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Protective effect of diltiazem against ischemia-induced decreases in regional myocardial flow in rat heart

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Abstract

Diltiazem has cardioprotective properties following myocardial ischemic injury. However, there are controversial results regarding the beneficial effects of diltiazem on regional myocardial flow after ischemia. Therefore, we investigated the effect of diltiazem on changes in regional myocardial flow due to ischemia for different periods. Non-radioactive colored microspheres were used for this measurement in isolated rat heart. After 20 or 40 min of global ischemia and 40 min of reperfusion, regional myocardial flow was decreased, especially in the endocardial layer. The endocardial/epicardial ratio was also decreased. The decreases in endocardial flow and the endocardial/epicardial ratio were more remarkable after 40 min of ischemia than after 20 min of ischemia. Diltiazem (10^{-6} M), which was administered 15 min before ischemia, prevented only the decrease in endocardial flow and endocardial/epicardial ratio after 20 min of ischemia, whereas it did not prevent that after 40 min of ischemia. Nifedipine (2×10^{-6} M) did not exert a cardioprotective effect. These findings suggested that the effect of ischemia is marked in the endocardium and, also, that the protective effect of diltiazem is seen only during a decrease in endocardial flow following short-term and reversible ischemia.

Keywords: Diltiazem; Coronary flow; Heart, isolated, rat; Ischemia; Microsphere; Reperfusion

1. Introduction

Ca^{2+} channel antagonists are used for a variety of cardiovascular diseases including heart disease and have become one of the standard first-choice drugs for essential hypertension and have also become established as therapeutic drugs for angina pectoris, together with β-adrenoceptor antagonists and nitrates (Opie, 1991).

Ca^{2+} channel antagonists have several features that may relate to myocardial protection during ischemia and reperfusion. Myocardial oxygen demand may be reduced by a decrease in heart rate and in myocardial contractility (Opie, 1991). Interference with neutrophil mobilization and activation may protect against the production of free radicals and the release of proteolytic enzymes (Azuma et al., 1986; Rousseau et al., 1994). A direct protective effect may also be produced by interference with ischemia-induced intracellular Ca^{2+} overload (Ver Donck et al., 1986, 1988; Tamura et al., 1996). Collateral blood flow into the ischemic area may improve following vasodilation by Ca^{2+} channel antagonists (Weishaar et al., 1979). Alternatively, Ca^{2+} channel antagonists may reduce the driving pressure for collateral flow by dilating the resistance vessels of the non-ischemic region.

There have been controversial observations on the change in regional myocardial flow after ischemia and reperfusion with Ca^{2+} channel antagonist administration. The myocardial transmural blood flow after ischemia and reperfusion was within the normal range in Ca^{2+} channel antagonist-treated dogs, whereas the flow deteriorated gradually in the middle layer and endocardium in untreated dogs (Rousseau et al., 1994). In contrast, another study reported that the endocardial/epicardial flow ratio recovered to normal levels after reperfusion only in Ca^{2+} channel antagonist-treated pigs, although the change in the regional myocardial flow was not statistically significant (Tadokoro et al., 1996). Another study showed no significant difference in myocardial regional blood flow between Ca^{2+} channel antagonist-treated dogs and untreated dogs

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(Suzuki et al., 1998). In these studies, ischemia was induced for over 60 min to provoke myocardial infarction. We expect that the duration of ischemia is related to these results, because the symptoms of angina pectoris caused by transient ischemia occur within 30 min of coronary occlusion and because Ca^{2+} channel antagonists are effective against angina pectoris, but not against myocardial infarction (Scholant and Alexander, 1994).

To clarify the effect of diltiazem, a Ca^{2+} channel antagonist, the influence of global myocardial ischemia on regional myocardial flow in isolated rat heart was characterized. Under these conditions, the collateral flow from the non-ischemic area to the ischemic area did not occur during the global ischemic period, and there was no effect of circulating neutrophils. We used non-radioactive colored microspheres and investigated the effects of diltiazem on the change in regional myocardial flow after different durations of ischemia to estimate the time dependence of its cardioprotective effect.

2. Materials and methods

2.1. Preparation

Male Wistar rats (250–350 g) were anesthesized with diethyl ether. Hearts were removed and perfused at a constant pressure (75 cm H_2O). The perfusion medium was modified Krebs–Henseleit solution (118 mM NaCl, 4.5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 23 mM $NaHCO_3$, 11 mM glucose), which was equilibrated with 95% O_2 and 5% CO_2 (pH 7.4, 37°C) min before perfusion and which was bubbled during the experiment. The isolated heart was covered with glass heater and the temperature was maintained at 37°C. Total coronary flow was measured using an electromagnetic flow meter (Statham SP 2201), which was calibrated by collection of coronary effluent. Left ventricular pressure, end-diastolic pressure and dP/dt of the left vetricule were measured with saline-filled latex balloons connected to a pressure to a pressure transducer (Statham P-50) with a poyethylene tube. The mean myocardial wet weight in all groups was between 1.24 and 1.35.

