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Attenuation by isosorbide dinitrate of coronary occlusion-induced acidosis in the dog myocardium

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1 In dogs anaesthetized with pentobarbitone, the thorax was opened and myocardial pH measured continuously by the use of a glass pH electrode inserted in the left ventricular wall.

2 The left anterior descending coronary artery (LAD) was partially occluded so that the LAD flow could be reduced to a half or one-third of the original flow (partial occlusion). LAD partial occlusion was continued for 90 min, drug or saline being infused for the last 60 min of this period.

3 LAD occlusion decreased myocardial pH significantly by 0.41 to 0.67 pH units, and increased ST segment of the surface electrocardiogram from 11.7 to 12.1 mV.

4 In dogs with non-ischaemic normal hearts, isosorbide dinitrate (ISDN; 1 mg kg^{-1}) did not change markedly either the LAD flow, myocardial pH or heart rate, whereas it decreased myocardial contractile force (determined by a strain gauge arch) slightly and both the systolic and diastolic blood pressure markedly.

5 In dogs with partial LAD occlusion, ISDN (1 mg kg^{-1}) increased myocardial pH significantly and decreased blood pressure, but did not change ST segment elevation in an epicardial lead.

6 These results indicate that ISDN attenuates ischaemia-induced acidosis without attenuating ischaemia-induced ST elevation in the dog myocardium.

Introduction

There are some difficulties in evaluating antianginal drugs in animal models, because anginal attacks cannot be observed in animals. According to Parratt (1974), antianginal drugs can be evaluated in animal models by assessment of their venodilator effect, by their effects on the microcirculation and on oxygen handling in the ischaemic myocardium. Winbury (1975) postulated that intramyocardial oxygen tension can be used to investigate antianginal drugs, because oxygen tension of the myocardium reflects the balance between oxygen supply and demand in the tissue. Insomuch as the purpose of the use of antianginal drugs is to improve oxygen balance in the ischaemic myocardium, intramyocardial oxygen tension is a reasonable indicator for evaluation of antianginal drugs.

Another approach used to assess antianginal drugs is to measure changes in myocardial metabolism. This is because myocardial metabolism shifts rapidly from the aerobic to anaerobic type during ischaemia of the heart; there are decreases in the levels of myocardial glycogen, adenosine triphosphate, and creatine phosphate, and increased release of lactate and protons during ischaemia (Ichihara & Abiko, 1975; Gevers, 1977). The increase in the level of protons in the ischaemic myocardium is of particular interest, because myocardial proton levels can be monitored continuously by the use of a pH electrode inserted in the myocardium. In fact, myocardial pH can be measured continuously, and using this method it has been confirmed that coronary occlusion produces acidosis of the myocardium (Ichihara *et al.*, 1979).

Recently we have proposed that myocardial pH can be used as an indicator of myocardial ischaemia, and this method can be employed for evaluation of antianginal drugs (Abiko *et al.*, 1984a). This is based on the findings that clinically effective antianginal drugs such as nitroglycerin, propranolol, diltiazem and nicorandil attenuate myocardial pH that has been reduced by partial occlusion of the coronary artery (Abiko & Sakai, 1980; Ichihara & Abiko, 1982; Shibano & Abiko, 1983). The purpose of the present study was to examine whether isosorbide dinitrate, a long acting nitrate, also attenuates myocardial pH during ischaemia.

