMP levels and cardiovascular parameters have not been reported. This study was designed to examine these relationships after a single oral dose of nicorandil, and to examine the influence of pretreatment with the K_{ATP}-channel blocker, glibenclamide (Cavero et al 1989; Clapham et al 1991), on these effects in conscious rats.

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Materials and Methods

Chemicals

Nicorandil and SG-86 (*N*-(2-hydroxyethyl)-nicotinamide) were synthesized in the Chugai Organic Chemistry Laboratory. [Carbonyl-^{14}C]nicorandil was synthesized by Amersham International (Little Chalfont, Buckinghamshire, UK). The specific activity was 9·58 MBq mg^{-1} and the radiochemical purity 99%. Unless otherwise stated, unlabelled nicorandil was used for experiments. Glibenclamide (Wako Junyaku, Osaka, Japan) was dissolved in NaOH (0·1 M; 1 mL) then glucose solution (5%, 4 mL) was added slowly with sonication to give a final concentration of 5 mg mL^{-1} (Furukawa et al 1993). Other compounds were dissolved in and diluted with distilled water. In this experiment the oral dose of nicorandil (3 mg kg^{-1}) was chosen according to information from previous experiments (Sakai et al 1980, 1984, 1998); this

dose was pharmacologically effective in animal models, and as the duration of its plasma concentration was not very long, we could relatively easily evaluate the mechanism for nicorandil-induced vasodepression in relation to its plasma concentration and vascular cGMP content.

Animals

All experiments were performed according to the Guiding Principles for the Care and Use of Laboratory Animals approved by The Japanese Pharmacological Society. Male Sprague–Dawley rats (Charles River Japan, Hino), 7–8 weeks, were used. The animals were fasted overnight before the experiment but water was freely available.

Blood pressure and femoral blood flow measurements in conscious rats

Animal preparations. Under pentobarbitone-Na anaesthesia (55 mg kg^{-1}, i.p.) rats (367·9 ± 4·4 g, n = 20) were surgically implanted with an indwelling polyethylene cannula for direct monitoring of blood pressure and heart rate. A PE-50 polyethylene cannula with a PE-10-tip was inserted via the left femoral artery into the abdominal aorta below the renal arteries and above the original of the iliac arteries. Arterial blood pressure was determined with a Nihon Kohden (Tokyo, Japan) DX-360 pressure transducer and heart rate with a Nihon Kohden AT-601G heart rate counter. A miniature Crystal Biotech (Hopkinton, MA, USA) VF-1 pulsed Doppler flow probe was sutured around the right iliac artery and connected to a Crystal Biotech (Northborough, MA, USA) model HVPD-20 20-MHz high-velocity pulsed Doppler module. Changes in blood flow velocity (kHz), measured as Doppler shifts, are directly proportional to volume flows (mL min^{-1}) (Haywood et al 1981). The femoral vascular resistance (mmHg mL^{-1} min^{-1}) determined from the mean arterial blood pressure and the mean femoral blood flow was calculated with a Nihon Kohden EO-601G analogue multiplier. For intravenous and intragastric injections of drugs, polyethylene cannulae (PE-10) were inserted via the jugular vein into the abdominal vena cava and into the stomach, respectively. The cannulae were extended subcutaneously and exteriorized between the scapulae and fitted to a tubular spring guard. The mobility of each rat was facilitated by connecting the indwelling cannula to an Instech (PA, USA) model 375/20 swivel. After surgery, each animal was individually caged and the indwelling cannula was flushed with saline solution (0·9%, 0·3 mL) by means of a syringe. All recordings were made on a chart with a Graphtec (Tokyo, Japan) WR-3101 Linearcorder under unanaesthetized and unrestrained conditions. Peak responses to drugs were expressed as percent changes from the pre-drug level.

Experimental protocols

The animals were divided into four groups (n = 5 in each group). Groups I and II were given intragastric vehicle (distilled water; 5 mL kg^{-1}) and nicorandil (3 mg kg^{-1}), respectively; groups III and IV were given intragastric vehicle (5 mL kg^{-1}) and nicorandil (3 mg kg^{-1}), respectively, after intravenous glibenclamide (20 mg kg^{-1}). Before drug administration a period of at least 60 min was allowed for stabilization of preparations. About 20 min after intravenous administration of glibenclamide (20 mg kg^{-1}, 5 mg mL^{-1}) over 5 min, nicorandil (3 mg kg^{-1}) was given directly into the stomach. For intragastric injection of nicorandil (3 mg kg^{-1}, 0·6 mg mL^{-1}), the drug solution (5 mL kg^{-1}) was administered over 60 s and flushed over 10–20 s with distilled water (0·4 mL).

