Biological Activity of Angiotensin-(1-7) Heptapeptide in the Hamster Heart

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Angiotensin II has been reported to have both a positive inotropic effect and a coronary constrictor action in the hamster heart. To study the contribution to these responses of phenylalanine in position 8, we assessed the direct cardiac effects of angiotensin-(1-7), which lacks phenylalanine in position 8. Syrian hamsters were used to determine the effects of angiotensin-(1-7) on cardiac performance in the diseased and normal hearts. We used the isolated isovolumic heart preparation perfused either at a constant pressure of 50 mm Hg or at a constant coronary (myocardial) flow rate of 7 ml/min (seven cardiomyopathic hamsters [CMH] and seven normal hamsters [NH] in each subgroup). At constant perfusion pressure, coronary (myocardial) flow rate decreased (p<0.01) in both CMH and NH (-31±8% vs. -39±4% of baseline, respectively); but the percent decrease in left ventricular pressure and the first derivative of left ventricular pressure over time (LV +dP/dt) was significant only in NH (-8±1% and -9±4%) but not in CMH (-14±5% and -21±5%). On the other hand, at a constant coronary (myocardial) flow rate, left ventricular pressure and LV +dP/dt tended to increase in both CMH and NH (+10±3% and +6±2% of baseline vs. +7±7% and +7±5%, respectively) but these changes were not significant. In comparison, angiotensin II, given under the same conditions of constant coronary (myocardial) flow rate, increased left ventricular pressure and LV +dP/dt in both CMH and NH (65±12% and 71±11% of baseline vs. 155±84% and 119±50%, respectively; n=3, in each subgroup). Data suggest that, in the hamster heart, angiotensin-(1-7) has 1) a selective coronary vasoconstrictor effect and 2) no significant direct positive inotropic action that can be related to the absence of phenylalanine in position 8. (Hypertension 1990;15(suppl I):I-29-I-33)

It has recently been shown that homogenates of dog brainstem incubated with radiolabeled angiotensin I produced the amino terminal angiotensin-(1-7) heptapeptide as the principal metabolite.1 This fragment of angiotensin II possesses novel biological actions that include stimulation of vasopressin release almost equal to the effect of angiotensin II.2 On the other hand, contrary to the parent compound angiotensin II, angiotensin-(1-7) has been shown to be almost inactive as a pressor agent, and it had little effect on renin inhibition or aldosterone release in vivo.3,4 Its direct cardiac effects, however, have not yet been studied. In view of the cardiovascular importance of the renin-angiotensin system, we planned this study to assess the direct cardiac effects of angiotensin-(1-7) in the normal and the diseased (myopathic) heart under constant perfusion pressure as well as under constant coronary (myocardial) flow rate (MFR). We hypothesized that, in the absence of a pressor effect in vivo, a positive inotropic action would be physiologically important to support the functional performance of the diseased heart.

Methods

Animals

Fourteen 200-day-old male Syrian hamsters of a cardiomyopathic hamster line from the Canadian Hybrid Farm, Halls Harbour, Nova Scotia, Canada, and 14 normal random-bred hamsters of the same age from the Canadian Hybrid Farm were used. All were fed a regular diet of Purina Rodent Lab Chow 5001 (ICN Biochemicals, Cleveland, Ohio), which contains protein 23.4%, fat 4.5%, gross energy 4.25 Kcal/g, sodium 3.0 mg/g, potassium 11.0 mg/g, calcium 12.0 mg/g, magnesium 2.1 mg/g, chloride 5.8 mg/g, and phosphorus 8.6 mg/g, with ad libitum intake of water in a climate-controlled room. The hamsters were killed by excision of the heart under pentobarbital anesthesia as will be explained. Baseline cardiac function was
assessed in the isolated isovolumic heart using the Langendorff technique.

To test cardiac responses to angiotensin-(1–7) at a concentration of 10^{-6} M, hamsters were divided into two subgroups. In one subgroup, the dose-response curve to angiotensin-(1–7) was obtained under constant MFR of 7 ml/min (seven cardiomyopathic hamsters [CMH] and seven normal hamsters [NH]), and in the other subgroup, the dose-response curve to angiotensin-(1–7) was tested at a preset perfusion pressure of 50 mm Hg (seven CMH and seven NH).

