Enhancement of the Inotropic Action of Glucagon by Small Concentration of Angiotensin

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SUMMARY

Under pentobarbital sodium anesthesia and bilateral vagotomy, studies were carried out in 2 groups of 3 dogs and another group of 4 and 7. Group 1 (4 dogs): Continuous i.v. infusion of angiotensin (5 ng/Kg/10 ml/min) produced no effects on LV contractility (LV dp/dt, maximum aortic flow velocity), LV stroke volume, heart rate or mean systemic BP. Group 2 (7 dogs): This concentration of angiotensin produced a greater increase in LVdp/dt following i.v. glucagon than those produced by glucagon alone. This potentiation with angiotensin, however, did not occur using epinephrine or isoproterenol, doses of which were chosen to provide a similar increase in LVdp/dt. Augmentation by angiotensin of chronotropic effects of these 3 drugs was not observed. Group 3 (3 dogs): Modification by angiotensin of inotropic effect of glucagon could be reproducible in propranolol pretreated dogs, and in 6 papillary muscle preparations (Group 4-3 dogs). The results suggest that small concentration of angiotensin (probably physiological level) may act on the cardiac contraction by indirect fashion.

Additional Indexing Words:

Beta adrenergic blockade Catecholamine Calcium ion Cyclic
3', 5' AMP Essential hypertension Cardiac output

ANGIOTENSIN has been known as a potent vasopressor agent which acts directly on the arteriolar smooth muscles. Other actions of this agent have been demonstrated: Angiotensin, for example, acts to release adrenal catecholamines¹⁾ and stimulates both the central²⁾⁻⁴⁾ and peripheral⁵⁾⁻⁸⁾ nervous systems. Recently, angiotensin has also been shown to produce a significant positive inotropic effect on mammalian ventricular myocardium.⁹⁾⁻¹³⁾ The

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This study was supported by research grants from the U.S. Public Health Service, National Institute of Health (HE 05410), and the Heart Association of Upstate New York, Inc. (PN-10046).

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This work was in part presented at the 43rd Scientific Session of the American Heart Association in November 1970 at Atlantic City, N.J.

Received for publication February 7, 1973.

vasopressor and the positive inotropic actions are of importance in the pathogenesis of high cardiac output hypertension as demonstrated in the chronic stage of Goldblatt animals^{14),15)} or in some groups of patients with renovascular,¹⁶⁾ or essential hypertension.¹⁷⁾

However, no increase in blood angiotensin activity could be demonstrated, and this led us to speculate that a low level of angiotensin (probably variations within physiological levels) may play a role in this type of hypertension. Actually, angiotensin doses, either insufficient to produce a pressor effect or much less concentrated (approaching circulating physiological levels) have been demonstrated to produce a stimulant effect on peripheral alpha adrenergic vasoconstrictor mechanisms,^{6),8),18)–20)} and to facilitate the sympathomimetics at various organ levels.^{21)–24)} There have been no systematic studies of the effect of low concentration of angiotensin on the heart. For this reason, this study was designed to determine the cardiac action of low concentration of this agent.

METHOD

A total of 17 adult mongrel dogs weighing between 13 and 26 Kg were studied. Experiments were performed in 4 groups. There are 2 groups of 3 dogs and another group of 7 and 4.

Dog with open heart preparation—Group 1 (4 dogs): Hemodynamic effects of small doses of angiotensin were studied. Dogs were anesthetized with intravenous pentobarbital sodium (30 mg/Kg). The cervical vagi were severed bilaterally. Positive pressure respiration was maintained by a Harvard respirator pump. Systemic arterial pressure was measured from the femoral artery using a Statham model P23-Db strain gauge. The chest was opened through a midline incision, the pericardium opened and the heart suspended in a pericardial cradle. The left ventricular (LV) intracavitary pressure was obtained by a large bore cannulae inserted into the ventricle through the apical dimple. The output on LV pressure was connected to an R.C. circuit in order to measure the maximum rate of LV pressure rise (LVdp/dt). An electromagnetic flowmeter (Statham model 4001), placed around the root of aorta, sensed aortic flow velocity. This signal was integrated to give LV stroke volume. The zero flow was taken as the diastole. Changes in LV contractility was assessed by either LVdp/dt or aortic flow velocity and expressed as percentage change from control which was taken as 100 percent. Mean systemic arterial pressure, LVdp/dt, aortic flow velocity, LV stroke volume and electrocardiogram were recorded simultaneously using an Electronics for Medicine model DR-8 multichannel oscilloscopic photographic recorder at a paper speed of 100 mm/sec. After the control tracing, angiotensin (valyl-5-octa peptide, Ciba) in the doses of 5 ng/Kg/10 ml/min was administered intravenously. Recordings were made repeatedly during a 3 hours period of the infusion, which was subsequently followed by 50, 100, 50 ng/Kg/10 ml/min of angiotensin.