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Methods

Eighteen mongrel dogs of either sex, weighing 13.9 ± 0.9 kg (mean ± s.e.), were anaesthetized with sodium pentobarbitone (30 mg kg^{-1}, i.v.). The dogs were ventilated with a positive-pressure respirator using room air. The left side of the thorax was opened, and an electromagnetic flow probe (FR-020T, Nihon Kohden) and an occluder were placed around the left anterior descending coronary artery (LAD). The occluder, consisting of a polyethylene tube and a thread (Abiko & Sakai, 1980), was placed immediately distal to the flow probe. The LAD flow was recorded on a polygraph (Model 146, San-Ei Instrument). The pH in an area of myocardium expected to become ischaemic after occlusion of the LAD, was measured by the use of a micro glass pH electrode (MI-410, Microelectrodes, Londonderry, NH, U.S.A.) and displayed on a pen recorder (KA-62H, Rikadenki Electronics). The tip of the pH electrode, about 1.2 mm in diameter, was placed in the subendocardial layers of the myocardium at a depth of about 8 mm. These procedures have been described previously (Abiko & Sakai, 1980). The electrocardiogram (standard limb lead II) and arterial blood pressure (from the left carotid artery) were also recorded on the polygraph. After a stabilization period of 60 min, the experiment with myocardial pH was started. By the use of an occluder, the LAD flow was reduced to about a half or one-third of the original flow (partial occlusion) and was kept constant. Thirty minutes after partial occlusion of LAD, isosorbide dinitrate (1 mg kg^{-1}) or saline solution was injected through a catheter placed in the left femoral vein. Partial occlusion was continued until 60 min after the drug or saline injection. Before, during, and after partial occlusion, myocardial pH and haemodynamic parameters were continuously measured and recorded. In some experiments, a surface electrocardiogram was recorded from the area of myocardium to become ischaemic and myocardial contractile force of the ischaemic area was also recorded by the use of a strain gauge arch sutured onto the epicardium.

All the data were expressed as mean ± s.e., and differences between means were analysed using Student's t test for paired observations and analysis of variance for unpaired observations. A P value of 0.05 or less was considered significant. Isosorbide dinitrate (ISDN) was dissolved in saline solution immediately before use at a concentration of 2 mg ml^{-1}.

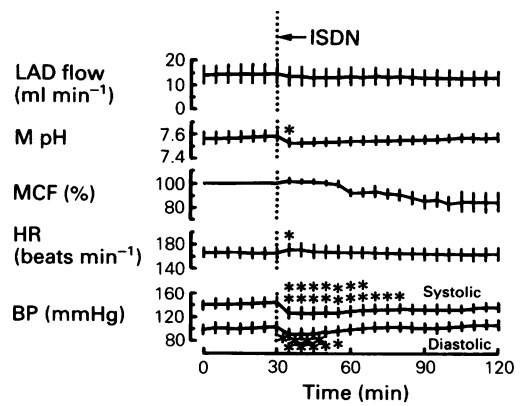

Figure 1 Effect of an injection of isosorbide dinitrate (ISDN; 1.0 mg kg^{-1} i.v.) on blood flow of the left anterior descending coronary artery (LAD flow), myocardial pH (M pH), myocardial contractile force (MCF), heart rate (HR), and systolic and diastolic blood pressure (BP) in dogs with non-ischaemic normal hearts. Values are expressed as mean ± s.e. (vertical lines), $n = 6$. *$0.01 < P < 0.05$; **$P < 0.01$ compared with the values at '30 min' (paired data analysis).

Results

The effect of isosorbide dinitrate on non-ischaemic normal myocardium

Figure 1 illustrates the effect of ISDN (1 mg kg^{-1}) on the cardiovascular system in the dog with a non-ischaemic normal heart, in which LAD occlusion was not performed. The LAD flow, myocardial pH, myocardial contractile force, heart rate and systolic and diastolic blood pressure immediately before ISDN injection were 14.7 ± 4.3 ml min^{-1}, 7.58, 100% (myocardial contractile force immediately before ISDN injection was defined as 100%), 165 ± 9 beats min^{-1}, and 145.0 ± 8.8 and 104.2 ± 9.2 mmHg, respectively. Injection of ISDN did not change LAD flow significantly, but decreased myocardial pH slightly immediately after the injection. Myocardial contractile force decreased gradually after the ISDN injection, but the decrease was not statistically significant.

ISDN decreased both systolic and diastolic blood pressure. Because ISDN decreased systolic and diastolic blood pressure without changing the LAD flow, it transiently decreased coronary resistance in the LAD area, although the value immediately before the ISDN injection (9.8 ± 1.4 mmHg ml^{-1} min) was not significantly different from that 5 min after the injection (9.6 ± 1.5 mmHg ml^{-1} min).