2.2. Colored microsphere method

Non-radioactive colored polystyrene microspheres (EZ-Trac, West Los Angeles, US) with a mean diameter of about 15 μm were used as a convenient means to measure flow in isolated rat hearts.

Microspheres were injected just proximal to the aorta. Before injection, the microspheres were centrifuged (3000 rpm, 15 min) and separated from the Tween 80 storage solution containing antibiotic agent at concentrations of 0.01% v/v. Tween 80 was added to the microspheres at a concentration of less than 0.01% as protection against embolism caused by nonspecific aggregation of microspheres. The stock solution of microspheres was sonicated for 5 min and dispersed by vortex mixing. Mixing was checked by calculating the number of aggregates with a hemocytometer.

The concentration of microspheres in the stock solution was adjusted to about 30,000 beads/50 μl. The injection volume of the microsphere solution was 50 μl. After the last injection of microspheres, the heart was removed from the aortic cannula and cut into the following parts: atrium, left ventricle, septum and right ventricle. Furthermore, the left ventricle was cut into slices. Slices were separated into the endocardial layer, the epicardial layer and the middle layer using a thin knife under a light microscope. The six rat heart parts were collected and weighed.

For radiolabelled microspheres, there is a report that a minimum of 400 spheres/sample is required for accurate flow measurement (Buckberg et al., 1971). For colored microspheres in solution, we could count >9 spheres/mm^3 and >450 spheres/sample. However, to maintain this level of accuracy, we injected maximal possible number of microspheres without negative effect on hemodynamic parameter (Fig. 1).

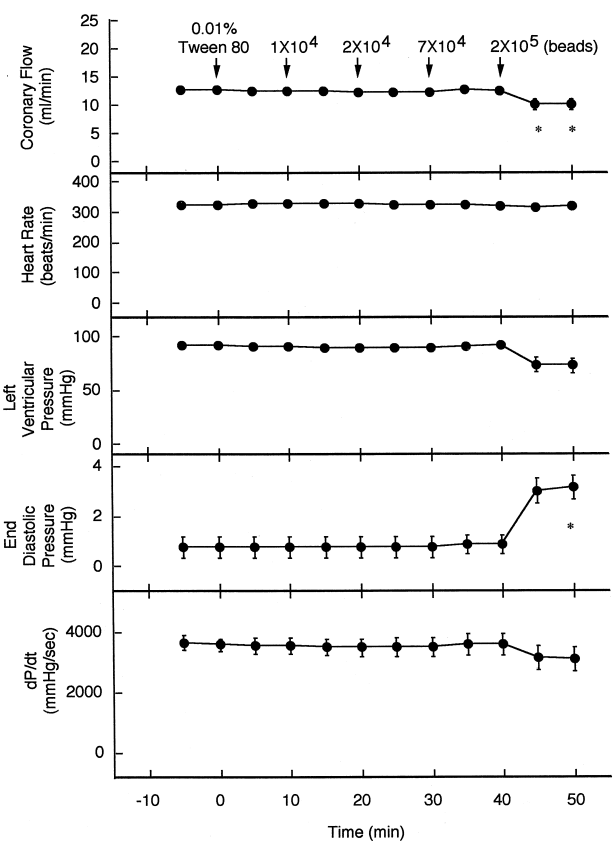

Fig. 1. The effect of cumulative injections of microspheres on coronary flow, heart rate, left ventricular pressure, end diastolic pressure and dP/dt of the left ventricule. Each arrow shows the point of microsphere injection and the data are presented as means and S.E.M. $^{*}P < 0.05$ vs. pretreated data ($n = 5$).

2.2.1. Homogenate

Myocardium was dissociated completely in 1 N NaOH solution in hot water (75°C). The homogenate was centrifuged (3000 rpm, 15 min) and the supernatant was removed. Tween 80 detergent solution (tissue digest reagent II EZ Trac) was added and mixed with a vortex mixer. The solution was centrifuged and the pellet was dissolved in 0.01% Tween 80.

2.2.2. Microscopic observation

The number of microspheres in the solution was counted on a hemocytometer. The regional flow values for each sample were calculated as total flow values and regional flow distributions (Kamitomo et al., 1995) according to the following equation:

$$\mathrm{RMF}_{\mathrm{sample}}\,(\mathrm{ml/min/g}) = \mathrm{CF}_{\mathrm{total}} \cdot \left(\mathrm{NM}_{\mathrm{sample}}/\mathrm{NM}_{\mathrm{total}}\right)/W_{\mathrm{sample}}$$

where $\mathrm{RMF}_{\mathrm{sample}}$ is the regional myocardial flow of each sample, $\mathrm{CF}_{\mathrm{total}}$ the coronary flow (measured using an electromagnetic flow meter, ml/min), $\mathrm{NM}_{\mathrm{sample}}$ the number of microspheres in each sample, $\mathrm{NM}_{\mathrm{total}}$ the number of the microspheres in all heart samples, and W_{sample} the weight of each sample (g).

