Comparative Study of Vasodilator Effects of the Potassium Channel Openers NIP-121 and Levocromakalim in Dogs and Rats

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ABSTRACT—The effects of potassium channel openers NIP-121 ((+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidine-1-yl)-6H-pyrano[2,3-f]benz-2,1,3-oxadiazole) and levocromakalim were examined in vitro and in vivo. In isolated canine vascular beds, NIP-121 (3 x 10^-9 to 10^-7 M) and levocromakalim (3 x 10^-8 to 10^-6 M) produced a concentration-dependent reduction in the vasoconstrictor responses to U46619. The effects were antagonized by glibenclamide, an ATP-sensitive potassium channel blocker. The maximal relaxation was more than 70% of the maximal vasodilation induced by papaverine (10^-4 M), except in the basilar artery. These compounds had very potent effects on the coronary and cranial mesenteric arteries and saphenous vein. In the coronary perfused rat heart, both compounds (10^-7 M) also increased coronary perfusion flow. The effects were also inhibited by glibenclamide (10^-6 M). In anesthetized dogs, NIP-121 (1 to 10 ng/kg (3.2 to 32 nmol/kg), i.v.) and levocromakalim (3 to 30 ng/kg (10.5 to 105 nmol/kg), i.v.) dose-dependently increased coronary and renal blood flow. NIP-121 and levocromakalim at higher doses produced the greatest increase in coronary blood flow among the blood vessels examined, in spite of the hypotensive effect. In conclusion, NIP-121 and levocromakalim were similarly selective vasodilators on the canine isolated coronary and cranial mesenteric arteries and saphenous vein, and they selectively increased coronary blood flow in vivo. With respect to increasing the coronary blood flow, NIP-121 had a fourfold greater potency than levocromakalim. This effect might be related to the glibenclamide-sensitive potassium channels.

Keywords: Potassium channel opener, NIP-121, Levocromakalim, Coronary artery, Coronary blood flow

Potassium channel openers such as cromakalim have been shown to cause relaxation in a variety of smooth muscle preparations (1–7) and the effect is generally found to be associated with an increase in the ^86Rb+ or ^42K+ efflux (3, 5, 7) via ATP-sensitive (8) and other types of K+ channels (9, 10). The increase in efflux is associated with hyperpolarization of the membrane towards the calculated K+ equilibrium potential (1, 3, 4, 7).

NIP-121, (+)-7,8-dihydro-6,6-dimethyl-7-hydroxy-8-(2-oxo-piperidine-1-yl)-6H-pyrano[2,3-f]benz-2,1,3-oxadiazole, has also been reported to produce arterial (11–15) or tracheal (16, 17) smooth muscle relaxation, to lower blood pressure (18), and also to shorten action potential duration (19). The effects were thought to be associated with an increase in the ^86Rb+ efflux that passes through potassium channels and is competitively inhibited by glibenclamide, an ATP-sensitive potassium channel blocker (11, 13–15). Similar findings have been reported for cromakalim (1–7). The smooth muscle relaxation and negative inotropic effect of NIP-121 have been reported to be about 10 times more potent than those of cromakalim in rat aorta (11, 13–15), guinea pig trachea (16) and heart (19), respectively.

In this study, the effects of NIP-121 and levocromakalim, an active enantiomer of cromakalim, were investigated using isolated canine blood vessels precontracted with the thromboxane A2 mimetic U46619 and anesthetized dogs. In addition, the effects of these compounds on coronary perfusion flow rate were also studied using coronary perfused rat heart.

