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Captopril and L-Arginine Have a Synergistic Cardioprotective Effect in Ischemic-Reperfusion Injury in the Isolated Rat Heart

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Objectives: The present study was designed to evaluate the possible effect of the combined administration of both captopril (Cap) and L-arginine (L-a) in the isolated ischemic rat heart model.

Background: Recent studies suggest that L-arginine and angiotensin-converting enzyme (ACE) inhibitors possess independent cardioprotective effects in ischemic hearts. The pharmacological effect of the combination of both drugs has not yet been investigated in the ischemic myocardium.

Methods: Using the modified Langendorf model, rats were perfused with either Cap 360 μmol/L (n = 6) or (L-a) 3mmol/L (n = 6), both captopril and L-arginine (Cap+L-a) (n = 8), or saline control (Con) (n = 8). The study design included 30 minutes of perfusion, 30 minutes of global ischemia, and 30 minutes of reperfusion thereafter.

Results: Hearts treated with both Cap+L-a demonstrated an improved performance in all parameters. After 10 minutes of reperfusion, the P_{max} in the Cap+L-a group was 98 ± 8 mmHg ($P < .001$), 59 ± 14 mmHg in the Cap group ($P < .02$), and 44.3 ± 10 mmHg in the L-a group (P = NS), compared with only 42 ± 8 mmHg in the control. After 10 minutes of reperfusion the dP/dt_{min} was: in the Cap+L-a group: −1,650 ± 223 mmHg/s ($P < .006$); in the Cap group: −1,051 ± 302 mmHg/s ($P < .03$); in the L-a group: −870 ± 131 mmHg/s (P = NS), compared with only −487 ± 131 mmHg/s in the control. Coronary flow was significantly increased in all 3 groups: Cap+L-a group: 22.3 ± 1.5 mL/min ($P < .001$); Cap group: 18 ± 1.6 mL/min ($P < .01$); L-a group: 19.8 ± 0.9 mL/min ($P < .02$), compared with 12.6 ± 0.9 mL/min in the Con group. Total NO level was significantly increased in the Cap+L-a group: 13.4 ± 2 μmol ($P < .03$) vs. 6.1 ± 1 μmol for the L-a group. NO levels of both the Cap group and the Con group were beneath detectable values.

Conclusion: Combined administration of captopril and L-arginine has a synergistic, protective effect on heart function and coronary flow that may be mediated by enhanced NO production.

Key Words: captopril, L-arginine, ischemia-reperfusion, isolated heart.

Recent studies suggest that captopril and L-arginine, each employed separately, have cardioprotective effects in ischemia-reperfusion injury (1,2). Angiotensin-converting enzyme (ACE) inhibitors are beneficial in severe heart failure, because they reduce mortality and improve ventricular (LV) function (3). They were shown to ameliorate the remodeling of the myocardium and to reduce infarct size in animal models of experimental myocardial infarction (4). This observation is supported by clinical studies demonstrating the favorable effects of ACE inhibitors following myocardial in-

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farction (5). Moreover, it has been reported that infarct expansion can be impeded and survival improved as a result of ACE inhibition (6). The optimal time for ACE-inhibitor administration after acute myocardial infarction remains controversial. Several experimental studies suggested that early intravenous administration of captopril after myocardial infarction may exacerbate myocardial injury and have a significant adverse effect on infarct size (7,8). Recently, Gurevitch et al. (9) showed that captopril administration of 360 μmol/L before and after induced ischemia in an isolated rat heart model resulted in improved coronary flow and hemodynamic performance during reperfusion. The above observations are probably mediated by several mechanisms, ascribed to ACE inhibitors (10–13). Similar results were obtained when a direct angiotensin II blocker (losartan) was used (14).

The effect of L-arginine administration in ischemia is more controversial (15). Maulik et al. (16) reported that administration of 3 mmol L-arginine before ischemia in a working isolated heart model reduced ischemia-reperfusion injury as evidenced by enhanced recovery of myocardial function. Administration of L-arginine can exert a beneficial effect on ischemic recovery principally by maintenance of nitric oxide (NO) production. This assumption is based on recent studies (17) showing that, while administration of L-arginine alone had a cardioprotective effect on the heart, the addition of NO inhibitors abolished this effect. In addition to its vasodilatory effect, NO can reduce ischemia-reperfusion injury by blocking the formation of hydroxyl (OH^-) radicals generated during the reperfusion of the ischemic myocardium (18). NO was also shown to increase cyclic adenosine monophosphate level during ischemia and result in improved myocardial performance (18). Koifman et al. (19) showed that in patients with chronic congestive heart failure (CHF), L-arginine increased NO levels and had a beneficial hemodynamic effect.

Conversely, other studies have suggested that NO accumulation at the initial phase of reperfusion may have a toxic effect. The latter is a result of free radical generation from NO that interferes with mitochondrial oxidation processes (20,21).

The combined effect of captopril and L-arginine was previously investigated by Van Gilst et al. (22), who showed that administration of both agents in an isolated heart model significantly increased coronary flow. The present study aimed to extend these findings by studying the combined effect of captopril and L-arginine on ischemic heart hemodynamic recovery, creatine kinase (CK), and NO levels.

