Direct myocardial effects of angiotensin II

PETER J. DEMPSEY, ZENA T. MCCALLUM, KENNETH M. KENT, AND THEODORE COOPER
National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014

DEMPSEY, Peter J., ZENA T. MCCALLUM, KENNETH M. KENT, AND THEODORE COOPER. Direct myocardial effects of angiotensin II. Am. J. Physiol. 220(2): 477–481. 1971.—There still remains speculation concerning the existence of a direct effect of angiotensin II on ventricular myocardium. Evaluation has been complicated by the sensitivity of the peripheral circulation in intact preparations, reflex mechanisms, and disagreement concerning the role of ganglia and endogenous catecholamine stores. Two series of experiments were designed that used hearts from normal cats and from cats that had undergone extrinsic cardiac denervation to deplete catecholamines. In the first series, 19 isolated perfused hearts were studied in a Langendorff apparatus. In the second series, tension and transmembrane action potentials were recorded from 20 right ventricular papillary muscles. In normal and denervated ventricular myocardial angiotensin (10^{-19} \text{g/ml} -10^{-4} \text{g/ml}) elicited a positive inotropic effect characterized by an increased maximum rate of tension rise and rate of relaxation, but not in a shortened time to peak tension. A change in the form of phase 2 of the action potential was noted. These data indicate that angiotensin elicits a direct positive inotropic effect which is independent of intact adrenergic nerves or endogenous catecholamine stores.

**Methods**

**General**

Thirty-three adult male cats weighing 2.2–4.1 kg were used for this study. Twelve cats were subjected to extrinsic cardiac denervation by the technique of mediastinal neural ablation (2). The operations were performed through a right lateral thoracotomy under halothane anesthesia. Cats with extrinsic cardiac denervation were studied between 22 and 60 days after operation.

Completeness of the denervation was verified by measurement of catecholamine content of samples of ventricular myocardium by fluorometric methods (3).

**Isolated Whole Heart**

The cats were anesthetized with sodium thiamylal (Surital) 30 mg/kg, and their hearts were removed quickly and mounted on a cannula. Retrograde perfusion of the coronary arteries was carried out at flow rates of about 20 ml/min with a modified Krebs-Ringer solution of the following composition in millimoles per liter: \(\text{Na}^+, 146; \text{K}^+, 3.6; \text{H}_2\text{PO}_4^-, 1.2; \text{Ca}^{2+}, 2.5; \text{Mg}^{2+}, 1.2; \text{Cl}^-, 128; \text{SO}_4^{2-}, 1.2; \text{HCO}_3^-, 25; \text{glucose} 5.6. \) The pH was 7.4, and the temperature was \(34 \pm 0.5 \text{C} \). The solution was oxygenated with 95% \(\text{O}_2\), 5% \(\text{CO}_2\). Fluid was not recirculated.

Mean perfusion pressure was monitored with a Statham P23 Db strain gauge through a sidearm on the perfusion cannula and was maintained at 28 mm Hg. Peak pressure developed by the isovolumically beating left ventricle was used as an index of contractility. For this purpose a small latex rubber balloon, mounted on an 18-gauge cannula, was introduced through a purse-string suture into the left ventricle and filled with a small amount of saline. The pressure developed in the balloon was measured by means of a Statham P23Db strain gauge. A previous study demonstrated that within a wide range, variation in balloon pressure was not an important determinant of the magnitude of the observed responses (4). The first derivative of the pressure tracing (dp/dt) was monitored on line by means of an active R C circuit differentiator with a time constant of 1 msec. The perfusion pressure, balloon pressure, and dp/dt were recorded simultaneously on a direct-
writing oscillograph. Angiotensin II was added to a volume of perfusate just prior to a run so that a perfusion with a constant concentration was achieved.

With the perfusion pressure monitored closely, these preparations were stable for up to 3 hr, although the experiments reported seldom extended over 2 hr. The reactivity of the Langendorff preparation was verified by reproducibility of dose-response curves to standard inotropic agents, such as calcium after varying lengths of time.

