# DISCUSSION

Angiotensin is formed when the blood supply to the kidney tends to be decreased, and when the compound appears in the circulation it produces changes in blood pressure and heart rate. The direct effects of angiotensin on smooth muscle in the vascular tree are prominent, and the question arises concerning the extent to which the changes in heart rate are secondary to the changes in blood pressure incident to arteriolar constriction.

In this study dogs were premedicated with morphine and anaesthetized with chloralose since these compounds do not interfere with the cardioinhibitory reflex elicited from the sinoaortic zones (84). The baroceptor reflexes retain their sensitivity in dogs under chloralose, whereas sodium pentobarbital largely prevents the vagal component of the cardioinhibitory response to vasoconstrictor agents (39). The repeated rapid intravenous injections of angiotensin in the doses used in this study did not produce tachyphylaxis. Page et al. (71), and Goldblatt et al. (40) also have reported similar results.

Following rapid intravenous injection of 10 µg of angiotensin the heart rate decreased. This occurred

at the time when the blood pressure was rising rapidly. When the blood pressure reached a maximum level and stayed there for awhile the heart rate started rising, reached the basal level, and continued rising above it although the blood pressure was still elevated.

The initial slowing of heart rate was proportionately less with slow rates of infusion. With an infusion rate of 1.25 µg/min. there was no initial decrease in heart rate. In these cases the blood pressure rose slowly and to a lesser degree than when 10 µg of the compound was given rapidly, and instead of producing a high peak of pressure there was a gradual rise and fall (Dog 15, Table 6 and fig. 5).

In another experiment (fig.6, Dog 16) initially a small dose was given and then the rate of injection was doubled stepwise until a dose of 5 µg/min. was reached. The first dose caused an increase in heart rate while the bigger doses caused initial decreases in heart rate but cardiac inhibition was less marked when compared with the initial inhibition produced by the same total dose (Dog 15, Table 6). The subsequent rise of heart rate, however, was proportional to the magnitude of the dose.

It is demonstrated that angiotensin exerts an accelerator action shortly after the beginning of the injection, as shown by the initial small dose of angiotensin in Dog 16, but this is counteracted in response to the sudden rise of blood pressure when bigger doses are used. When angiotensin is injected at rates such as to cause a very gradual rise in blood pressure the cardioinhibitory phase is less or absent. In the intact animal under physiologic conditions probably large amounts of angiotensin would not be released suddenly. Hence, it seems reasonable to postulate that when angiotensin is liberated in response to stimuli which develop in the intact animal it will not elicit a decrease in heart rate at any time.

The rise of blood pressure was buffered in these studies by the use of a compensator to determine the effect of angiotensin in the absence of baroceptor reflexes. The cardioinhibitory action of angiotensin was prevented proportionately as the rise in blood pressure was counteracted, and even though the rise in arterial pressure was not completely buffered there was no initial slowing of heart rate after injection of angiotensin. The heart rate increased steadily after injection.

When all four moderator nerves are cut the medullary sympathetic centers are released from inhibition and neurogenic hypertension is produced. The hypertension depends in part upon acceleration of the heart rate and augmentation of the cardiac output and in part on increased peripheral vasoconstriction (81). Thomas and McLean (81) also demonstrated that the samples of angiotonin available to them had a cardioaccelerator action in sinoaortic denervated dogs. Angiotensin was injected before and after sinoaortic denervation. In the latter case heart rate and blood pressure rose from the basal level and there was no initial slowing although the blood pressure rose rapidly. Thus it is demonstrated that in an animal which has the sinoaortic zones denervated angiotensin not only is incapable of producing a decrease in heart rate but produces marked cardiac acceleration eventhough the rate already is high.

The actions of Pitressin upon blood pressure and heart rate were studied in order to make a comparison with the actions of angiotensin. Both of these compounds have a vasopressor action. When given to dogs under experimental conditions similar to those used in the study of effects of angiotensin, Pitressin

caused a marked rise of blood pressure and a decrease in heart rate. When equipressor doses of the two compounds were given the maximum amount of slowing produced by Pitressin was about twice that produced by angiotensin. Also, the bradycardia produced by Pitressin was still present 15 - 20 min. after the injection. Prolonged bradycardia after the injection of Pitressin also has been observed in unanaesthetised dogs (87). It is apparent that the cardioinhibitory effect of Pitressin is more marked and much more prolonged than that of angiotensin.

