Effect of Angiotensin on the Cardioaccelerator Response to Sympathetic Nerve Stimulation in Isolated Rabbit Hearts[1] (35152)

J. L. THOMPSON[2]
(Introduced by W. B. Youmans)
Department of Physiology, University of Wisconsin Medical School, Madison, Wisconsin 53706

Angiotensin is known to influence the cardiovascular system in a number of different ways. In addition to its powerful direct vasoconstrictor properties, angiotensin affects the cardiovascular system by interacting with the sympathoadrenal system at various sites. Angiotensin is a potent releaser of catecholamines from the adrenal medullae of some species (1); it stimulates the sympathetic nervous system at central sites (2), as well as at some ganglia (3, 4); and also it has been reported that angiotensin interacts with some adrenergic neuroeffector sites in the peripheral circulation (5, 6).

Reports concerning the interaction of angiotensin with cardiac neuroeffector sites have been variable. Krasney *et al.* (7) observed consistent cardioacceleration after intracoronary injections of angiotensin in the dog. They concluded that this acceleration was dependent on an intracardiac source of catecholamines. However, other investigators have reported that the release of catecholamines at cardiac neuroeffector sites was not altered during sympathetic nerve stimulation by angiotensin treatment (8, 9). The experiments described here were designed to clarify the nature of the interaction of angiotensin with cardiac neuroeffector sites.

Methods. The innervated, Langendorff perfused hearts of 15 Dutch rabbits, ranging in weight from 1.5 to 2.5 kg, were used in this study. A modified Tyrode solution (NaCl 137, KCl 2.7, $CaCl_2$ 2.7, $MgCl_2$ 0.5, NaH_2PO_4 0.9, $NaHCO_3$ 12.0, dextrose 5.5 m*M*/liter, equilibrated with 95% O_2–5% CO_2) containing 2 μg/ml of atropine was pumped from a reservoir by a Harvard multispeed peristaltic pump through a Thelco precision water bath and delivered at 37° to the ascending aorta by means of a cannula inserted through the left common carotid artery. A cannula for recording perfusion pressure was inserted through the right common carotid artery. Other vessels were tied off so that all of the perfusion fluid was forced through the coronary arteries. The heart remained *in situ*. A force transducer was attached to the apex of the heart, and tension development, perfusion pressure, and heart rate were recorded on a Gilson polygraph. Once the infusion rate was initially adjusted, it was kept constant throughout the experiment. The infusion rates ranged from 10.5 to 15 ml/min.

Stimulation of the sympathetic nerves to the heart was accomplished by dissecting the right caudal cervical ganglion, and placing a bipolar platinum stimulating electrode under branches leaving this ganglion. An American Electronics Laboratory stimulator was used to deliver supramaximal square-wave pulses of 1-msec duration at a frequency of 2/sec. In all 15 preparations studied, 30-sec periods of sympathetic nerve stimulation were given at 5-min intervals. In three of these preparations, the heart rate response to stimulation at 5-min intervals was observed for 60 min to determine if there was any noticeable change in response with time. In the other 12, after two to four initial stimulation periods, an angiotensin (Hypertensin, Ciba) infusion (5 ng/ml) was begun. Three periods of

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[2] Present address: Department of Pharmacology, Medical Research Laboratories, Pfizer, Inc., Groton, Conn. 06340.

