Direct myocardial effects of angiotensin II

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An early report by Hill and Andrus (11) and later reports by Vogin and Buckley (17) and Koch-Weser (12) presented evidence that angiotensin possessed a direct myocardial effect. Subsequently, however, reports have appeared that attribute changes in cardiac parameters following administration of this hormone to the effects of peripheral vascular reflexes (10), ganglionic stimulation (1, 6, 13), or release of catecholamines from the intramyocardial adrenergic nerve terminals (14, 16). In addition, a negative inotropic effect has been attributed to angiotensin (5, 8).

The chronically denervated, catecholamine-depleted heart offered a unique opportunity to study the direct effect of angiotensin on myocardial tissue, and determine more precisely the role played by intrinsic stores of catecholamines. Although some previous work had been done using reserpine pretreatment (7, 10, 12), its direct effects on the myocardium, other than catecholamine depletion, have occasionally made interpretation of results difficult. The present study consists of two series of experiments that used hearts from normal cats and from cats that had undergone extrinsic cardiac denervation to deplete the myocardial catecholamine stores. In one series, isolated perfused hearts were studied by means of a modified Langendorff apparatus. In the second, tension as well as transmembrane action potentials were recorded from isolated right ventricular cat papillary muscles.

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: Na+, 146; K+, 3.6; H2PO4−, 1.2; Ca++, 2.5; Mg++, 1.2; Cl−, 128; SO4−, 1.2; HCO3−, 25; and glucose 5.6. The pH was 7.4, and the temperature was 34 ± 0.5°C; the solution was oxygenated with 95% O2-5% CO2. 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 P23 Db 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 ± .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 baseline was not altered by angiotensin.

Since the baseline parameters of isovolumic pressure and
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In blocking the given type of autonomic response in this Langendorn preparation.

Action Potentials

Table 1 summarizes the electrophysiology data obtained. As can be seen resting potential and total voltage were unchanged following any of the doses tested. The 50% repolarization time was lengthened only at the highest dose given, and is consistent with the major finding. Within the dose ranges tested, angiotensin produced a change in the configuration of phase 2, the plateau phase, in action potentials of both normal (n = 5) and denervated (n = 4) papillary muscles (Figs. 7 and 8). This occurred at the same time as the positive inotropic effect was taking place. This change can best be described as an exaggeration or a “peaking” of the plateau phase. This peaking was most pronounced at the highest dose, and, therefore, accounts for the slight prolongation of the 50% repolarization time. It should be emphasized that, although the peaking is present at lower dose ranges of angiotensin, it is not reflected in the measured parameters in Table 1.

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 and not secondary to the inotropic effect produced by this fore, that the observed change is due to angiotensin itself but not by a.

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 positive inotropic effect in papillary muscles is characterized by an increased rate of tension development, but not by a.

In the case of angiotensin, the inotropic effect is also demonstrated that the same pattern of response is true in a whole-heart preparation. In addition, it was observed 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 adrenal 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 complicating 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.

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REFERENCES

8. Frank, M. J., N. Manouchehr, P. Gaasbecka, P. Stein, and