Materials and Methods

Experimental Procedures

Male Wistar rats (300 to 400 g) were anesthetized by intraperitoneal injection of pentobarbital sodium (30 mg/kg body weight) and heparinized. Their hearts were rapidly excised, immersed in a cold saline solution with heparin, and mounted on a stainless-steel canula of modified Langendorf perfusion apparatus. Retrograde aortic perfusion was initiated at a perfusion pressure of 85 mmHg with oxygenated modified Krebs-Henseleit (KH) buffer solution of the following composition (mmol/L): NaCl 118, KCl 4.7, $CaCl_2$ 2.0, $MgSO_4$ $7H_2O$ 1.2, KH_2PO_4 1.2, glucose 11.1, and $NaHCO_3$ 25. The perfusate was bubbled continuously with 95% O_2 and 5% CO_2, maintaining a pH of 7.4–7.5.

The heart temperature was monitored by a transmitter implanted in the right ventricular wall, and the temperature was carefully maintained at 37°C by water-jacketing the reservoir and the isolated hearts. The right atrium was removed, and the heart was paced to 300 beats/min at 4 V with the use of an external pacemaker (Grass S-44 stimulator, Medical Instruments, Portland, OR), ensuring identical heart rates for all hearts. A water-filled latex balloon was placed in the left ventricular cavity through a small incision in the left atrium and was connected to a HP-1290C universal (Hewlett-Packard, Palo Alto, CA) pressure transducer. The balloon was tied and inflated to a volume that produced 5-mmHg diastolic pressure. Zero calibration of the pressure transducer was examined throughout the experiment. Global ischemia was induced by closing the perfusion to the heart.

Hemodynamic Measurements

Left ventricular peak systolic pressure (P_{max}), the first derivative of the rise (dP/dt_{max}) and fall in left ventricular pressure (dP/dt_{min}), the area calculated under the left ventricular–developed pressure curve (pressure-time integral) that correlates with oxygen consumption, were obtained. These variables were continuously recorded, and measurements were taken at 10 minute intervals.

Protocol

Twenty-eight rats were randomly divided into 4 groups: isolated hearts receiving KH solution

(group 1, n = 8) served as control; isolated hearts receiving 360 μmol/L captopril in perfusate solution (group 2, n = 6); isolated hearts receiving 3 mmol/L L-arginine (group 3, n = 6); and hearts receiving both 360 μmol/L captopril and 3 mmol/L-arginine (group 4, n = 8).

Preischemic measurements were recorded after a 30-minute period of stabilization, and thereafter, the hearts underwent a 30-minute period of global ischemia at 31°C. CK activity was measured spectrophotometrically in the effluent at the first minute of reperfusion. Thereafter measurements of LV function were taken at 10-minute intervals. Throughout the study, experiments were alternated between the control group and the experimental limbs to minimize bias or differences in the results.

NO Measurements

The total amount of NO was measured in the effluent perfusate at the first minutes of reperfusion as previously described (23). NO_2 was determined with the Griess reaction after reduction of NO_3 with a nitrate reductase prepared in our laboratory from *Escherichia coli* incubated in anaerobic conditions with NO_3. The total level of NO_2^+ NO_3 in the perfusate is given in micromoles.

Ethics

All animals received humane care as described in *Principles of Laboratory Animal Care* formulated by The National Society for Medical Research and *Guide for the Care and Use of Laboratory Animals* prepared by the National Academy of Sciences, published by the National Institutes of Health (NIH publication 85-23, revised 1985).

Statistics

Results are presented as mean values ± SE. Two-way ANOVA with repeated measurements for drug and time effects was calculated for all variables. Because of the difference in effluent flow during the first postischemic minute, CK and NO values were normalized to units per flow. Differences between groups regarding the baseline values, CK, and NO levels were analyzed by the Student's paired or unpaired *t* test. Significance was established at the level of $P < .05$.

Results

Preischemic Period (Baseline)

During the first 30 minutes preceding ischemia, all experimental groups were found to maintain constant hemodynamic values. All 3 treated groups (captopril, L-arginine, and their combination) exhibited a significant improvement in comparison with the control group (Table 1). At the initial 30-minute baseline period (preischemia), coronary flow values were : 24.4 ± 2 mL/min in the captopril group ($P < .001$), 21.6 ± 2 mL/min in the L-arginine group ($P < .01$), and 23.5 ± 1.4 mL/min ($P < .001$) in the combination groups, as compared with control group values of 13.2 ± 1.4 mL/min. The hearts treated with captopril had a significant improvement in left ventricular systolic performance, with a P_{max} of 145 ± 14 mmHg vs. 104 ± 5.6 mmHg in the control ($P < .03$). Hearts receiving both captopril and L-arginine had an improvement in P_{max} values to 114 ± 5 mmHg ($P < .04$) in comparison with the control. dP/dt_{max} was significantly higher in the captopril group (4,045 ± 496 mmHg/s; $P < .05$) versus the control group (2,228 ± 136 mmHg/s; $P < .03$). The hearts receiving L-arginine had a significant improvement in dP/dt_{max} (3,960 ± 759 mmHg/s; $P < .03$) as compared with controls. With respect to the dP/dt_{min} and pressure-time integral, no significant differences were observed between the 4 groups at this stage of the study.