Dog with intact circulation—Group 2 (7 dogs): Modification by angiotensin of the cardiac effects of epinephrine, isoproterenol and glucagon was studied. Anes-

thesia, vagotomy and measurement of systemic arterial pressure were made by similar techniques as described in the previous group. The left ventricular pressure was obtained by a catheter inserted into the ventricle through the right common carotid artery, and $LVdp/dt$ was measured by a similar method. Heart rate was measured with an Electronics for Medicine model cardiograph. Mean systemic arterial pressure (MSAP), $LVdp/dt$ and heart rate were simultaneously recorded using the recorder at a paper speed of 1 mm/sec. After the control tracing, epinephrine (0.01, 0.1, or 1.0 $\mu g/Kg$), isoproterenol (0.01, 0.03, or 0.05 $\mu g/Kg$) or glucagon (1, 4, or 16 $\mu g/Kg$) was administered intravenously, and the changes in $LVdp/dt$, heart rates or MSAP to each dose were measured. These doses of 3 different drugs were chosen in order to provide a similar concentration-dependent change in $LVdp/dt$ and HR. Repeated injections of each subsequent dose or drug were only made when all data had returned to control. After these measurements, all data were allowed to return to control, when the infusion of angiotensin (5 $\mu g/Kg/10$ ml/min) was carried out. During angiotensin infusion, similar injection of 3 drugs with 3 different doses were repeated. Group 3 (3 dogs): Effects of propranolol on the effect of angiotensin plus glucagon were evaluated. The response to glucagon during angiotensin infusion obtained in the previous group was compared with that obtained in dogs which were pretreated by propranolol (0.5 mg/Kg).

Dog papillary muscle preparation—Group 4 (3 dogs): Effects of glucagon singly or used in combination with angiotensin on the development tension of papillary muscles were evaluated in 6 papillary muscles. Dogs were anesthetized with pentobarbital sodium (30 mg/Kg i.v.). Either right or left ventricular papillary muscles were removed rapidly and suspended in a 30 ml organ bath by a silver wire. One end attached to a muscle holder and stimulator, and the other to a force displacement transducer (Grass model FT 10 C) model. The papillary muscles were placed under a resting tension of 2 Gm and stimulated through the electrode with a Grass model S4 stimulator at a rate of 30/min, with square wave stimuli of 10 msec duration at a voltage of 25% above threshold. Recordings were made on a Grass model 7 recorder at a paper speed of 1 mm/sec. The organ bath used in the present study was of following composition expressed as Gm/L; $CaCl_2 \cdot 2H_2O$, 0.24; KCl 0.42; dextrose 2.0 and $NaHCO_3$, 0.5 which was equilibrated with 95% O_2 -5% CO_2 and had a pH of 7.4. The changes in the development tension by adding glucagon (0.05, 0.2, and 0.8 $\mu g/ml$) into the solution were compared with those of angiotensin (1 ng/Kg) alone and those of glucagon and angiotensin in combination.

RESULTS

Hemodynamic effects of low concentration of angiotensin—(Group 1 dogs): Intravenous infusion of angiotensin at a rate of 5 ng/Kg/10 ml/min during a period of 3 hours did not produce any changes in the left ventricular contractility as assessed by $LV dp/dt$ or maximum aortic velocity, LV stroke volume, heart rate or mean systemic arterial pressure. When more concentrated doses (50, 100, 500 ng/Kg/10 ml/min) were used for the infusion, a dose dependent increases in systemic arterial pressure were obtained (13 mmHg $\pm$

