Nature of the Inotropic Action of Angiotensin on Ventricular Myocardium

By Jan Koch-Weser, M.D.

Angiotensin II has a significant positive inotropic action on mammalian ventricular myocardium. In the intact circulation this action is modified or masked by the vascular effects of angiotensin which lead to coronary vasoconstriction and to marked increases of total peripheral resistance.1-6 On the other hand, when myocardial contractility is determined directly and when secondary effects of the vascular changes on the heart are prevented or taken into account, angiotensin is found to have a consistent, concentration-dependent, direct action on the strength of ventricular contraction.1,5-7 Tension development by isolated cat papillary muscles is doubled in optimal concentrations of angiotensin; this increase is more than half the maximum obtainable with levarterenol in the same preparations.7 The positive inotropic effect of angiotensin is clearly a significant component of the overall circulatory response to this agent.

The mechanism of the inotropic action of angiotensin has not been established. Recently it has been suggested that a large part of the stimulant action of angiotensin on the heart is the result of liberation of catecholamines from the cardiac stores.8 Other workers found that the positive inotropic effect of angiotensin did not depend upon myocardial catecholamine content.6 One of the aims of the present study was to resolve this uncertainty.

The inotropic effects of many drugs are influenced by the physical conditions under which the myocardium contracts and by the ionic composition of the fluid surrounding the muscle.8,10 Studies on the effect of changes in physical factors on the action of a drug which affects myocardial contractility can lead to a better understanding of the mechanism of action of the drug.8-11 We therefore determined the influence of temperature, of frequency of contraction, and of changes in the external concentrations of calcium and of sodium on the inotropic action of angiotensin.

Methods

Papillary muscles were obtained from the right ventricles of 36 kittens varying in weight from 0.4 to 1.25 kg (median 0.64 kg). Only muscles less than 1 mm² in cross-sectional area were chosen in order to insure adequate oxygenation.12 Most ventricles contained two satisfactory papillary muscles, occasional hearts yielded one or three.

The animals were killed by a sharp blow on the skull and the hearts immediately removed. Papillary muscles were dissected in oxygenated solution at room temperature. The mural end of each muscle was fixed to a muscle holder by a stainless steel clamp, the tendinous end was tied with a silk thread to a wire extending upwards to a strain gauge transducer. The muscles were kept at a resting tension approximately one-half of that initially determined in each muscle to be associated with maximum development of tension. Resting tension varied between 0.2 and 0.4 g depending on the cross-sectional area of the preparation.

The normally quiescent muscles were stimulated through two punctate platinum electrodes. Stimuli were square-wave pulses of 5 msec duration and of voltage 50% above threshold. Except when the influence of frequency was studied, the muscles were stimulated at a frequency of 12/min. At this low frequency the strength of contraction of papillary muscles is far from maximum and positive inotropic effects are prominent.10 Furthermore, oxygen requirements are relatively low, and the release of endogenous...

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Effects of angiotensin and levarterenol on time course of tension development by kitten papillary muscle. 37°C, contraction frequency 12/min. Superimposed tracings from oscilloscope records at sweep speed of 200 mm/sec.

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.

The papillary muscles were suspended in 50 ml of a modified Krebs solution of the following composition (before equilibration with CO₂): Na⁺ 140 mEq/liter, K⁺ 5 mEq/liter, Ca²⁺ 4.5 mEq/liter, Mg²⁺ 2 mEq/liter, Cl⁻ 98.5 mEq/liter, SO₄²⁻ 2 mEq/liter, HCO₃⁻ and H₂CO₃ 29 mM, HPO₄⁻ and H₂PO₄⁻ 1 mM, fumarate 5 mM, pyruvate 5 mM, l-glutamate 5 mM, glucose 10 mM, and insulin 5 IU/liter. This solution was continuously oxygenated and stirred by passing through it finely dispersed bubbles of a mixture of 95% O₂ and 5% CO₂. After equilibration with this mixture the pH was 7.4. The osmolar concentration was 285 mosmol/liter (determined by Fiske cryoscope). In studies on the effect of low external Na⁺ concentrations sucrose was substituted for sodium chloride to maintain the same osmotically effective concentration. Except when the temperature of temperature was studied, the bath was maintained at 37°C.