**Langendorff Preparation**

The Langendorff preparation used in our laboratory has been described in detail previously.2 The balloon inserted in the left ventricle by the mitral valve was prepared in a 37° C water bath from a thin rubber material (Akwell Industries Inc., Dothan, Alabama) fastened around the tip of a catheter system. The catheter system consisted of a 3F Millar transducer tip catheter (Mikro-Tip, N/A, Millar Instruments, Houston, Texas) and a PE-50 tube attached together by a silk thread placed 1 cm above the tip of the Millar catheter. The Millar catheter was used to record intraventricular pressures, and the PE-50 tubing served to fill the balloon with water for volume and preload adjustment.

The hamster was given 1,000 units heparin i.p., 30 minutes before anesthesia. Under pentobarbital anesthesia (30 mg/kg body wt. i.p.) and an adequate ventilation with positive end expiratory pressure by tracheostomy (Rodent Respirator, Harvard Apparatus, Millis, Massachusetts), the heart was rapidly dissected out and placed immediately in an ice-cold Krebs-Henseleit bicarbonate buffer solution saturated with oxygen. The heart was then securely attached to the plastic grooved-tipped cannula of the Langendorff apparatus through the aortic stump. The heart was then immediately perfused retrogradely at 37° C with an oxygenated modified Krebs-Henseleit bicarbonate buffer solution, either at a constant perfusion pressure or a constant MFR, depending on the protocol of the study. In the instance of constant perfusion pressure, the perfusate was run at a pressure of 50 mm Hg, which was monitored with a MP-150 pressure transducer (Micron Instruments, Los Angeles, California), situated at the level of the aortic valve. In the case of constant MFR, the rate of infusion was set at 7 ml/min by a roller pump (MasterFlex, Universal Electric Co., Owes, Michigan) and was also monitored by collecting the effluent for consecutive periods of 5 minutes each. MFR was calculated in milliliters per gram of left ventricular weight per minute (ml/g LV/min). The perfusate contained the following (mM): NaCl 117, KCl 4.7, CaCl2 2.5 plus 0.5 to balance EDTA, KH2PO4 1.2, MgSO4 1.2, NaHCO3 25, Na2EDTA 0.5, and dextrose 11.0. pH of the buffer was 7.4, when oxygenated with 95% O2 and 5% CO2 at 37° C.

At the start of the experiment, the balloon-catheter system was inserted into the left ventricle through the left atrium with the balloon deflated; the volume of the balloon was then adjusted so that the end-diastolic left ventricular pressure was set at 0 mm Hg; the volume of the balloon was then left unchanged throughout the experiment. The atrioventricular node was then crushed and the isolated heart paced at a constant rate of 260 beats/min, which was maintained throughout the experiment with an S-9 stimulator (Grass Instr. Co., Quincy, Massachusetts); duration of the stimulus was 5 msec at 2–3 V. Twenty-five to 30 minutes were allowed for equilibration. During this time, the left ventricular systolic pressure (LVP), the rate of increase in pressure (LV +dP/dt), and the left ventricular end-diastolic pressure were continuously monitored and recorded at a paper speed of 0.05 mm/sec (Brush Recorder, Gould Inc., Cleveland, Ohio). At the end of the equilibration period, the paper speed was increased to 50 mm/sec to obtain baseline readings.

At the end of the experiment, heart weight was obtained with a Mettler PC440 precision balance (Mettler Instrument Corp., Hightstown, New Jersey). Left ventricular weight was normalized both by body weight and by brain weight because brain weight was reported to be constant irrespective of physiologic or nutritional stimuli.6

**Protocol of the Study**

Angiotensin-(1–7) dose–left ventricular performance response curve was obtained in both cardiomyopathic and control hearts at either a constant perfusion pressure of 50 mm Hg (seven CMH and seven NH) or at a constant MFR of 7 ml/min (seven CMH and seven NH). These responses were compared with those obtained in preliminary experiments using the parent compound, angiotensin II, under similar conditions of constant MFR or constant perfusion pressure in both CMH and NH (three in each group). The hamsters used in these preliminary experiments were 300 days old although several previous reports had defined end-stage cardiomyopathy as occurring when the animal is about 200 days old and continuing beyond that age.7

