Although beneficial effects of angiotensin converting enzyme (ACE) inhibition have been demonstrated in ill (ischemic, failing) hearts, it has not been proved that ACE inhibition induces changes in healthy hearts. The question is of clinical relevance, as many hypertensive patients do not display cardiac damage at the onset of treatment with ACE inhibitors, and possible changes in cardiac work might turn out more or less advantageous in the development of hypertensive heart disease. In a refined working heart preparation allowing measurement of cardiac work, including the contribution of atrial work and paracrine cardiac regulation, effects of captopril on cardiac dynamics were assessed. Coronary overflow of bradykinin, norepinephrine, and lactate was measured. Hearts were perfused for 20 min with vehicle or captopril at $3 \times 10^{-7}$, $3 \times 10^{-6}$, and $3 \times 10^{-5}$ mol/L. At the highest concentration, captopril increased coronary flow. Extending previous studies, the present study demonstrates that, in a concentration-dependent manner, captopril decreased oxygen consumption and maximal left ventricular pressure although the bradykinin outflow was not affected. From these influences of the drug on cardiac work and metabolism in healthy hearts, a protective influence of captopril in acute, critical situations of cardiac malnourishment or cardiac overload may be derived. Am J Hypertens 1998;11:1290–1296 © 1998 American Journal of Hypertension, Ltd.

KEY WORDS: Angiotensin converting enzyme inhibitor, isolated working rat heart, cardiac function, bradykinin, norepinephrine.

Due to their reducing influence on vascular resistance and systemic blood pressure, angiotensin converting enzyme (ACE) inhibitors are presently widely prescribed to hypertensive patients, irrespective of the presence of heart damage. Although beneficial cardiac effects of ACE inhibition have been shown in hypertrophic and ischemic hearts, independent from the normalization of blood pressure, the effects of such a treatment on healthy, ie, normotensive and normoxemic, hearts have not been systematically examined, except for a captopril-dependent increase in coronary flow (CF). However, the hemodynamic effects of ACE inhibition in healthy hearts are of considerable clinical relevance, as, at the onset of antihypertensive therapy with ACE inhibitors, many hypertensive patients do not suffer from any heart damage, and the same type of drug effect that has been found to be beneficial in the diseased heart may not necessarily develop in healthy hearts.
ACE inhibition has been shown to influence the local renin-angiotensin system in various tissues. Two major mechanisms have been proposed to mediate the effects of ACE inhibition. It inhibits the synthesis of angiotensin II, a peptide that increases inotropy and chronotropy, thereby interacting with sympathetic activation.\(^\text{9}\) Over longer periods angiotensin II may act as a growth factor within myocardial tissue.\(^\text{10}\) Inhibition of angiotensin II synthesis is accompanied by accumulation of angiotensin fragments, which per se have dilatory effects on the coronary arteries.\(^\text{11}\)

The second major mechanism of ACE inhibition is in reducing the degradation of bradykinin (BK), which, in ischemic and hypertrophic hearts, has been shown to enhance coronary flow and inotropy, and to reduce the degradation of energy-rich phosphates by improving endothelial functions.\(^\text{1,12}\) BK induces the release of potent vasoactive modulators such as endothelium-derived relaxing factor (or NO), prostaglandins, and endothelium-derived hyperpolarizing factor.\(^\text{13,14}\)

Considering the heterogeneous processes triggered by ACE inhibition, its resultant influence on cardiac work and metabolism in healthy hearts is difficult to predict. With respect to this issue, results from previous studies examining the effects of ACE inhibition in isolated ischemic and hypertrophic hearts remain inconclusive.\(^\text{1,15}\) Moreover, besides using ill hearts, those studies employed the Langendorff or isolated working heart technique adopted from Bardenheuer and Schrader.\(^\text{16}\) In this preparation contributions of mechanic and also paracrine influences of the atria to cardiac dynamics cannot be assessed. However, atrial activity seems of particular relevance if the role of the local renin-angiotensin system is examined for normal cardiac functioning, because the number of angiotensin receptors is far greater in the atria than in the ventricles.\(^\text{17}\) The present study adopted an improved working heart technique, which preserved the regular anterograde perfusion of atria and ventricles, and consequently also the contribution of atrial work to cardiac dynamics. The direct flow of the perfusion medium through the heart allowed a paracrine hormonal response to local ACE inhibition not only in the coronary and right ventricle, but also in the left atrium and ventricle.