The drugs used in this study were angiotensin II (l-asparaginyl-5-L-valyl angiotensin octapeptide), nethalide (1-(2-naphthyl)-2-isopropylaminoethanol hydrochloride), 3,4-dichloroisoproterenol, l-norepinephrine bitartrate monohydrate, tyramine hydrochloride, and reserpine phosphate. Dilutions from stock solutions were made in such a way that the volume of drug solution added never exceeded 0.3% of the organ bath volume. All concentration-effect curves were determined by allowing the muscle to return to a steady state in drug-free solution before each increase in drug concentration.

Results

ROLE OF NOREPINEPHRINE RELEASE IN THE INOTROPIC ACTION OF ANGIOTENSIN

The positive inotropic effect of angiotensin results almost entirely from an increase in the rate of development of tension (fig. 1). The time required to reach peak tension and the total duration of tension development are increased very slightly. Levarterenol also raises the rate of tension development by ventricular myocardium, but at the same time causes a significant decrease in the duration of contraction (fig. 1). Thus, both compounds raise myocardial contractility by increasing the de-
degree of activation of the contractile element, i.e., by shifting the force-velocity curve to the right, but their effects on the duration of the active state are quite different. Compounds such as tyramine, which produce their positive inotropic effect by releasing norepinephrine from the cardiac stores, shorten the time to peak tension and the total duration of contraction just as does levarterenol (personal observations). The failure of angiotensin to shorten the duration of the active state of ventricular muscle suggests that the drug is not an effective releaser of myocardial catecholamines.

The inotropic action of angiotensin is not significantly altered by pretreatment of the experimental animal with reserpine (fig. 2). An injection of 1 mg of reserpine per kg administered 24 hours prior to the experiment practically abolished the inotropic response of the papillary muscle to $10^{-5}$ M tyramine. In contrast, the increases in tension development due to angiotensin were insignificantly greater after depletion of the myocardial catecholamine stores than in normal myocardium.

FIGURE 3
Effect of nethalide on the inotropic responses of kitten papillary muscle to tyramine and angiotensin. 37°C, contraction frequency 12/min, chart speed 0.25 mm/sec. In IA and IB nethalide $5 \times 10^{-6}$ M was added 15 min prior to the record shown. Addition of other drugs indicated by arrows. IA and IB tyramine $10^{-4}$ M; II A and II B angiotensin $2 \times 10^{-6}$ M.

FIGURE 4
Influence of nethalide on the inotropic log concentration-effect curve of angiotensin. Kitten papillary muscles, 37°C, contraction frequency 12/min. Mean and standard error of 9 muscles; all effects expressed as percentage of maximum effect in same concentration-effect curve; means obtained by averaging logarithms of values determined from individual curves.

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3.0,
1.5
0.5

FREQUENCY - beats/min

DEVELOPED TENSION - gram/mm²

ANGIOTENSIN 10⁻⁶ M

NO DRUG

106.8 103.6 103.2 91.4 54.1 21.0 14.6

2 6 12 30 60 120 240

** Influence of frequency of contraction on the inotropic action of angiotensin. Mean of 8 kitten papillary muscles, each contracting at 7 frequencies (logarithmic scale). 37°C. Numbers are mean and standard error of angiotensin-induced increase in developed tension, expressed as per cent of control tension.

The inotropic effect of angiotensin on the cat papillary muscle is greatly influenced by the frequency of contraction (fig. 5). The degree of activation of mammalian ventricular muscle increases over a wide range with the frequency of contraction so that the cat papillary muscle develops its highest tension at high contraction frequencies. The absolute and, even more strikingly, the relative increase in the strength of contraction caused by a nearly optimal concentration of angiotensin (10⁻⁶ M) decreases steadily with increasing frequency of contraction (fig. 5).