Pitressin injected into dogs with blood pressure partially or completely buffered still produced slowing. The pattern of slowing remained of the same type as in the intact dog but the degree was less. This shows that the modes of action of angiotensin and Pitressin upon heart rate are different. Angiotensin has a cardioaccelerator effect while Pitressin not only has no cardioaccelerator effect but seems to have a cardioinhibitory effect which is not completely prevented by buffering the rise of blood pressure.

Acceleration of the heart as the result of injection of angiotensin after sinoaortic denervation or during buffering of the rise of blood pressure

theoretically could be attributed either to direct action of the drug on the heart or to an action exerted through the cardioaccelerator center. Experiments concerning the effect of angiotensin on the denervated heart have been performed by several groups of investigators. In a single experiment the injection of angiotensin resulted in a 3% decrease in rate of the denervated heart of an unanaesthetized dog (43). Lorber and Visscher (61) and Lorber (62), who worked on completely isolated, blood perfused dog and cat hearts, found that the usual response to angiotensin was slight slowing coincident with the decrease of coronary flow. Hill and Andrus (44,45), working on isolated cat hearts perfused with Ringer-Locke solution, reported that no significant effects upon heart rate were produced. In some instances the heart rate diminished slightly during the period of reduced coronary flow, and in a few others there was an evanescent slight acceleration at the height of this effect. On the basis of these experiments it can be concluded that angiotensin has no direct cardioaccelerator effect.

The other possibility is that angiotensin may act on the cardioaccelerator center either directly

or from higher centers or via the changes in blood chemistry in the brain which are secondary to its effects upon blood pressure. Since the vasoconstrictor effect of angiotensin on blood vessels in the central nervous system would be expected to be less than the effects on other parts of the body one would anticipate that the compound might produce an increase in blood flow in the brain. If this should occur there might be an increase in $P_{O_2}$ and decrease in $P_{CO_2}$ in the centers. As a result of this a mild decrease in cardioaccelerator tonus might occur (84). The phenomenon of a decrease in accelerator tonus in sinoaortic denervated animals has been observed following injections of vasopressin (1,88) and methoxamine (3). If angiotensin, in some way, causes a decrease in blood flow to the brain this could cause an increase in activity of the center; however, as indicated above, there seems to be no reason for postulating that angiotensin would cause a decrease in blood flow in any part of the brain. The possibility which remains is that angiotensin acts directly on neural structures and perhaps directly on the centers in the medulla to cause an increase in heart rate via the nerves to the heart.

The accelerator effect on heart following angiotensin, with mechanical buffering of the blood pressure, may be attributed through the cardio-accelerator center. Angiotensin has been reported to have no effect on the denervated heart (11,12,9,10). After vagotomy, a marked increase in the heart rate was observed with angiotensin. When the sympathetic supply to the heart was also removed, the acceleration was reduced. This would suggest the possible direct effect of angiotensin on the cardioaccelerator center, which brings about the increase in the heart rate via the sympathetic pathways (69).

The accelerator effect was more marked after vagotomy but was of smaller magnitude after transverse section of the spinal cord. This also points towards the central stimulating action of angiotensin on cardiac accelerator center, sending impulses to the heart via sympathetic pathways. After the use of pentolinium as ganglion blocking agent, the cardiac accelerator action of angiotensin was present and was of greater magnitude (about 60 beats/minute). When angiotensin was injected after pentolinium and serpasil, this accelerator action of angiotensin was reduced, when angiotensin was injected after serpacil only and

after bretylium tosylate only the cardiac accelerator effect of angiotensin was negligible.(Table 10).

The full ganglion blocking effect of pentolinium could not be demonstrated in these sets of experiments. The dosage of 5 mg/kg of body weight was repeated upto four times. The blocking effect was most marked in the first instance and subsequently, it was reducing. As such, the central accelerator effect of angiotensin could never be checked completely in these cases (Fig.8).