2.7 SE, 27 mmHg $\pm$ 5.8 SE, and 41 mmHg $\pm$ 6.6 SE). This BP increases manifested within several minutes, the peak responses attained and stabilized in 30 min. At this time, the heart rate increased (11 beats/min $\pm$ 3.2, 18 beats/min $\pm$ 5.1 and 9 beats/min $\pm$ 3.6), while both LV dp/dt and LV stroke volume were unaffected. This BP elevation, therefore, appears to be due to an increase in peripheral vascular resistance, in which the changes in cardiac output may have a minor contribution,

Modification by angiotensin of inotropic and chronotropic effects of epinephrine, isoproterenol and glucagon—(Group 2 dogs): Each dose of epinephrine, isoproterenol and glucagon was chosen to provide a similar increase in LV dp/dt and the heart rate. The increases in LV dp/dt and heart rate manifested within 5 sec after the injection and returned to control levels in 5 min. On the contrary, the response to glucagon lasted approximately 60 min following injection. Therefore, glucagon administration was made in cumulative doses. Each dose of glucagon was given when the peak inotropic and chronotropic effects to the previous dose had been attained. The interval between each injection was approximately 10 min. The increases in heart rate and LV dp/dt produced by epinephrine, isoproterenol and glucagon, administered singly or in combination with low concentration of angiotensin (5 ng/Kg/10 ml/min) were compared and shown in Table I. There were no significant differences between the positive inotropic effects of epinephrine or isoproterenol, used singly

Table I. Effects of Epinephrine, Isoproterenol and Glucagon on LV dp/dt and Heart Rate Before and During Infusion of Small Angiotensin Doses (5 ng/Kg/10 ml/min)

Dose (μ g/Kg)	Mean increase in LV dp/dt (%)			Mean increase in heart rate (beats/min)		
	Before	During	P value	Before	During	P value
Epinephrine						
.01	14 $\pm$ 3.1	13 $\pm$ 2.7	> .1	13 $\pm$ 2.1	15 $\pm$ 4.1	> .1
.1	40 $\pm$ 5.1	38 $\pm$ 3.3	> .1	53 $\pm$ 4.6	48 $\pm$ 5.6	> .1
1.0	50 $\pm$ 8.4	60 $\pm$ 9.8	> .1	78 $\pm$ 6.3	70 $\pm$ 7.1	> .1
Isoproterenol						
.01	18 $\pm$ 3.1	16 $\pm$ 4.2	> .1	17 $\pm$ 3.2	19 $\pm$ 4.3	> .1
.03	43 $\pm$ 7.2	39 $\pm$ 5.6	> .1	58 $\pm$ 5.1	60 $\pm$ 6.8	> .1
.05	55 $\pm$ 8.3	57 $\pm$ 9.3	> .1	80 $\pm$ 7.2	79 $\pm$ 9.3	> .1
Glucagon						
1.0	16 $\pm$ 3.2	27 $\pm$ 4.1	< .05	15 $\pm$ 2.1	17 $\pm$ 4.3	> .1
4.0	41 $\pm$ 6.4	59 $\pm$ 5.8	< .05	50 $\pm$ 4.1	48 $\pm$ 7.6	> .1
16.0	51 $\pm$ 7.3	81 $\pm$ 9.8	< .05	70 $\pm$ 6.5	81 $\pm$ 9.8	> .1

The values are the means $\pm$ SE obtained from a group of 7 dogs.

or during infusion of angiotensin. However, the increase in LV dp/dt which was produced by glucagon during angiotensin infusion was much greater than that obtained by glucagon alone. This difference was statistically significant ($P < .05$). The differences between the positive chronotropic actions of each 3 drugs, used singly and during angiotensin infusion, were insignificant. The mean blood pressure responses to the doses of each drug used singly were transient, were seen within 20 sec after injection, and consisted of average decrease of 3 mmHg $\pm$ 0.8 SE (epinephrine), 11 mmHg $\pm$ 2.3 SE (isoproterenol) and 6 mmHg $\pm$ 1.7 SE (glucagon). When these drugs were administered during infusion of low concentration of angiotensin, the mean BP changes by epinephrine or isoproterenol were similar to those obtained by single injection. Glucagon, during infusion of angiotensin, however, produced an increase in mean BP by an average of 8 mmHg $\pm$ 2.1 SE. Since this BP elevation was dose dependent and occurred with simultaneous potentiation of the left ventricular contractility (LV dp/dt), the increase in BP may be due to an increase in cardiac output, although the latter was not measured in this study.