The effect of isosorbide dinitrate on ischaemic myocardium

The LAD flow, myocardial pH, change in ST segment (ΔST), myocardial contractile force, heart rate and systolic and diastolic blood pressure immediately before partial occlusion ('0 min' in Figure 2) were

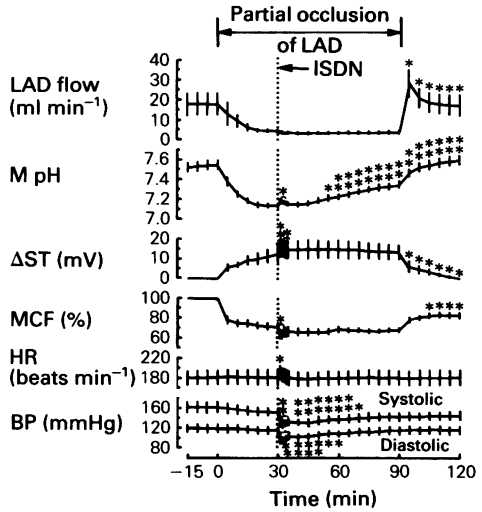

Figure 2 Effect of an injection of isosorbide dinitrate (ISDN; 1.0 mg kg^{-1} i.v.) on the left anterior descending coronary artery (LAD) flow, myocardial pH (M pH), change in ST segment (ΔST), myocardial contractile force (MCF), heart rate (HR) and blood pressure (BP) in dogs with hearts in which the LAD was partially occluded. LAD flow was reduced by an occluder so that the flow could be reduced to about a half or one-third of the original flow (partial occlusion). LAD partial occlusion continued for 90 min, and ISDN was injected 30 min after the start of LAD partial occlusion ('30 min'). Values are expressed as mean ± s.e. (vertical lines), $n = 6$. For explanation of symbols see legend of Figure 1.

17.7 ± 5.6 ml min^{-1}, 7.54, 0 mV, 100% (myocardial contractile force immediately before LAD occlusion was defined as 100%), 181 ± 15 beats min^{-1}, and 161.7 ± 11.7 and 118.3 ± 8.5 mmHg, respectively. LAD occlusion decreased both LAD flow and myocardial pH; LAD flow and pH 30 min after occlusion were 4.2 ± 0.8 ml min^{-1} (a decrease of about 76%) and 7.13 (a decrease of 0.41 pH units), respectively. LAD occlusion for 30 min also produced an elevation of ST segment (an increase by 12.1 ± 4.1 mV) and a decrease in myocardial contractile force (a decrease of 28.6%). When ISDN (1 mg kg^{-1}) was injected 30 min after LAD occlusion, myocardial pH increased gradually but significantly. However, ISDN did not attenuate the elevation of ST segment or modify the decrease in myocardial contractile force during ischaemia. Nevertheless, it decreased both the systolic and diastolic blood pressure as in the experiments with non-ischaemic normal hearts.

Effects of saline on ischaemic myocardium

In this series of control experiments, saline was injected instead of ISDN 30 min after the institution of myocardial ischaemia. LAD flow, myocardial pH, ΔST, myocardial contractile force, heart rate, and systolic and diastolic blood pressure immediately before partial occlusion ('0 min' in Figure 3) were 19.5 ± 3.0 ml min^{-1}, 7.55, 0 mV, 100% (myocardial contractile force immediately before LAD occlusion was defined as 100%), 147 ± 8 beats min^{-1}, and 143.3 ± 7.6 and 103.3 ± 7.4 mm Hg, respectively. LAD occlusion decreased both LAD flow and myocardial pH; LAD flow and pH 30 min after occlusion were 7.5 ± 1.4 ml min^{-1} (a decrease of about 62%) and 6.88 (a decrease of 0.67 pH units). LAD occlusion for 30 min also produced an elevation of ST segment (an increase by 11.7 ± 3.4 mV) and a decrease in myocardial contractile force (a decrease of 26.5%). When saline (0.5 ml kg^{-1}) was injected 30 min after LAD occlusion, myocardial pH and other parameters did not change significantly. After release of partial occlusion, the LAD flow and the myocardial pH returned to the pre-occlusion levels.