Ischemia and Reperfusion

Hemodynamic parameters. After 30 minutes of global ischemia, the hearts of all 4 groups demon-

Table 1. Baseline Measurements (Preischemia)

	Control	L-Arginine	Captopril	Combination
Animals weight (g)	359 ± 10.6	341 ± 9.3	353.2 ± 7	333 ± 5.3
P_{max} (mmHg)	104 ± 5.6	122.5 ± 15.5	145 ± 14.2*	114.7 ± 5*
dP/dt_{max} (mmHg/s)	2,228 ± 136	3,960 ± 759*	4,045 ± 496*	3,040 ± 502
dP/dt_{min} (mmHg/s)	−1,825 ± 123	−3,158 ± 642	−3,241 ± 248*	−2430 ± 212
Pressure-time integral ($mmHg/s^2$)	6.1 ± 0.3	6.6 ± 0.6	8.4 ± 0.8	6.7 ± 0.5
Coronary flow (mL/min)	13.2 ± 1.4	21.6 ± 2*	24.4 ± 2*	23.5 ± 1.4*

Note. Data are shown as mean ± SE.
* $P < .05$.

strated significant deterioration in left ventricular function. All postischemic measurements were better in hearts receiving both captopril and L-arginine (group 4) compared with the other groups, suggesting improved recovery. After 10 minutes of reperfusion, P_{max} was 93 ± 8 mmHg in the combination group and 42 ± 8 mmHg in the control ($P < .001$; Fig. 1). Values pf dP/dt_{max} and dP/dt_{min} at this stage were 2,068 ± 347 mmHg/s and −1,650 ± 223 mmHg/s for the combination group (4), compared with 684 ± 177 mmHg/s and −487 ± 131 mmHg/s for the control group, respectively ($P < .001$; Fig. 2) and ($P < .006$; Fig. 3). Pressure-time integrals were mildly increased in the combination-therapy group (5.6 ± 0.7 mmHg/s^2), compared with control (2.5 ± 0.3 mmHg/s^2; $P = .06$) (Fig. 4).

At the same stage (10 minutes of reperfusion), P_{max} was 59 ± 14 mmHg in the hearts receiving captopril and 42 ± 8 mmHg in the control ($P < .02$; Fig. 1), dP/dt_{max} was 1,275 ± 278 mmHg/s, and dP/dt_{min} was −1,051 ± 302 mmHg/s in the captopril group, compared with 684 ± 177 mmHg/s and −487 ± 131 mmHg/s in the control group ($P < .03$; Figs. 2 and 3, respectively). There were no significant differences in pressure-time integrals (3.38 ± 0.9 mmHg/s^2 vs 2.5 ± 0.3 mmHg/s^2) between these groups (Fig. 4).

Hearts subjected to L-arginine treatment had improved postischemic parameters in comparison with controls. After 10 minutes of reperfusion, P_{max} was significantly increased to 44.3 ± 10 mmHg ($P < .02$), whereas a statistically nonsignificant trend of improvement was achieved in other hemodynamic parameters: dP/dt_{max} of 1,026 ± 210 mmHg/s, dP/dt_{min} of −870 ± 249 mmHg/s, and pressure-time integral of 2.6 ± 4 mmHg/s^2.

No significant differences were observed in all hemodynamic parameters when comparing the groups that received L-arginine or captopril to the groups that received the combination therapy.

Coronary flow. Coronary flow was significantly increased during reperfusion in all 3 treated groups as compared with the control group. The most significant improvement was observed in the combination group. After 10 minutes of reperfusion, coro-