Effect of propranolol on response to glucagon in combination with low concentration of angiotensin—(Group 3 dogs): The ability of glucagon, used alone, to produce positive inotropic and chronotropic effects was demonstrated in 4 dogs with propranolol (0.5 mg/Kg) pretreatment. The potentiation of the positive inotropic effect of glucagon by low concentration of angiotensin (5 ng/Kg/10 ml/min) could also be reproduced. This potentiation was similar to that obtained in dogs without propranolol (Group 2 dogs) as described above. The increases in heart rate which were produced by glucagon alone were 7 beats/min $\pm$ 1.1 SE, 31 beats/min $\pm$ 2.6 SE, and 66 beats/min $\pm$ 5.8 SE in dogs with propranolol, which were slightly lower than those observed in dogs without propranolol (Group 2 dogs). The modification of the chronotropic effect of glucagon by angiotensin could likewise not be demonstrated.

Responses to glucagon, singly or in combination with angiotensin in papillary muscle preparation—(Group 4 dogs): Six papillary muscles of 3 dogs were exposed to 3 different doses of glucagon (0.05, 0.2, and 0.8 μ g/ml). These glucagon concentrations were chosen to provide a similar positive inotropic response as obtained in Group 2 dogs. The isometric tension increased progressively as the concentration of glucagon was increased. This inotropic effect was sustained but it could be reversed by washing the muscle with a glucagon free medium. Repeated exposure to glucagon resulted in a similar increase in the isometric tension. The exposure of this muscle to the solution which contained angiotensin at the concentration of 0.1 ng/ml did not provide any effect on the isometric tension. The addition of glucagon of similar doses in this solution, however, resulted in a greater augmentation of isometric ten-

Fig. 1. Effect of an increase in the concentration of glucagon upon the development of isometric tension in the isolated dog papillary muscle. Solid line=glucagon, Dotted line=glucagon after angiotensin (0.1 ng/ml) was in the bathing solution, vertical line=SE.

sion when compared with those occurring with glucagon alone. This difference was statistically significant ($P < 0.001$). These responses to glucagon alone and to glucagon in combination with angiotensin were compared and illustrated in Fig. 1. The recording shown in Fig. 2 is the one of the typical tracings and demonstrates the action of angiotensin for the enhancement of the inotropic effect of glucagon. These effects could be reproduced and the removal of angiotensin from the solution provided a similar responses to the subsequent addition of glucagon as those observed in these muscles during the initial exposure to glucagon.

Fig. 2. Dog papillary muscle myogram showing the responses to glucagon, angiotensin, singly and in combination. The positive inotropic effect after glucagon, used alone (panel A) is significantly potentiated by angiotensin (panel C), which did not produce any effect when used singly (panel B).

DISCUSSION

Angiotensin, in doses which produce a pressor effect when given intravenously, has been known to produce a positive inotropic effect on the heart. This effect was demonstrated in the papillary muscles of experimental animals.⁹⁾⁻¹³⁾ In the intact circulation, however, this effect could be greatly modified by the baroreceptor responses occurring simultaneously with the

elevation of systemic arterial pressure.^{12), 13), 25)–28)} The pressor effects produced by intravenous administration of angiotensin are not accompanied by an increase either in myocardial contractility or cardiac output for this reason. Therefore, the increase in vascular resistance appears to be a primary factor responsible for the elevation of systemic arterial pressure. Since the action of angiotensin on myocardial contraction has been only significant at high concentrations which produce pressor effect, it is obscure whether the positive inotropic action of this agent has any contribution to the overall circulatory response to this agent. Recently, however, Koch-weser¹⁰⁾ has reported that an augmentation of the development tension of cat papillary muscles could be produced by much less concentrated angiotensin doses (10^{-8} and 10^{-9} M). These angiotensin concentrations appear to be insufficient to raise systemic arterial pressure when such concentration is achieved by intravenous administration. In the present study, similar low angiotensin doses were given intravenously in dogs with intact circulation. Three hours of infusion, however, produced no measurable changes in LV dp/dt, LV stroke volume or systemic arterial pressure. Dickinson and Lawrence²⁹⁾ in rabbits and McCubbin et al³⁰⁾ in unanesthetized dogs have experimentally produced a sustained hypertension by several days' continuous intravenous infusion of low angiotensin doses, which was insufficient at the beginning to raise arterial blood pressure. They noted that this hypertension was not accompanied by any increases in cardiac output. From these data, it seems unlikely that there is any direct action of angiotensin on the myocardial contractility to account for an increase in cardiac output and participate in the elevation of systemic arterial pressure.

