Beneficial Effect of Nipradilol (K-351) on Acute Myocardial Ischemia
Study of the Relationship between Regional Myocardial Blood Flow and Energy Metabolism

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Abstract—To examine the effects of nipradilol on ischemic myocardium, experiments were performed on regional myocardial blood flow (MBF) and energy metabolism in anesthetized, open-chest dogs. Nipradilol at a dose of 0.3 mg/kg was i.v.-administered 10 min after coronary ligation. MBFs at various sites, including ischemic and non-ischemic areas, were determined by the hydrogen gas clearance method. The levels of ATP and creatine phosphate (CP) at the site of MBF determination were measured 60 min after ligation, and mitochondrial function (RCI, QO₂) in the ischemic and non-ischemic areas was determined. Following nipradilol administration, aortic pressure and heart rate were significantly lowered. In ischemic areas with MBF below 40 ml/min/100 g, nipradilol had no influence on MBF. However, the tissue level of ATP in nipradilol treated hearts was significantly higher as compared with untreated hearts. In the area of mild ischemia with MBF of 40–60 ml/min/100 g, nipradilol preserved the tissue ATP and CP levels in spite of a decrease in MBF. Moreover, an inhibition of the decrease in mitochondrial respiratory function was observed in ischemic areas with MBF below 20 ml/min/100 g. Thus, nipradilol administered following ischemia preserved ATP content and mitochondrial function in the ischemic myocardium with reduction of heart rate and aortic pressure. This suggests that nipradilol exerts a cardioprotective effect in acute ischemia. It seems that the cardioprotective effect is due to a decrease in myocardial oxygen demand and preservation of mitochondrial function.

Nipradilol is a new β-adrenoceptor blocking agent having a vasodilating action (1–3), and it is expected that these pharmacological characteristics will bring about beneficial effects on acute ischemic myocardium.

In general, factors such as reduction in myocardial oxygen demand, improvement in the myocardial blood flow to the ischemic area, and prevention of disturbances in myocardial metabolism are suggested as the mechanism that protects ischemic myocardium. However, little has been reported about the effects of nipradilol on regional myocardial blood flow (MBF) of ischemic myocardium and disturbed cardiac metabolism.

Therefore, we examined the influence of nipradilol on MBF and myocardial contents of ATP and CP in coronary artery-obstructed dogs. We also investigated its influence on the mitochondrial respiratory function in ischemic myocardium and examined whether nipradilol could improve disturbances of energy metabolism in ischemic myocardium.
Materials and Methods

Forty adult mongrel dogs, weighing 7–15 kg, were anesthetized by intra-peritoneal administration of 50 mg/kg of pentobarbital sodium, and subsequently, the pericardium was incised by thoracotomy from the left 5th intercostal space under artificial respiration. The left anterior descending coronary artery (LAD) was dissected free immediately distal to the first diagonal branch for ligation (Fig. 1).

The forty dogs were divided into two groups, namely, 20 animals of the control group and 20 animals of the nipradilol group. Ten minutes after coronary ligation, 0.3 mg/kg nipradilol was i.v.-administered over a 3-min period to the nipradilol group, while physiological saline solution was similarly administered to the control group.

The 0.3-mg/kg dose of nipradilol was selected so that a decrease in heart rate and a decrease in coronary vascular resistance and left ventricular enddiastolic pressure, in other words, a β-adrenoceptor blocking action and a vasodilating action, would be obviously exhibited based on the report of Uchida (2).

A catheter inserted into the femoral artery was connected to a pressure-transducer, and then aortic pressure and heart rate were consecutively recorded.

MBFs were recorded before ligation and at 5 min, 15 min, 30 min and 60 min after ligation. As shown in Fig. 1, a wire-type platinum electrode, 0.08 mm in diameter, was introduced into the heart muscle and fixed into the left ventricular wall middle layer of the ischemic area (I), the border at the visible ischemic edge (II, III, IV), and the non-ischemic area (V). MBFs were determined by the hydrogen gas clearance method (4) using a PHG 201-type tissue blood flow meter (Unique Medical Co.).

