INHIBITION OF MYOCARDIAL CALCIUM ACCUMULATION DURING ISCHEMIA AND REPERFUSION BY RESERPINE IN ISOLATED GUINEA PIG HEARTS

Minoru HIRATA, Hiroshi FUKUI, Norio SHIMAMOTO and Noriko GOTO
Biology Laboratories, Central Research Division, Takeda Chemical Industries, Osaka 532, Japan

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Reperfusion of the ischemic myocardium with an oxygenated medium aggravates ischemia-induced cellular damage (1-5), and the cellular damage is assumed to be correlated to myocardial calcium accumulation (4, 6, 7). Reserpine has been reported to protect against myocardial cell damage due to hypoxia and subsequent reoxygenation as evidenced by inhibition of myocardial enzyme release (8, 9) and ultrastructural changes (10). However, the effects of reserpine on the myocardial calcium accumulation during ischemia and reperfusion have not yet been revealed. The present authors were interested in clarifying the effects of reserpine on the myocardial function and calcium accumulation during reperfusion.

According to the Langendorff's technique, an isolated guinea pig heart preparation was made, and perfused with oxygenated Krebs-Ringer bicarbonate solution (pH 7.4) at 75 cm H2O and 37°C. The coronary arterial flow (CAF) was measured with an electromagnetic flowmeter (Nihon Kohden, MF-26). A flaccid, saline filled rubber balloon was inserted into the left ventricular cavity to measure the left ventricular pressure (LVP) and its dp/dt with a pressure transducer (Nihon Kohden, MPU-0.5). The heart rate (HR) was registered with a cardiotachometer (Nihon Kohden, RT-2) triggered by the LVP pulses.

After an equilibration period of about 30 min, the heart preparation was exposed to global ischemia for 60 min under constant flow perfusion at a rate of 0.1 ml/min, followed by reperfusion for varying periods under the constant pressure of 75 cm H2O. After a given period of perfusion, the heart was subjected to measurement of the myocardial calcium content.

About 400 mg of the left ventricular free wall was dried at 95 °C for 24 hr, and ashed at 650°C for 5 hr in a furnace. The ashes were dissolved in an equal volume mixture of 3M TCA and glacial acetic acid, and the calcium concentration was measured with an atomic absorption spectrometer (Hitachi, 201) after adequate dilution.

Reserpine (Raupina®, Yamanouchi) was intraperitoneally administered at doses of 4 mg/kg, 2 days before; 2 mg/kg, 24 hr before; and 2 mg/kg, 2 hr before the experiment. Control animals were intraperitoneally treated with physiological saline in the same schedule as in the reserpine group. For measurement of the myocardial norepinephrine content freshly excised left ventricular muscle was homogenized in an acidic condition. Norepinephrine was adsorbed on a mixture of alumina and Amberlite CG-50,
extracted by ether, and measured by high performance liquid chromatography employing an electrochemical detector. The reserpine pre-treatment depleted the myocardial norepinephrine stores by about 99%, from 1.54±0.14 in the control (n=10) to 0.02±0.01 μg/g wet weight of ventricular tissue in the reserpine group (n=6).

Immediately after the isolated heart preparation became ischemic, the contractility of the left ventricle rapidly decreased, and the preparation ceased to contract in a few min. When the heart preparations were reperfused, contractions were restored within seconds, although the beating was irregular. The beating rate in a stable stage during reperfusion was not significantly different from the pre-ischemia level in the control and reserpine groups, except that the rate tended to be higher in the reserpine than in the control group (Table 1).

