The effect of an endothelin (ET) A/ETB receptor antagonist, TAK-044, and/or an angiotensin converting enzyme (ACE) inhibitor, temocaprilat, on myocardial metabolism and contraction during ischemia and reperfusion was examined by phosphorus 31-nuclear magnetic resonance (31P-NMR) in Langendorff rabbit hearts. After normothermic 15 min global ischemia, 60 min of posts ischemic reperfusion was carried out. TAK-044 and/or temocaprilat was administered from 40 min prior to the global ischemia. Adenosine triphosphate (ATP), creatine phosphate, inorganic phosphate, pH, left ventricular systolic developed pressure (LVDev.P), left ventricular end-diastolic pressure (LVEDP) and coronary flow were measured. Twenty-eight hearts were divided into 4 experimental groups consisted of seven hearts each: Group I consisted of controls, Group II was perfused with TAK-044 (10⁻⁶ mol/L), Group III was perfused with temocaprilat (10⁻⁶ mol/L), and Group IV was perfused with TAK-044 (10⁻⁶ mol/L) in combination with temocaprilat (10⁻⁶ mol/L). Group II showed a more early recovery of ATP during posts ischemic reperfusion (82±3%) compared with Group I (71±3%). Group III showed a significant inhibition of the decrease in ATP during global ischemia (54±3%) compared with Group I (45±3%). Group IV also showed a significant marked inhibition of the decrease in ATP during global ischemia (59±5%) and a more significant improvement on recovery of ATP during posts ischemic reperfusion (86±3%) compared with the other 3 groups. There were no differences in LVDev.P, LVEDP and coronary flow among these groups.

In conclusion, TAK-044 in combination with temocaprilat had a significant potentiation on myocardial metabolism during both ischemia and reperfusion. (Jpn Circ J 1999; 63: 770–774)

Key Words: Angiotensin converting enzyme inhibitor; Endothelin receptor antagonist; Ischemia; Myocardial contraction; Myocardial metabolism

Methods

Isolated Heart Preparation

Male Japanese white rabbits weighing 1.6–1.7 kg were anesthetized by intravenous injection of sodium pentobarbital (30 mg/kg). The chest was opened and heparin sodium (1,000 IU/kg) was injected through the right atrial appendage. The heart was quickly removed and after aortic cannulation the retrograde Langendorff perfusion was initiated without delay.

The hearts were perfused at a constant perfusion pressure of 80 mmHg with modified Tyroide’s solution at 37°C of the following composition (in mmol/L); NaCl 108, KCl 5, MgCl₂ 1, HEPES 5, CaCl₂ 2, glucose 10, sodium acetate 20. The perfusate was oxygenated with 100% O₂ using a bubble-type artificial lung (Japan Medical Supply Co, Ltd, Hiroshima, Japan) adjusted to pH 7.40. The perfusate was recycled to an artificial lung after passing through the heart. A 40-μm Swank filter (Nipro Co, Ltd, Tokyo, Japan) was placed within this route to remove any contaminants.

To measure left ventricular (LV) pressure, a latex balloon was inserted into the left ventricle via a left atrial incision. The balloon was large enough so that no pressure was generated by itself over the range of left ventricular volumes used in the experiment. The balloon was filled with bubble-free saline and attached to a pressure transducer (Millar Instruments Inc, Houston, TX, USA) connected to a polygraph system RM-6000 (Nihon Kohden Co, Tokyo, Japan).
The balloon volume was set to produce a left ventricular end-diastolic pressure (LVEDP) of 10 mmHg. The isovolumic measurement of left ventricular developed pressure (LVDev.P) was employed as the index of LV contraction.

To drain effluent from the left ventricular Thebesius vein, a polyethylene tube was inserted into the left ventricle via a left atrial incision. The heart was paced through the right ventricle with an agar wick soaked in saturated KCl and encased in polyethylene tubing at 180 beats/min throughout all experiments. The pacing was performed by an electronic stimulator SEN-3301 and isolator SS-302 J (Nihon Kohden Co). Coronary flow was measured by an electromagnetic flow probe (Nihon Kohden Co) attached around the ascending aorta. These data were recorded using a thermal-array recorder WS-681G (Nihon Kohden Co).