Here, this refined working heart preparation was used to assess the effects of the ACE inhibitor captopril on cardiac function, including oxygen consumption of isolated, nonischemic, nonhypertensive rat hearts, which were perfused with the ACE inhibitor at different pharmacologic concentrations. To differentiate effects mediated by changes in the endogenous release of BK and norepinephrine (NE), overflow of these substances into the coronary effluvate was measured.

**MATERIALS AND METHODS**

**Animals, Surgery, and Apparatus**

Forty-two unrestrained male Wistar rats (weight, 175 to 250 g) were anesthetized with pentobarbital (6 mg/100 g, intraperitoneal [ip]), and heparin (600 i.E./100 g, ip) was injected. The heart was removed during artificial respiration and mounted on a Langendorff perfusion apparatus, switchable to a standard working heart arrangement. The hearts were perfused with modified Krebs-Henseleit solution (in mmol/L: NaCl 130, KCl 4.7, CaCl\(_2\) 2.4, MgSO\(_4\) 1.6, NaHCO\(_3\) 25, KH\(_2\)PO\(_4\) 1.2, glucose 11.1; and insulin 8 IU/L) equilibrated with 95% O\(_2\) and 5% CO\(_2\) at 37°C, so that pH was 7.4, and osmolarity 285 mOsm. The solution was filtered through a Millipore (Nolsheim, France) filter (1.2 μm pore diameter). The venae cavae and pulmonales were ligated. The left auricle was cannulated by a polyethylene catheter (diameter, 1.2 mm) to allow anterograde perfusion. The pulmonary artery was cannulated to sample coronary flow. Then, retrograde perfusion was switched to anterograde perfusion. In this working heart modification the pressure of the left atrium was held constant at 7.5 mm Hg by a PC-controlled peristaltic pump (Ismatec SA, Zurich, Switzerland), regulating the flow of the Krebs-Henseleit solution into the left atrium. The intrathoracic pressure was registered by a Statham P 23 Db (Hellige, Freiburg, Germany). The afterload was set to 60 mm Hg. The left ventricle was cannulated to measure the left ventricular pressure continuously (by a Statham P 23 Db, Hellige). \(P_{\text{max}}\), \(P_{\text{mean}}\), and heart frequency were recorded by a digital electromanometer (type 330, Hugo Sachs Elektronik, Freiburg, Germany); \(dP/dt_{\text{max}}\) was measured by a differentiator (Hugo Sachs Elektronik). Aortic flow and coronary flow (CF) were measured by electromagnetic flowmeters (Hellige), and cardiac output (CO) was recorded. Clark oxygen microelectrodes (Diamond, Ann Arbor, MI) in closed, temperature-controlled chambers were used to continuously measure the pO\(_2\) of the coronary effluvate, ie, the arteriovenous difference of the perfusion solution before and after passage through the isolated heart. Oxygen extraction (supply) and oxygen consumption were calculated as described by Neely et al.\(^\text{18}\) All hemodynamic parameters were registered continuously. To ensure normal physiologic cardiac functioning, hearts displaying initial heart rates of less than 200 beats/min or a cardiac output of less than 15 mL/min were excluded. The protocol was accepted by the Ethical Committee for animals of the Land Schleswig Holstein.