**Isolated Papillary Muscle**

The cats were anesthetized as before, the hearts were removed, and the thin right ventricular papillary muscle was removed and placed in a horizontal 25-ml Lucite muscle bath. One end was held in a fixed position, and the other end attached to a Minneapolis-Honeywell force-displacement transducer for measurement of tension. The first derivative of the tension tracing was monitored on line as described above. The muscle bath was perfused with a Krebs-Ringer solution of the same composition as above at a rate of 10 ml/min. Temperature was 25 ± 0.5°C. The muscles were stimulated to contract isometrically at 12 beats/min by means of fine platinum electrodes placed in close proximity on either side of the base of the muscle. Voltage used was 3–5% above threshold. Action potentials were recorded by means of 3 M KCl-filled glass microelectrodes, handpulled, so that the tip resistance was from 5 to 20 megohms. The recording system consisted of a Bak standard wide-band electrometer (Electronics for Life Sciences) and a Tektronix model 502 dual-beam oscilloscope. All data during an experimental run were recorded on a multichannel Hewlett-Packard 3955 tape system.

**Drugs**

Drugs used were angiotensin amide (supplied as Hypertensin-Ciba), phentolamine methanesulfonate, dl-propranolol, atropine sulfate, and lidocaine hydrochloride. Doses were expressed as the salt.

**Statistical Analysis**

In the Langendorff preparation, results were expressed as percentage of the maximum response. The maximum response of each heart was obtained at some time during each run by either Ca++ or norepinephrine. The results of the run were then expressed as a percent of this maximum response, and thus each heart served as its own control. Papillary muscle results were expressed as percent change from control.

Group means were compared with the Student t test, and the statistical significance of the differences was expressed as a P value.

**RESULTS**

**Langendorff Isolated-Heart Perfusion**

A total of 19 cats (13 normal and 6 denervated) was used for this part of the study. In both normal and chronically denervated hearts, angiotensin always elicited a positive inotropic effect in doses from $10^{-10}$ to $10^{-6}$ g/ml (Fig. 1). This positive inotropic effect was dose related, and was characterized by an increase in the maximum dp/dt, but not in a shortened time to peak pressure development. As can be seen from Fig. 2, angiotensin also changed the pattern of relaxation. Specifically, the velocity of relaxation (the negative phase of the dp/dt tracing) was increased. The total contraction time from the onset of tension development to return to base line was not altered by angiotensin.

Since the base-line parameters of isovolumic pressure and...
FIG. 3. Dose-response curves for angiotensin in isolated perfused heart. Ordinate: percent of maximum response of left ventricular isovolumic pressure on the left, and of the dp/dt on the right. Abscissa: dose of angiotensin in g/ml.

Myocardial Norepinephrine Stores

Myocardial norepinephrine content of the chronically denervated myocardium was 0.01 ± 0.008 µg/g as compared with 1.88 ± 0.03 µg/g in normal myocardium.

DISCUSSION

These results indicate that angiotensin II within a wide dose range is capable of eliciting a direct positive inotropic effect on mammalian ventricular myocardium which is independent of intact adrenergic ganglia, nerves, or endogenous catecholamine stores. The dose-response curves for two quite different types of experimental preparations showed a high degree of correlation in magnitude of response as well as dose ranges. The data pertaining to electrophysiology serve to strengthen this argument, for it would seem unlikely that a substance which has no direct effect on the myocardial cell would produce a change in the action potential which is totally coincident in time with the positive inotropic effect, and which is seen in the normal and catecholamine-depleted hearts alike. In addition, since phentolamine, propranolol, and atropine failed to modify the observed responses, it is unlikely that they were mediated through components of the autonomic nervous system.
changes, however, are usually changes of duration (mainly action potential of ventricular myocardium (9). These of phase 2 and phase 3), not of form. It would seem, there-
fore, that the observed change is due to angiotensin itself
and not secondary to the inotropic effect produced by this
hormone.