**Measurement of High Energy Phosphates and Intracellular pH by 31P-NMR**

The heart was placed in a NMR sample tube 25 mm in diameter. The temperature of the 31P-NMR device was maintained at 37°C. The 31P-NMR spectra were recorded with a JNM-GX 400 FT NMR spectrometer (JEOL, Tokyo, Japan) operating at 161.7 MHz. Radiofrequency pulses of 45°C were repeatedly applied at an interval of 1.0 s. The spectra were obtained by accumulating 300 free induction decays. Quantitative analysis was performed by the relative intensities of the α-ATP (adenosine triphosphate), creatine phosphate (CrP), and inorganic phosphate (Pi) peaks. The areas under each peak were integrated 5 times with a planimeter. The mean of 5 such readings was normalized as a percentage of the value given during the initial basal period. The distance of the intracellular Pi peak relative to the intracellular CrP peak (pH-independent) was measured and the intracellular pH was determined using the following equation:

$$pH = pk - \log_{10} \left( \frac{\Delta - \Delta_b}{\Delta_a - \Delta_b} \right)$$

where $pk = 6.79$, $\Delta_a = 3.25$, $\Delta_b = 5.75$, and $\Delta$ is the chem-
then showed a transient increase after reperfusion (ie, over-

A transient increase of Pi was significantly lower (84±7) in the TAK-044 group than that in the control group (111±5). After reperfusion, Pi was significantly lower (82±3%) in the TAK-044 group than in the control group, and in the TAK-044 in combination with temocaprilat group, it was significantly higher (86±3%) during the same period after reperfusion than in the other 3 groups.

Experimental Protocol

The hearts were divided into the following 4 groups: (1) control group with no administration of either TAK-044 (Takeda Chemical Industries, Ltd, Japan) or temocaprilat (Sankyo Co, Tokyo, Japan) throughout all experiments (n=7), (2) TAK-044 group with TAK-044-containing solution (10–6 mol/L) alone for all experimental time periods from 40 min prior to the global ischemia (n=7), (3) temocaprilat group with temocaprilat-containing solution (10–6 mol/L) alone for all experimental time periods from 40 min prior to the global ischemia (n=7), and (4) TAK-044 plus temocaprilat group with TAK-044-containing solution (10–6 mol/L) in combination with temocaprilat-containing solution (10–6 mol/L) for all experimental time periods from 40 min prior to the global ischemia (n=7). After a 30-min warm-up period, we successively performed 10 min of basal perfusion, 15 min of continuous normothermic global ischemia (caused by clamping the perfusion line) and 65 min of posts ischemic reperfusion (caused by opening the perfusion line). LV pressure and coronary flow were measured at 5-min intervals, and the NMR spectra were recorded continuously.

Statistical Analysis

Statistical analysis was performed by ANOVA with Tukey’s post hoc test. All values were expressed as mean±SEM, and p<0.05 was considered significant.

Results

Myocardial Metabolism During Ischemia and Reperfusion

Pi (Fig 1) Pi increased to 441±37% during the 10–15 min period of global ischemia in the control group. After reperfusion, Pi was significantly lower (84±7) in the TAK-044 group than that in the control group (111±5).

CrP (Fig 2) CrP decreased to 5±1% during the 10–15 min period of global ischemia in the control group, and then showed a transient increase after reperfusion (ie, over-

Coronary Flow During Ischemia and Reperfusion

Coronary flow was maintained about 4.5 ml min−1 g−1 wet weight during basal perfusion. After reperfusion, coronary flow showed a small rebound increase (ie, hyperemia: 5.0 ml min−1 g−1 wet weight) in all 4 groups. There were no significant differences in coronary flow during basal perfusion, global ischemia and reperfusion among the 4 groups.

Discussion

In the present study, global ischemia decreased LVDev.P, ATP, CrP, and pH while it increased Pi. These parameters recovered to pres ischemic values approximately 60 min after the start of reperfusion with the exception of ATP, which did not recover sufficiently in the control group.