However, there seems to be a synergism between pentolinium and angiotensin either at chemical level or at the 'effector mechanism' level, because angiotensin produced a marked cardiac acceleration after pentolinium, after pentolinium and serpasil, but not after serpasil alone. Serpasil depletes the adrenergic nerves from their catecholamine stores. It is possible that angiotensin liberates epinephrine from adrenal medulla as reported by Feldberg and Lewis (33) and this effect is potentiated by pentolinium by the reflex, elicited by the fall of blood pressure. This reflex would increase the sympathetic activity through partially blocked sympathetic ganglia, at sympathetic nerve ending level and at adrenal medullary level. This

increased sympathetic activity will accelerate the heart rate to a greater magnitude rather than when angiotensin is given alone.

Atleast half of this effect is blocked at post-ganglionic sympathetic nerve endings when serpasil is given after pentolinium.

When serpasil is given alone and then angiotensin is injected, there is very little cardiac acceleration. This slight acceleration could be either due to incomplete blocking of central effect at post-ganglionic sympathetic nerves or due to its stimulating effect at sympathetic nerve endings or at sinoatrial node. A marked reduction in cardiac acceleration action of angiotensin was observed in experiments where the drug was injected after the stabilization of heart rate was achieved following injection of bretylium tosylate.

Bicherton and Buckley (12) have shown a central effect of angiotensin on vasomotor center. This influence is carried to peripheral smooth muscle cells of arterioles via sympathetic pathways. In these cross circulation experiments, angiotensin was injected to the donor. The donor's blood circulated in the recipient's head only. The recipient's head was

connected to the rest of the body only via spinal cord. An increase in blood pressure in the recipient was shown due to an increase in peripheral resistance.

The action on isolated cardiac papillary muscle was blocked by dichlorisoproterenol (DCI)(8). This is a Beta cell blocking compound. The action of angiotensin on heart rate as shown in our experiments has been very much reduced by sympatholytic compounds, such as serpasil or bretylium tosylate. These results suggest that a part of cardiac accelerator effect of angiotensin is because of liberation of catecholamines at sympathetic post-ganglionic nerve endings to the heart and a part of the action could be because of its direct stimulating effect on Beta cells of the heart.

Thus angiotensin over and above having its central accelerator effect is found to have a peripheral basis of cardiac acceleration. This acceleration is present after the administration of ganglionic blocking agents but reduced after administration of sympatholytic compounds (70).

This action could also be because of liberation of adrenaline from adrenal medulla as reported by

Feldberg and Lewis (loc. cit.), or it could be because of liberation of catecholamines from cardiac sympathetic nerve endings or because of stimulation of Beta cells of the heart.

One of the above possibilities of this peripheral basis of cardiac accelerator mechanisms of angiotensin could be that it liberates catecholamines from adrenal medulla which in their own turn increase the heart rate. The results of Gordon and Fred (37,41) indicate that catecholamines liberated from the adrenal medulla play an important role in the cardiovascular responses of angiotensin. They concluded that angiotensin releases epinephrine from the adrenal medulla.

In these groups of experiments with a dose of 10 µg of angiotensin injected at one time it is observed that tying of adrenal veins on either side has got no effect on cardiac accelerator action of angiotensin. There is no significant difference in increase in heart rate between two conditions as a result of angiotensin injection (Table 11, fig. 9 and 13).

The other possible source of catecholamine release may be from the sympathetic supply to the heart.

Reserpine was injected to deplete the catecholamine stores. It is observed that either the cardioaccelerator effect of angiotensin is absent or is very limited after reserpine (Fig.12). This difference in increase in heart rate in two conditions is statistically significant (P = 0.01). This points towards the fact that probably the cardioaccelerator action of angiotensin is by the release of sympathin from the sympathetic terminals to the heart.

The cardiac accelerator action of sympathin can be checked by using a substance which could block the Beta cells of pace maker, so that they do not respond to the sympathin.

Inderal was injected for this purpose and afterwards angiotensin is given. It is observed that with inderal the cardiac accelerator action of angiotensin is very much reduced and is of the magnitude of 0 to 7 beats/min. This reduction is also statistically significant (P = 0.01) (Fig.10,11 and 13).

These observations show that angiotensin increases the heart rate by releasing catecholamines from the sympathetic supply to the heart.