Measurement of plasma concentrations of nicorandil and its main metabolite, SG-86

Five rats received oral [carbonyl-^{14}C]nicorandil (3 mg kg^{-1} containing 28·74 MBq radioactivity) dissolved in distilled water (3 mL kg^{-1}). Before dosing and 15 and 30 min and 1, 2, 3, 4 and 5 h after dosing blood samples were obtained from the tail vein and plasma was separated by centrifugation at 3000 rev min^{-1} at 4 °C. The concentrations of nicorandil and SG-86 in the specimens were determined according to a procedure described elsewhere (Sakai et al 1980, 1998). Briefly, ethanol (99·5%, 200 μL) was added to the plasma (50 μL) and the mixture was shaken and then centrifuged at 4°C for 5 min at 3000 rev min^{-1}. The supernatant (200 μL) was evaporated, redissolved in methanol (10%, 150 μL) and analysed by high-performance liquid chromatography (HPLC) (LC-Module-1, Waters Associates, Milford, MA). HPLC was conducted on a 250 mm × 4·6 mm i.d. TSK gel ODS 80Tm column (Tosoh), with 10% methanol as mobile phase at a flow rate of 1 mL min^{-1}. The radioactivity of the eluent was measured by liquid scintillation counting (Packard, Meriden, CT; Tri Carb 2500TR).

Measurements of cGMP levels in the thoracic aorta

The rats were trained for five days continuously by gastric intubation of a Teflon stomach tube (1·6 mm o.d., 11 cm length). On the day before the experiments animals were weighed and randomly divided into two groups. Drug solutions, prepared fresh daily, were given between 0900 and 1100 h. The

first group received oral nicorandil (3 mg kg^{-1}; as nicorandil solution, 3 mL kg^{-1}). The second group, which served as control, was given oral distilled water (3 mL kg^{-1}). In both groups, rats were killed by time-matched decapitation before dosing and 15 min and 1, 2 and 3 h after drug administration. The thoracic aorta was quickly removed, and tissue cGMP content was determined with a competitive radioimmunoassay (Amersham International), according to a procedure reported elsewhere (Sakai et al 1998). Tissue cGMP levels were expressed as pmol (mg protein)$^{-1}$.

Statistical analysis

Data are expressed as means ± s.e.m. Differences between paired or unpaired mean values were analysed by Student's *t*-test. Analysis of variance was used for multiple comparisons of data. When multiple comparisons were made with a single control, Dunnett's test was used to determine the level of statistical significance. Probabilities of less than 5% ($P < 0.05$) were considered statistically significant.

Results

Effects of nicorandil on arterial blood pressure, heart rate, femoral blood flow and femoral vascular resistance in conscious rats

Basal values of mean arterial blood pressure, heart rate, femoral blood flow and femoral vascular resistance, just before vehicle or nicorandil administration were: in the absence of glibenclamide; vehicle-treated group, 99·0 ± 3·7 mmHg, 398·0 ± 15·6 beats min^{-1}, 2·3 ± 0·2 mL min^{-1} and 44·3 ± 2·9 mmHg mL^{-1} min^{-1} (n = 5), respectively; nicorandil-treated group, 103·0 ± 3·0 mmHg, 418·0 ± 8·0 beats min^{-1}, 2·1 ± 0·1 mL min^{-1} and 49·2 ± 2·7 mmHg mL^{-1} min^{-1} (n = 5), respectively: in the presence of glibenclamide; vehicle-treated group, 115·5 ± 3·1 mmHg, 352·3 ± 16·2 beats min^{-1}, 1·4 ± 0·1 mL min^{-1} and 75·3 ± 2·5 mmHg mL^{-1}min^{-1} (n = 5), respectively; nicorandil-treated group, 113·3 ± 2·1 mmHg, 341·7 ± 19·0 beats min^{-1}, 1·5 ± 0·1 mL min^{-1} and 76·4 ± 2·4 mmHg mL^{-1} min^{-1} (n = 5), respectively. There were no significant differences between the corresponding values.