2.2.3. Cumulative injection of microspheres

To investigate whether the loaded microspheres affected cardiac function, microspheres were injected cumulatively every 10 min after the effects on hemodynamic parameters had disappeared. The number of microspheres injected was as follows: 1000, 2000, 7000, 20,000, 70,000 and 200,000 beads (Fig. 1). The summed number of injected microspheres at each injection time was as follows: 1000, 3000, 10,000, 30,000, 100,000 and 300,000, respectively.

The same number of colored microspheres (30,000 beads each) was injected three times at intervals of 10 min (Fig. 2).

2.2.4. Measurement of regional myocardial flow at pre- and post-ischemia and reperfusion

Thirty thousand microsphere beads were injected. After 10 min of diltiazem, nifedipine or saline treatment, the same number of microspheres was injected again and the infusion was continued for 5 min (15 min in total). After 20 or 40 min of global ischemia and 40 min of reperfusion, a third 30,000 aliquot of beads was injected.

2.3. Drugs

Diltiazem hydrochloride and nifedipine were obtained from Sigma. Diltiazem was dissolved in modified Krebs–Henseleit solution (final concentration 10^{-6} M) and administered 15 min before ischemia. In preliminary studies, 10^{-5} M diltiazem caused marked bradycardia and cardiac arrest, so that 10^{-6} M diltiazem was used in the present study. Nifedipine was dissolved in distilled water under a sodium lamp in a dark room. The solution was diluted with modified Krebs–Henseleit solution (final concentration 2×10^{-6} M).

2.4. Data analysis

All data are shown as mean values and the standard error of the mean (S.E.M.). Statistical testing was carried

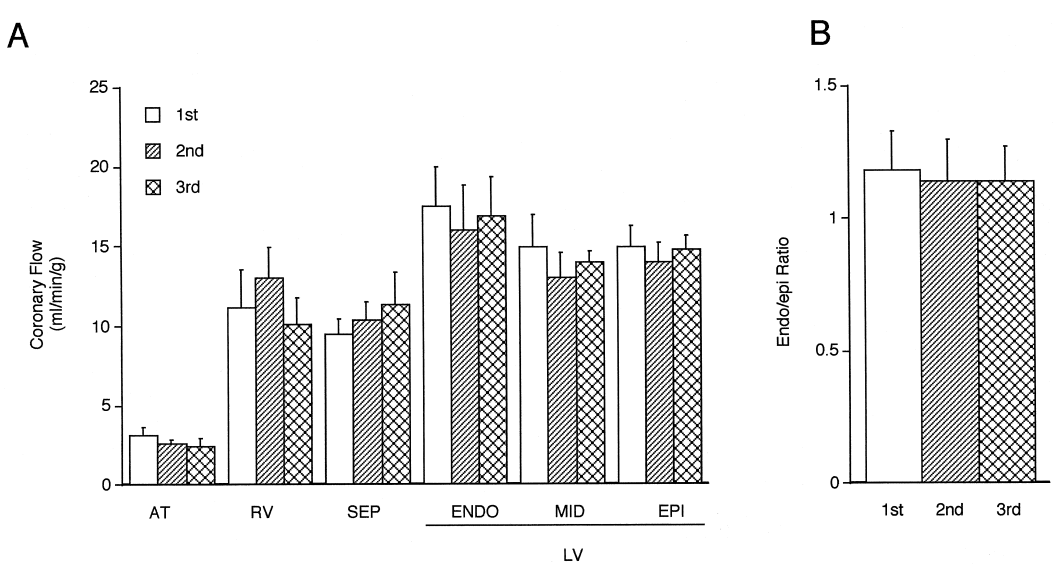

Fig. 2. Constancy of myocardial regional flow and the endocardial/epicardial ratio during microsphere injection. The columns and bars indicate means and S.E.M. ($n = 5$) of the data after three successive injections in the indicated region. AT, atrium; RV, right ventricle; SEP, interventricular septum; ENDO, endocardial layer of left ventricle; MID, middle layer of left ventricle; EPI, epicardial layer of left ventricle; LV, left ventricle. The same abbreviations are used in all of the following figures.

out using analysis of variance (ANOVA). If the probability value was less than 0.05, a parametric post hoc test (Dunnet's test) was carried out. In comparing the two groups, Student's test was performed. Differences were considered to be significant if the probability value was less than 0.05.

3. Results

3.1. Effect of microsphere injection on total coronary flow, heart rate, left ventricular pressure and dP / dt

Cumulative injections of microspheres from 1×10^4 to 2×10^5 beads had no effect on global coronary flow, heart rate, left ventricular pressure, end-diastolic pressure and dP/dt of the left ventricule (Fig. 1). These data showed that the cumulative injection of microspheres up to 10^5 beads did not affect hemodynamic function. Additional injection of 2×10^5 beads resulted in a decrease in coronary flow. Therefore, fewer than 10^5 beads (usually 3×10^4 beads, three times) were used in this study.