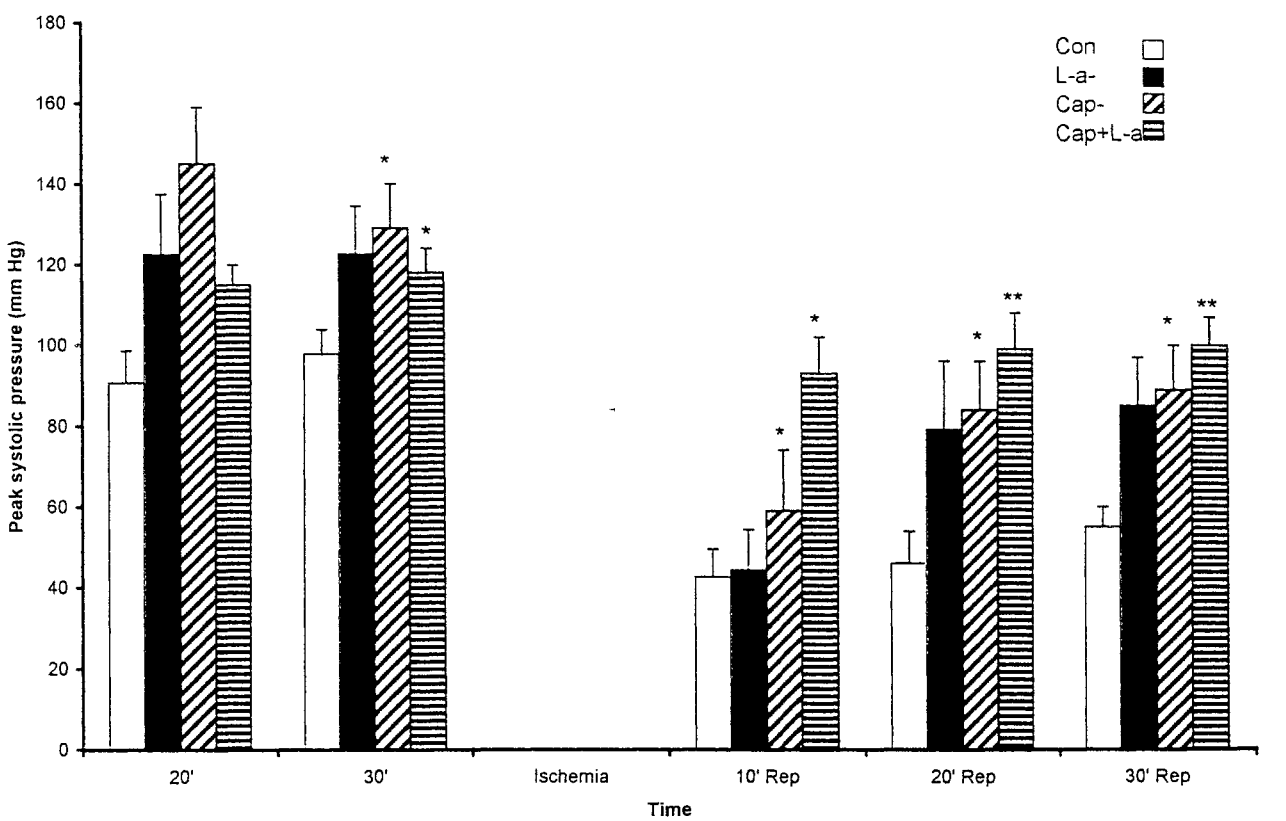

Fig. 1. Left ventricular peak systolic pressure before and after ischemia. Results are presented as mean ± SE. Before ischemia, the hearts receiving captopril (Cap; ▨) and the combination (Cap+L-a; ▤) had a better performance compared with control (Con; □). After ischemia, hearts from the combination group had the best left ventricular performance as compared with the control group or the L-arginine (L-a; ■) and captopril groups. *$P < .05$; **$P < .01$ compared with the control group.

