Improvement of Ischemic Myocardial Dysfunction by Nisoldipine in Relation to Its Coronary Vasodilating Action

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ABSTRACT—We examined the cardioprotective effect of nisoldipine against myocardial dysfunction during ischemia and reperfusion in comparison with those of diltiazem and nifedipine in rabbit hearts perfused at constant pressure. These calcium antagonists were administered to the hearts before 60 min of ischemia. They inhibited the increase of end-diastolic pressure during ischemia in a dose-dependent manner. Diltiazem at 1.0 μM, nifedipine at 3.0 μM and nisoldipine at 0.01 μM produced the maximal cardioprotective effect. Nisoldipine had a beneficial effect with less negative inotropic effect than those of diltiazem and nifedipine and it produced a significant increase of coronary flow during reperfusion. When the vascular component was eliminated under constant flow perfusion, nisoldipine also showed the cardioprotective effect. Nisoldipine did not produce any beneficial effect without the inhibition of the increase in end-diastolic pressure during ischemia nor did it do so without the increase of reperfusion flow. Therefore, the nisoldipine-increased coronary flow during reperfusion as well as the inhibition of ischemic contracture by nisoldipine seems to play a crucial role in improving the myocardial dysfunction of ischemic-reperfused hearts.

Keywords: Nisoldipine, Ischemic myocardial function, Coronary vasodilating action, Heart (rabbit, perfused)

Nisoldipine is a 1,4-dihydropyridine calcium antagonist with a high vascular selectivity (1, 2). This property was observed in experiments on human tissues as well as animal ones (3). The coronary artery selectivity of nisoldipine was at least 10 times more pronounced than that of nifedipine (3), and the cardiodepressant effect was less potent in human tissues (3). From such a pharmacological point of view, nisoldipine seems to be a promising drug for the treatment of ischemic heart disease. Actually, recent studies have been focused on the use of nisoldipine for cardioprotection (4–8). However, the mechanism of its beneficial effect has not been fully understood, and little is known about the potency of this effect in comparison with those of other calcium antagonists. In the present study, therefore, we examined the effect of nisoldipine on the cardiac function during ischemia and reperfusion in comparison with those of diltiazem and nifedipine using rabbit hearts perfused at constant pressure. Furthermore, we examined the effect of nisoldipine on the ischemic-reperfused heart under constant flow perfusion to eliminate the effect of change in coronary flow.

MATERIALS AND METHODS

Male Japanese white rabbits (1.8–2.2 kg) were heparinized (1000 IU) and anesthetized with sodium pentobarbital (100 mg, i.v.). The heart was excised and rapidly perfused according to the method of Langendorff with an oxygenated (95% O₂–5% CO₂) Krebs-Henseleit solution containing 118 mM NaCl, 4.7 mM KCl, 2.55 mM CaCl₂, 1.18 mM MgSO₄, 24.88 mM NaHCO₃ and 11.1 mM D(+)-glucose, pH 7.4, at 37°C. The perfusate was filtered through a membrane filter (0.45 μm) before use. A water-filled latex balloon which was connected to a pressure transducer (SPB-105, Nihondenkisanei, Tokyo) was fitted into the left ventricle via the atrium. Left ventricular developed pressure, which was defined as systolic left ventricular–end-diastolic pressure (LVP), was recorded from the transducer onto a polygraph (340, Nihondenkisanei). Heart rate (HR) was measured by a HR counter.

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triggered by the LVP pulse. Intraballoon pressure was adjusted with atmospheric pressure to obtain a stable preparation. Constant pressure perfusion was performed at a perfusion pressure of 75 cmH₂O. Constant flow perfusion was performed at a flow rate of 30 ml/min. Coronary flow (CF, at constant pressure perfusion) was measured by means of an electromagnetic flow meter (MFV-3100, Nihon Kohden, Tokyo). Coronary perfusion pressure (at constant flow perfusion) was recorded from the transducer onto the polygraph.

The heart preparation was placed inside a water-jacketed chamber to maintain the temperature at 37°C. The heart was perfused under normal conditions for 40–60 min. After the heart was treated with drug or vehicle for 10 min, global ischemia was induced by stopping the perfusion flow. During ischemia, which lasted for 60 min, the maximal value of the left ventricular end-diastolic pressure (EDPm) was measured. Reperfusion was performed in the absence of the drug for 90 min. During reperfusion, the recovery of LVP, HR and CF were determined. The post-ischemic recovery of LVP, HR and CF were expressed as a percentage of the pre-ischemic values before drug treatment.