After LAD ligation for 60 min, cardiac muscles corresponding to the respective sites of electrode implantation were collected in about one sec, using a biopsy drill with a diameter of 3 mm, and specimens were rapidly frozen in liquid nitrogen. The myocardial middle layer was used as a sample for determination of tissue ATP and CP. The contents of ATP and CP were determined by the luciferin-luciferase method (5), using a BLR 102 bioluminescence reader (Aloka Co.).

The cardiac muscles of the ischemic area (I) and non-ischemic area (V) conforming to the regions of blood flow determination, were isolated, and the mitochondria were isolated using alkaline protease according to Hatefi’s method (6). The mitochondrial sample (0.3 ml) was added to 2.8 ml of mannitol reaction solution (0.3 M mannitol, 10 mM potassium chloride, 10 mM magnesium chloride, 10 mM potassium chloride, 0.25 mM EDTA), and 0.2 M succinic acid (0.1 ml), the substrate, and 0.01 M ADP (0.05 ml) was added; the respiratory activity of mitochondria was observed using a bioxygraph (Sensonix Japan Co.). Its respiratory control index (RCI) and state III O2 consumption rate (ΔO2) were calculated.

Aortic pressure and heart rate were represented by the mean±standard error and other results, by the mean±standard deviation. The statistical analysis for significant difference was made by Student’s t-test in the case of between-groups comparison, and the paired t-test used for within-groups comparison.
Results

Heart rate and aortic pressure: In this experiment, no significant change due to LAD ligation was observed in heart rate and aortic pressure. The heart rate and systolic and diastolic aortic pressure were significantly lowered to 87±4 beats/min, 76±5 mmHg and 51±4 mmHg from the pre-administration values of 118±5 beats/min, 98±6 mmHg and 67±4 mmHg, respectively. The heart rate, systolic aortic pressure and diastolic aortic pressure showed decreases of 26%, 22% and 24%, respectively. Thereafter, until the completion of the experiment, the decreases in heart rate and aortic pressure were consistently maintained. In comparison with the control group, the heart rate and systolic and diastolic aortic pressures in the nipradilol group showed significantly lower values than the control group at any point in time (Fig. 2).

MBF: Figure 3 shows time-course changes in MBFs, divided into four subgroups according to the level of MBF at 5 min after ligation (different subgroup for each increment of MBF of 20 ml/min/100 g). In areas with an MBF less than 40 ml/min/100 g, no significant change was shown due to nipradilol, but in areas with an MBF of 40–60 ml/min/100 g and more than 60 ml/min/100 g, significant decreases of 18% and 26%, respectively, due to nipradilol were observed. In the case of between-groups comparison, the blood flow in the area with an MBF of 40–60 ml/min/100 g in the nipradilol group was significantly lower than the control group at any point following the administration.

Myocardial contents of ATP and CP:
Fig. 3. Effect of nipradilol on the regional myocardial blood flow (MBF). In the area with MBF less than 40 ml/min/100 g, there were no influences of nipradilol on MBF. With MBF more than 40 ml/min/100 g, the nipradilol group showed a significant decrease in MBF compared with the controls. 0≤MBF <20, C: n=24, N: n=26; 20≤MBF<40, C: n=19, N: n=20; 40≤MBF<60, C: n=18, N: n=19; 60≤MBF, C: n=20, N: n=20. *: P<0.01 vs. 5-min ligation, **: P<0.005 vs. 5-min ligation. N.S.: Not significant.