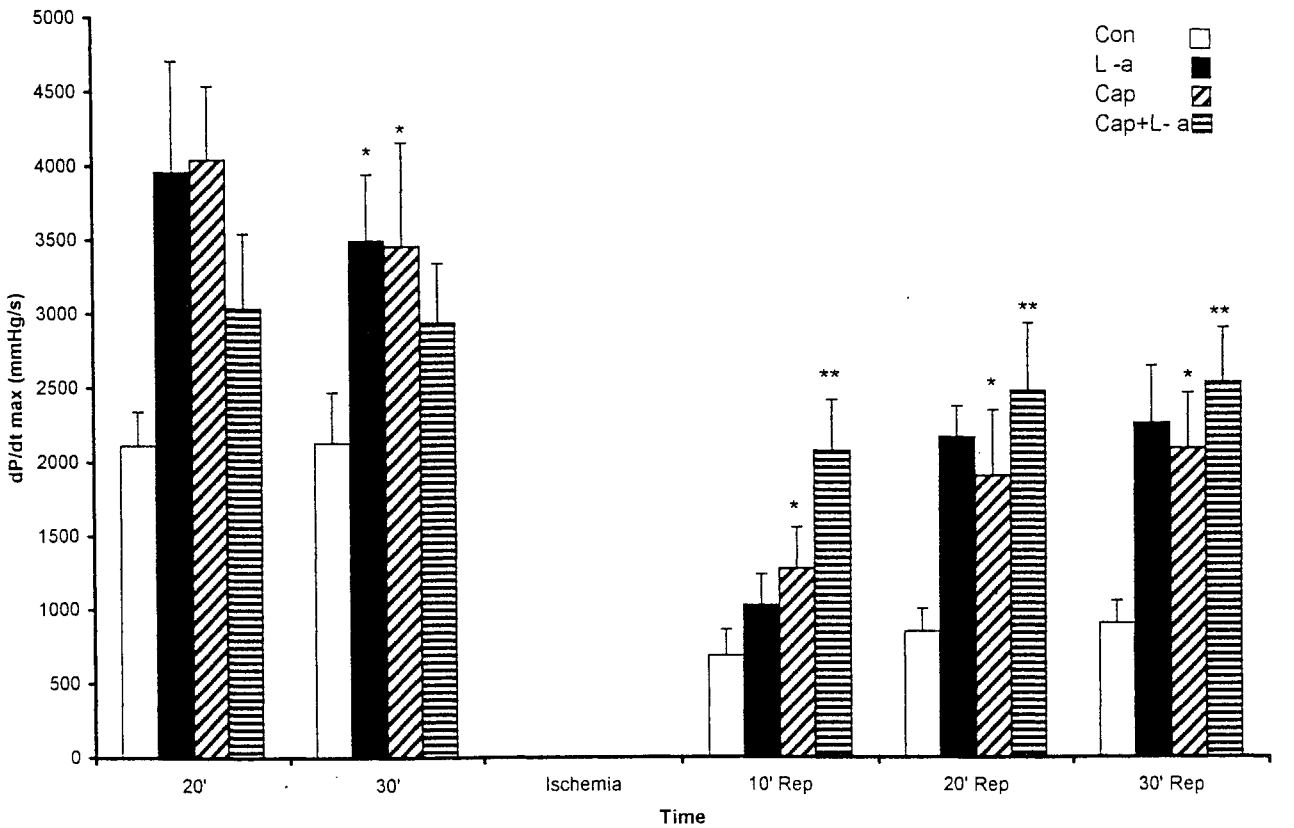

Fig. 2. dP/dt_{max} before and after ischemia. Result are presented as mean ± SE. Before ischemia, hearts receiving captopril (Cap; ▨) had an improved performance compared with the controls (Con; □). After ischemia, hearts treated with both captopril and L-arginine (Cap+L-a; ▤) had the best recovery of dP/dt values as compared with the control group and the L-arginine (L-a; ■) or the captopril group. *$P < .05$; **$P < .01$ compared with the control group.

nary flow measured in the hearts receiving both captopril and L-arginine was 22.3 ± 1.5 mL/min compared with only 12.6 ± 0.9 mL/min in the control group ($P < .001$; Fig. 5).

In the hearts receiving captopril, coronary flow was 18 ± 1.6 mL/min ($P < .01$ as compared with controls), whereas in hearts receiving L-arginine, coronary flow was 19.8 ± 0.9 mL/min ($P < .002$ as compared with controls).

No significant difference was observed comparing the groups that received L-arginine or captopril to the group that received the combination therapy.

CK Levels

The total amount of CK tended to be lower in all 3 treated groups in comparison with the control group (Table 2). In the control group, the total CK level in the effluent of the first minute of reperfusion was 3,971 ± 1,578 U, compared with 662 ± 131 U in the combination therapy ($P = .062$), 613 ± 129 U in the captopril group ($P = .06$), and 908 ± 96 U in the L-arginine group ($P = .064$).

NO Levels

The total amount of NO measured in the effluent of the first minute of reperfusion were significantly higher in the combination group (13.4 ± 2 μmol; Table 2) compared with the L-arginine group (6.1 ± 1 μmol; $P < .03$). In both the captopril and control groups, NO values were beneath the detectable threshold levels.

Discussion

In this study, we have shown that combined administration of captopril and L-arginine has a synergistic beneficial effect on the hemodynamic performance and coronary flow values after ischemia-reperfusion injury in the rat heart. Separate administration of captopril or L-arginine, however, had a limited cardioprotective coronary flow–enhancing effect.