MATERIALS AND METHODS

Tissue bath experiments

Mongrel dogs of either sex weighing 8 to 15 kg purchased from Saitama Experimental Animals Supply (Saitama) were used. All dogs were sacrificed by venesection under deep anesthesia induced by intravenous injec-
tion of an excess dose of sodium pentobarbital. The canine arteries (large left circumflex (LCX) and left anterior descending (LAD) portions of coronary, basilar, cranial mesenteric, renal and femoral arteries) and saphenous vein were dissected free and cleaned of adherent fat and connective tissue; Then they were each cut into a spiral strip 1 to 3 mm in width and 10 to 15 mm in length while being maintained in Krebs Henseleit solution (KHS) of the following composition: 118.4 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 25 mM NaHCO₃ and 11.7 mM glucose. KHS was maintained at 37°C and bubbled with 95% O₂/5% CO₂ (pH 7.4). Each experiment was performed with preparations isolated from the same dogs.

The arteries and vein were suspended in 20-ml organ baths containing KHS. Isometric tension was recorded under a resting tension of 1.5 g for LCX and LAD, 1 g for the basilar and cranial mesenteric arteries, 2 g for the renal and femoral arteries, and 0.7 g for the saphenous vein (TB-611T; Nihon Kohden, Tokyo). Each resting tension was adjusted to generate the U46619-induced maximal contraction for each vascular bed, and each resting tension was also maintained at a constant basal-tension level. The tissue was allowed to equilibrate for a period of 1 hr during which the KHS was changed every 20 min. After the equilibration period, the arteries and vein were contracted with a sub-maximal concentration (3 x 10⁻⁹ (cranial mesenteric artery and saphenous vein) or 10⁻⁸ M (other blood vessels)) U46619 to produce similar developed tension. In the preliminary study, the dose-response curves or the pD₂ values for U46619 in different blood vessels were approximately similar. Maximal contractions were obtained at about 10⁻⁷ to 10⁻⁸ M. We confirmed that the contractions by U46619 at the concentration used here were sub-maximum. NIP-121 and levcromakalim were cumulatively added to the preparation in half log unit increments after the contractile responses were allowed to reach a stable plateau. At the end of the experiment, papaverine (10⁻⁴ M) was added to induce maximal relaxation. The relaxation produced is expressed as a percentage of the maximal relaxation induced by papaverine. For each drug, the concentration required for half maximal relaxation (IC₅₀) was determined by drawing a straight line between two points, one on each side of the 50% relaxation level relative to the maximal relaxation induced by papaverine, in the steepest portion of the dose-response curve. Antagonist pA₂ estimates were calculated by Schild analysis.

Coronary perfusion experiments

Male Wistar rats weighing 270 to 380 g, purchased from Charles River Japan (Kanagawa), were used. All rats were anesthetized with sodium pentobarbital (30 mg/kg, i.p.) and injected with sodium heparin (500 units/kg, i.p.). After 10 min, the hearts were excised and placed in ice-cold KHS until contractions had ceased. Each heart was then cannulated through the aorta and quickly placed in a Langendorff apparatus where it was perfused with KHS containing pyruvic acid (2 mM) and bubbled with 95% O₂/5% CO₂ at a constant pressure (80 mmHg). The perfusion pressure was the best condition for maintaining a constant perfusion flow rate, developed tension and heart rate. The perfusion was carried out at 37°C. The developed tension was monitored by a force transducer (TB-611T, Nihon Kohden) attached near the apex of the left ventricle by a nylon ligature. A resting tension of 2 g was applied at the start of the perfusion. Heart rate was triggered by the pulse of tension-development (AT-600G, Nihon Kohden). The coronary flow rate was monitored with an electromagnetic blood flowmeter (MFV-1100, Nihon Kohden) via a coronary probe (FF-030T, Nihon Kohden) attached to the Langendorff apparatus. Drugs were applied in the chamber (6 ml) just before the heart preparation. In the competition assay, the perfusing KHS solution containing glibenclamide (10⁻⁶ M) was changed for 10 min before the application of NIP-121 or levcromakalim (10⁻⁷ M). We confirmed that the vehicle had virtually no effect.