Table 1. Influence of nipradilol on the correlation between the regional myocardial blood flow (MBF) and tissue ATP, CP contents

| at 60 min after ligation | ATP     | CP      |
|-------------------------|---------|---------|
| 0≤MBF<20                | Control (n) | Nipradilol (n) | Control (n) | Nipradilol (n) |
| 1.37±0.71 (24)          | 2.66±0.85*** (26) | 1.87±0.73 (24) | 2.43±1.11 (26) |
| 2.85±0.81 (18)          | 3.61±1.01** (20)  | 4.54±1.97 (19) | 5.50±1.24 (20) |
| 3.89±0.62 (18)          | 4.54±0.65** (19)  | 6.10±1.82 (18) | 8.35±1.36*** (19) |
| 5.15±0.52 (20)          | 5.25±0.89 (20)    | 9.45±2.01 (20) | 9.34±0.92 (20)  |

Table 1 shows ATP and CP contents in each area of the myocardium 60 min after ligation. As mentioned above, each area was divided into four subgroups according to the level of MBF at 5 min after ligation. The ATP contents showed no significant difference in the non-ischemic area with an MBF of more than 60 ml/min/100 g between both groups. The area with an MBF of 40–60 ml/min/100 g showed a significantly (P<0.05) higher value of ATP contents. 4.54±0.65 μmoles/g w.w., in the nipradilol group as compared with 3.89±0.62 μmoles/g w.w. of the control group. In the area with an MBF of 40–60 ml/min/100 g, the ATP in the nipradilol group showed a significantly (P>0.025) higher value of 3.61±1.01 μmoles/g w.w. than 2.85±0.81 μmoles/g w.w. in the control group. Also in the area with an MBF of 0–20 ml/min/100 g, the nipradilol group showed a
Table 2. Respiratory function of mitochondria isolated from non-ischemic (N.I.) and ischemic (I.) areas

|                     | Respiratory control index | State III $O_2$ consumption rate (natoms/mg protein/min) |
|---------------------|---------------------------|----------------------------------------------------------|
| Control group       |                           |                                                          |
| N.I.                | 3.75±0.43                 | 309±37                                                   |
| I.                  | 2.35±0.52                 | 193±58                                                   |
| Nipradilol group    |                           |                                                          |
| N.I.                | 3.69±0.29                 | 333±36                                                   |
| I.                  | 3.14±0.31**               | 266±36*                                                  |

In each group, respiratory control index and state III $O_2$ consumption rate in the I. area were significantly decreased compared with those in the N.I. area. In the I. area, the nipradilol group showed significant increase, in respiratory control index and state III $O_2$ consumption rate compared with the controls. There was no significant difference between the two groups in the N.I. area. *: $P<0.01$ vs. control, **: $P<0.005$ vs. control. Mean±S.D.

significantly ($P>0.01$) higher value of $2.65±0.85$ μmoles/g w.w. as compared to $1.37±0.71$ μmoles/g w.w. in the control group.

The CP level in the area with an MBF of $40–60$ ml/min/100 g was $6.10±1.82$ μmoles/g w.w. in the control group and $8.35±1.36$ μmoles/g w.w. in the nipradilol group, so the value in the nipradilol group was significantly ($P<0.01$) higher. No significant difference was observed in the other areas.

**Mitochondrial respiratory function:** The RCIs in the ischemic area with an MBF less than $20$ ml/min/100 g at 60 min after ligation were $2.35±0.52$ in the control group and $3.14±0.31$ in the nipradilol group, so the nipradilol group showed a significantly higher value ($P<0.005$) (Table 2).

The $O_2$ of the nipradilol group was $266±36$ natoms/mg protein/min, being than that of the control group, $193±58$ natoms/mg protein/min. On the other hand, no significant difference was observed between both groups in these parameters at the non-ischemic area.

**Discussion**

Nipradilol is a $\beta$-adrenoceptor blocking agent having a nitroglycerin-like vasodilating action (1, 7) and an $\alpha$-blocking action, although this is weak (1, 8, 9). It has been reported (10) that nipradilol increases coronary blood flow under dynamic stenosis by dilation of the proximal coronary artery (11) as well as a reduction in myocardial oxygen demand (3, 11). It is well-known that conventional $\beta$-adrenoceptor blockers reduce myocardial oxygen demand and at the same time reduce coronary blood flow. There may be cases in which the reduction in coronary blood flow exceeds the reducing effect of the myocardial oxygen demand. Therefore, such $\beta$-blockers are occasionally inappropriate for the treatment of ischemic myocardium (12, 13). Nipradilol is expected to be useful as a new drug for the treatment of ischemic myocardium by obviating this undesirable effect.

In our experiment, nipradilol reduced the MBF in non-ischemic areas and in mildly ischemic areas (MBF $40–60$). These phenomena seem to have occurred accompanying decreases in blood pressure and heart rate or in other words, a decrease in the myocardial oxygen demand. In both moderately ischemic areas (MBF $20–40$) and severely ischemic areas (MBF $0–20$), nipradilol did not affect MBF. One of the reasons why the MBF in the ischemic areas did not decrease in spite of a decrease in myocardial oxygen consumption by nipradilol is considered to be that nipradilol dilates the collateral blood vessels through its nitroglycerin-like vasodilating action (1, 2, 14). An increase in blood flow following the dilation of collateral vessels may compensate for a decrease in blood flow in ischemic areas accompanying a decrease in myocardial oxygen consumption. Furthermore, $\beta$-adrenergic blockers are known to redistribute blood flow from normal to ischemic areas (15).
Although nipradilol reduced the MBF in mildly ischemic areas and did not affect the MBF in moderately and severely ischemic areas, it improved the decrease in ATP and CP contents. Moreover, nipradilol preserved mitochondrial respiratory function in severely ischemic myocardium.

In general, many metabolic changes such as disturbance of mitochondrial structure and function (16, 17), loss of high energy phosphate (18), and the accumulation of various metabolites are observed in the ischemic myocardium. Furthermore, it seems that the following are involved in the maintenance mechanism of high energy phosphate: 1) an increase in MBF in ischemic myocardium, 2) a decrease in ATP consumption (energy utilization) due to a reduction in ischemic myocardial oxygen demand, and 3) maintenance of ATP production (energy liberation) due to the protection of mitochondria.

Especially, the ATP content in the myocardium seems to be the parameter that best reflects cellular activity and the energy kinetics of ischemic myocardium. Kübler and Spieckermann (19) reported that when the ATP level of the myocardium became lower than 2 µmoles/g w.w., irreversible disturbances were caused in the cell. In our study, the ATP content in the severely ischemic area of the myocardium was 1.37±0.71 µmoles/g w.w. in the control group, and was less than the critical level of viability. However, the ATP content in the nipradilol group was 2.65±0.85 µmoles/g w.w., exceeding the critical level of viability. Although nipradilol did not influence the MBF in the severely and moderately ischemic areas, the decrease of ATP was inhibited in every ischemic area. Nipradilol protected mitochondrial respiratory function in the severely ischemic area.

These results suggested that the maintenance of high energy phosphate by nipradilol was due to the reduction in ischemic myocardial oxygen demand based on the decreased heart rate and blood pressure as observed in the present experiment and also based on the reduction of left ventricular preload reported by Uchida (2). Furthermore, it seemed that nipradilol might maintain the production of ATP by preserving mitochondrial respiratory function in ischemic myocardium. With regard to the preservation of mitochondrial function, we have previously reported (20) that metoprolol (beta blockade) inhibited the accumulation of long chain acyl-CoA in mitochondria and significantly preserved their respiratory function in ischemic myocardium. In the present study, the reduction of ischemic myocardial work by nipradilol is thought to be one of the mechanisms for the preservation of mitochondria.

In conclusion, this study suggests that nipradilol is effective in maintaining ATP content in the ischemic myocardium by reducing myocardial oxygen demand and by preserving mitochondrial function. Furthermore, it is of great importance, from the clinical standpoint, that we can obtain these beneficial effects with the administration of nipradilol after the onset of ischemia.
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ERRATA

Y. OKAMOTO, T. MATSUBARA, N. IYEDA, K. MIYAJIMA, K. IIDA, T. NISHIDA, S. KOYABASHI, Y. KAKINUMA, K. ITOH, N. HIBI, T. KAMBE and N. SAKAMOTO:
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page 372, legend for Fig. 1, line 5, left should read left

page 374, column 2, line 6, (P>0.025) should read (P<0.025)

page 375, column 1, line 1, (P>0.01) should read (P<0.01)