The observation that ACE inhibitors have cardio-

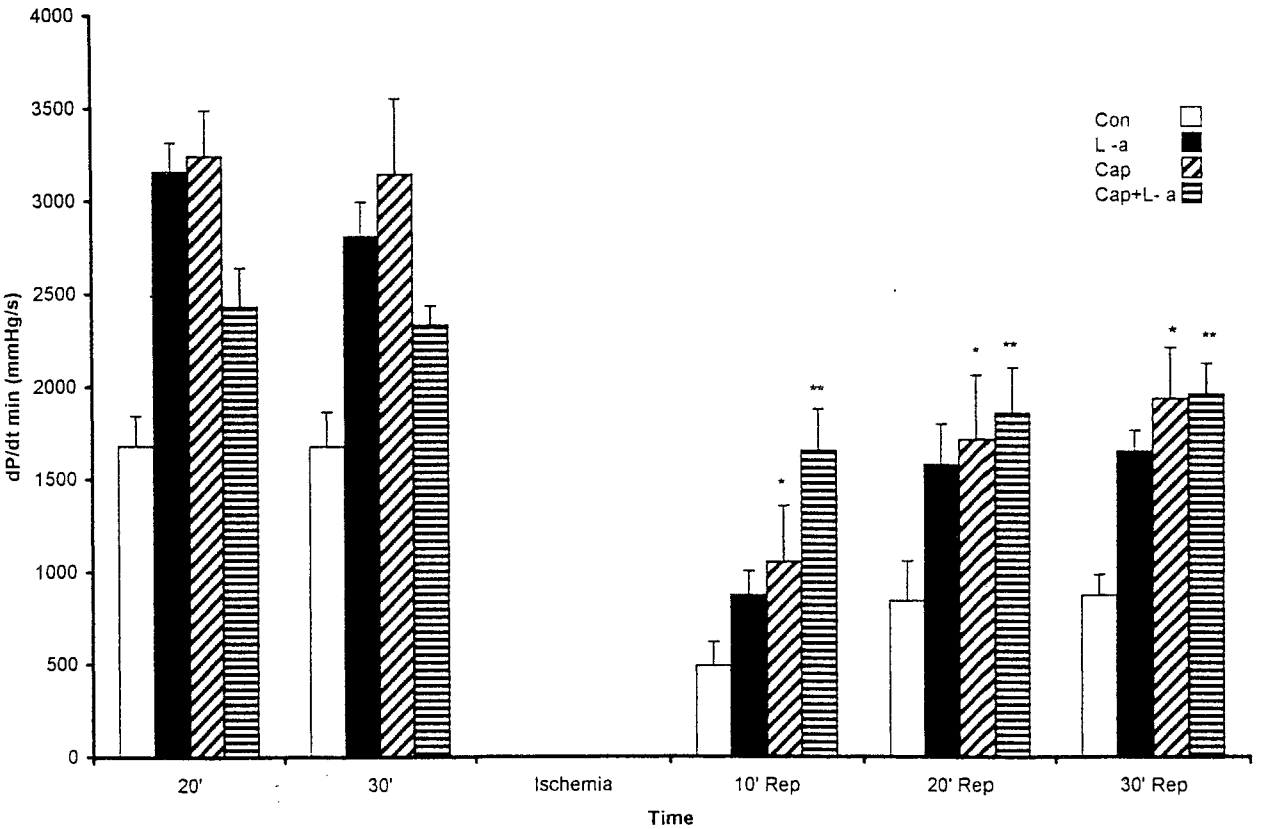

Fig. 3. dP/dt_{min} before and after ischemia. Result are presented as mean ± SE. Before ischemia, hearts receiving captopril (Cap; ▨) and L-arginine (L-a; ■) had an improved performance. But after ischemia, hearts treated with both captopril and L-arginine (Cap+L-a; ▤) had the best recovery of dP/dt_{min} as compared with the control group (Con; □) and the L-arginine or the captopril group. $*P < .05$; $**P < .01$ compared with the control group.

protective properties is well documented (24). One possible underlying effect entails improvement in coronary flow that may be mediated by 2 mechanisms: 1) an effect on myocardial autocrine function of the angiotensin system, resulting from local inhibition of the vasoconstrictive angiotensin II (25); and 2) potentiation of bradykinin, facilitated by the mimicry between ACE and the bradykinin degrading enzyme kinase-2 (12,26). Recent data attribute the increase in coronary flow to local release of endothelium-derived relaxing factor by captopril (27). Additional cardioprotective mechanisms of ACE inhibitors include: inhibition of sympathetic stimulation (angiotensin II being a potentiator of the response to sympathetic stimulation) (10) and a free radical–scavenging effect (the latter playing an important role in the pathogenesis of reperfusion damage) attributed to the sulfate group in captopril (10).

In the current study, we found that hearts receiving captopril improved hemodynamic performance after 30 minutes of initial perfusion. This result is somewhat unpredictable, because angiogensin II has been shown to possess positive inotropic effects, and captopril would have been expected to decrease myocardial contractility. In our study, the most noteworthy effect of the drug was the improvement in coronary flow, and only consequently, the enhancement of myocardial contractility. We also found that captopril improved hemodynamic performance and coronary flow during heart recovery, 30 minutes following the induction of global ischemia. This observation is in line with a previous report (9) that showed a cardioprotective effect employing similar drug dosages in an isolated rat heart model.

The effect of L-arginine on ischemic myocardium is more controversial. Maulik et al. (16) found that ischemia-reperfusion is followed by a decrease in NO content that can be reversed by pretreatment of the heart with L-arginine. In addition to its vasodilatory effect, NO can neutralize free radicals (29), and inhibit neutrophils (30,31) and platelet aggregation (32). In biological systems, NO is produced during a conversion of the amino acid, L-arginine, into L-cirtulline by the action of nitric oxide syn-