The decrease in ATP and the fall in pH during ischemia were significantly reduced by temocaprilat, and TAK-044 in combination with temocaprilat. During reperfusion, early recovery of ATP was observed in the TAK-044 group and better restoration of ATP was noted for TAK-044 in combination with temocaprilat. The inhibition of CrP overshoot was seen in the TAK-044 group, temocaprilat group and especially in the TAK-044 in combination with temo-
caprilat group. These findings show that temocaprilat had a protective effect on myocardial metabolism only during ischemia, and TAK-044 had a beneficial effect on metabolism during reperfusion only. However, TAK-044 in combination with temocaprilat had a potentiation on metabolism during both ischemia and reperfusion.

On the other hand, myocardial contraction did not show any differences during ischemia and reperfusion among the 4 groups in spite of the beneficial effect on myocardial energy metabolism. Hoffmeister et al reported that an experimental promotion of the recovery of the ATP content in the myocardium did not produce any improvement in contractility. It seems there may be more of a delay in the recovery of contraction than in the ATP content. Myocardial relaxation and coronary flow also showed no significant differences during ischemia and reperfusion among the 4 groups. Generally speaking, the clinical benefit of TAK-044 and temocaprilat is a vasodilator effect, but in the present experiment, these agents did not show this effect against the coronary flow of the perfused rabbit heart.

ET-1 is an extremely potent vasoconstrictor peptide derived from vascular endothelial cells. ET-1 can also be produced by other cell types such as smooth cells and cardiomyocytes. During myocardial ischemia and reperfusion, the production and release of myocardial ET-1 is stimulated and plasma ET-1 levels are increased, which suggests that plasma ET-1 levels may be related to the extent of the ischemia-reperfusion injury. The mechanism of action of the ET receptor antagonist on myocardial ischemia and reperfusion has not yet been fully elucidated and possible mechanisms include prevention of the no-reflow phenomenon, inhibition of ET-induced neutrophil activation, interruption of the interference of ET with the renin-angiotensin system, inhibition of the release of intracellular calcium, membrane stabilization, anti-oxidative effects or that interaction of oxygen-derived free radicals. Activation of ET-1 receptors on myocytes may trigger phospholipase C activity, resulting in hydrolysis of inositol phosphates and the subsequent release of intracellular calcium, which leads to an increase in the susceptibility of the myocardium to ischemia-reperfusion injury.

ET-1 is activated during ischemia and reperfusion, so the Ca2+ content of the myocyte and mitochondria increases, resulting in inhibition of ATP production. In addition, ET-1 may promote mitochondrial Ca2+ accumulation in the presence of intracellular Ca2+ overload and may inhibit both oxidative phosphorylation and ATP production. Accordingly, oxidative phosphorylation and ATP production may be increased by inhibiting the ET-1 effect during ischemia and reperfusion.

Moreover, ET-1 interacts with the renin-angiotensin system. Thus, synergistic actions between ET-1 and angiotensin II seem to exist at various levels in the regulation of the cardiovascular system. It has been shown that ET-1 augments the production of angiotensin II, and angiotensin II stimulates the synthesis of ET-1. Because angiotensin II seems to contribute to the ischemia-reperfusion injury, as suggested by the beneficial effects of angiotensin II receptor antagonists, there is the possibility that part of the effect of ET-1 on the ischemia-reperfusion injury is mediated through interference with the renin-angiotensin system.

On the other hand, it has been reported that ACE inhibitors reduce ischemic myocardial injury. ACE inhibitors have a variety of effects on the ischemic myocardium: (1) increasing bradykinin, (2) stimulation of nitric oxide formation, (3) enhancing prostacyclin production, and (4) reduction of catecholamines. In the present study, the ACE inhibitor, temocaprilat, had a cardioprotective effect and, furthermore, the ACE inhibitor in combination with the ETA/ETB receptor antagonist, TAK-044, had a marked cardioprotective effect during ischemia and reperfusion, which suggests that the renin-angiotensin system may be involved in the development of myocardial ischemia and reperfusion injury due to ET-1.

TAK-044 in combination with temocaprilat had a beneficial potentiation in protecting the myocardium from ischemic injury due to abnormalities of energy metabolism that occur during ischemia and subsequent reperfusion, which suggests future clinical applications.

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References

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