When cat papillary muscles contracting at a low frequency are cooled from 38 to 32 and 26°C and are allowed to equilibrate at the new temperature, tension development increases with cooling (fig. 6). This increase in contractility results from a greatly increased duration of the active state which more than compensates for a lower rate of development of tension. The positive inotropic effect of angiotensin expressed as per cent of control developed tension decreases with cooling, but

Influence of physical factors on the inotropic action of angiotensin

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the absolute increment in tension development due to the drug increases slightly (fig. 6).

Over a wide range increases in the concentration of calcium in the extracellular fluid raise the tension developed by ventricular myocardium. Increases in the sodium concentration have the opposite effect (fig. 7). Both these changes in contractility are caused by corresponding changes in the degree of activation of the contractile element with little or no alteration in the duration of the active state. In an ionic environment in which the rate of development of tension is low (low Ca or high Na) the inotropic effect of angiotensin is greater than under conditions (high Ca or low Na) in which the degree of activation of the contractile element is already high before the addition of the drug (fig. 7).

Discussion

The results of the present study indicate that the inotropic action of angiotensin on ventricular muscle does not result from release of cardiac catecholamines. If any such release does occur, it is insignificant in comparison with the direct action of angiotensin. The failure of pretreatment with reserpine to influence the positive inotropic effect of angiotensin is in agreement with results in the dog heart-lung preparation. Two reports of partial block of the cardiac effect of angiotensin by DCI are difficult to evaluate since no quantitative data are given. DCI has marked stimulant actions of its own on the myocardium and in its presence positive chronotropic and inotropic responses to many drugs are reduced. This is true even when these compounds act neither directly nor indirectly by stimulating adrenergic β-receptors. The failure of nethalide to alter the inotropic effect of angiotensin confirms that angiotensin is not a releaser of norepinephrine in the heart and also shows that it does not act directly on β-receptors.

In addition to the findings presented, there is much indirect evidence against a significant release of catecholamines from the heart by angiotensin. 1) In isolated preparations the...
positive chronotropic effect of angiotensin is very small compared to its positive inotropic action.\(^6\) \(^7\) 2) Angiotensin has no effect on the cardiac membrane and action potentials and does not depress the ST segment in the electrocardiogram.\(^5\) \(^1\) 3) The drug causes no ectopic impulse formation in isolated heart muscle preparations,\(^7\) a common occurrence when these are exposed to effective catecholamine releasers such as tyramine (fig. 3). 4) Angiotensin increases ventricular contractility far more than that of atrial muscle.\(^7\) 5) The positive inotropic effect of angiotensin is almost completely inhibited by cinnarizine (N-benzyl-N'-cinnamylpiperazine).\(^1\)

The present study shows that angiotensin per se does not release catecholamines from the cardiac stores. Electrical stimulation of the intensity and frequency employed in our experiments does not release significant amounts of endogenous norepinephrine from papillary muscles.\(^1\) Thus, our results do not rule out the possibility that angiotensin may sensitize the receptor to the action of released norepinephrine or that the release of norepinephrine due to stimulation of sympathetic nerves may be increased by angiotensin.\(^2\) \(^0\) \(^2\) \(^2\) \(^2\) Evidence for the operation of either of these mechanisms in the heart has not been found. Rather, Smyth found no enhancement by angiotensin of the actions of epinephrine on the isolated rabbit heart.\(^2\) Furthermore, the release of norepinephrine from sympathetic nerves in the myocardium which results from intense electrical stimulation of heart muscle\(^8\) is not increased by angiotensin (unpublished observation). Angiotensin has been shown to be an effective releaser of catecholamines from the adrenal medullas of dogs and cats\(^2\) \(^4\) \(^2\) \(^8\) and it may also have ganglion-stimulating activity.\(^2\) These effects may account for the cardio-acceleration seen when angiotensin is infused intravenously after the sino-aortic baroreceptors have been inactivated.\(^2\)

