OBJECTIVES We examined whether the combination of an angiotensin-converting enzyme (ACE) inhibitor and an angiotensin II receptor blocker (ARB) synergistically mediates coronary vasodilation and improves myocardial metabolic and contractile dysfunction in ischemic hearts.

BACKGROUND Either an ACE inhibitor or ARB mediates coronary vasodilation in ischemic hearts.

METHODS In dogs with myocardial ischemia, we infused an ACE inhibitor (temocaprilat, 10 μg/kg/min) or ARB (RNH-6270, 10 μg/kg/min) into the coronary artery.

RESULTS Perfusion pressure of the left anterior descending coronary artery was reduced from 104 ± 8 to 42 ± 2 mm Hg, so that coronary blood flow (CBF) decreased to one-third of the baseline value. Ten minutes after starting the infusion of temocaprilat, the cardiac bradykinin level increased (from 32 ± 6 to 98 ± 5 pg/ml). Coronary blood flow (29 ± 2 to 44 ± 3 ml/100 g/min) and the cardiac level of nitric oxide (NO) (7.8 ± 1.9 to 17.5 ± 3.2 μmol) also increased, with these changes being attenuated by either N^G-nitro-L-arginine methyl ester or HOE140. RNH-6270 alone caused a modest increase in CBF (34 ± 3 ml/100 g/min), with no increase in the cardiac NO or bradykinin levels. Both temocaprilat and RNH-6270 caused a further increase in both CBF (51 ± 4 ml/100 g/min) and cardiac NO levels, without increasing the bradykinin level, and these changes were inhibited by HOE140. In the nonischemic heart, RNH-6270 augmented bradykinin-induced increases in CBF.

CONCLUSIONS The combination of an ACE inhibitor and ARB mediates greater increases in CBF and more potent cardioprotective effects through bradykinin-dependent mechanisms than either drug alone. (J Am Coll Cardiol 2002;40:162–6) © 2002 by the American College of Cardiology Foundation

Angiotensin–converting enzyme (ACE) produces angiotensin II (1), a potent coronary vasoconstrictor. The ACE inhibitors are reported to inhibit the degradation of bradykinin by inhibiting kinase II (2,3). Bradykinin generates nitric oxide (NO) through B2 receptor activation (4,5), which increases coronary blood flow (CBF) through B1 receptor activation (6,7), both of which contribute to an increase in CBF (8). Accordingly, the concomitant administration of an ACE inhibitor and angiotensin II receptor blocker (ARB) may cause further increases in CBF. However, the infarct size-limiting effect of both ACE inhibitors and ARBs is reported to be blunted by HOE140 (9), and the effects of ARBs are related to bradykinin (10), suggesting that concomitant administration of ACE inhibitors and ARBs may have overlapping effects on CBF in the ischemic myocardium. In contrast, the interaction between bradykinin and angiotensin II receptors was reported recently, which involved heterodimerization with the activation of Gαq and Gαi proteins (11). This suggests that antagonizing angiotensin II release may increase the sensitivity of bradykinin receptors. If this is the case, ARBs would potentiate ACE inhibitor- or bradykinin-induced coronary vasodilation. At present, it is not clear whether the coronary effects of either ACE inhibitors or ARB are identical or different, and both drugs cause a further increase in CBF in ischemic hearts.

To answer these questions, we tested the effects of an ACE inhibitor, ARB or combined therapy on CBF in the ischemic myocardium.