In the absence of glibenclamide, a single intragastric dose of nicorandil (3 mg kg^{-1}) induced prominent reductions in blood pressure and femoral vascular resistance, accompanied by pronounced increases in heart rate and femoral blood flow. Onset of the effect of nicorandil was rapid, and the maximum was reached within 5 min. The hypotensive and femoral vasodilator effects of nicorandil lasted for at least 1 h (Figure 1A). In the presence of glibenclamide (20 mg kg^{-1} over 5 min), the effects of nicorandil were barely apparent (Figure 1B). As shown in Figure 2, in the absence of glibenclamide a significant correlation was observed between reductions in blood pressure and femoral vascular resistance ($y = 1.095x + 47.793$, $r = 0.976$, $P < 0.001$, $n = 8$, paired).

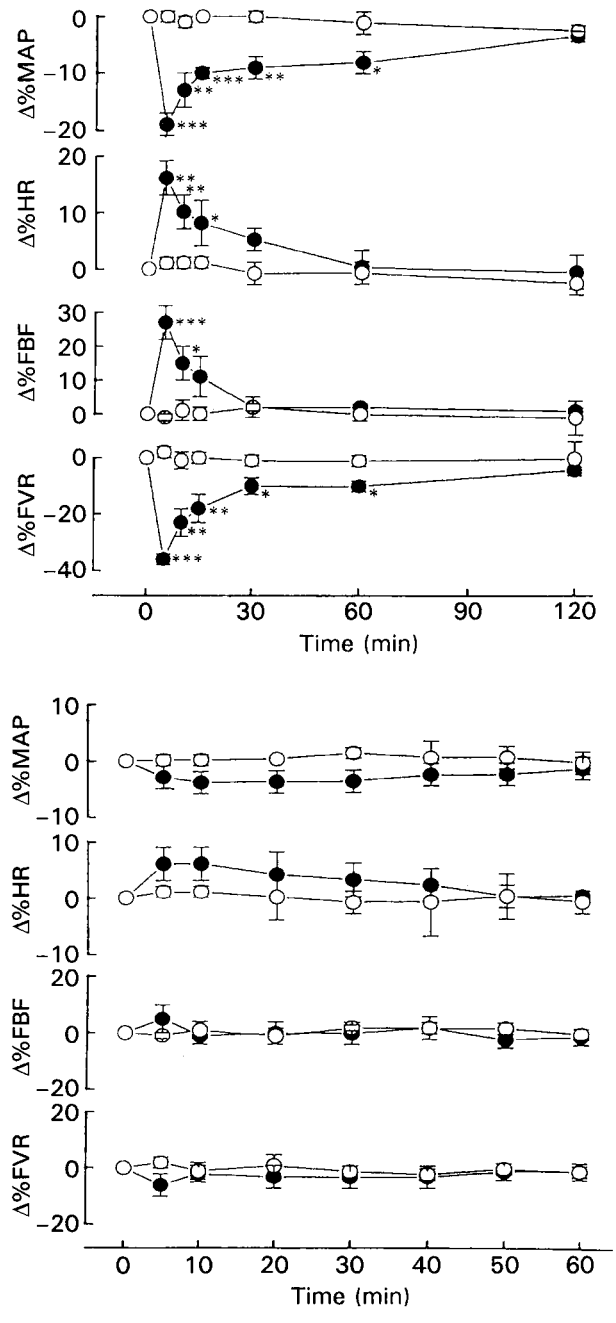

Figure 1. Effects of intragastric nicorandil (3 mg kg^{-1}) on mean arterial blood pressure (MAP), heart rate (HR), femoral blood flow (FBF) and femoral vascular resistance (FVR) in the absence (upper) and presence (lower) of glibenclamide (20 mg kg^{-1} i.v.) in conscious rats. Vertical bars represent means ± s.e.m. of results from five observations. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, significantly different from the corresponding values from the vehicle-treated (○) group.