In vivo experiments

Mongrel dogs of either sex weighing 9 to 18 kg purchased from Saitama Experimental Animal Supply were used. All dogs were anesthetized with sodium pentobarbital (30 mg/kg, i.v.), ventilated by a respirator (20 breaths/min with a tidal volume of 20 to 25 ml/kg) and prepared for recording of hemodynamic variables as described previously in detail (20). During the experiment, the anesthesia was maintained at a constant level by supplemental injection of sodium pentobarbital (3 to 4 mg/kg/hr) into the right femoral vein with an infusion pump (STC-503; Terumo, Tokyo).

Arterial pressure was measured by means of a catheter inserted into the right femoral artery, advanced to the thoracic aorta and attached to a pressure transducer (TP-400T, Nihon Kohden). The heart rate was measured by a tachometer (AT-601G, Nihon Kohden). Each blood flow rate was monitored with an electromagnetic blood flowmeter (MFV-1100, Nihon Kohden) via a calibrated electromagnetic flow probe (FB-030B or FB-025B, Nihon Kohden) attached to the left circumflex coronary, vertebral, renal and left femoral artery. A left cephalic vein was cannulated for the i.v. injection of the drugs.

The doses of NIP-121 and levcromakalim required to elicit a decrease in the mean aortic pressure (−5% to −25%) and an increase in the coronary blood flow (10% to 60%) were calculated from the equation obtained by
the least squares method (21).

Compounds

The following compounds were used: NIP-121 and levromakalim (Central Research Laboratories of Nissan

Fig. 1. Effects of NIP-121 (top) and levromakalim (bottom) on isolated canine left circumflex coronary artery (●), left anterior descending coronary artery (▲), basilar artery (○), renal artery (▲), cranial mesenteric artery (○), femoral artery (▲) and saphenous vein (×). The arteries and vein were contracted with a sub-maximal concentration (final concentration: 3 x 10⁻⁹ M in cranial mesenteric artery and saphenous vein or 10⁻⁸ M in other blood vessels) of U46619 for producing the same tension-development of U46619. Relaxations produced are expressed as a percentage of the maximal relaxation induced by 10⁻⁴ M papaverine. Each point represents the mean ± S.E.M. derived from four experiments.

Table 1. IC₅₀ values of NIP-121 and levromakalim on U46619-induced contraction of isolated canine blood vessels

| Vascular bed | IC₅₀ value (×10⁻⁹ M) | Ratio |
|--------------|----------------------|-------|
|              | NIP-121 | Levromakalim |       |
| LAD          | 1.58 ± 0.10 (1) | 14.45 ± 0.38 (1) | 9.1  |
| LCX          | 1.60 ± 0.12 | 17.25 ± 1.40 | 10.8 |
| BA           | >100     | >100         | —    |
| RA           | 4.06 ± 1.12* (1/2.6) | 38.35 ± 7.19** (1/2.7) | 9.4  |
| MA           | 2.45 ± 1.40 (1/1.6) | 8.91 ± 1.39 (1.6) | 3.6  |
| FA           | 5.89 ± 1.07** (1/3.7) | 31.67 ± 4.69** (1/2.2) | 5.4  |
| SV           | 1.33 ± 0.51 (1.2) | 3.46 ± 0.53 (4.2) | 2.6  |

Abbreviations used are: left anterior descending coronary artery (LAD), left circumflex coronary artery (LCX), basilar artery (BA), renal artery (RA), cranial mesenteric artery (MA), femoral artery (FA) and saphenous vein (SV). The values in parentheses are the ratio against the IC₅₀ for the LAD of each compound. Ratio values are the ratio between NIP-121 and levromakalim. Each value represents the mean ± S.E.M. of four preparations. *P<0.05, **P<0.01, compared with the value in the LAD group (Tukey's multiple comparison test).
cromakalim ($P < 0.05$), and the maximal relaxations were more than 70% of the maximal response induced by papaverine ($10^{-4}$ M). The vasodilating effects of both compounds in LAD were more potent than those in renal and femoral arteries. In the coronary and renal arteries, the vasorelaxant effects of NIP-121 was about 10 times more potent than those of levcromakalim. Glibenclamide ($10^{-8}$ to $10^{-6}$ M) concentration-dependently antagonized the effects of these compounds in the canine arteries and vein except for the basilar artery. The $pA_2$ values of glibenclamide for NIP-121 and levcromakalim were 7.2 and 7.3 (LCX), 7.4 and 7.3 (LAD), 7.1 and 7.4 (renal artery), 7.2 and 7.3 (cranial mesenteric artery), 7.0 and 7.3 (femoral artery) and 7.2 and 7.5 (saphenous vein), respectively (the mean of four experiments). The slope value of the Schild plot for each type of blood vessel was about 1.