Restoration of myocardial [H^+] during LAD occlusion

The values of myocardial pH in Figures 2 and 3 were converted to those of myocardial [H^+], and the values

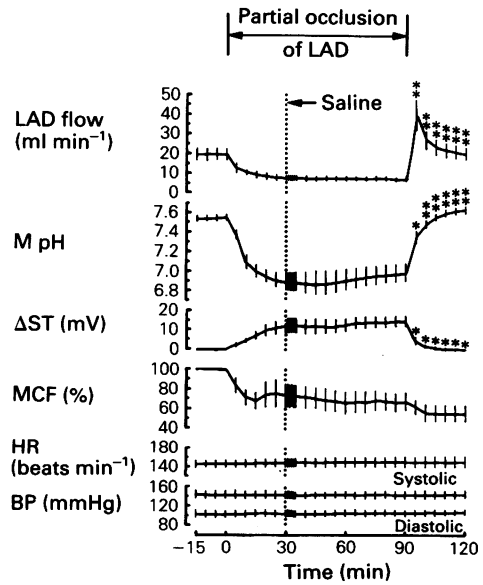

Figure 3 Effect of saline solution (0.5 ml kg^{-1} i.v.) on the left anterior descending coronary artery (LAD) flow, myocardial pH (M pH), ΔST, myocardial contractile force (MCF), heart rate (HR) and blood pressure (BP) in dogs with hearts in which the LAD was partially occluded. Values are expressed as mean ± s.e. (vertical lines), $n = 6$. For explanation of symbols see legend of Figure 1.

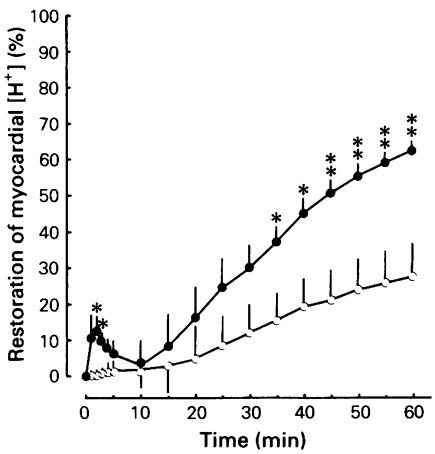

Figure 4 The restoration of myocardial [H^+] after an injection of saline (0.5 ml kg^{-1} i.v.) (○) or isosorbide dinitrate (ISDN, 1.0 mg kg^{-1} i.v.) (●). This figure was constructed from the data shown in Figures 2 and 3. The myocardial [H^+] immediately before partial occlusion ('0 min' in Figures 2 and 3) was taken as 100%, and that immediately before injection ('30 min') as 0%. The value of myocardial [H^+] obtained at a given time in ISDN experiments was compared with that obtained at the same time in saline solution experiments. Each value is expressed as mean with vertical lines indicating s.e. $*0.01 < P < 0.05$, $**P < 0.01$ (unpaired data analysis).

of myocardial [H^+] immediately before and 30 min after LAD occlusion were defined as 100% and 0%, respectively. The % restoration of myocardial [H^+] induced by saline or ISDN was then calculated. (Saline or ISDN was injected 30 min after LAD occlusion, and the results are shown in Figure 4).

In the saline group, the restoration of myocardial [H^+] gradually increased; the % restoration 60 min after the saline injection was 27.4 ± 9.1%, suggesting that there is a spontaneous recovery of myocardial [H^+] during ischaemia. This spontaneous recovery in myocardial pH may be due to a spontaneous increase in blood flow in the collateral circulation or diffusion of fluid from the non-ischaemic to the ischaemic area. Nevertheless, the mechanism of this phenomenon is still unclear. The restoration of myocardial [H^+] in the ISDN group, however, was invariably higher than that in the saline group; the % restoration 60 min after the ISDN injection was 62.2 ± 2.6%, being significantly higher than that in the saline group, and the % restoration 2, 3, 35, 40, 45, 50, and 55 min after the ISDN injection was also significantly higher than that in the saline group. These results indicate that ISDN accelerates recovery of myocardial [H^+] from the effects of ischaemia, and that the ISDN-induced actual recovery of myocardial [H^+] (recovery in the presence of ISDN minus recovery in the absence of ISDN) was about 34.8% 60 min after the injection.