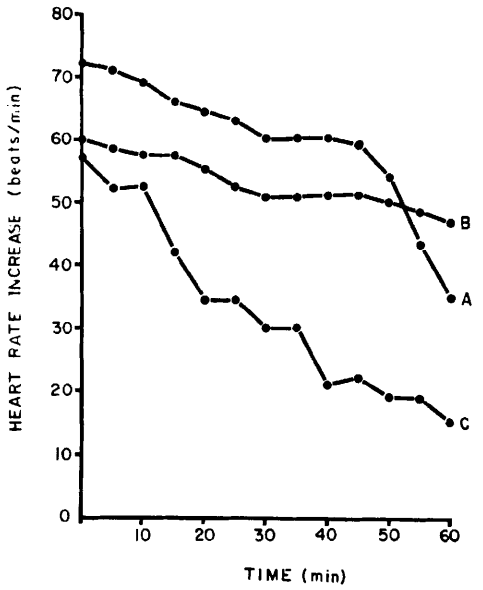

FIG. 1. Increases in heart rate in response to sympathetic nerve stimulation given at 5-min intervals at a frequency of two/sec. Records (A, B, C) are the responses of three different isolated heart preparations. Each period of stimulation was for 30 sec at a maximal voltage of 1-msec duration.

stimulation were given during the angiotensin infusion. Infusion was then discontinued and the effects of additional periods of stimulation were determined. In five of 12 preparations the responses to stimulation during a second angiotensin infusion were observed, while the responses to a third infusion were observed in two preparations.

In three additional preparations the chronotropic responses to norepinephrine (Levophed, Winthrop) injections (0.1–1 μg) were determined before and during angiotensin infusions (5 ng/ml).

Results. The chronotropic responses to sympathetic nerve stimulation in the three preparations in which angiotensin was not given are shown in Fig. 1. A progressive decrease in the responses was observed in all three preparations. The rate of decrease varied considerably from preparation to preparation; although the rate was quite consistent for any one preparation for at least the initial 45 min. Allowing longer periods between stimulations did not prevent the progressive decrease in responses. Because of these wide variations, rather than determining an average rate of decrease in the responses with time, it was considered preferable to compare responses during angiotensin infusions with the one immediately preceding the infusion.

The results from the 12 preparations in which angiotensin was infused are summarized in Table I. In 10 there were greater increases in heart rate during angiotensin infusion than before the infusion. In the other two preparations, there was no increase in the magnitude of the responses as compared to the response immediately preceding the infusion, but the rate of decrease in the responses was less. The average increase in heart rate during sympathetic nerve stimulation in all 12 preparations immediately preceding the initial angiotensin infusion was 51 $\pm$ 6.4 beats/min. The average increase in response to the initial stimulation during angiotensin was 63 $\pm$ 7.9 beats/min. As analyzed by the paired t test, the responses during angiotensin were significantly ($p < .005$) greater than those before angiotensin, and this difference was significant without considering the progressive decrease in response to be expected with time and repeated stimulations alone.

Figure 2 shows the results from two preparations in which second angiotensin infusions were given. As indicated by the data for the second and third infusions in Table I, the magnitude of the potentiation during succeeding angiotensin infusions was less than during the initial infusions. In those five preparations in which a second infusion was given, the average potentiation of the accelerator responses during the initial angiotensin infusion was 44%, but only 28% during the second infusion. In the two preparations in which three angiotensin infusions were given, the magnitude of the potentiation progressively decreased with succeeding infusions in each preparation (30%, 28%, 11% and 103%, 59%, 29%).

No differences were observed in the chronotropic responses to norepinephrine injections before and during angiotensin infusions in any of the three preparations studied.

Discussion. From the results of this study

TABLE I. Chronotropic Responses of the Isolated Rabbit Heart to Sympathetic Nerve Stimulation (2/sec) Before and During Angiotensin Infusions (5 ng/ml).

Rabbit no.	First angiotensin infusion			Second angiotensin infusion			Third angiotensin infusion		
	Before[a]	During[b]	% Change	Before[a]	During[b]	% Change	Before[a]	During[b]	% Change
1	66	85	+30	50	63	+28	45	50	+11
2	75	73	−3						
3	51	63	+24						
4	49	100	+103	66	105	+59	72	93	+29
5[c]	36	51	+42	29	39	+35			
6	30	35	+17						
7	88	94	+7						
8	21	26	+24						
9	33	30	−10						
10	27	32	+19						
11[c]	66	87	+32	70	77	+10			
12	73	82	+12	78	86	+10			
Mean ± SE (all 12)	51 ± 6.4	63 ± 7.9	27 ±10.3						
Mean ± SE (5 with second infusions)	58 ± 6.8	81 ± 8.1	44 ± 5.6	59 ± 8.7	74 ± 11.1	28 ± 9.1			

[a] Increase in heart rate (beats/min) in response to sympathetic nerve stimulation immediately preceding the beginning of angiotensin infusion.