In the control group, the left ventricular diastolic pressure (DP) reached maximum around 5 min after reperfusion and remained high throughout the period of reperfusion (Table 1). The left ventricular systolic pressure (SP) did not recover to the pre-ischemia level, and the pulse pressure (PP) remained low at about 40% of the pre-ischemia level. The left ventricular pressure dp/dt also remained low at 50 to 70% of the pre-ischemia level. In reserpinized hearts, the contractility was relatively low even before the ischemic intervention as reflected in the significant decrease in the left ventricular pressure dp/dt as compared with that in the control group (Table 1). The SP and DP, however, did not differ markedly from those in the control group. The cardiac depression seen after reperfusion was relatively slight in the reserpinized hearts. Even 5 min after reperfusion, the DP was not elevated significantly, and the SP and PP recovered to more than 80 and 65% of the pre-ischemia values, respectively. The left ventricular pressure dp/dt almost completely recovered to the pre-ischemia level. The recovery in these cardiac parameters was more prominent 60 min after reperfusion.

The calcium contents of the myocardium freshly prepared from the non-treated and reserpine-treated guinea pigs were 5.24±0.31 (n=5) and 5.24±0.14 (n=7) mmoles/kg dry weight, respectively, and there was no statistically significant difference between these two groups. The myocardial calcium content was significantly increased after the preparative procedure and constant pressure perfusion period for about 30 min as shown in Fig. 1, although the causative mechanisms

| Table 1. Effects of reserpine treatment on physiological parameters of isolated heart preparations |
|-----|-----|-----|-----|-----|-----|
|     | n   | Before | 5 min | 60 min | 5 min | 60 min |
|     |     | SP (mmHg) | DP (mmHg) | PP (mmHg) | dp/dt (mmHg/sec) | HR (beats/min) |
| Control | 10 | 76.7±4.2 | 6.6±0.9 | 70.1±3.8 | 1173±106 | 240±7 |
|         | 5 min | 52.5±3.1*** | 25.5±5.0* | 27.9±4.3*** | 705±112** | 246±5 |
|         | 60 min | 49.6±2.3*** | 21.7±6.5* | 27.9±6.5*** | 774±101*** | 230±9 |
| Reserpine 8 mg/kg i.p. | 6 | 60.0±5.5 | 10.5±4.5 | 50.3±5.1 | 695±72** | 260±5 |
|         | 5 min | 49.7±2.5 | 17.2±5.5 | 32.5±5.6 | 665±90 | 267±3 |
|         | 60 min | 51.6±2.2 | 6.4±1.1 | 45.2±3.1 | 920±117 | 258±8*** |

Before: immediately before intervention of ischemic episode; 5 and 60 min: 5 and 60 min after reperfusion. SP, DP and PP: left ventricular systolic, diastolic and pulse pressure, respectively. HR: heart rate. *P<0.05, **P<0.01, as compared with the control group. + P<0.05, ++ P<0.01, +++ P<0.001, as compared with the values of "Before" in each group.
were not clear. Constant pressure perfusion for another 2-hr period, however, did not affect the myocardial calcium content (Fig. 1, Time control). The myocardial calcium content tended to be decreased by an ischemic episode of 60 min as shown in Fig. 1. In the control group, the content steeply increased until a plateau was reached at 15 min of reperfusion, and the content was about 2.5 times higher than the ischemia level. The kinetics of the myocardial calcium accumulation during reperfusion was essentially the same as reported by Shon and Jennings in anesthetized dog hearts (6). In the reserpine group, the myocardial calcium content was significantly lower at 15 and 60 min of reperfusion as compared with the control group.

Subcellular distribution of calcium massively taken up by the myocardial cells during reperfusion has not yet been precisely revealed so far, although intracellular calcium has been inferred to be mainly immobilized in the mitochondria (6, 7). Cytosolic distribution, however, is also implied as visualized by the "hypercontraction band" in the ultrastructural investigations (3) and by sustained elevation of the left ventricular diastolic pressure in the present study since the process of muscular contraction is triggered by cytosolic calcium ions (11). The association of the well preserved myocardial function with lesser degrees of myocardial calcium accumulation in the reserpinized hearts suggests that reserpine alleviates the deranged handling of calcium by the cell membrane to result in preservation of the myocardial function. Further studies are underway to elucidate the subcellular distribution of calcium and the mechanisms of reserpine actions.

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