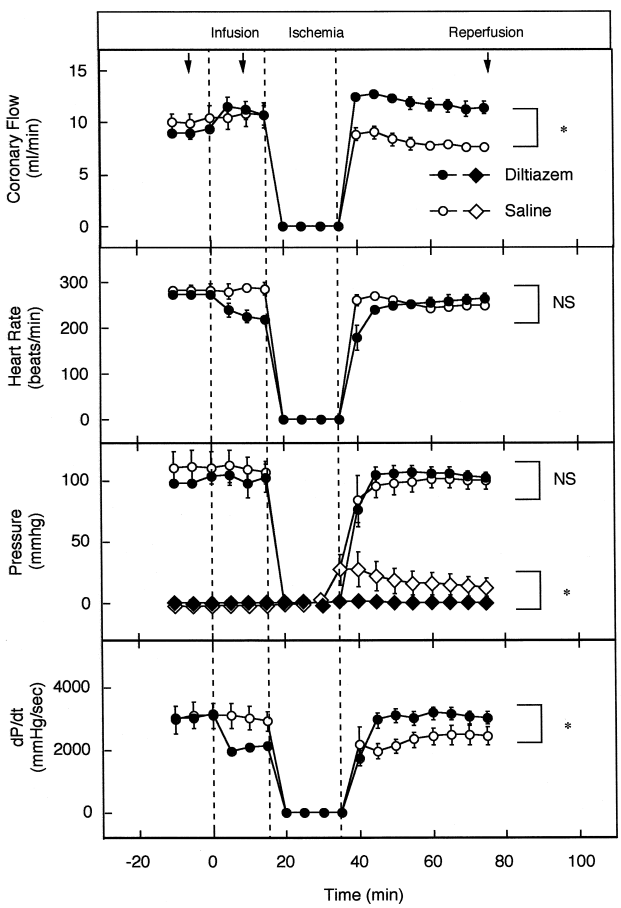

Fig. 3. The effect of diltiazem (closed circles) and saline (open circles) on changes in coronary flow, heart rate, left ventricular pressure, end diastolic pressure and dP/dt of the left ventricule induced by 20 min of ischemia and 40 min of reperfusion. Results are shown as means and S.E.M. ($n = 5$). The diltiazem and saline were administered for 15 min before ischemia. The upper and lower circles in the pressure panel show the systolic and diastolic pressures, respectively. Arrows indicate injection of microspheres. * The diltiazem group is significantly different from the saline group ($P < 0.05$).

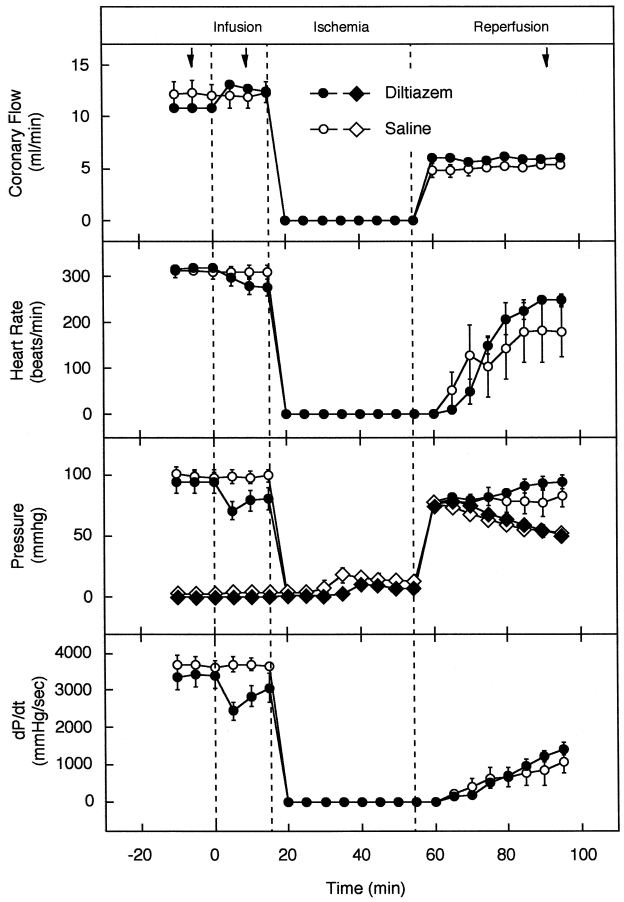

Fig. 4. The effect of diltiazem (closed circles) and saline (open circles) on changes in coronary flow, heart rate, left ventricular pressure, end diastolic pressure and dP/dt of the left ventricule induced by 40 min of ischemia and 40 min of reperfusion. Results are shown as means and S.E.M. ($n = 5$). The diltiazem and saline were administered for 15 min before ischemia. Arrows indicate injection of microspheres.

3.2. Effect of microsphere injection on regional myocardial flow

There was no significant difference in the regional myocardial flow in each part of the heart when 3×10^4 microspheres were injected three times without ischemia (Fig. 2). Furthermore, endocardial/epicardial flow ratios were calculated in the left ventricle and showed no change following injection of fewer than 10^5 microsphere beads (Fig. 2B).