**Effects of NIP-121 and levcromakalim on perfusion flow rate in the coronary perfused rat heart**

Table 2 summarizes the basal hemodynamic data prior to application of NIP-121 or levcromakalim to coronary perfused rat heart. After a bolus injection, NIP-121 and levcromakalim (final concentration was $10^{-7}$ M) increased the coronary perfusion flow rate (Fig. 2). The peak increases with both compounds occurred 1 min after application. The duration of the NIP-121-induced dilation was longer than that by levcromakalim at the same concentration ($10^{-7}$ M). The increase in coronary perfusion flow rate by NIP-121 and levcromakalim were completely inhibited by glibenclamide ($10^{-6}$ M), an inhibitor of ATP-sensitive potassium channels (Fig. 3). Basal values of cardiac wall tension in the NIP-121 and levcromakalim groups were 1.81±0.12 and 1.82±0.13 g, respectively ($n=4$). Wall tension after application of NIP-121 and levcromakalim was 1.48±0.06 g at 3 min and 1.73±0.06 g at 2 min, respectively. The decrease in tension-development induced by NIP-121 was inhibited by glibenclamide.

**Hemodynamic effects of NIP-121 and levcromakalim in anesthetized dogs**

Table 3 summarizes the basal hemodynamic data prior to application of NIP-121 or levcromakalim. The differences of the control values among the groups were not significant ($P > 0.05$ by Tukey’s multiple comparison test). After a bolus i.v. injection, NIP-121 (1, 3 and 10 $\mu$g/kg (3.2, 9.6 and 32 nmol/kg)) and levcromakalim (3, 10 and 30 $\mu$g/kg (10.5, 35 and 105 nmol/kg)) significantly increased the coronary perfusion flow rate in the coronary perfused rat heart. Each value represents the mean±S.E.M. derived from four experiments. Significantly different from pre-values, *$P<0.05$ and **$P<0.01$.

**Table 2. Control values in the coronary perfused rat heart**

|                 | NIP-121 group | Levcromakalim group |
|-----------------|---------------|---------------------|
| CPF (ml/min)    | 8.5±1.2       | 7.8±1.1             |
| HR (beats/min)  | 310.0±11.5    | 307.5±10.3          |
| TD (g)          | 1.8±0.1       | 1.8±0.1             |

Abbreviations used are: coronary perfusion flow rate (CPF), heart rate (HR) and tension-development (TD). Each value represents the mean±S.E.M. of four preparations.
reduced the mean aortic pressure, in a dose-dependent manner (Fig. 4, data not shown at the highest doses). The peak responses in mean aortic pressure, at higher doses, occurred 3 to 5 min after NIP-121 or levcromakalim. The dose-responses for the hypotensive effects at the three concentrations for each drug were characterized by a significant linear relationship (P<0.001, r = 0.929 and P<0.001, r = 0.937 for NIP-121 and levcromakalim, respectively) between the logarithm of the dose and the % decrease in mean aortic pressure. Table 4 shows the calculated doses of NIP-121 and levcromakalim required to elicit a decrease in the mean aortic pressure (−5% to −25%). NIP-121 showed about 3 times higher hypotensive activity than levcromakalim. Both compounds at all concentrations significantly increased the heart rate during the reduction in aortic pressure. NIP-121 dose-dependently increased the blood flow in all vascular beds examined (Fig. 4). However, levcromakalim dose-dependently increased only the coronary and renal blood flow but not vertebral or femoral blood flow. NIP-121 and levcromakalim, at a higher dose, had the greatest effect on coronary blood flow, in spite of the hypotensive effect. The maximal effects on coronary blood flow of NIP-121 and levcromakalim were obtained at 3 and 10 μg/kg,