Increases in the frequency of contraction and in the external calcium concentration and decreases in sodium concentration all raise myocardial contractility by increasing the degree of activation of the contractile element, i.e., by shifting the force-velocity curve to the right.\(^9\) \(^1\) The positive inotropic effect of angiotensin results from the same change. When heart muscle is contracting under conditions which cause the degree of activation to be high before addition of angiotensin, the drug produces relatively minor increases in tension development (figs. 5 and 7). In other words, the magnitude of the inotropic effect of angiotensin is inversely proportional to the intensity of the degree of activation in its absence. Very similar observations have been made on the inotropic action of the cardioactive steroids.\(^1\) \(^6\)

It is obvious that the degree of activation of heart muscle cannot be increased ad infinitum by combining the influence of various agents and experimental conditions with positive inotropic effects. Several factors may place an upper limit on the amount of tension which a given number of heart muscle fibers can generate.\(^1\) 1) There must be an intrinsic limit to the amount of tension which the contractile elements can develop even under the most favorable circumstances.\(^2\) This limit was never reached in these experiments since at all times further increases in the strength of contraction could be achieved with norepinephrine. 2) The maximum rate at which energy can be supplied to the contractile elements by the metabolic processes can also be a limiting factor.\(^2\) \(^8\) \(^0\) However, under our experimental conditions, papillary muscles contracting at high resting tension and rapid rates are capable of much higher rates of energy expenditure than were ever reached in these experiments. 3) More specific limits on the extent to which a drug can raise the degree of activation of heart muscle may be set by the mechanism of its inotropic effect. Angiotensin may act by increasing in certain cellular sites the concentration or availability of a substance which favors activation of the contractile element. It is possible that angiotensin can raise the concentration of this substance only to a certain level. Alternatively, increasing concentrations of such a substance may not continue to increase contractility once an optimum concentration has been reached.
If increases in calcium concentration and in contraction frequency increase contractility by the same mechanism as does angiotensin, these changes would then be expected to diminish the inotropic effect of the drug.

There is evidence that the positive inotropic effect of increases in external calcium concentration and of decreases in sodium concentration are both the result of increased exchange or accumulation of calcium by heart muscle. The increases in the strength of myocardial contraction with increases in frequency also appear to be due to increased entry of calcium into the muscle fiber. The effects of angiotensin and of increases in external calcium concentration on the degree of activation of the contractile element, on the duration of the active state, and on the interval-strength relationship of cat papillary muscle are very similar. This suggests that angiotensin may also produce its positive inotropic effect by influencing the myocardial exchange and balance of the calcium ion.

Cooling of kitten papillary muscles contracting at a low frequency increases their tension development. In contrast to changes in frequency of contraction and in ionic concentrations, this inotropic effect results from marked prolongation of the active state and occurs despite a considerably lower rate of development of tension. Significantly, this type of increase in contractility does not lead to a decrease in the inotropic action of angiotensin (fig. 6). However, when expressed in terms of the control tension the inotropic effect is greatly influenced by temperature, just as it is by the other experimental conditions studied. The present study again emphasizes the importance of physical factors when the inotropic effects of drugs are studied and compared quantitatively.

**Summary**

The effect of changes in various chemical and physical factors on the positive inotropic action of angiotensin was studied in isolated papillary muscles of kittens. Pretreatment of the animals with reserpine did not diminish the positive inotropic effect of angiotensin. Concentrations of nethalide which strongly antagonize the response of the papillary muscle to levarterenol or tyramine had no effect on the inotropic concentration-effect curve for angiotensin. It is concluded that angiotensin does not release significant amounts of catecholamines from the cardiac stores and that its inotropic action on ventricular myocardium does not result from stimulation of adrenergic β-receptors.

Increases in external calcium concentration, decreases in sodium concentration, and shortening of the interval between contractions diminish both the absolute and the relative inotropic effect of angiotensin. All these maneuvers and angiotensin itself raise myocardial contractility by increasing the degree of activation of the contractile element. Cooling of papillary muscles does not decrease the absolute inotropic effect of the drug. Angiotensin and increases in external calcium concentration affect the mechanical properties of papillary muscles in a similar fashion. Angiotensin may raise contractility by augmenting the entry of Ca++ into the myocardial fiber.

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