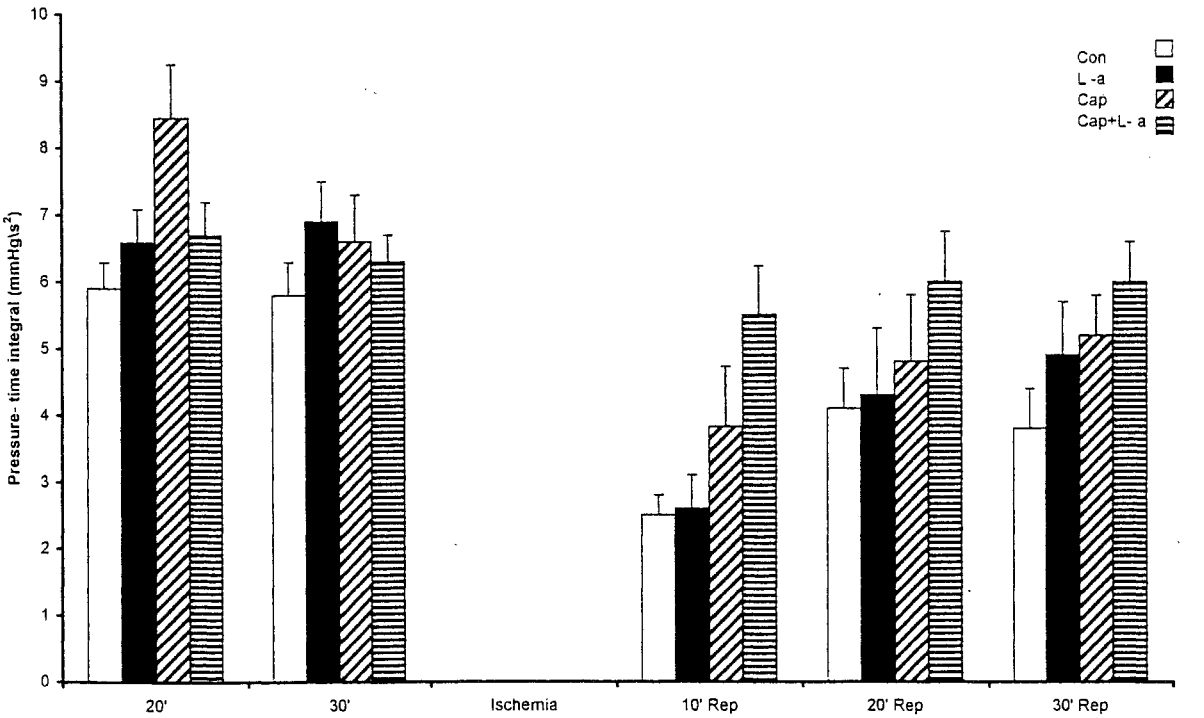

Fig. 4. Pressure-time integral before and after ischemia. Result are presented as mean ± SE. No significant improvement was observed in all treated groups before ischemia compared with the control. After ischemia, the combination group (Cap+L-a; ▤ b) had the best improvement (P = .06) compared with control (Con; □). L-Arginine (L-a; ■) and captopril (Cap; ▨) caused only a mild improvement.

thase (NOS). In the nonischemic myocardium, NO concentration could be maintained primarily by L-citrulline recycling to L-arginine. However, the conversion to L-arginine from L-citrulline requires adenosine triphosphate, and it is likely that the opposite cycle is inhibited or significantly reduced in energy-deprived ischemic myocardium.

Several studies indicate that L-arginine administration has a deleterious effect on the ischemic-reperfused heart (18,20). It has been shown that administration of NOS inhibitors such as N^{ω}-nitroso-L-arginine methyl ester may improve hemodynamic performance and reduce infarct size, an effect that was reversible upon addition of L-arginine. These contradicting effects may result from the fact that NO-free biological systems can rapidly react with superoxide anions (O^{-}) to form highly reactive peroxynitrite radicals with cytotoxic activity. Thus, when the concentrations of both O_2 and NO are significantly increased, ($ONOO^{-}$) may become the primary metabolite of NO. $ONOO^{-}$ is a highly reactive radical, which may account for its cytotoxic activity. Nevertheless, there is no direct evidence that this radical plays an active role in ischemia-reperfusion injury (33,34).

Further studies indicated that NO and its activated second messenger cyclic guanosine monophosphate (cGMP) may also play a role in ischemia-reperfusion injury. Toit et al. (18) found that administration of low doses of NO inhibitors improves heart recovery after ischemia without significantly influencing NO and peroxynitrite levels. Maulik et al. (16) reported that cGMP levels were increased during ischemia after administration of L-arginine. cGMP is a well-known second messenger that regulates calcium/calmodulin-dependent kinase. Antagonism of calmodulin was previously shown to result in reduced infarct size (35). In addition, ischemia-reperfusion was shown to induce phosphodiesteric breakdown and turnover of phosphinositides in the rat heart. Pretreatment with L-arginine significantly blocked phosphinositide loss, providing an alternative mechanistic insight for its action (16).

Our findings indicate that administration of L-arginine increased coronary flow, and as a result, enhanced cardiac contractility in the normal heart. However, following induced ischemia, despite the marked improvement in coronary flow, L-arginine administration caused only a partial recovery of heart function. We have also shown that L-arginine increases NO levels after ischemia, possibly ex-