METHODS

Instrumentation. Twenty-nine beagle dogs weighing 11 to 15 kg were used. Each animal was anesthetized with pentobarbital sodium (30 mg/kg intravenously). The methods for the preparation of the experiments were previously described (2).
Experimental protocols. PROTOCOL I: EFFECTS OF TEMOCAPRILAT OR RNH-6270, OR BOTH, ON MYOCARDIAL ISCHEMIA PRODUCED BY CORONARY HYPOPERFUSION (N = 24). Coronary arterial and venous blood was sampled for blood gas analysis and for determination of the levels of lactate, plasma NO metabolites (i.e., nitrate and nitrite) and bradykinin, as well as plasma ACE activity. After the dogs’ hemodynamics became stable, we infused either saline (n = 7, group A), HOE140 (n = 7, group B) or Nω-nitro-L-arginine methyl ester (L-NAME) (n = 7, group C). Five minutes after starting the infusion, the coronary perfusion pressure (CPP) was reduced by using an occluder attached to the extracorporeal bypass tube, so that CBF was decreased to one-third of its control value. After low CPP was reached, the occluder was adjusted precisely to keep the CPP constant at this level. We have confirmed that 5 min is required to obtain a stable state in the hypoperfused myocardium. Once these measurements were obtained at 5 min, temocaprilat (10 μg/kg/min; Sankyo K.K., Tokyo, Japan) was infused into the left anterior descending coronary artery (LAD), and all hemodynamic and metabolic parameters were measured again after 10 min. The coronary hemodynamic and metabolic parameters, as well regional myocardial contraction, were studied at 10 min after starting the infusion of temocaprilat or RNH-6270. After recording all of the data, we discontinued the infusion of all drugs and the occluder was released for 10 min. After confirming that all of the hemodynamic and metabolic parameters had returned to baseline levels, we again reduced the CPP and decreased CBF to one-third of baseline. After 5 min, RNH-6270 (10 μg/kg/min; Sankyo K.K.) was infused for 10 min, and the protocol described earlier was repeated. Finally, after discontinuation of drugs and release of the occluder for 10 min, we again reduced the CPP and infused RNH-6270 plus temocaprilat (both at 10 μg/kg/min) for 10 min. The dose of temocaprilat or RNH-6270 was determined as the minimal dose that caused maximal vasodilation.

PROTOCOL II: EFFECT OF TEMOCAPRILAT OR RNH-6270 ON BRADYKININ-INDUCED CORONARY VASODILATION (N = 5). We infused saline, one of three doses of RNH-6270 (3.3, 6.7 or 10 μg/kg/min) or one dose of temocaprilat (10 μg/kg/min). In each group, we measured CBF and CPP during the infusion of three doses of bradykinin (5, 10 and 20 μg/kg/min).

Assays. Lactate levels were measured by an enzymatic assay, and the lactate extraction ratio (LER) was calculated as the coronary arteriovenous difference of the lactate concentration multiplied by 100 and divided by the arterial lactate concentration. The method used for measurement of bradykinin has been described previously (12). The total amount of plasma NO metabolites (i.e., nitrate and nitrite) was analyzed by an automated procedure based on the Griess reaction (13). The differences of the nitrate and nitrite concentrations between coronary venous and arterial blood were used to quantify the cardiac NO level. The method used for measurement of plasma ACE activity has been described previously (14).

Statistical analysis. Statistical analysis was performed with repeated measures analysis of variance, followed by the modified Bonferroni multiple comparison (15,16). All results are expressed as the mean value ± SEM, and a value of p < 0.05 was considered significant.

RESULTS

The mean blood pressure (102 ± 2 mm Hg) and heart rate (142 ± 2 beats/min) did not differ significantly between the groups (p = 0.76) or before and during coronary hypoperfusion with or without pharmacologic intervention. RNH-6270 increased CBF, but temocaprilat increased CBF (p < 0.01) more. Both RNH-6270 and temocaprilat further increased CBF (p < 0.01). The increase in CBF due to temocaprilat, but not RNH-6270, was blunted by either HOE140 or L-NAME (p < 0.01). Interestingly, the synergistic increase in CBF caused by RNH-6270 plus temocaprilat was blunted by either HOE140 or L-NAME (p < 0.01). RNH-6270 and temocaprilat increased the percent CBF to ~40% and 10%, respectively, which predicted that administration of both agents could cause an increase of ~50% in CBF. In fact, the combination of RNH-6270 and temocaprilat caused a 70% increase in CBF. The severity of myocardial ischemia, based on the changes of regional fractional shortening and LER, was parallel with the changes in CBF (data not shown).

Temocaprilat caused a marked decrease in plasma ACE activity in coronary venous blood (from 5.9 ± 0.7 to 0.12 ± 0.04 IU/l) during coronary hypoperfusion (p < 0.01). In contrast, RNH-6270 did not decrease the plasma ACE activity (6.3 ± 0.5 IU/l), and the combination of temocaprilat plus RNH-6270 did not cause a further decrease in ACE activity (0.15 ± 0.06 IU/l).