It has been demonstrated that small doses of angiotensin are capable of facilitating the action of sympathomimetics at various organ levels,^{21)–24)} although such angiotensin doses alone do not produce any hemodynamic changes. Several workers have reported that subpressor amounts of angiotensin enhance the vasoconstrictor effect of endogenous norepinephrine,^{6), 8), 18)–20)} and have postulated that such action of low angiotensin doses on the peripheral alpha adrenergic vasoconstrictor mechanism may be of importance regarding the pathogenesis of essential hypertension. There is controversy regarding similar action of angiotensin in enhancing the stimulant effect of inotropic agents on the heart. Beulnes et al²¹⁾ have observed the enhancement by small doses of angiotensin on cardiac action of norepinephrine, metaraminol and tyramine. Koch-weser,¹⁰⁾ however, has described that angiotensin does not release catecholamines from cardiac stores and does not stimulate adrenergic beta receptors. Peach et al²²⁾ have shown that very small doses of angiotensin inhibit norepinephrine uptake in the heart and produce an ino-

tropic effect. Smyth²³⁾ has failed to demonstrate the enhancement by angiotensin of positive inotropic effect of epinephrine on the heart. In the present study, angiotensin did not show any significant potentiation of inotropic effect of either epinephrine or isoproterenol. However, it significantly augmented the action of glucagon on LV dp/dt, although such doses of angiotensin alone as used in this study did not produce any hemodynamic changes. Since this augmentation could be reproduced in dogs pretreated with propranolol, small doses of angiotensin may act on the heart in indirect fashion and this action appears to be independent of beta adrenergic receptor sites, which is in agreement with previous observation.^{10),11)} Since glucagon and epinephrine have a similar metabolic action,³¹⁾ the differences found between these 2 agents are difficult to explain. Recent studies, however, have demonstrated that the inotropic effects of these 2 drugs are not mediated by the same putative receptor sites.^{32)–34)} The Ca^{++} exchangeability on excitation-contraction coupling in cardiac muscles could involve several components of Ca^{++} exchange,^{35),36)} the one of which influenced by catecholamines may be different from those which are affected by glucagon, although both agents increase cyclic AMP concentration and are dependent on Ca^{++} for the conversion of phosphorylase b to phosphorylase a.^{31)–34)}

Using papillary muscle preparations, the augmentation of the inotropic effect of glucagon could be reproduced in the present study. The doses of angiotensin used for this study did not produce any changes on the development of tension in papillary muscles when used alone. These doses were chosen to provide similar concentrations as achieved by intravenous administration, and were very small, probably approaching the circulating physiological level.³⁷⁾ This mechanism of action of angiotensin, if applicable to those postulated for the nature of inotropic action of this agent, would be compatible with those made by Koch-weser.¹⁰⁾ He postulated that angiotensin may raise contractility by augmenting entry of Ca^{++} into the myocardial fiber. These small amounts of angiotensin and glucagon may interact through a common pathway at a Ca^{++} exchangeability on excitation contraction coupling mechanism.

Zimmerman⁸⁾ and Hodge et al³⁷⁾ have postulated that physiological amounts of angiotensin may act to regulate vascular tone by facilitating biochemical stimuli in several vascular beds. From the data obtained in the present study, it is possible to speculate that angiotensin affects both vascular smooth muscles and ventricular myocardium at high concentrations, producing its pressor effect; yet it acts on both vascular beds and cardiac muscles by indirect fashion. The latter may be of importance regarding its role in the regulation of vascular tone and cardiac output. Some abnormality in this re-

gulatory mechanism might be related to the etiology of high blood pressure occurring in association with increased cardiac output either in the chronic stage of experimental Goldblatt animals, or renovascular or labile hypertension in man.

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