**Peptides and Dosages**

The solution of angiotensin-(1–7) was prepared anew just before the experiment by dissolving the peptide in the oxygenated Krebs-Henseleit bicarbonate buffer. After recording the baseline data, angiotensin-(1–7) solution was infused continuously by a Harvard pump (model 940, Harvard Apparatus, Millis, Massachusetts) into the cannula just above the tip of the Millar catheter system. The infusion rate was 0.0051, 0.0103, 0.0206, 0.051, 0.103, and 0.206 ml/min, each for 5 minutes. The doses of the drug infused were calculated as 4.6, 9.2, 18.5, 45.8, 92.6, and 185.2 µg/min for angiotensin-(1–7). These doses were then normalized for MFR and left
ventricular weight, and the units were expressed as molar and moles per gram left ventricle. Angiotensin II was infused by a Harvard pump into the cannula just above the aortic stump at the same six graded infusion rates as those of angiotensin-(1-7). The doses of angiotensin II were calculated as 0.53, 1.07, 2.14, 5.30, 10.71, and 21.4 μg/min; they were then expressed as molar and moles per gram left ventricle.

### Statistical Analysis

All statistical analyses were done using an SAS computer. Comparison between the groups was evaluated by analysis of variance (ANOVA) and covariance including repeated measures. A two-way ANOVA for repeated measures was used to compare different groups and the responses to different doses of angiotensin-(1-7). If the interaction was significant, unpaired t test or one-way ANOVA followed by Bonferroni t test was done between two or more groups, respectively.8

### Results

#### Baseline Data

In the groups in which hearts were subjected to constant perfusion pressure of 50 mm Hg (Table 1), body weight and brain weight were significantly smaller in CMH as compared with NH. Although left ventricular weight of CMH was not different from NH, left ventricular weights normalized by body weight (LV/BW, where LV is left ventricular weight and BW is body weight) and brain weight (LV/brain, where brain is brain weight) were significantly higher in CMH as compared with NH (p<0.05). In the groups in which the hearts were subjected to constant MFR (Table 1), body weight and brain weight were again significantly smaller in CMH as compared with NH (p<0.05). LV/BW was also significantly higher in CMH as compared with NH (p<0.05).

Baseline cardiac indexes in the four groups are summarized in Table 1. Left ventricular developed pressure (LVP) and LV +dP/dt were significantly lower in CMH as compared with NH at constant perfusion pressure and at constant MFR. Note, however, that baseline MFR was not different between CMH and NH when perfusion pressure was kept at 50 mm Hg.

### Cardiac Responses to Angiotensin-(1-7)

As illustrated in Figure 1 (top), the reduction in MFR was significant in both CMH and NH (-31±8% vs. -39±4% of baseline, respectively) at a constant perfusion pressure of 50 mm Hg; however, the percent decrease in LVP and LV +dP/dt (Figure 1 [bottom]) was significant in NH (-8±1% and -9±4%, respectively; p<0.05, for both) but of borderline significance in CMH (-14±5% and -21±8%, respectively; p<0.05, by paired t test, but p=NS, by Bonferroni test) (Figure 1 [bottom]) although the magnitude of the effect was even larger than that in NH. On the other hand, at a constant MFR, there was a tendency to increase LVP and LV +dP/dt (Figure 2) in both CMH and NH (+10±3% and 6±2% of baseline vs. +7±7% and 7±5%, respectively) but these changes did not attain statistical significance.

Angiotensin II increased LVP and LV +dP/dt in both CMH (n=3) and NH (n=3) (65±12% and 71±11% of baseline vs. 155±84% and 119±50%, respectively) at a constant MFR. At a constant perfusion pressure, although MFR decreased substantially in both CMH and NH (-30±9% and -21±12% from baseline, respectively), LVP and LV +dP/dt increased in both CMH and NH (75±19% and 57±14% of baseline vs. 137±51% and 93±29%, respectively).

### Discussion

Angiotensin II has a direct positive inotropic effect in isolated cat papillary muscles,9 isolated perfused rabbit hearts,10 bathed guinea pig atrium,11 and isolated hamster hearts.12 This effect in isolated tissues or organs was shown to be independent of intact adrenergic ganglia, nerves, or endogenous
catecholamine stores. It is not clear, however, which segments in the angiotensin II molecule are involved in the binding to the myocardium. From studies of structure-activity relations of angiotensin II analogues, the phenylalanine group in position 8 reportedly contains the signal for biological responses. To study the contribution to these effects of phenylalanine in position 8, we assessed the direct cardiac responses to angiotensin-(1-7), which lacks phenylalanine in position 8. These studies also help in understanding the biological effects of angiotensin-(1-7). This fragment of angiotensin II was found to be almost inactive as a pressor agent, and it had little effect on renin-inhibition or aldosterone release in vivo.