Discussion

It has been shown that isosorbide dinitrate dilates coronary vessels, resulting in an increase in coronary flow (Goldberg, 1948; Wendt, 1972; Willerson *et al.*, 1975). In the present study, however, isosorbide dinitrate at a dose of 1 mg kg^{-1} did not increase coronary flow in the non-ischaemic normal myocardium although coronary resistance was decreased. This is in contrast to the studies of Wendt (1972), who demonstrated a coronary flow increase in the dog by infusing the drug at the dose of 0.25 to 0.5 mg kg^{-1} min^{-1} for 5 min, and of Willerson *et al.* (1975), who observed an increase in flow (microsphere method) in dogs anaesthetized with chloralose. Nevertheless, isosorbide dinitrate decreased blood pressure markedly in the present study as in other studies (Wendt, 1972; Fiedler & Nitz, 1981).

The results demonstrate that partial occlusion of LAD produced a decrease in myocardial pH, and that isosorbide dinitrate, 1 mg kg^{-1}, attenuated the decrease in myocardial pH that had been induced by occlusion of the LAD. The extent of recovery of myocardial [H^+] induced by isosorbide dinitrate during ischaemia was 34.8% 60 min after the injection. According to our previous studies, the extent of recovery of myocardial [H^+] induced by nitroglycerin (Shibano & Abiko, 1983), diltiazem (Ichihara & Abiko, 1982), and nicorandil (Abiko *et al.*, 1984b) during ischaemia is in a range of 20 to 60% 60 min after the injection, while the extent of recovery induced by propranolol (Ichihara & Abiko, 1982), atenolol (Sakai & Abiko, 1985), and sotalol (Izumi *et al.*, 1982) during ischaemia is more than 60% (see Abiko *et al.*, 1984a). Therefore, it can be concluded that isosorbide dinitrate attenuates the ischaemia-induced acidosis of the myocardium to an extent similar to that induced by nitroglycerin, diltiazem, and nicorandil. These findings provide a pharmacological basis for the antianginal action of isosorbide dinitrate (Danahy & Aronow, 1977; Elkayam & Aronow, 1982). However, it is unclear as to why elevation of ST segment during ischaemia was not attenuated by isosorbide dinitrate. One explanation may be that myocardial pH reflects information from the subendocardium (because the pH electrode is in the subendocardium) and ST segment reflects that of the subepicardium (because of the surface electrode), and that isosorbide dinitrate improves only subendocardial ischaemia.

In addition, there is no apparent reason why isosorbide dinitrate attenuates the myocardial acidosis induced by occlusion of the coronary artery. A decrease in arterial blood pressure could be responsi-

ble for the ischaemia-induced acidosis of the myocardium, as a decrease in blood pressure causes a reduction in myocardial work which results in a decrease in oxygen consumption of the heart. However, a decrease in blood pressure does not always attenuate the ischaemia-induced myocardial acidosis, as shown in the experiments with dipyridamole (Shibano & Abiko, 1983). A decrease in myocardial contractile force may also be responsible for the beneficial effect of isosorbide dinitrate on the ischaemic myocardium. Nevertheless, the decrease in myocardial contractile force induced by isosorbide dinitrate was not very prominent, indicating that the decrease in myocardial contractile force may not be a predominantly causative factor in producing the pH-attenuating effect of isosorbide dinitrate. Alternatively, it is conceivable that isosorbide dinitrate produces its beneficial effect as a result of a direct effect on the myocardium as demonstrated for nitroglycerin (Borow *et al.*, 1981; Szekeres & Udvary, 1983). This effect is thought to be due to an increase in the intracellular level of cyclic GMP in the ischaemic myocardial cells (Kukovetz *et al.*, 1979; Laustiola, 1984). Also, it is possible that the action of isosorbide dinitrate depends on the degree of myocardial acidosis, as after LAD occlusion myocardial pH decreased by 0.67 units in the saline-control experiments (Figure 3), and by 0.41 units in the experiments with isosorbide dinitrate (Figure 2). It is conceivable that isosorbide dinitrate would not have been able to attenuate myocardial pH against a more acidic background. However, this view is not plausible because drugs such as propranolol and atenolol can attenuate myocardial pH against a more acidic background (being acidic by more than 0.6 pH units) (Ichihara & Abiko, 1982; Sakai & Abiko, 1985).

In conclusion, our results demonstrate that isosorbide dinitrate attenuates myocardial acidosis during ischaemia in the dog heart.

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