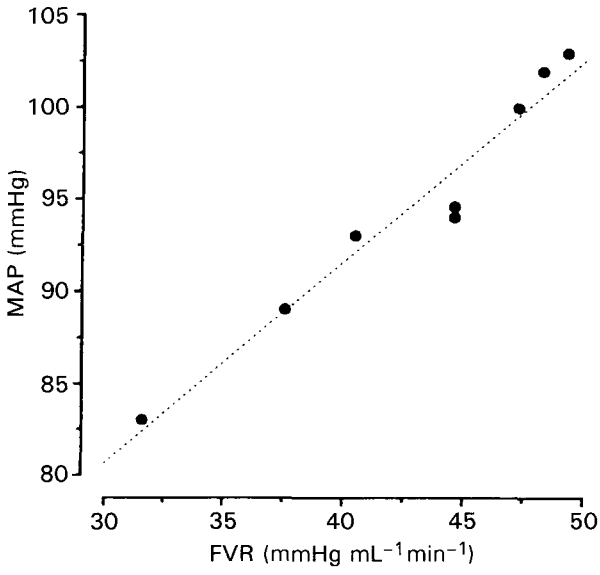

Figure 2. Correlation between mean arterial blood pressure (MAP) and femoral vascular resistance (FVR) from time 0 to 3 h (data not shown in Figure 1A) after intragastric administration of nicorandil (3 mg kg^{-1}). See Figure 1A.

Plasma levels of nicorandil and its main metabolite, SG-86

As shown in Figure 3, [^{14}C]nicorandil (3 mg kg^{-1}) was rapidly absorbed after oral dosing, reaching a maximum plasma concentration (C_{max}) within 30 min. The C_{max} of nicorandil was approximately 2·6 μg mL^{-1}, and T_{max} 0·45 h. Approximately 2 h after dosing the plasma level of nicorandil fell by approximately 0·9 μg mL^{-1} (4·2 μM). On the other hand, the C_{max} (approx. 1·7 μg mL^{-1}) of SG-86 was attained at 2 h, and thereafter decreased gradually. As shown in Figure 4, there was a correlation between blood pressure decrease and plasma nicorandil levels ($y = -0{\cdot}004x + 103{\cdot}457$, $r = -0{\cdot}913$, $n = 5$, unpaired).

Effects of nicorandil on vascular cGMP formation

After oral nicorandil (3 mg kg^{-1}), changes in vascular cGMP formation were examined for 3 h. As depicted in Figure 3, the cGMP formation was maximum 15 min after the nicorandil dose, and thereafter a steady-state level was maintained for at least 2 h. There was no significant difference between the cGMP increases occurring 15 min and 2 h after administration of nicorandil. Even 2 h after administration, the vascular cGMP level in the group treated with nicorandil was significantly higher (approx. 1 pmol mg^{-1} increase from the control level) compared with that in the vehicle-treated (control) group.

Discussion

These results reveal that nicorandil given intragastrically to conscious rats elicited pronounced vasodepression lasting for at least 1 h, accompanied by increases in heart rate and femoral blood flow and decreases in femoral vascular resistance. Treatment with glibenclamide almost abolished these changes.

In this experiment it was noted that there is a significant correlation between reductions in blood pressure and femoral vascular resistance after nicorandil administration, indicating that the nicorandil-induced vasodepression was dependent on the reduction of peripheral vascular resistance.

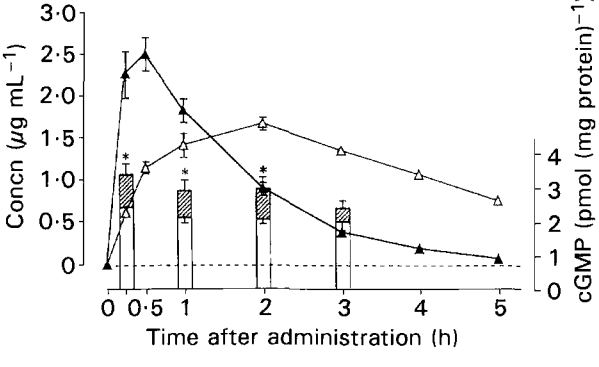

Figure 3. Plasma levels of nicorandil and its main metabolite SG-86, and vascular cGMP formation after oral administration of nicorandil (3 mg kg^{-1}). Vertical bars represent means ± s.e.m. of results from five measurements of plasma levels (▲ nicorandil; △ SG-86) and from ten measurements of cGMP levels (▨ nicorandil; □ vehicle). *P<0·05, significantly different from the result from the vehicle-treated group.