Figures 2 and 3 show the cardiac levels of NO and bradykinin. The cardiac NO level was increased by temocaprilat (p < 0.01) but not by RNH-6270. Interestingly, both RNH-6270 and temocaprilat caused a further increase
in cardiac NO levels ($p < 0.01$). Because the CBF response was identical to the changes in the cardiac NO level and L-NAME blunted ($p < 0.01$) the synergistic increase in CBF in the RNH-6270 plus temocaprilat group, bradykinin may have been increased in this group, as compared with its level in the temocaprilat group. However, the bradykinin level seen after administration of temocaprilat was not enhanced in the RNH-6270 plus temocaprilat group, al-

**Figure 1.** Coronary blood flow (CBF) before the reduction of coronary perfusion pressure (C) and during myocardial ischemia with (I + Drug) or without (I) RNH-6270, temocaprilat or both. The results were obtained with saline (left), HOE140 (middle) or N-$\text{NO}_2$-nitro-L-arginine methyl ester (L-NAME) (right) infusion.

**Figure 2.** Nitric oxide (NOx) (cardiac NO$_2^- +$ NO$_3^-$) level before the reduction of coronary perfusion pressure (C) and during myocardial ischemia with (I + Drug) or without (I) RNH-6270, temocaprilat or both. The results were obtained with saline (left), HOE140 (middle) or N-$\text{NO}_2$-nitro-L-arginine methyl ester (L-NAME) (right) infusion.
though the enhancement of CBF in this group was completely blocked by HOE140 ($p<0.01$). To explain this discrepancy, we hypothesized that RNH-6270 may enhance the CBF response to bradykinin produced by temocaprilat. When we tested this hypothesis, we found that bradykinin-induced coronary vasodilation was enhanced by RNH-6270 but not by temocaprilat (Fig. 4).

**DISCUSSION**

Augmentation of bradykinin-induced coronary vasodilation by ARB. We showed that ARB could enhance ACE inhibitor-induced NO production and cause increases in CBF in the ischemic myocardium. We first thought that the addition of an ARB might cause a slight increase in the cardiac bradykinin level and thus raise the NO level. Indeed, Tsutsumi et al. (10) observed bradykinin-induced increases in NO and cyclic guanosine monophosphate in the aorta of mice. However, we showed that an ARB plus ACE inhibitor did not increase cardiac bradykinin levels. This result may seem to be contradictory, but it can be explained as follows. First, the species, target tissue and experimental conditions were different between the Tsutsumi et al. (10) study and the present study. Tsutsumi et al. (10) administered an ARB to the isolated aorta of mice, whereas we infused it into the coronary artery of dogs with ischemic myocardium. Also, because we observed that an ARB can enhance bradykinin-induced increases in CBF, if bradykinin was present in the experiment of Tsutsumi et al. (10), the present result predicts that an ARB increases cyclic guanosine monophosphate levels even in the isolated aorta.

In ischemic heart, cardiac NO production was partially attenuated by either $l$-NAME or HOE140, suggesting that bradykinin contributes to the production of NO due to ischemic stress. Furthermore, cardiac bradykinin levels were augmented by HOE140 in ischemic hearts. This suggests that the blockade or stimulation of bradykinin receptors augments or reduces cardiac bradykinin production, respectively.

Although the cellular mechanisms remain unclear, this is the first evidence, to the best of our knowledge, that ARB infusion enhances bradykinin-induced coronary vasodilation and that it increases ACE inhibitor-induced bradykinin-mediated coronary vasodilation.

**Study limitations.** The major assumption in all of the experimental protocols used in the present study was that intracoronary infusion of agents such as temocaprilat, RNH-6270, $l$-NAME and HOE-140 did not have any effect on the peripheral vessels and that the observed changes in the LAD territory also were only due to a local effect on the coronary vasculature. If our pharmacologic interventions in the LAD territory also had an influence on systemic hemodynamics, the beneficial effects of temocap-
rilat or RNH-6270 may have been secondary to systemic vascular effects, such as afterload reduction. However, in the preliminary study, we observed that systemic vascular resistance is not altered by temocaprilat or RNH-6270 in ischemic hearts (data not shown).

**REFERENCES**

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