[b] Increase in heart rate (beats/min) in response to initial period of stimulation during angiotensin infusion.

[c] Responses of these rabbits during the first and second angiotensin infusions are graphed in Fig. 2.

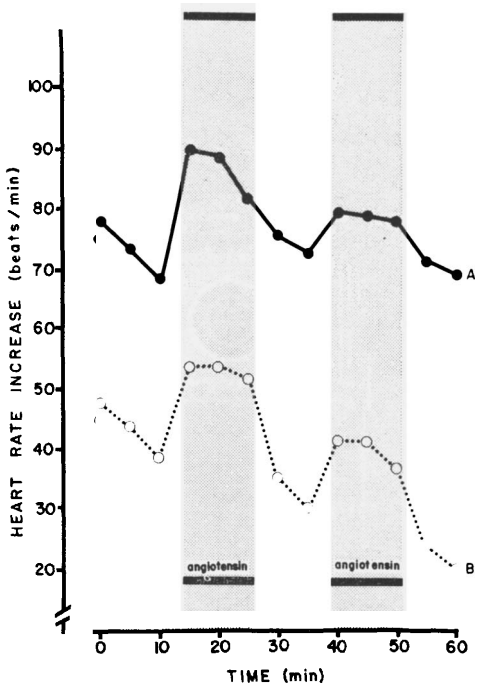

FIG. 2. Increases in heart rate in response to sympathetic nerve stimulation given at 5-min intervals at a frequency of two/sec before and during angiotensin infusions (5 ng/ml). Each period of stimulation was for 30 sec at a maximal voltage of 1-msec duration. Durations of angiotensin infusions are indicated by shaded areas.

it can be concluded that angiotensin interacts with the cardiac neuroeffector sites in the rabbit heart under these conditions in such a way that the accelerator response to sympathetic nerve stimulation is potentiated. A similar influence of angiotensin has been observed previously to occur at some other adrenergic neuroeffector sites. The constrictor response of vascular smooth muscle to sympathetic nerve stimulation has been reported to be increased during angiotensin treatment in the perfused paw (5) and renal vascular bed (6) of the dog, perfused cat mesenteric vessels (10), perfused central ear artery of the rabbit (11), and the perfused renal artery stump of the rat (12). In addition, it has been reported that angiotensin increases the responses to sympathetic nerve stimulation of the cat spleen and guinea pig vas deferens (13).

Theoretically, there are a number of ways in which angiotensin could modify events at the adrenergic neuroeffector site with a resultant potentiation of the response to nerve stimulation. Some evidence has been reported for at least three possible mechanisms of action: a—Sensitization of the receptor site to the action of norepinephrine, b—facilitation of the release of norepinephrine, c—inhibition of the normal re-uptake of neurally released norepinephrine. There are reports in the literature both supporting and refuting all of these mechanisms of action. It is possible that there are real differences in various species, and even at different neuroeffector sites within the same animal. However, it is more probable that the inconsistencies are due in most cases to varying levels of activity in the adrenergic nerves, experimental difficulties in distinguishing between increased release and inhibition of re-uptake, or to the use of widely different doses of angiotensin. For instance, Zimmerman and Whitmore (5) noted an increased pressor response to sympathetic nerve stimulation in the dog paw during angiotensin infusions, and this increased response was associated with increased levels of norepinephrine in the venous effluent. In the perfused femoral bed of epidurally blocked dogs, Lowe (14) observed no significant differences in the pressor responses to angiotensin injections before and after alpha and beta receptor blockade; therefore, he concluded that angiotensin was not causing release of norepinephrine from inactive sympathetic nerves. Although some investigators have measured only tissue retention of norepinephrine as an indication of the effects of angiotensin on uptake of catecholamines, it has been pointed out (15) that this is not sufficient to distinguish between re-uptake and release, since either an inhibition of re-uptake or increased release of norepinephrine would result in decreased retention of norepinephrine. Dosage also appears to be important in determining by what mechanism angiotensin interacts with adrenergic neuroeffector sites. Kiran and Khairallah (16) observed in the isolated rabbit aorta that angiotensin released norepinephrine, but only in pharmacological doses, the amount required being 1000-fold in excess of that needed to inhibit norepinephrine uptake.