**Experimental Design**

The rats were randomly assigned to five groups. Twenty minutes after the beginning of recordings the hearts were perfused either with vehicle (placebo, \(n = 8\)) or with captopril (mo-
lecular weight, 271 g/L, Squibb-Heyden, München, Germany) at four different concentrations (final concentrations: $3 \times 10^{-8}$, $n = 6$; $3 \times 10^{-7}$, $n = 7$; $3 \times 10^{-6}$, $n = 7$; $3 \times 10^{-5}$, $n = 8$) for 20 min. After another 20-min period of washout the recordings were stopped. The wet heart weight was determined. Captopril was added to the Krebs-Henseleit solution right before entry into the left atrium. The 10-min interval preceding the perfusion with, respectively, captopril or vehicle, was used as baseline.

**Measurement of Bradykinin and Norepinephrine Overflow Into the Coronary Perfusate** For BK measurement CF was pooled over subsequent 10-min intervals (baseline, drug perfusion phase 1, drug perfusion phase 2, washout phase 1, washout phase 2). Then, 10 mL of each sample was mixed with 0.65 mL of an inhibitor solution (containing 10 mL aprotinin, 100 mL hexadimethrine bromide [0.61 g/100 mL], 0.1 mol/L EDTA, and 20 mL 5% albumin and 20% hydrochloric acid). After heat enzyme degradation (10 min at 95°C) samples were stored at −20°C. Samples were concentrated by solid phase extraction (phenyl-silica column) and lyophilization. Samples were reconstituted with radioimmunoassay buffer, and incubated (24–36 h) with rabbit antibradykinin serum and 125I-Tyr3-bradykinin. After charcoal adsorption and centrifugation of the tubes (3000 rpm for 15 min at 4°C), the supernatants were aspirated and the pellets were counted in a γ-counter. The minimal detection limit of BK detection was 0.3 fmol/mL.

For NE measurement CF was pooled during the baseline (10 min), drug administration (20 min), and washout (20 min). We filled iced test tubes containing EDTA (10 mmol/L final concentration) with 10 mL of each sample. The tubes were stored at −20°C. NE was assayed by reverse-phase HPLC with electrochemical detection. The sensitivity of NE detection was 0.25 pg/mL.

**Data Analysis** For statistical evaluation, measures of cardiac function (CF, CO, Pmax, dP/dtmax, heart rate (HR), and oxygen extraction), and parameters derived from these measures (oxygen consumption: oxygen extraction * CF; left ventricular peak pressure-volume work index: CO * Pmax; and tension-time-index: HR * dP/dtmax) were determined for subsequent 30-s epochs during the 10-min baseline, the 20-min drug perfusion interval, and for the final 20-min washout period. Average concentrations of BK and NE were determined for 10- and 20-min intervals, respectively. Mean values (± SEM) were calculated and differences between the effects of captopril and vehicle were statistically confirmed using nonparametric tests (Mann-Whitney U tests) on baseline-adjusted values. A P value <.05 was considered significant.

**RESULTS**

The lowest concentration of captopril, $3 \times 10^{-8}$ mol/L, did not induce any significant changes in the parameters assessed, therefore this report will focus on the effects of the three higher concentrations.

**Coronary Flow, Oxygen Extraction, and Oxygen Consumption** The highest ($3 \times 10^{-5}$ mol/L) concentration of captopril, corresponding to maximum plasma levels reached in humans after a high antihypertensive dose of 100 mg captopril (orally), substantially increased CF (during the first 12 min, $P < .05$; Figure 1). The effect decreased during continuous perfusion and completely disappeared during the washout period. Changes in CF at the lower concentrations of captopril ($3 \times 10^{-6}$, $3 \times 10^{-7}$ mol/L) did not reach significance.