3.3. Effects of ischemia on total coronary flow, heart rate, left ventricular pressure and dP / dt

Global ischemia was induced by shutting off the perfusion. The total coronary flow, heart rate, left ventricular

pressure and dP/dt of the left ventricule were reduced by this treatment, while the end-diastolic pressure was increased. Reperfusion was started 20 or 40 min after global ischemia. Total coronary flow, heart rate, left ventricular pressure, dP/dt of the left ventricule and end-diastolic pressure gradually recovered to almost preischemic levels following 20 min of ischemia. The hemodynamic disturbances following 20 min of ischemia were reversed following reperfusion, recovering up to 70% of the preischemic values for total coronary flow and dP/dt of the left ventricule, while 40 min of ischemia caused more profound effects, which could not be reversed following reperfusion (Figs. 3 and 4). These data indicated that 40 min of ischemia might cause irreversible changes in the isolated rat heart.

Interestingly, diltiazem (10^{-6} M) treatment completely restored the coronary flow and dP/dt of the left ventricule following 20 min of ischemia to pre-ischemic levels. This drug also blocked the increase in end-diastolic pressure following 20 min of ischemia (Fig. 3). In contrast, diltiazem did not affect the heart rate and systolic blood

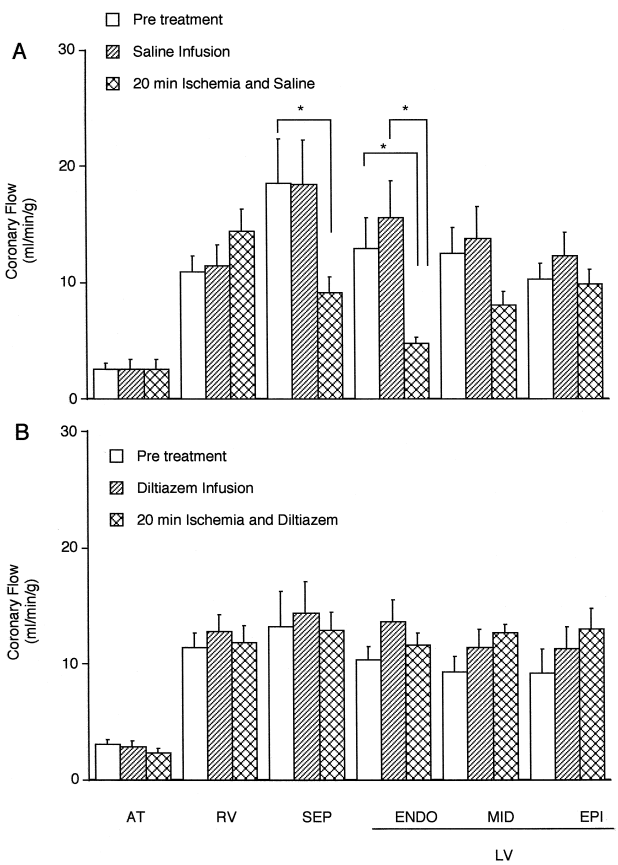

Fig. 5. The effect of diltiazem (B) on the changes in the regional coronary flow induced by 20 min of ischemia. Panel A shows treatment with vehicle (saline). Results are shown as means and S.E.M. ($n = 5$). The abbreviations are as in Fig. 2. After ischemia for 20 min, reperfusion was performed for 40 min. The coronary flow was measured as described in Section 2. * Significantly different from the control data of the pretreatment and saline groups ($P < 0.05$).

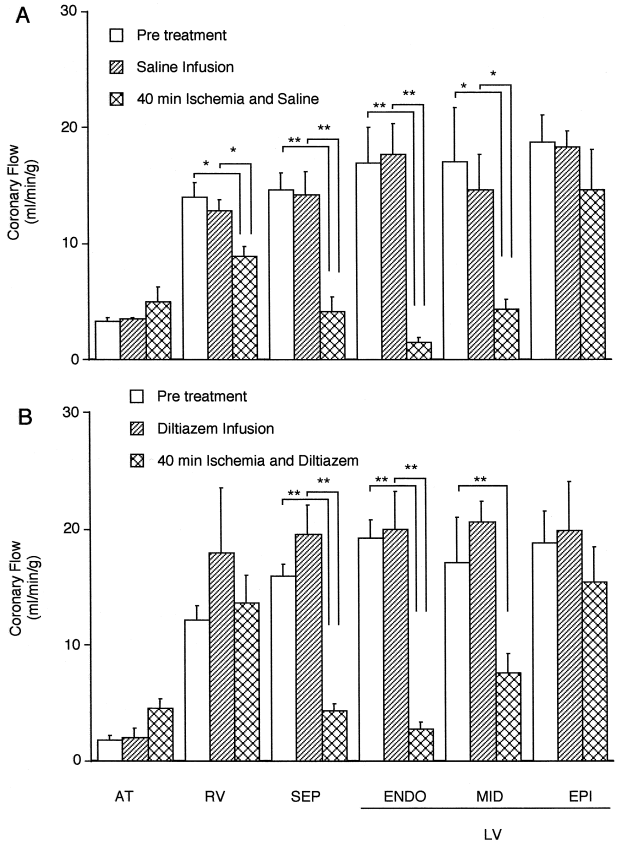

Fig. 6. Lack of influence of diltiazem (B) on the changes in regional coronary flow induced by 40 min of ischemia. Panel A shows treatment with vehicle (saline). Results are shown as means and S.E.M. ($n = 5$). The abbreviations are as in Fig. 2. After 40 min of ischemia, reperfusion was performed for 40 min. * Significantly different from the control data of the pretreatment and saline groups ($P < 0.05$), * * $P < 0.01$.

pressure. As shown in Fig. 4, the longer ischemia (40 min) caused a profound decrease in cardiac function, i.e., coronary flow, heart rate, left ventricular pressure and dP/dt of the left ventricule. Under these conditions, diltiazem could not restore myocardial function.