|                | NIP-121 group | Levocromakalim group |
|----------------|---------------|----------------------|
| 1 μg/kg        | 3 μg/kg       | 3 μg/kg              | 10 μg/kg               |
| MAP (mmHg)     | 113.0± 3.7    | 110.8± 5.8           | 102.5± 9.7             | 100.3± 8.0             |
| HR (beats/min) | 97.0± 8.4     | 111.0± 7.8           | 118.3± 7.4             | 123.5± 3.6             |
| CBF (ml/min)   | 14.8± 2.4     | 15.0± 2.4            | 39.0± 15.0             | 27.0± 4.0              |
| VBF (ml/min)   | 14.3± 3.0     | 15.3± 3.3            | 19.8± 4.7              | 20.3± 5.2              |
| RBF (ml/min)   | 102.8± 33.0   | 105.5± 31.5          | 129.0± 24.9            | 140.0± 27.8            |
| FBF (ml/min)   | 54.5±10.5     | 60.5±10.3            | 38.0± 8.3              | 35.0± 7.8              |

Abbreviations used are: mean aortic pressure (MAP), heart rate (HR), coronary blood flow (CBF), vertebral blood flow (VBF), renal blood flow (RBF) and femoral blood flow (FBF). Each value represents the mean ± S.E.M. of six animals.
Table 4. Calculated doses of NIP-121 and levromakalim required to elicit a 5% to 25% decrease in mean aortic pressure (MAP) in anesthetized dogs

| Reduction in MAP (% of initial value) | Calculated doses (µg/kg) | NIP-121 | Levromakalim |
|--------------------------------------|--------------------------|---------|--------------|
| -5                                   | 1.35 (1.04 - 1.74)       | 3.82 (2.97 - 4.92) |
| -10                                  | 1.67 (1.33 - 2.10)       | 4.75 (3.79 - 5.94) |
| -15                                  | 2.08 (1.69 - 2.54)       | 5.90 (4.83 - 7.20) |
| -20                                  | 2.58 (2.13 - 3.11)       | 7.33 (6.11 - 8.80) |
| -25                                  | 3.20 (2.66 - 3.85)       | 9.11 (7.65 - 10.84) |

Values are geometric means (95% confidence limits) for six animals.

Table 5. Calculated doses of NIP-121 and levromakalim required to elicit a 10% to 60% increase in coronary blood flow (CBF) in anesthetized dogs

| Increase in CBF (% of initial value) | Calculated doses (µg/kg) | NIP-121 | Levromakalim |
|--------------------------------------|--------------------------|---------|--------------|
| 10                                   | 1.06 (0.75 - 1.48)       | 3.66 (2.78 - 4.81) |
| 20                                   | 1.22 (0.91 - 1.63)       | 4.18 (3.28 - 5.34) |
| 30                                   | 1.41 (1.10 - 1.80)       | 4.78 (3.82 - 5.98) |
| 40                                   | 1.63 (1.30 - 2.05)       | 5.47 (4.40 - 6.78) |
| 50                                   | 1.88 (1.49 - 2.37)       | 6.25 (5.00 - 7.81) |
| 60                                   | 2.17 (1.69 - 2.80)       | 7.14 (5.60 - 9.10) |

Values are geometric means (95% confidence limits) for six animals.

respectively. Table 5 shows the doses of NIP-121 and levromakalim required to elicit an increase in coronary blood flow (10% to 60%) calculated from the equation obtained by the least squares method. NIP-121 was about 4 times more potent than levromakalim.