We used the 200-day-old Syrian hamster model of cardiomyopathy as a unique model of heart failure, with some resemblance to human cardiac dysfunction in its response to treatment with digitalis and diuretics. Moreover, this hamster model offers the advantage of spontaneous development of normotensive cardiac dysfunction without the need to expose the animal to surgery or to administration of myocardial depressant agents. The Langendorff technique was used in this study to assess the direct effects of the angiotensin-(1-7) on the heart under constant heart rate without the influence of circulating humoral factors or other factors such as respiration and autonomic reflexes.

At a constant perfusion pressure of 50 mm Hg, baseline LVP and LV +dP/dt were significantly lower in CMH as compared with NH. Our data regarding cardiac performance in the isolated perfused hearts of 200-day-old hamsters are similar to those data of Sievers et al and Wikman-Coffelt et al, who studied 240-250-day-old hamsters, using the Langendorff preparation with a perfusion pressure of 90-100 mm Hg. We used a perfusion pressure of 50 mm Hg in our studies to reduce cardiac tissue edema during perfusion throughout the duration of our experiments. The same perfusion pressure was used in both normal and myopathic hearts. Under these conditions, we identified a depressed baseline cardiac performance in the CMH. Baseline MFR was not different between CMH and NH, as also shown by Sievers et al.

At a constant perfusion pressure, angiotensin-(1-7) decreased MFR in both CMH and NH. The decrease in LVP and LV +dP/dt, however, was significant only in the NH hearts. On the other hand, at constant MFR, angiotensin-(1-7) caused a slight but nonsignificant increase LVP and LV +dP/dt in both NH and CMH. Although there is no report about the effects of angiotensin-(1-7) on CMH hearts, Bonnarderaux and Regoli studied the effects of this fragment of angiotensin II on isolated perfused rabbit hearts. The rabbit hearts were perfused through an aortic cannula at a constant rate of 18 ml/min, using Krebs solution at 32°C. Under these conditions, they infused angiotensin-(1-7) at a concentration of 10^-3 M into rabbit hearts for 5 minutes. The maximum percent change of LVP from the baseline was 4±2% in rabbit hearts. This
change was not statistically significant, leading to the conclusion that angiotensin-(1-7) was ineffective on LVP of rabbit hearts. These results are concordant with ours obtained at constant MFR. In our experiments at constant perfusion pressure, angiotensin-(1-7) decreased MFR in both CMH and NH. Although the magnitude of decrease in MFR was larger in myopathic hearts as compared with normal hearts, the decrease in MFR did not attain statistical significance in the myopathic hearts, probably because of the variance in diseased hamsters. The reduction in LVP and LV +dP/dt in both CMH and NH under these conditions is, therefore, interpreted as flow related in view of the lack of any depressant effect when MFR was maintained constant. A methodological discrepancy should be discussed at this point. At constant MFR, coronary perfusion pressure increased in response to angiotensin-(1-7) because of increased coronary resistance. One can argue that this increased coronary perfusion pressure, in turn, might have caused cardiac tissue edema leading to impaired cardiac performance and suppression of myocardial responses to angiotensin-(1-7). Unsupportive of this possibility, however, is that cardiac responses to angiotensin II were examined under similar experimental conditions, and a positive inotropic effect was clearly seen.

Compared with the parent compound angiotensin II, angiotensin-(1-7) had coronary vasoconstrictive effect, whereas its inotropic effect was markedly blunted (8.5% that of angiotensin II in CMH and 5.9% in NH) when MFR was maintained constant.

There are few reports about the effects of angiotensin-(1-7) on various blood vessels. Klossa et al reported that angiotensin-(1-7) has no contractile activity in isolated blood vessels. Recently, Kono et al said that the pressor action of angiotensin-(1-7) in humans was very weak and less than 0.028% of the pressor action of angiotensin II; there is no report, however, about the effect of angiotensin-(1-7) on coronary vessels of hamster hearts. From our results it seems that angiotensin-(1-7) selectively constricts the coronary arteries, and that leads to decreased coronary blood flow.

Angiotensin-(1-7) has a selective coronary vasoconstrictor effect and no direct inotropic action in hamster hearts. This lack of positive inotropic effect might be related to the absence of phenylalanine in position 8 of angiotensin II.

References

Key Words • angiotensin • hamster studies • coronary perfusion • ventricular function
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