During the whole interval of high-dose perfusion with captopril oxygen extraction was significantly reduced ($P < .01$; after 11 min of perfusion, $P < .05$; Figure 1). During the washout period values approximated baseline value. Although the reduction in oxygen extraction failed to reach significance at a concentration of $3 \times 10^{-6}$ mol/L captopril, a somewhat stronger decrease in captopril-induced oxygen extraction at the concentration of $3 \times 10^{-7}$ mol/L was statistically confirmed for the period between the 5th min of captopril perfusion and the 7th min of washout ($P < .05$).

Oxygen consumption proved to be reduced by captopril in a concentration-dependent manner. Reduction of oxygen consumption was most pronounced during the highest concentration of captopril (reaching significance after 15 min of perfusion) and became gradually weaker and nonsignificant during perfusion of the $3 \times 10^{-6}$ and $3 \times 10^{-7}$ mol/L concentrations (Figure 1).

**Cardiac Function** Pmax was decreased during infusion of captopril at the high concentration (2–16 min after onset of captopril perfusion, $P < .05$; Fig. 2). A similar decrease during infusion of the lower concentration of $3 \times 10^{-6}$ mol/L captopril failed to reach significance, and the low concentration ($3 \times 10^{-7}$ mol/L) even appeared to enhance Pmax towards the end of the perfusion epoch. Like Pmax, cardiac work and partially cardiac output (CO) were also reduced during the highest concentration of captopril ($P < .05$, Figure 2). Changes in these parameters during the lower concentrations of captopril remained nonsignificant, except for a relative enhancement of work at the end of the perfusion period during the lowest concentration of captopril ($3 \times 10^{-7}$ mol/L).

Neither concentration of captopril changed HR significantly ($230 \pm 10$ beats/min during captopril perfusion [$3 \times 10^{-5}$ mol/L]) or $245 \pm 3$ beats/min during...
vehicle perfusion). Also inotropy (dP/dt\text{max}) and heart-rate–adjusted inotropy (dP/dt\text{max} \times HR) remained unaffected by the ACE inhibitor.

**Bradykinin, and Norepinephrine Release** An enhancement in BK overflow during the first 10-min interval of captopril perfusion was too variable to reach significance. Mean (± SEM) BK concentrations during perfusion of captopril and vehicle are shown in Figure 3. NE overflow was not significantly affected by captopril administration, except for a slight increase in NE overflow during perfusion of the highest concentration of captopril (+120 pg/20 min v vehicle 80 pg/20 min, \(P < .08\)).

**DISCUSSION**

Inhibition of ACE by captopril showed a concentration-dependent response on coronary perfusion, inducing prompt coronary dilation at a concentration of \(3 \times 10^{-5}\) mol/L, which is consistent with previous reports. The present study adds to this knowledge in that it demonstrates for the first time that captopril gradually decreased oxygen consumption and extraction in a concentration-dependent manner and reduced maximal left ventricular pressure without critically reducing cardiac output. Effects were most pronounced at the highest concentration of captopril (\(3 \times 10^{-5}\) mol/L), which is comparable to a therapeutic concentration in humans, reached after ingestion of a single, high antihypertensive dose of 100 mg captopril orally. At that concentration, the prompt increase in CF was accompanied by a continuous reduction in oxygen extraction and oxygen consumption, indicating a decrease in oxygen demand. Thus, the metabolic consequences of ACE inhibition in normoxemic hearts may well be similar to those in ischemic hearts, where ACE inhibition reduced the loss of

![Figure 1. Mean (± SEM) coronary flow, oxygen extraction, and oxygen consumption in isolated hearts perfused with captopril (thick lines, \(n = 8\)) at concentrations of \(3 \times 10^{-5}\) mol/L (top), \(3 \times 10^{-6}\) mol/L (middle), \(3 \times 10^{-7}\) mol/L (bottom), and vehicle (thin lines, \(n = 13\)). Infusions of captopril started after a 10-min baseline period and lasted for 20 min. Recordings were continued for a 20-min washout period. Intervals of significant differences between the effects of vehicle and captopril are marked by thick lines (\(P < .05\) and \(P < .01\)).](image)
energy-rich phosphates. However, BK outflow during and after captopril perfusion was variable and failed to change significantly, suggesting that, under normoxemic normotensive conditions BK is not the primary mediator of vasodilation.