3.4. Ischemic changes in regional myocardial flow

Next, regional myocardial flow was measured using the colored microsphere method. As shown in Fig. 5A, the regional myocardial flow in the endocardial layer was as high as that in the middle portion and epicardial layer.

As shown in Figs. 5 and 6, 20 and 40 min of ischemia decreased the regional myocardial flow, especially in the endocardial layer and the septal part of the isolated rat heart.

The regional flow in the left ventricular endocardial and middle cardiac layers, the septal part and right ventricular part was significantly reduced ($P < 0.05$). The reduction of endocardial flow after 40 min of ischemia was more profound than that after 20 min of ischemia ($P < 0.05$) (Figs. 5 and 6). In contrast, the regional flow in the

epicardial layer and other parts was not reduced significantly.

These ischemic changes were more obvious when viewed as the endocardial/epicardial ratio. The endocardial/epicardial ratio was reduced dramatically following 20–40 min of ischemia. The endocardial/epicardial ratio after 40 min of ischemia showed a greater reduction than after 20 min of ischemia (Fig. 7, $P < 0.05$). These changes showed the more profound effect of the longer period (40 min) of ischemia on regional myocardial flow.

3.5. Effect of diltiazem pretreatment on ischemia-induced changes in regional myocardial flow

As shown in Fig. 5B, diltiazem pretreatment completely reversed the reduction in septal and endocardial myocardial flow induced by 20 min of ischemia. This pretreatment also improved the slight decrease of middle cardiac and epicardial regional myocardial flow.

As shown in Fig. 6B, the longer ischemic treatment (40 min) produced more profound effects on regional myocardial flow. The diltiazem pretreatment could not reverse the severe decrease in regional myocardial flow in the septal, endocardial or middle myocardial layers (Fig. 6B). This inability to restore regional myocardial flow following 40 min of ischemia was more obvious when regional myocardial flow was expressed as the endocardial/epicardial ratio (Fig. 7B).

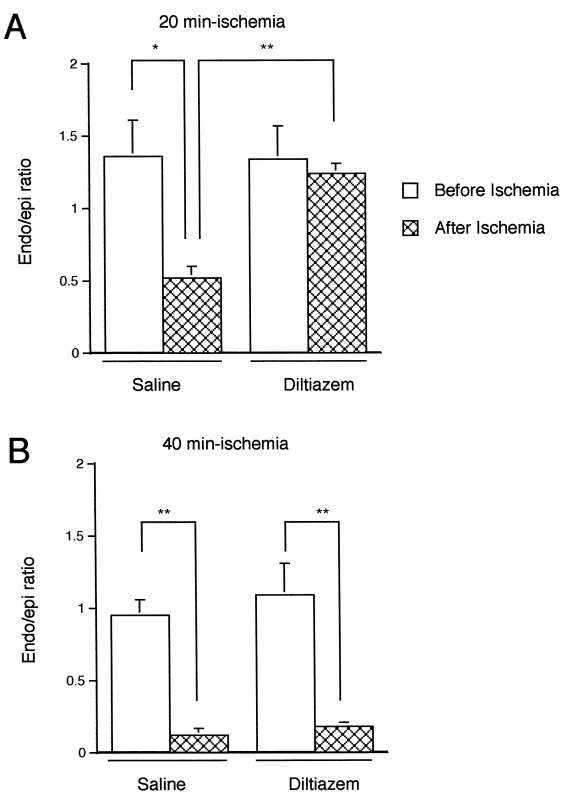

Fig. 7. The effect of diltiazem (right side) on the decreases in the endocardial/epicardial ratio of the regional myocardial flow induced by 20 min (A) or 40 min (B) of ischemia. Results are shown as means and S.E.M. ($n = 5$). * Significantly different from the control data for the ischemia and the saline groups, respectively ($P < 0.05$), ** $P < 0.01$.

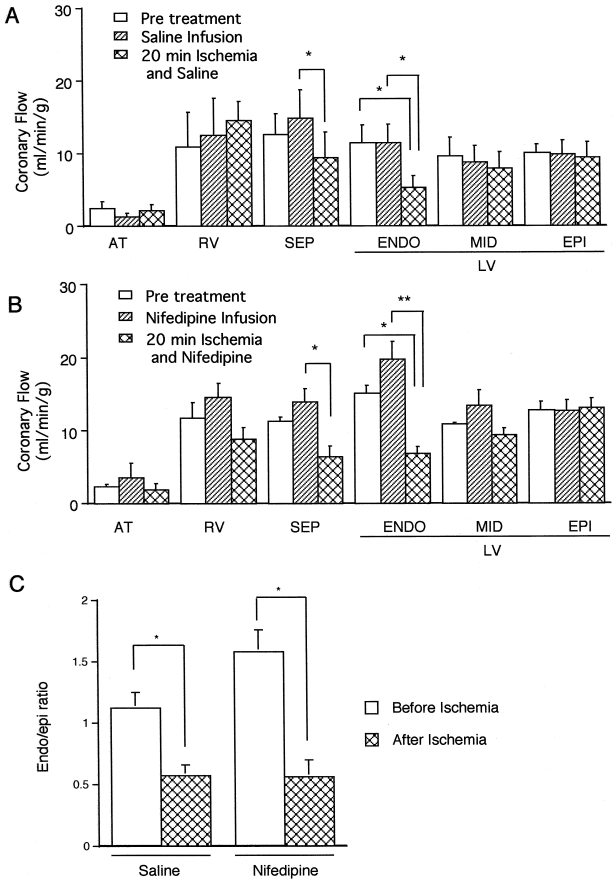

Fig. 8. Lack of influence of nifedipine (B) on changes in the regional coronary flow induced by 20 min of ischemia. Panel A shows treatment with vehicle (saline). The abbreviations are as in Fig. 2. After 20 min of ischemia, reperfusion was performed for 40 min. Lack of influence of nifedipine (C) on the decreases in the endocardial/epicardial ratio of the regional myocardial flow induced by 20 min of ischemia. Results are shown as means and S.E.M. ($n = 4$).

3.6. Effect of nifedipine pretreatment on ischemia-induced changes in regional myocardial flow

As shown in Fig. 8, nifedipine, a dihydropyridine derivative of Ca^{2+} antagonist, could not restore the decreased septal and endocardial myocardial flow and endocardial/epicardial ratio following both short-term (20 min) and long-term ischemia (40 min). These data indicate the selectivity of diltiazem to protect against the ischemia-induced reduction of myocardial flow (Fig. 8).

4. Discussion

In the present study, with the focus on the effects of diltiazem on changes in regional myocardial flow after

ischemia, we showed that the protective effects of diltiazem depended on the duration of myocardial ischemia. Diltiazem reversed the reduction of regional myocardial flow in the endocardial layer following 20 min of ischemia, whereas it hardly affected the reduction in regional myocardial flow after the longer, 40-min period of ischemia in the endocardial and septal layers of the left ventricle. The results suggested that 40 min of ischemia caused irreversible damage to the myocardial tissue and/or microcirculation of coronary blood vessels, which could no longer be prevented by diltiazem. However, nifedipine, another type of Ca^{2+} channel antagonist with dihydropyridine structure, did not show the preventive effect on the reduction of regional myocardial flow after a short period (20 min) of ischemia.

4.1. Ca^{2+} channel antagonist and reduction of regional blood flow after ischemia

There are controversial observations on the effects on regional coronary flow changes of ischemia and reperfusion. Tadokoro et al. showed that the difference in regional myocardial flow on reperfusion between diltiazem-treated and untreated dogs was not statistically significant (Tadokoro et al., 1996). Suzuki et al. reported no significant difference between the myocardial regional blood flow of Ca^{2+} channel antagonist-treated dogs and untreated dogs (Suzuki et al., 1998). On the other hand, two groups have indicated that myocardial blood flow in the middle layer and endocardium was reduced in control animals and that the decrease in the endocardial/epicardial flow ratio was restored by treatment with Ca^{2+} channel antagonists in pigs (Rousseau et al., 1994; Tadokoro et al., 1996). This discrepancy might depend on the differences in experimental conditions or species (Tadokoro et al., 1996).

In the former negative results, however, the length of coronary occlusion (60 and 90 min) was longer than the length of ischemia in the present study (20 and 40 min). Our results indicated that diltiazem could not reverse the reduction of regional myocardial flow after a long period (40 min) of ischemia, probably due to irreversible changes in the heart, whereas it could reverse the reduction after a short period (20 min) of ischemia. Thus, the protective effect of diltiazem was dependent on the duration of ischemia, which is related to the extent of ischemic damage. These data are in good agreement with the clinical fact that diltiazem is useful for protecting against angina pectoris, but not myocardial infarction.

Moreover, because the former investigators studied partial ischemia in the blood supplied heart in vivo, there is a possibility that diltiazem exerted other effects on the collateral blood flow and/or neutrophils (Tadokoro et al., 1996; Suzuki et al., 1998).

4.2. Effect of diltiazem on coronary flow, heart rate, ventricular pressure and dP/dt

Treatment with diltiazem alone without ischemia caused a slight increase in coronary flow and a decrease in heart rate, left ventricular pressure and dP/dt. This change in myocardial function is supposed to reduce oxygen demand in the myocardium. The reduction in oxygen demand may be a myocardial protective effect of diltiazem (Opie, 1991). Therefore, the effect on oxygen demand may contribute to the beneficial effect on the reduction of regional myocardial flow in this study.