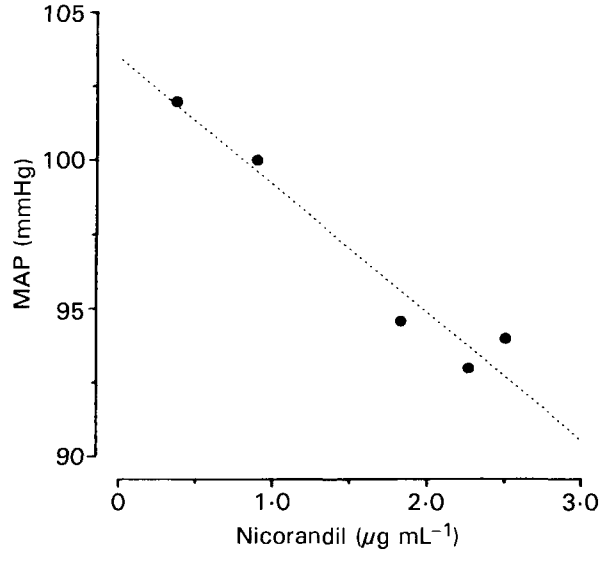

Figure 4. Correlation between mean arterial blood pressure (MAP) and plasma nicorandil levels after oral administration of nicorandil (3 mg kg^{-1}). Plot at 15, 30 min, 1, 2 and 3 h (data not shown in Figure 1A) after nicorandil administration. See Figures 1A and 3.

Nicorandil is a hybrid between a nitrate and a potassium-channel activator (Taira 1989)—it relaxes the resistance vessels by activating K_{ATP} channels (Yanagisawa & Taira 1980) and also acts on conductance vessels through a nitrate-like action, stimulating cGMP (Kukovetz & Holzmann 1987). Indeed, in the current experiment glibenclamide, an antagonist of K_{ATP} channels (Cavero et al 1989; Clapham et al 1991), prevented the reductions in blood pressure and femoral vascular resistance induced by nicorandil. The dose of glibenclamide ($20\,mg\,kg^{-1}$) used was enough to abolish the vasodepressor effect of cromakalim ($30\,\mu g\,kg^{-1}$), a K_{ATP}-channel opener (Hamilton & Weston 1989), but not that of acetylcholine ($0{\cdot}1\,\mu g\,kg^{-1}$), in anaesthetized rats (Saito & Sakai 1998). It seems that the increase in heart rate was a reflex response to the reduction in blood pressure, because it was blocked by treatment with propranolol (data not shown). It has been reported that nicorandil barely affects the central nervous system in anaesthetized dogs (Shiraki et al 1981) and that doses which double the coronary flow in the isolated canine heart ($<30\,\mu M$) were not seen to have any effect on electrophysiological parameters and contractility (Taira et al 1979). Thus, it seems that nicorandil-induced vasodepression occurs by acting on peripheral resistance vessels.

In this study, nicorandil ($3\,mg\,kg^{-1}$) given orally was rapidly absorbed (C_{max} approx. $2{\cdot}6\,\mu g\,mL^{-1}$; T_{max} 0·45 h) and gradually metabolized to a pharmacologically inactive, denitrated compound, SG-86. This result is in accord with previous findings (Sakai et al 1984). In the current experiment a correlation was observed between blood-pressure reduction and the plasma level after nicorandil administration. It was noted that 2 h after dosing the plasma level of nicorandil was approximately $0{\cdot}9\,\mu g\,mL^{-1}$ ($4{\cdot}2\,\mu M$), which produces a cGMP increase of approximately $1\,pmol\,mg^{-1}$ from the basal level in the in-vivo thoracic aorta, sufficiently developing pronounced muscle relaxation, when tested in in-vitro aortic preparations (Sakai et al 1998). Thus, nicorandil dilates conductance vessels (involving large coronary arteries) by stimulation of guanylate cyclase. It has been confirmed that dilation of large coronary arteries and antispasmodic action of nicorandil are a result of its nitrate-like properties, whereas a sustained increase of peripheral blood flow and a reduction of systemic vascular resistance result primarily from dilation of resistance arterioles caused by K_{ATP}-channel activation (Taira 1989; Kinoshita & Sakai 1990). Thus, the beneficial clinical effect of nicorandil in patients with coronary artery disease seems to be attributable to the combination of nitrate-like and K_{ATP}-channel-opening activity (Kinoshita & Sakai 1990).

These results confirm that nicorandil reduces blood pressure through its K_{ATP}-channel opening action on resistance vessels, whereas it dilates conductance vessels through its nitrate-like properties, probably related to spasmolytic effects. Thus, nicorandil seems to regulate the redistribution of blood flow and vascular tone through its balanced effect on vasculature.

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