Drugs used in the present study were as follows: nisoldipine and nifedipine (Bayer, Leverkusen, Germany) and diltiazem hydrochloride (Nacalai Tesque, Kyoto). Nisoldipine and nifedipine were dissolved in ethanol and diltiazem hydrochloride was dissolved in purified water. Ethanol at a final concentration of 0.03% had no effect on the indexes of cardiac function. The experiments with nisoldipine and nifedipine were carried out under a sodium vapor lamp to prevent possible photochemical degradation of the compounds.

RESULTS

Effect of calcium antagonist on cardiac function before and during ischemia

Hearts had initial values of LVP (79 ± 2 mmHg), HR (164 ± 5 beats/min), and CF (31 ± 1 ml/min) under normal conditions. The effects of diltiazem, nifedipine, and nisoldipine on these indexes of function were observed for 10 min after application of drugs. Their cardiodepressant effects are summarized in Table 1, and their effects on CF are shown in Fig. 2. After 10 min of observation, the ischemia was induced. The end-diastolic pressure (EDP) in hearts treated with no drug gradually increased and reached a maximal level at 50 min during ischemia. As shown in Fig. 1, calcium antagonists inhibited the EDPm during ischemia in a dose-dependent manner. The IC₅₀ for diltiazem, nifedipine and nisoldipine was 1.0 μM, 3.0 μM and 0.01 μM, respectively.

Effect of calcium antagonist on cardiac function during ischemia and reperfusion

After 60 min of ischemia, the vehicle-treated hearts were reperfused for 90 min during which the CF reached

| Table 1. Effect of nisoldipine on cardiac function prior to ischemia |
|----------------------|---------|---------|
| Drug                | IC₅₀ (M) |         |
|                     | LVP     | HR      |
| Nisoldipine         | 7.87 × 10⁻⁸ | 2.58 × 10⁻⁷ |
| Nifedipine          | 1.37 × 10⁻⁷ | 2.05 × 10⁻⁷ |
| Diltiazem           | 1.82 × 10⁻⁶ | 2.83 × 10⁻⁶ |

Fig. 1. Effect of calcium antagonist on EDPm during ischemia. Each value indicates the mean ± S.E. (N = 6). *P < 0.05 vs. control.
Fig. 2. Effect of calcium antagonist on CF before and after ischemia. ●, control; calcium antagonist: ○, 0.001 μM; △, 0.01 μM; □, 0.1 μM; ×, 1.0 μM; ◊, 3.0 μM. Points are means ± S.E. (N = 6). *P<0.05 vs. control.

Fig. 3. Effect of calcium antagonist on HR before and after ischemia. ●, control; calcium antagonist: ○, 0.001 μM; △, 0.01 μM; □, 0.1 μM; ×, 1.0 μM; ◊, 3.0 μM. Points are means ± S.E. (N = 6). *P<0.05 vs. control.

Fig. 4. Effect of calcium antagonist on LVP before and after ischemia. ●, control; calcium antagonist: ○, 0.001 μM; △, 0.01 μM; □, 0.1 μM; ×, 1.0 μM; ◊, 3.0 μM. Points are means ± S.E. (N = 6). *P<0.05 vs. control.
to 80±3 and 56±2% of the preischemic value at 10 and 90 min, respectively (Fig. 2). In contrast, treatment of hearts with either diltiazem (1.0 and 3.0 μM) or nisoldipine (0.01 and 0.1 μM) before induction of ischemia increased the CF to higher levels than those of treatment with the vehicle during the first 10 min of reperfusion (Fig. 2). It should be noted that the treatment with 0.1 μM nisoldipine maintained nearly the same CF as that before ischemia during the 90-min reperfusion period.

The effects of three calcium antagonists on recovery of HR and LVP are shown in Figs. 3 and 4. After 90 min of reperfusion, HR recovered to its preischemic level in the vehicle-treated heart (Fig. 3), whereas these calcium antagonists at the highest concentration showed a negative chronotropic effect because of a slow washout from the myocardium. On the other hand, the heart treated with vehicles showed a recovery of LVP to 71±3% of the preischemic value (Fig. 4). In contrast to the results in the vehicle treatment, the treatment with calcium antagonists before induction of ischemia enhanced the recovery of LVP after reperfusion. The ED90 that showed a 90% recovery of the LVP at 90 min of reperfusion was as follows: diltiazem (1.0 μM), nifedipine (3.0 μM) and nisoldipine (0.01 μM).

Figure 5 shows the effects of calcium antagonists on EDP at the end of reperfusion. There was a significant elevation of EDP in the vehicle-treated heart during reperfusion. In contrast, diltiazem (1.0–3.0 μM), nifedipine (3.0 μM) and nisoldipine (0.01–0.1 μM) reduced the increase of EDP.

Comparison between negative inotropic effect and cardioprotective effect

Figure 6 shows the dose-response curves for the negative inotropic effect (NIE) of calcium antagonists and their improving effect on the recovery of LVP. For nifedipine, the negative inotropic effect was stronger (0.1 μM) or equal (3.0 μM) in comparison with the effect on the LVP recovery during reperfusion. In contrast, diltiazem and nisoldipine showed a stronger effect on the recovery of the LVP at lower concentrations. The recovery/NIE ratio for diltiazem at 1.0 μM, nifedipine at 3.0 μM and nisoldipine at 0.01 μM was 2.3, 1.0 and 5.3, respectively.

Is the cardioprotective effect of nisoldipine attributed to the increase of coronary flow?

The following experiments were conducted to clarify whether the effect of nisoldipine on the recovery of LVP could be attributed to the increase of coronary flow. The experimental condition was changed from constant pressure perfusion to constant flow perfusion. The perfusion was conducted at a constant flow rate of 30 ml/min, which corresponded to the flow rate (31±1 ml/min) used before in the previous experiments done at the constant pressure perfusion. Nisoldipine at 0.01 μM, the most effective dose under constant pressure perfusion, was used in this series of experiments. The experimental protocol was the same as that performed at constant pressure perfusion except for the perfusion system. After 60 min of ischemia, the hearts were reperfused at 15 ml/min and 30 ml/min for 90 min. Figure 7 shows the effect of nisoldipine on the EDP during ischemia and reperfusion. During ischemia, nisoldipine inhibited the EDPm by 50% in comparison with the control. During reperfusion, nisoldipine significantly reduced the increase of EDP at both flow rates, compared to that of the vehicle-treated hearts (Fig. 7). Figure 8 shows the effects of nisoldipine on HR, LVP and coronary perfusion pressure (PP) during reperfusion. Nisoldipine had no effect on HR during reperfusion at both flow rates. During 90 min of reperfusion at 30

![Fig. 5. Effect of calcium antagonist on EDP after 90 min of reperfusion. Each value indicates the mean±S.E. (N = 6). *P<0.05 vs. control.](image-url)
ml/min, the PP in the vehicle-treated hearts reached to 141 ± 8% and 185 ± 14% of the preischemic value at 10 and 90 min, respectively. In contrast, treatment of hearts with nisoldipine before induction of ischemia reduced the PP to lower levels than that of treatment with the vehicle during the reperfusion. For the recovery of LVP in the vehicle-treated hearts, the improvement in the recovery of LVP did not depend upon the flow rate of reperfusion. In contrast, keeping the flow rate at 30 ml/min, the recovery of LVP in the nisoldipine-treated hearts reached 100% of the preischemic value at 90 min of reperfusion. Reducing the flow rate to 15 ml/min led to a 76% recovery of LVP, and nisoldipine at 0.01 µM showed no significant improvement the LVP recovery.

**DISCUSSION**

It is generally accepted that timely administration of calcium antagonists is essential for producing their cardioprotective effects on the ischemic-reperfused heart in the experimental studies (9-11). In the present study, diltiazem, nifedipine, and nisoldipine were administered to perfused rabbit hearts before 60 min of ischemia. Judging from the release of lactate dehydrogenase, this dura-
tion of ischemia was not associated with severe myocardial injury. This problem will be discussed in detail elsewhere. Diltiazem at 1.0 μM improved the recovery of LVP after ischemia with a negative inotropic effect. Nifedipine at 3.0 μM produced cardiodepression prior to the cardioprotective effect. Pretreatment of hearts with nisoldipine at 0.01 μM showed the most potent effect on the recovery of LVP during reperfusion in this study. Here we discuss the mechanism of cardioprotection produced with nisoldipine.

The cardioprotective effects of nisoldipine have been discussed on the basis of its action on either myocardial components or vascular components. Several investigators described that the beneficial effect of this drug may be due to the inhibition of Ca^{2+} influx into myocardium during ischemia and reperfusion (9, 12–14). Some of them (9, 12) emphasized that the beneficial effect of nisoldipine was attributed to the energy conservation as a result of its negative inotropy. In the present study, nisoldipine inhibited the ischemic contracture at much lower concentrations than those of diltiazem and nifedipine. This inhibitory effect might be attributed to the energy conservation as a result of the inhibition of Ca^{2+} influx into the myocardium. The hearts treated with no drug showed no significant improvement in the recovery of LVP, although the reperfusion flow rate was kept at 100% of the pres ischemic value. In contrast, the hearts treated with nisoldipine prior to ischemia reduced the ischemic contracture and showed the complete recovery of LVP during reperfusion under this condition. Thus, the inhibitory effect of nisoldipine on the ischemic contracture seemed to contribute to the recovery of cardiac function during reperfusion. The increase in EDP during reperfusion was also reduced by the pretreatment with nisoldipine. Although the precise mechanism of this phenomenon remains unknown, it may be due to an increase of Ca^{2+} influx (14–16). The inhibition by nisoldipine of EDP development during reperfusion might reflect the inhibitory effect of nisoldipine on the ischemic contracture during ischemia.