In conjunction with the increase in CF, $P_{\text{max}}$ and myocardial work were decreased in a concentration-dependent manner by captopril. $dP/dt_{\text{max}}$, indicating early systolic performance of the left ventricle, remained unchanged. This pattern of effects contrasts with results from ischemic isolated working hearts and also from isolated hypertrophic hearts of stroke-prone spontaneously hypertensive rats. Administration of ramiprilat (at a concentration equivalent to the high concentration of captopril used here) increased $P_{\text{max}}$ and $dP/dt_{\text{max}}$, which under baseline conditions were substantially lower than in the healthy hearts of the present study. However, in hearts from spontaneously hypertensive rats also a decrease in maximal left ventricular pressure has been observed when these hearts were treated for a long time interval (11 weeks) with the ACE inhibitor cilazapril.

Actions of ACE inhibition on cardiac work have been shown to involve an improved left ventricular distensibility and regional relaxation, with the actions possibly reflecting influences of NO and cGMP-mediated endothelial agents such as BK. Examining normoxemic isolated working hearts during perfusion of captopril at a concentration of $1 \times 10^{-6}$ mol/L Anning et al revealed an enhanced ventricular relaxation, which—confounding expectations—was not associated with reduced peak left ventricular pressure. This pattern of changes appears to be very comparable to that observed in the present study at the concentration of $3 \times 10^{-6}$ mol/L captopril. Significant decreases in ventricular maximal pressure and cardiac work, in the present study, were observed only with the higher concentration of captopril ($3 \times 10^{-5}$ mol/L), suggesting that the emergence of reducing effects on left ventricular maximal pressure requires a concentration of captopril higher than that.
examined by Anning et al.\textsuperscript{23} On the other hand, left ventricular relaxation may become manifest even with lower concentrations of captopril. Although the present experiments did not assess left ventricular relaxation, concentration-dependent decreases in oxygen extraction and consumption (accompanied by adequate coronary perfusion) were observed during ACE inhibition. These decreases reflect an improved metabolic condition, which—as pre- and afterload, and heart rate, remained constant—was probably due to reduced cardiac work. Thus, the data further complement the view that ACE inhibition protects healthy hearts from the detrimental consequences of cardiac overload.

The effects of captopril cannot be explained by catecholaminergic mechanisms, because at the high concentration of captopril used (decreasing $P_{\text{max}}$) NE overflow was not reduced but rather tended to be increased. This result agrees with other studies, which also failed to find an enhancement of noradrenergic overflow during ACE inhibition,\textsuperscript{25,26} but differs from results in ischemic hearts, where ACE inhibition reduced the enhanced baseline norepinephrine release.\textsuperscript{27} These results also correspond with data in humans: in patients with failing hearts the increased norepinephrine release was reduced during ACE inhibition.\textsuperscript{28} Considering the multiple factors influencing sympathetic activity during ACE inhibition (such as the facilitated norepinephrine release by BK, and the inhibition of norepinephrine release by prostaglandins\textsuperscript{29}), it may well be that in isolated but otherwise healthy hearts these effects compensate each other.

In sum, apart from coronary dilation, ACE inhibition by captopril decreases maximal left ventricular pressure and oxygen consumption in nonhypertensive, nonischemic hearts. These results support the view of a potential protective action of captopril in healthy hearts at risk for cardiac overload.

\section*{ACKNOWLEDGMENT}

We thank Dr. W. Guenther for helpful advice. We thank C. Zinke for excellent technical assistance, and A. Otterbein, K. Jarowitz, S. Baxmann, and B. Fink for substantial technical support.

\section*{REFERENCES}

8. van Gilst W, van Wijngaarden J, Scholtens E, et al:


