Myocardial Actions of Angiotensin

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The cardiovascular effects of angiotensin have been studied extensively since its identification 25 years ago.1-3 The synthesis of various angiotensins and the demonstration of the pharmacological identity of the naturally occurring and the synthetic octapeptides have facilitated such studies in recent years. It has been found consistently that angiotensin is a powerful constrictor of arteriolar smooth muscle, increases total peripheral resistance, and raises the systemic arterial pressure. No such agreement exists concerning the actions of the drug on heart muscle.

Of 32 published studies on changes in the normal human or animal circulation following administration of angiotensin, 19 have found that cardiac output is decreased and 13 that it remains unchanged. Increases of diastolic volume of the heart, of left ventricular end diastolic pressure, of diastolic pressures in the other cardiac chambers, of pulmonary wedge pressure, of central venous pressure, and of circulation time have also been observed following introduction of angiotensin into the intact circulation.4-13 These observations have led some workers to conclude that angiotensin depresses the strength of contraction of heart muscle or that it lacks inotropic action on the heart.4,5,13-15

On the other hand, studies in which myocardial contractility was evaluated more directly suggest that at least some concentrations of angiotensin exert positive inotropic effects on mammalian myocardium.8,17-25 Some of these reports describe only a few observations or yield no quantitative data; the increases in contractility observed in the other studies varied from minor24 to pronounced.8,17 These discrepancies reflect in part the widely different and narrow ranges of angiotensin concentration studied by different workers. The evidence for the positive inotropic effect of angiotensin has received little attention, and it is still widely held that the increase in peripheral resistance is solely responsible for the pressor effect of the drug in the intact circulation.

The present study was undertaken to determine the effects of synthetic angiotensin over a wide range of concentrations on the strength and time course of contraction of mammalian ventricular and atrial muscle. The drug had consistent, concentration-dependent, positive inotropic actions on mammalian ventricular myocardium. The actions of angiotensin on impulse formation and on resting fiber length of mammalian myocardium were also studied.

Methods

Heart muscle preparations were obtained from the hearts of 46 kittens weighing 0.48 to 1.52 kg (median 0.82 kg). Right ventricular papillary muscles (average length 1 cm, cross-sectional area less than 1 mm²) and left atrial strips (average length 1 cm, width 0.5 cm, maximum thickness less than 0.5 mm) were used in the studies on contractility. These preparations were quiescent unless stimulated electrically. The chronotropic effects of angiotensin were determined on right atria which included the SA node and contracted spontaneously. The effect of angiotensin on the diastolic pressure-volume relationship was studied by means of whole left atria, distended with oxygen, surrounded by physiological salt solution, and coupled with a volume recorder which measured the fluid displaced by the atrium.26

The animals were killed by a sharp blow on the skull and the preparations immediately dissected in oxygenated solution at room temperature. One end of each muscle was fixed to a muscle holder by a stainless steel clamp, the other end was tied with a silk thread to a wire extending upwards to a strain gauge transducer. The preparations were kept at a resting tension approximately one-half of that initially determined in each muscle to be associated with near-maximum.
development of tension. Resting tension varied between 0.2 and 0.5 g depending on the cross-sectional area of the preparation.

The preparations were stimulated through two punctate platinum electrodes placed in contact with the tissue just above the clamp. Stimuli were square-wave pulses of 5 msec duration and of voltage approximately twice threshold. Isometric mechanograms were recorded on a Sanborn direct-writing oscillograph, and high-speed tracings were photographed with a Grass kymograph camera from a Tektronix dual-beam oscilloscope. Details of the stimulating and recording devices and of the performance of the preparations have been described previously.7-8

All preparations were suspended in a modified Krebs solution whose temperature was maintained at 37°C. Its composition before equilibration with CO₂ was: Na⁺ 140 mEq/liter, K⁺ 5 mEq/liter, Ca²⁺ 4.5 mEq/liter, Mg²⁺ 2 mEq/liter, Cl⁻ 98.5 mEq/liter, SO₄= 2 mEq/liter, HCO₃⁻ and H₂CO₃ 24 mM, HPO₄= and t.POr 1 mM, fumarate 5 mM, pyruvate 5 mM, L-glutamate 5 mM, glucose 10 mM, and insulin 5 units/liter. The solution was continuously oxygenated and stirred by passage of finely dispersed bubbles of a mixture of 95% O₂ and 5% CO₂. After equilibration the pH was 7.4.

The synthetic angiotensin studied was 1-L-asparaginyl-5-L-valyl angiotensin octapeptide (mol wt 1031.2).* Dilutions from a 10⁻² M aqueous stock solution were made prior to each experiment because very dilute solutions were found to decrease in activity over several days.2 The volume of angiotensin solution added to the bath never exceeded 0.2% of the bath volume. Concentration-action curves were determined by allowing the muscle to return to a steady state in drug-free solution before each increase in drug concentration.

Results

**INOTROPIC EFFECTS**

The inotropic effect of angiotensin on kitten papillary muscles is shown in figure 1. Angiotensin 10⁻¹⁰ M caused a very slight increase in contractility of a few muscles. The maximum positive inotropic effect was reached with 10⁻⁷ M angiotensin, the mean increase in the strength of contraction with this concentration was 120.6% (SE 8.1%). No further increase in tension development was caused by solu-

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* Hypertensin-Ciba generously furnished by Dr. A. J. Plummer, Ciba Pharmaceutical Company, Summit, New Jersey.
tropic effect of the most effective concentration of angiotensin results almost entirely from an increase in the rate of development of tension. This is also true for lower concentrations of the drug. Thus angiotensin raises myocardial contractility by increasing the degree of activation of the contractile elements (i.e., by altering their force-velocity relationship). The time required to reach peak tension increases only slightly: in 32 preparations the mean increase was 4.2% (SE 0.9). The relaxation time is also changed little: the mean time required for 90% relaxation in 32 preparations increased by only 5.6% (SE 1.1). The finding that angiotensin causes only slight increases in the time to peak tension and in the total time during which tension is developed indicates that the drug has no significant effect on the duration of the active state.

The onset of the positive inotropic action of angiotensin is rapid. The first effect appears within a few seconds after addition of the drug. The full effect of any concentration up to the optimum is reached in three to four minutes. The time required for the development of the full effect is independent of the number of contractions during exposure to the drug, and the positive inotropic effect develops in the absence of any contractions. The full action of all those concentrations of angiotensin which produced less than the maximum inotropic effect lasts approximately ten minutes. This is followed by a very slow disappearance of the action, with contractility remaining well above normal for more than two hours. The gradual decline of the effect is due to inactivation of the drug rather than to loss of responsiveness of the preparation, since replacement of the solution with fresh solution of the same angiotensin concentration immediately restores the full effect. The inotropic action of angiotensin is completely reversible by washing with drug-free solution, and the time course of disappearance of the effect is similar to that of its development.

Concentration-action curves for both angiotensin and l-norepinephrine were determined on 15 papillary muscles and are compared in figure 3. The ED$_{50}$ of angiotensin is smaller than that of levarterenol: 3.6 x 10$^{-6}$ mol/liter.
Effect of angiotensin on atrial contractility. Mean and standard error of 18 left atrial strips, kitten. 37°C. Contraction frequency 12/min. Abscissa: molar concentration of angiotensin, logarithmic scale; ordinate: increase in developed tension.

(see $0.9 \times 10^{-8}$) and $7.2 \times 10^{-8}$ mol/liter (see $0.7 \times 10^{-8}$) respectively. Levarterenol shows a steeper concentration-action curve than angiotensin, and in any concentration above $7 \times 10^{-9}$ M the amine causes a greater inotropic effect than equimolar concentrations of the octapeptide. The maximum increase in contractility obtained with levarterenol averaged 211% as compared to 116% for angiotensin.

The range of angiotensin concentrations which increase atrial contractility is similar to that determined on papillary muscles, but the magnitude of the positive inotropic effect is far less in the atrium (fig. 4). As indicated by the standard errors, the response of individual preparations varied considerably but the increase in contractility never exceeded 19%. Determination of the effect of angiotensin on the pressure generated by isochorically contracting whole atria confirmed its weakly positive inotropic action on atrial muscle.

In six experiments angiotensin in concentrations up to $10^{-4}$ M had no effect whatsoever on the mechanical performance of ventricular strips from Rana pipiens.

EFFECTS ON DIASTOLIC FIBER LENGTH

Diastolic pressure-volume curves were determined on six left atria of kittens. To avoid the influence of hysteresis and slow yielding, the curves were always determined by stepwise increases of distending pressure, and sufficient time to reach a constant volume was allowed. After an initial distension in normal solution, curves were determined alternatively with and without angiotensin in the bathing fluid.

Figure 5 shows the result of one such experiment. In all experiments $10^{-5}$ M angiotensin consistently failed to change the relationship between distending pressure and atrial volume. Lower concentrations also lacked any effect on resting atrial fiber length. The same lack of effect was observed in ventricular muscle: no concentration between $10^{-10}$ M and $10^{-4}$ M changed the relationship between resting tension and resting length of papillary muscles.

EFFECTS ON IMPULSE FORMATION

The effects of angiotensin on the frequency of impulse formation in SA node are negligible (fig. 6). In the absence of angiotensin the mean SA nodal rate in 16 preparations...
Effect of angiotensin on pacemaker rate. Mean and standard error of 16 right atria including SA node, kitten. 37°C. Abscissa: molar concentration of angiotensin, logarithmic scale; ordinate: change in pacemaker rate on addition of each concentration of drug to standard solution.

was 134.8 beats/min (SE 3.3). Concentrations of angiotensin from $10^{-10}$ M to $10^{-5}$ M failed to change this rate in a statistically significant fashion. Angiotensin $10^{-5}$ M significantly increased the rate ($P < 0.02$), but there was much variation (range of increase 1% to 16%) and the mean increase was only 3.9%. In contrast, maximally effective concentrations of levarterenol commonly doubled the frequency of contraction of the isolated kitten heart.

Equally striking is the failure of angiotensin to cause ectopic impulse formation in normally quiescent heart muscle. No concentration of angiotensin ever led to the appearance of spontaneous activity in left atrial strips or in papillary muscles. In contrast, levarterenol $10^{-6}$ M to $10^{-5}$ M induced ectopic impulse formation, ranging from occasional extrasystoles to rapid irregular rhythm, in 10 of 20 papillary muscles.

Even the highest concentrations of angiotensin tested did not change the threshold voltage required to excite papillary muscles. High-speed tracings of contractions showed no evidence of conduction disturbances due to angiotensin.

**Discussion**

**INOTROPIC EFFECTS**

The positive inotropic action of angiotensin on ventricular myocardium is the result of an increase in the degree of activation of the contractile elements, as is true also in the case of the sympathomimetic amines and the cardiac glycosides. Unlike the sympathomimetic amines, however, angiotensin produces no decrease in the duration of the active state, so that ventricular systole is not shortened.

The increase in myocardial contractility probably participates significantly in the pressor action of angiotensin. Appreciable increases in the strength of contraction appear with concentrations of $10^{-9}$ M (fig. 1). Considerably higher concentrations are obtained in the blood during therapeutic administration of angiotensin at rates of 1.6 to 5.2 µg/min. Ventricular function curves in dogs suggest that infusion of 1 µg/kg/min greatly increases myocardial contractility, and even such rates of administration have been used clinically. Some increase in cardiac contractility probably results from angiotensin infusion at all usual rates, and it will be marked at high rates. Thus, the pressor effect of angiotensin in the intact circulation is not due solely to its action on the smooth muscle of the resistance vessels, but results also from its positive inotropic action on the myocardium. The relative importance of the latter action may increase with the rate of administration.

The supposition that angiotensin has negative or no inotropic effects on the heart has been based mainly on determinations of cardiac output in the intact circulation. Changes in output after angiotensin have been variable; they appear to be influenced by the prior circulatory state and by the rate and duration of administration of the drug. Many workers have found no significant change in cardiac output despite large increases in blood pressure; in other studies cardiac output was decreased, but often the fall was due entirely to slowing of the heart rate, and stroke volume remained constant. In any case, changes in cardiac output are not a useful index of the inotropic effects of agents with other actions on the circulatory system. A drug which markedly increases peripheral resistance and de-
creases the heart rate may be expected to decrease cardiac output and to increase left ventricular end diastolic pressure; neither of these changes can be considered evidence for decreased myocardial contractility. In the intact circulation even such a powerful positive inotropic agent and venoconstrictor as levarterenol can decrease cardiac output. Another possible cause of decreased cardiac output during angiotensin infusion is coronary vasoconstriction which may result in inadequate myocardial perfusion. It seems certain that a direct negative inotropic effect of angiotensin is never a contributing factor. Rather, the increased myocardial contractility due to the drug tends to moderate any fall in cardiac output or to prevent the decrease completely. Significantly, angiotensin has been found to increase cardiac output in shock or when peripheral resistance was lowered by administration of tolazoline.

The maximum increase in ventricular contractility obtainable with norepinephrine is almost twice that produced by the optimum concentration of angiotensin. However, due to the different slopes of their concentration-action curves, the ratio of their positive inotropic actions varies greatly with concentration. The same may be true for the actions of these agents on vascular muscle and may largely explain the discrepant reports on their relative strength as pressor agents.

There is no ready explanation for the difference in the ventricular and atrial inotropic actions of angiotensin. Dissimilarities in the responses of atrial and ventricular myocardium to other inotropic drugs have been observed. It is interesting that the weak atrial inotropic effect of angiotensin resembles its atrial chronotropic effect rather than its strong ventricular inotropic action.

**Effects on Resting Fiber Length**

In this study no evidence was found of any effect of angiotensin on the diastolic length-tension relationship of mammalian heart muscle. Changes in diastolic volume of the isolated heart have been observed but reflect increased contractility rather than alterations in the resting pressure-volume relation. An increased "tonus diastolique" of papillary muscles after exposure to angiotensin has been reported, but the preparations were unstable and shortening even before the addition of the drug, perhaps due to rigor of insufficiently oxygenated central fibers.

**Effects on Cardiac Impulse Formation**

Only the highest concentration of angiotensin (10^{-7} M) increased the frequency of impulse formation in the atrial pacemaker, and the rise was very small. Previous workers have found angiotensin in various concentrations to have positive, negative or no direct chronotropic effects on the heart. All the reported changes have been small, and their statistical significance is impossible to evaluate from the data given. It seems certain that direct chronotropic effects play no role in the heart rate changes produced by angiotensin in vivo. The cardiac slowing consistently observed in the intact circulation is clearly due to a vagal reflex from the sino-aortic pressor receptors. The cardio-acceleration observed after sino-aortic denervation appears to result from central stimulation.

No concentration of angiotensin from 10^{-10} M to 10^{-4} M caused ectopic impulse formation in atrial or ventricular myocardium. This failure to increase ectopic myocardial automaticity is in marked contrast to the action of levarterenol and agrees with observations on the electrocardiogram of unanesthetized dogs. No effect of angiotensin on the cardiac resting membrane potential or on the action potential has been found. The absence of significant effects on the electrical properties of heart muscle is a valuable characteristic of angiotensin when it is used as a pressor agent.

**Summary**

Synthetic angiotensin II has pronounced inotropic effects on isometrically contracting, isolated papillary muscles of kittens. The response increases with drug concentration over the range from 10^{-10} M to 10^{-5} M. The mean maximum increase in tension development is 120%. Contractility is raised by angiotensin through an increase in the degree of activation of the contractile elements with no...
significant change in the duration of their active state. Low concentrations of angiotensin have a greater inotropic action than equimolar concentrations of l-norepinephrine; but the maximum inotropic effect obtainable with l-norepinephrine is almost twice that of angiotensin.

The inotropic effects of angiotensin on cat atrial muscle are slight, and the drug is inactive on frog ventricular myocardium. It has no effect on the resting length-tension relationship of mammalian atrial or ventricular muscle. The frequency of impulse formation in the SA node is not significantly changed by concentrations of angiotensin up to \(10^{-5}\) M. In marked contrast to levarterenol, angiotensin does not cause ectopic impulse formation in atrial or ventricular muscle.

It is suggested that the positive inotropic effect of angiotensin on ventricular myocardium is of importance for the pressor action of the drug in the intact circulation. The increase in myocardial contractility tends to minimize or prevent decreases in cardiac output in the face of increased resistance to cardiac ejection and thus supports the elevation of arterial pressure.

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