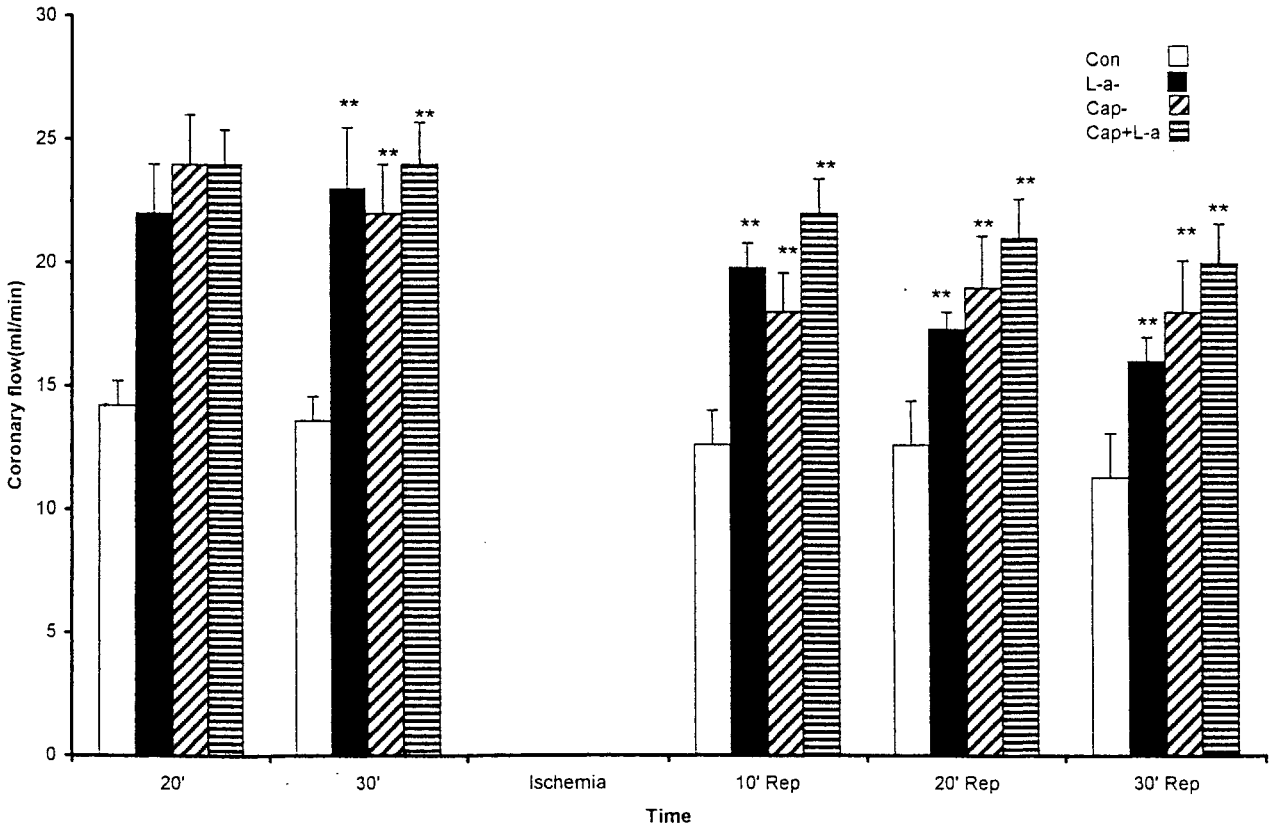

Fig. 5. Coronary flow before and after ischemia. Results are presented as mean ± SE. Before and after ischemia, all treated groups had a significant increase in coronary flow, L-arginine (L-a; ■), captopril (Cap; ▨), and the combination (Cap+L-a; ▤) compared with the control (Con; □). During reperfusion, at all stages, mean coronary flow was higher in the treated groups compared with control. The combination therapy tended to have the higher values. No statistically significant difference was found. $^{**}P < .01$ compared with the control group.

plaining the high rates of coronary flow during reperfusion.

The principal message of this study is that combined administration of captopril and L-arginine resulted in a nonsignificant trend toward an improved cardioprotective effect during reperfusion, in comparison with administration of each agent separately. The lack of effect on normal heart function of the combination therapy on one hand, and the improved recovery after ischemia on the other, indicates that better hemodynamic recovery is attributed to a direct involvement of both drugs in ischemic-reperfusion mechanisms.

The administration of both agents was previously tested only on a normal nonischemic heart. Van Gilst et al. (22) showed that both drugs given together improved coronary flow, and this effect was evident only when using sulfhydril-containing ACE inhibitors. Thus, they assumed that the presence of the sulfhydril moiety might potentiate the effect of the endogenous nitrovasodilator that is released as a result of an increase in bradykinin concentrations. But the effect of this reaction is probably limited, because high concentrations of L-arginine are already available. Another explanation for the combined effect of both drugs is based on the presence

Table 2. CK and NO Measurements After Ischemia

	Control	L-Arginine	Captopril	Combination
NO level (μmol · mL flow · min)	BDL	6.1 ± 1	BDL	13.4 ± 2*
CK level (U · mL flow · min)	3,971 ± 1,578	908 ± 96	613 ± 129	662 ± 131

Note. Data are shown as mean ± SE.
BDL, Beneath detectable level.
* $P < .05$.

of the sulfhydril group in the captopril molecules. Ignarro et al. (36) showed that an amino acid containing sulfhydril group had a vasodilatory effect similar to captopril, as a result of the fact that NO primarily activated gunanylate cyclase in the smooth muscle cells and in blood vessels. This activation is mediated by *S*-nitrosothiol and is dependent on the availability of a sulfhydril group. Captopril administration can improve the availability of the sulfhydril group and thus enhance L-arginine activity.

Our findings that NO concentrations are significantly increased following administration of both drugs, as compared with the administration of L-arginine alone, further support the possible effect of captopril in enhancing NO generation from L-arginine.

The most remarkable effect of both drugs was the improvement in left ventricular contractility. Because each of the agents has a significant beneficial effect on coronary flow, the slight improvement in coronary flow observed in the combination group probably did not account for the better hemodynamic recovery, which may emphasize the importance of the local effect of both drugs. The hemodynamic improvement following the combination therapy can be explained by radical-scavenging effects of captopril (reduced peroxynitrite), intensified by NO donation provided by L-arginine.

In summary, combined administration of captopril and L-arginine has a better cardioprotective effect on the recovery of the isolated rat heart after ischemia. These results call for additional experimental and clinical studies aiming at elucidating the protective effects of both drugs.

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