Tumas et al. (17) reported that nisoldipine produced its beneficial effect on the ischemic-reperfused heart without producing negative inotropic or negative chronotropic effects. Furthermore, Watts et al. (11, 18) and Tilton et al. (19) suggested that the beneficial effect did not appear to be induced by direct myocardial actions of nisoldipine, but might reflect improved vascular function which was associated with the vascular selectivity of this drug. Considering the LVP/negative inotropic effect ratio (Fig. 6), nisoldipine had the beneficial effect with less negative inotropic effect in comparison with diltiazem and nifedipine.

McDonagh and Roberts (20) suggested that the beneficial effect of nisoldipine may be due to the reduction of endothelial uptake of Ca^{2+} rather than the vasodilation during reperfusion. However, Tilton et al. (19) reported that nisoldipine prevented changes in coronary vascular hemodynamics induced by ischemia and reperfusion, but did not prevent the loss of endothelial cells during ischemia. Therefore, the beneficial effect of nisoldipine on endothelial cells during ischemia and reperfusion is still controversial.

The failure of tissue to reperfuse after a transient ischemia period has been called the no-reflow phenomenon (21). Sassen et al. (22) and Watts et al. (18) suggested that the beneficial effect of nisoldipine was associated with increased coronary flow during reperfusion, namely reduction in the no-reflow phenomenon. The present study also demonstrated that the cardioprotective effect of nisoldipine was associated with the significant increase of coronary flow during reperfusion under constant pressure perfusion. When the vascular component was eliminated under constant flow perfusion, nisoldipine also improved the increase of coronary perfusion pressure during reperfusion. Furthermore, the recovery of LVP in nisoldipine-treated hearts depended upon the flow rate of reperfusion (Fig. 8). Therefore, the nisoldipine-increased coronary flow during reperfusion seems to play an important role in improving the myocardial dysfunction of ischemic-reperfused heart. Nisoldipine may increase the oxygen supply to the post ischemic myocardium upon reperfusion by dilating coronary vessels. The increase of coronary flow during reperfusion could increase the removal rate of detrimental metabolites in and around the cell.

In this paper, the effect of nisoldipine on the myocardial dysfunction during ischemia and reperfusion was discussed in relation to the negative inotropic effect and coronary vasodilating action. Although nisoldipine was effective for producing such a cardioprotection as described above, other actions of this drug can not be entirely ruled out at present. One probable cause of the myocardial injury during ischemia and reperfusion could be free radical generation and lipid peroxidation. According to Janero and Burghardt (23) and Herbacynska-Cedro and Gordon-Majszak (24), nisoldipine inhibited the free radical-induced lipid peroxidation. Therefore, it will be interesting to examine whether nisoldipine has a cardioprotective effect on the myocardial injury induced by free radical generation and lipid peroxidation during ischemia and reperfusion.

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REFERENCES

1. Kazda, S., Garthoff, B., Meyer H., Schlossmann, K., Stoepel, K., Towart, R., Vater, W. and Wehinger, E.: Pharmacology of a new calcium antagonistic compound, isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (Nisoldipine, Bay K5552). Arzneimittelforschung 30, 2144–2162 (1980).

2. Kazda, S.: Inhibitory effects of nisoldipine on serotonin and potassium induced contractions of porcine coronary and femoral arteries. Arzneimittelforschung 41, 204–207 (1991).

3. Godfrained, T., Egleme, C., Finet, M. and Jaumin, P.: The actions of nifedipine and nisoldipine on the contractile activity of human coronary arteries and human cardiac tissue in vitro. Pharmacol. Toxicol. 61, 79–84 (1987).

4. Kimball, B.P., Watson, K.R., Bui, S. and Frankel, D.: Preservation of left ventricular performance with reduced ischemic dysfunction by intravenous nisoldipine. Am. J. Cardiol. 66, 400–405 (1990).

5. Lahiri, A., Rodrigues, E.A., Carboni, G.P. and Raftery, E.B.: Effects of long-term treatment with calcium antagonists on left ventricular diastolic function in stable angina and heart failure. Circulation 81, Supp. 2, 130–138 (1990).