Although 20 min of ischemia caused a slight reduction of dP/dt of the left ventricule, which reflects myocardial contractility, and an increase in end-diastolic pressure, which reflects diastolic function, the regional flow in the endocardial layer of the left ventricle was markedly decreased. Myocardial function failed more profoundly after 40 min of ischemia than after 20 min of ischemia. This suggested that 40 min of ischemia produced irreversible myocardial damage, against which diltiazem could not provide protection.

4.3. Mechanism of beneficial effect of diltiazem on regional myocardial flow

Ca^{2+} channel antagonists, including diltiazem, have several features related to the protection of myocardium against ischemia-induced changes. We induced global myocardial ischemia in the isolated rat heart perfused with blood-free solution. Under these conditions, collateral flow definitely did not occur during the ischemic period, because of the absence of a healthy cardiac area. Circulating neutrophils also could not be involved in this myocardial protection. The beneficial effect of diltiazem in this study may result from the direct protective effect, which is produced by interference with ischemia-induced intracellular Ca^{2+} overload (Ver Donck et al., 1986, 1988; Tamura et al., 1996). It might also be due to a decrease in the myocardial oxygen demand, because myocardial contractility seemed to be reduced, despite a constant heart rate (Fig. 4). However, the latter possibility is unlikely, because nifedipine, a dihydropyridine Ca^{2+} channel antagonist, which decreases the myocardial oxygen demand and reduces myocardial contractility, did not protect against the ischemia-induced decrease in myocardial regional flow (Fig. 8).

Recently, Sato et al. reported that diltiazem and verapamil, but not nifedipine, inhibited the elevation of extracellular potassium concentration in isolated guinea pig heart (Sato et al., 1999). They also showed that the negative inotropic potency of diltiazem and verapamil, but not that of nifedipine, depended on the potassium concentration in the perfusate. In their experiments, nifedipine did not block the decrease in adenosine triphosphate or creatine phosphate levels due to global ischemia for 30 min.

This phenomenon might result in the selectivity of the cardioprotective effect of diltiazem.

In addition, under the present conditions, the coronary vessels in the isolated heart might dilate considerably, but not maximally (data not shown), because the coronary flow rate is around 5 ml/g min in vivo. In this case, the cardioprotective effects of calcium antagonists would be weaker than their in vivo effects (Lamping and Gross, 1984; Abrahamsson et al., 1985; Pijl et al., 1993), although diltiazem had an obvious effect in this experiment (Figs. 5 and 7).

The protective effect of diltiazem on the reduction of regional myocardial flow following ischemia depended on the duration of ischemia. The mechanism is currently unknown. Therefore, the precise measurement of changes in regional flow following ischemia using colored microspheres will be important to elucidate both the pathophysiology of ischemic heart disease and the protective mechanism of Ca^{2+} channel antagonists.

4.4. Colored microspheres

The present study also showed the utility of the colored microsphere method for the measurement of regional myocardial flow and for the detection of damage to the coronary circulation following ischemia in the isolated rat heart. Although Prinzen and Glenny (1994) described the use of colored microspheres in experiments on myocardial ischemia, the changes in myocardial regional flow following ischemia were not investigated. Here, the colored microsphere method detected changes in regional myocardial flow after ischemia. Although there was a microsphere loss in necrotic myocardium (Jugdutt et al., 1979), the relatively marked differences between samples that had undergone ischemia and controls were obvious using colored microspheres (Figs. 5 and 6). Thus, the measurement of regional myocardial flow with colored microspheres is a useful technique to detect changes in the microcirculation.

Cook and Hof also reported that myocardial ischemia produced myocardial damage dependent on the duration of ischemia in the isolated rabbit heart (Cook and Hof, 1985). De Groot and Van der Vusse (1993) reported that myocardial lactate content and regional flow reduction depended on the duration of ischemia. Taken together, our data for ischemia and the reports from these investigators indicate that the selective reduction of endocardial flow occurs during short periods of ischemia. The endocardial layer might be vulnerable to ischemic attack due to a deficiency of the circulation in spite of a fairly good blood flow in the endocardial layer, which was shown by the endocardial/epicardial ratio of about 0.9–1.3 in the control samples, as reported by others (Anderson et al., 1987; Hiller et al., 1996; Walland et al., 1993). The vulnerability of the endocardium may be because collateral vessels are generally subepicardial in the heart.

There have been other reports regarding the measurement of regional myocardial flow with non-radioactive microspheres. However, the application of non-radioactive microspheres under conditions of myocardial ischemia has not been developed, and the detection of the effects of diltiazem using this convenient and useful technique has not previously been reported. We have shown the use of non-radioactive colored microspheres to be a convenient technique for measuring changes in regional myocardial flow due to ischemia in isolated rat heart preparations. This method seems to be more precise than the method using a Doppler flow meter (Prinzen and Glenny, 1994; Hiller et al., 1996).

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