In relation to the electrophysiologic findings, it should
be emphasized that the change in the action potential
occurred simultaneously with the onset of the positive
inotropic effect. It has been previously demonstrated that
changes in inotropic state alone may effect changes in the
action potential of ventricular myocardium (9). These
changes, however, are usually changes of duration (mainly of phase 2 and phase 3), not of form. It would seem, therefore, that the observed change is due to angiotensin itself and not secondary to the inotropic effect produced by this hormone.

As originally pointed out by Koch-Weser (12), the posi-
tive inotropic effect in papillary muscles is characterized
by an increased rate of tension development, but not by a
shortened time to peak tension. This study confirms this
and also demonstrates that the same pattern of response is
true in a whole-heart preparation. In addition, it was ob-
erved that the rate of relaxation increases, as well as the
rate of contraction, both in the papillary muscle and in the
whole heart. Koch-Weser had also observed that cooling
did not alter the absolute inotropic effect, and in the present
studies both the papillary muscle at 25 C and the whole
heart at 34 C did in fact exhibit responses of comparable
magnitude to angiotensin.

The conclusion by some authors that the cardiac effects
of angiotensin are in some way indirect has drawn apparent
support from the fact that angiotensin stimulates the adre-
nal medulla, causing a release of catecholamines (15), and
that it also increases levels of plasma and myocardial
catecholamines following an intravenous infusion in intact
dogs (14). More recently, Farr and Grupp (6) have stated
that, in the intact dog, the cardiac effects of angiotensin
are caused by stimulation of the caudal cervical ganglia.

The present experiments have eliminated the compli-
cating factors of reflex activities, sympathetic ganglia, and
endogenous catecholamine stores. We wish to emphasize,
however, that these results certainly do not obviate the
possibility of an additional component in intact animals
which could result from ganglionic stimulation, or other
types of indirect reflex stimulation.

The authors express their appreciation to Mr. James Hopkins
and Mr. James Dickens for their technical help at surgery.

Received for publication 3 June 1970.

REFERENCES

1. Åersen, J. W., and E. Reiff. Stimulation of the cat stellate ganglion
2. Cooper, T. T. W., Gilbert R. D. Bloodwell, and R. Croft.
Chronic exsanguine cardiac denervation by regional neural abla-
4. Dempsey, P. J., and T. Croft. Supersensitivity of the chronically
administration of angiotensin II on ventricular performance.
7. Fowler, N. O., and J. G. Holmes. Coronary and myocardial
8. Frank, M. J., N. Manoucheri, P. Ganesan, P. Stein, and

FIG. 6. Dose-response curves for angiotensin in papillary muscle.
Ordinate: percent change from base line of tension on left, and of dT/dt
on right. Abscissa: dose of angiotensin in g/ml.

TABLE 1. Effect of angiotensin on transmembrane
action potential at 25 C

<table>
<thead>
<tr>
<th>Measurement</th>
<th>10^-11 g/ml</th>
<th>10^-9 g/ml</th>
<th>10^-7 g/ml</th>
<th>10^-5 g/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Total action potential voltage, mv</td>
<td>108</td>
<td>105</td>
<td>107</td>
<td>105</td>
</tr>
<tr>
<td>50% Repolarization time, msec</td>
<td>±11</td>
<td>±11</td>
<td>±11</td>
<td>±11</td>
</tr>
</tbody>
</table>

Values are means ± SE of 64 impalements in 9 papillary muscles. A: control. B: after angiotensin. * P < .02; all other values are not significantly different.

FIG. 7. Unretouched tracings of response of action potential of a normal papillary muscle to angiotensin 10^-7 g/ml. Left panel: control tracing. Right panel: tracing following angiotensin.

FIG. 8. Unretouched tracings of response of action potential of a chronically denervated papillary muscle to angiotensin 10^-7 g/ml. Left panel: control tracing. Right panel: tracing following angiotensin.