In the present study, the concentration of

angiotensin used was within the range of blood concentrations that have been reported to result in dogs after hemorrhage (17) and in certain pathologic conditions in humans (18). At this concentration, the accelerator responses to norepinephrine injections were similar to the responses before angiotensin infusions. This is in agreement with the findings of Smyth (19) also in isolated rabbit hearts. If the disposition of exogenous norepinephrine is similar to neurally released norepinephrine, these findings indicate that in this preparation, angiotensin is neither sensitizing the receptor sites nor preventing the re-uptake of norepinephrine, both of which should result in potentiated responses to injected norepinephrine. Therefore, the increased accelerator response to sympathetic nerve stimulation during angiotensin is most likely due to facilitated release of norepinephrine. This action of angiotensin depends upon actively discharging adrenergic neurons in this preparation since there was no acceleration after angiotensin injection when the sympathetic nerves were inactive (unpublished observations).

These findings are not in agreement with the results of two previous preliminary studies of the interaction of angiotensin with cardiac neuroeffector sites. Koch-Weser (8) reported that the release of norepinephrine from sympathetic nerves in the myocardium which results from intense electrical stimulation of cat papillary muscles was not increased by angiotensin. And Zimmerman *et al.* (9) indicated that the amount of tritiated norepinephrine released by stellate nerve stimulation in the isolated rabbit heart did not establish that the release of norepinephrine was facilitated by angiotensin. However, there is some evidence from the work of Krasney *et al.* (7) that the increase in heart rate after intracoronary injections of angiotensin in the dog was due to the release of intracardiac norepinephrine.

The retention of exogenously infused tritiated norepinephrine has been studied in isolated rat (20) and rabbit (21) hearts. In both studies the retention of norepinephrine was decreased during angiotensin infusions, and these results were interpreted as indicating that angiotensin was acting by preventing the normal uptake process for norepinephrine at the nerve endings. However, it is difficult, on the basis of these studies alone, to determine whether the uptake of norepinephrine was decreased or its release increased, since either of these changes would result in decreased retention.

The results of the present studies indicate that the potentiated accelerator response to sympathetic nerve stimulation during angiotensin infusions in the isolated rabbit heart under the conditions of these experiments is due to an increased release of norepinephrine. Because of the low concentrations of angiotensin at which this action occurred, it is reasonable to postulate that in some conditions this interaction of angiotensin with the cardiac adrenergic nerve endings may be a factor in the cardiac response to endogenous angiotensin.

Summary. The accelerator response to sympathetic nerve stimulation in perfused rabbit hearts was significantly greater during than before angiotensin infusions. A similar effect of angiotensin at other adrenergic neuroeffector sites has been reported previously, and mechanisms of action that have been suggested for this influence include sensitization of the receptor site to the action of norepinephrine, prevention of the re-uptake or facilitated release of norepinephrine. Evidence is presented in the experiments reported here that at cardiac neuroeffector sites angiotensin does not sensitize the receptor sites nor prevent the uptake of norepinephrine, since the accelerator responses to norepinephrine were similar before and during angiotensin infusions. It appears most likely, therefore, that the mechanism by which angiotensin causes an increased accelerator response to sympathetic nerve stimulation in these preparations is to potentiate the release of norepinephrine from the active adrenergic nerve endings.

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