DISCUSSION

NIP-121 is a newly synthesized pyranobenzoxadiazole derivative possessing very potent vasorelaxant properties (11, 13 - 15). The effects of NIP-121 were about 10 times more potent than those of cromakalim (11, 13 - 15, 18). Levromakalim, an active enantiomer of cromakalim, is two times more potent than cromakalim (22). In this study, we examined the effect of NIP-121 in comparison with that of levromakalim in various blood vessels, especially the coronary artery.

The mechanism of vasorelaxation by a potassium channel opener is related to a reduction in the cell membrane potential (hyperpolarization) associated with an increase in outward K⁺ current (23, 24). The hyperpolarization produces vasorelaxation by inhibition of membrane Ca²⁺-permeability via not only voltage-dependent Ca²⁺ channels but also dihydropyridine-insensitive (13, 14, 25) or receptor-operated Ca²⁺ channels (25). The inhibitory actions of cromakalim on intracellular mechanisms, e.g., Ca²⁺-refilling into (26, 27) and Ca²⁺-release from the sarcoplasmic reticulum (28), on the agonist-induced vasoconstriction, have also been reported. The effects of both NIP-121 and levromakalim are considered to depend upon the mechanisms against thromboxane-receptor mediated cellular responses, because we previously confirmed that the effect of NIP-121 was associated with the increase in the ⁸⁶Rb⁺ efflux from vascular smooth muscle cells and is competitively inhibited by glibenclamide, an ATP-sensitive potassium channel blocker (11, 13 - 15, 29).

NIP-121 and levromakalim were potent coronary vasodilators in in vivo experiments. In the isolated tissue bath experiments, these compounds caused potent vaso-dilation not only in the coronary artery but also in the cranial mesenteric artery and saphenous vein. The blood flow rates in the cranial mesenteric artery and saphenous vein may be also increased. The effects were antagonized by glibenclamide. In the coronary perfused heart, the perfusion flow rate was also increased by each compound, and the effects were also inhibited by glibenclamide. The coronary vasodilator effects of other potassium channel openers, e.g., cromakalim (22) and pinacidil (30, 31), have also been reported. Uchida (32) reported that coronary vasospasms might be caused by impaired K⁺-conductance in coronary artery smooth muscle. The decrease in potassium permeability via glibenclamide-sensitive potassium channels may be very important for the development of a coronary vasospasm. It should also be noted that the coronary selective effects of these drugs in the in vivo experiments might also be explained by differences in rate and extent of absorption of these compounds in different vascular beds, rather than differences in receptor- (channel-) sensitivity.

The effects of NIP-121 and levromakalim on the canine basilar artery were weaker than those on other peripheral vessels. McPherson and Stork (33) reported the weak effect of cromakalim in cerebral arteries of spontaneously hypertensive and Wistar-Kyoto rats, and they suggested that cromakalim does not hyperpolarize the rat cerebral artery (34), since the artery was thought to be devoid of cromakalim-sensitive potassium channels that may tonically influence the resting membrane potential of certain vascular beds (33). The resting membrane potential of the cerebral artery (−38 mV in anterior cerebellar) was higher than those of other arteries such as the cranial mesenteric artery (−60 mV), because of the lack of the potassium channels in the cerebral artery (33). The weak effects of NIP-121 and levromakalim might be due to the absence of the potassium channels in the canine basilar artery.
In conclusion, we found that NIP-121 and levcromakalim were similarly selective vasodilators on the isolated canine coronary and cranial mesenteric arteries and saphenous vein. In addition, these compounds selectively increased coronary blood flow in vivo to a greater extent than in other vascular beds. The potency of NIP-121 for increasing the coronary blood flow was about 4 times higher than that of levcromakalim. This effect might be related to glibenclamide-sensitive potassium channels.

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