6. Kern, M.J.: Influence of calcium channel antagonist therapy on the ischemic response to acute coronary occlusion in humans. Clin. Cardiol. 12, Supp. 3, 77–85 (1989).

7. Duncker, D.J., van Woerkens, L.J., Serruys, P.W., Roelandt, J.R., Hugenholtz, P.G. and Verdouw, P.D.: Actions of nisoldipine in cardiovascular disease. Can. J. Cardiol. 5, 266–274 (1989).

8. Silke, B., Frais, M.A., Midtbo, K.A., Verma, S.P., Sharma, S., Reynolds, G., Jackson, N. and Taylor, S.H.: Comparative hemodynamic dose-response effects of five slow calcium channel blocking agents in coronary artery disease. Clin. Pharmacol. Ther. 42, 381–387 (1987).

9. de Jong, J.W.: Timely administration of nisoldipine essential for prevention of myocardial ATP catabolism. Eur. J. Pharmacol. 118, 53–59 (1985).

10. Nayler, W.G.: Calcium antagonists and myocardial ischemia. In Calcium Antagonists, pp. 157–176, Academic Press, San Diego (1988).

11. Watts, J.A., Hawes, E.M., Jenkins, S.H. and Williams, T.C.: Effects of nisoldipine on the no-reflow phenomenon in globally ischemic rat hearts. J. Cardiovasc. Pharmacol. 16, 487–494 (1990).

12. de Jong, J.W., Huizer, T. and Tijssen, J.G.P.: Energy conservation by nisoldipine in ischemic heart. Br. J. Pharmacol. 83, 943–949 (1984).

13. de Jong, J.W. and Huizer, T.: Reduced glycolysis by nisoldipine treatment of ischemic heart. J. Cardiovasc. Pharmacol. 7, 497–500 (1985).

14. du Toit, E.F. and Opie, L.H.: Modulation of severity of reperfusion stunning in the isolated rat heart by agents altering calcium flux at onset of reperfusion. Circ. Res. 70, 960–967 (1992).

15. Hearse, D.J., Garlick, P.B. and Humphrey, S.M.: Ischemic contracture of the myocardium: Mechanisms and prevention. Am. J. Cardiol. 39, 986–993 (1977).

16. Tani, M. and Neely, J.R.: Role of intracellular Na+ in Ca2+ overload and depressed recovery of ventricular function of reperfused ischemic rat hearts: Possible involvement of H+-Na+ and Na+-Ca2+ exchange. Circ. Res. 65, 1045–1056 (1989).

17. Tumas, J., Deth, R. and Klomer, R.A.: Effects of nisoldipine, a new calcium antagonist, on myocardial intact size and cardiac dynamics following acute myocardial infarction. J. Cardiovasc. Pharmacol. 7, 361–367 (1985).

18. Watts, J.A., Whipple, J.P. and Hatley, A.: A low concentration of nisoldipine reduces ischemic heart injury: enhanced reflow and recovery of contractile function without energy preservation during ischemia. J. Mol. Cell. Cardiol. 19, 809–817 (1987).

19. Tilton, R.G., Watts, J.A., Land, M.P., Larson, K.B., Sutera, S.P. and Williamson, J.R.: Discordant effects of nisoldipine on coronary vascular resistance and permeability changes during reflow after ischemia in isolated rabbit hearts. J. Mol. Cell. Cardiol. 23, 861–872 (1991).

20. McDonagh, P.F. and Roberts, D.J.: Prevention of transcoronary macromolecular leakage after ischemia-reperfusion by the calcium entry blocker nisoldipine. Circ. Res. 58, 127–136 (1986).

21. Gavin, J.B., Humphrey, S.M. and Herdson, P.B.: The no-reflow phenomenon in ischemic myocardium. Int. Rev. Exp. Pathol. 25, 361–383 (1983).

22. Sassen, L.M.A., Bezstarost, K., Verdouw, P.D. and Lamers, J.M.J.: Effects of nisoldipine on recovery of coronary blood flow, sarcoplasmic reticulum function and other biochemical parameters in post-ischemic porcine myocardium. Biochem. Pharmacol. 41, 43–51 (1991).

23. Janero, D.R. and Burghardt, B.: Antioxidant effects of dihydropyridine calcium antagonists. Biochem. Pharmacol. 38, 4344–4348 (1989).

24. Herbaczynska-Cedro, K. and Gordon-Majszak, W.: Nisoldipine inhibits lipid peroxidation induced by coronary occlusion in pig myocardium. Cardiovasc. Res. 24, 683–687 (1990).