Mechanisms by which Angiotensin II Affects the Heart Rate of the Conscious Sheep

WARWICK B. LEE, MANSEL J. ISMAY, AND EUGENIE R. LUMBERS

SUMMARY Intravenous infusions of angiotensin II were given to conscious sheep. During these infusions, the pressor action of angiotensin was antagonized by concomitant infusion of sodium nitroprusside. Under these conditions, angiotensin produced a dose-dependent tachycardia. This dose-dependent tachycardia was not affected by propranolol and therefore it was not due to an action of angiotensin on sympathoadrenal mechanisms. The dose-dependent tachycardia was reduced by atropine, and abolished by increases in systolic pressure. We conclude that iv infusions of angiotensin cause a central, dose-dependent reduction in vagal tone. This action is normally antagonized by the baroreceptor reflex response to the hypertensive action of angiotensin. Therefore, in those conditions in which endogenous angiotensin production is increased and blood pressure is not elevated (e.g., sodium deficiency and pregnancy), angiotensin may influence heart rate. Circ Res 47: 286-292, 1980

USING techniques similar to those described by Smyth et al. (1969), other scientists have shown that, in the conscious sheep and fetal lamb (Ismay et al., 1979) and in the anesthetized mongrel dog (Lumbers et al., 1979), the reflex cardiac slowing in response to increases in systolic pressure caused by intravenous injections of phenylephrine resulted from an increase in cardiac vagal tone. Phenylephrine has no direct cardiac effect (Varma et al., 1960). However, in the dog, the sheep, and fetal lamb, when arterial pressure was increased by intravenous injection of angiotensin II there was no progressive increase in pulse interval as systolic pressure rose (Ismay et al., 1979; Lumbers et al., 1979). In both species this lack of cardiac slowing seen with acute transient increases in systolic pressure caused by angiotensin II was due to a central inhibitory action

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of angiotensin on the vagus, an effect which was measured directly in anesthetized mongrel dogs by recording from cardiac vagal efferent nerves (Lumbers et al., 1979). In humans, when arterial pressure was increased by injection of angiotensin, there was an initial reflex bradycardia which was followed by a tachycardia (Smyth et al., 1969).

A similar effect may occur when angiotensin is infused. Wasserstrum and Herd (1977) showed that, in squirrel monkeys, the reflex cardiac slowing in response to infusions of pressor doses of angiotensin was less than that seen in response to infusions of pressor doses of phenylephrine.

Most workers claim that angiotensin affects heart rate through activation of sympathoadrenal mechanisms, e.g., release of catecholamines from the adrenal medulla (Feldberg and Lewis, 1964; Staszewska-Barczak and Vane, 1967), stimulation of autonomic ganglia (Lewis and Reit, 1965), alteration of noradrenaline stores at adrenergic nerve endings (Zimmerman et al., 1972), and stimulation of central sympathetic structures (Bickerton and Buckley, 1961; Ferrario et al., 1972). Relatively few workers have concluded that angiotensin has an effect on vagal tone (Scroop and Lowe, 1969). Therefore, experiments were carried out in conscious sheep to determine the mechanisms by which intravenous infusions of angiotensin affect heart rate.

**Methods**

Experiments were carried out in six conscious nonpregnant ewes and four conscious wethers.

Under general anesthesia (1 g thiopentone, Abbott), polyvinyl catheters (2.7 mm o.d., 1.5 mm i.d.) were inserted into a femoral vein and a femoral artery. A double lumen polyvinyl catheter (2 mm o.d., 2 X 0.89 mm i.d.) was inserted into a jugular vein. The sheep were housed in metabolic cages and fed lucerne chaff and oats and were allowed free access to water.

Cannulas were flushed once daily with heparinized saline (100 IU/ml). The animals were treated with intramuscular injections of 1 g of streptomycin sulfate (Glaxo) and 600 mg of penicillin (Crystapen, Glaxo) daily for 3 days postoperatively.

On the day of an experiment, a sheep was connected to the perfusion pumps and recording apparatus which were located outside the room in which the animals were housed. Food and water were withdrawn. During the course of an experiment, the sheep was unable to see the investigator and remained undisturbed.

Infusions were given to the sheep, using Braun Per fusor pumps and, if necessary, a Palmer perfusion pump. Arterial pressure was recorded with a Bell & Howell (4-327-0109) pressure transducer connected to a Devices M19 8-channel recorder. Arterial pressure was increased by infusing phenylephrine HCl (0.033–3 mg/min) or angiotensin II (0.11–33 µg/min). The systolic arterial pressure was not increased above 200 mm Hg.

In another series of experiments, the pressor action of angiotensin II (0.22–44 µg/min) was antagonized by a concomitant intravenous infusion of sodium nitroprusside (22–165 µg/min), a vasodilator drug with no direct cardiac effect (Page et al., 1955). In four sheep, 17 control experiments were carried out to confirm that sodium nitroprusside had no effect on heart rate independent of its effects on blood pressure. In these experiments, the effects of combined sodium nitroprusside and phenylephrine infusions on pulse interval were compared with measurements of pulse interval made at the same systolic pressures during intravenous infusions of phenylephrine alone. Propranolol (15 mg, iv followed by a continuous infusion of 0.5 mg/min) was used to produce β-adrenergic blockade. This dose of propranolol blocks the cardiac response to 2-4 µg of isoproterenol (see also Ismay et al., 1979). Atropine sulfate (2-4 mg, iv followed by a continuous infusion of 0.4 mg/min) was used to block the cardiac effects of the vagus (Ismay et al., 1979). Combined sympathetic and parasympathetic blockade was obtained by combining the doses and infusions of propranolol and atropine described above.

**Calculation of Results**

The beat-to-beat pulse intervals were recorded with the paper speed at 25 mm/sec, and were related to the preceding systolic pressure peak. The reproducibility of reading pulse intervals was 1.6 ± 1.4% (sb). Recordings of the systolic pressure and pulse interval were made only 3 minutes or more after infusions had commenced or 3 minutes or more after the changes in arterial pressure had stabilized. When arterial pressure and heart rate were steady, the pulse intervals and systolic pressures of six beats were measured at each level of systolic pressure, or dose of angiotensin. In those experiments in which the effects of angiotensin on pulse interval were studied with the pressor action of angiotensin antagonized by concomitant intravenous infusion of sodium nitroprusside, the changes in pulse interval produced with different doses of angiotensin were referred to the mean control pulse interval measured before infusions of angiotensin in the following manner: % change in pulse interval = control pulse interval – pulse interval during infusion × 100/control pulse interval. Data were analysed using Student’s two-tailed non-paired t-test.

**Results**

The Effect of Intravenous Infusions of Angiotensin on Pulse Interval When the Pressor Action of Angiotensin II was Prevented by Concomitant Infusion of Sodium Nitroprusside

When the pressor action of angiotensin II was prevented by concomitant intravenous infusion of
TABLE 1  Changes in Pressure and Pulse Interval*

<table>
<thead>
<tr>
<th></th>
<th>Systolic pressure (mm Hg)</th>
<th>RPI (msec)</th>
<th>Δ Systolic pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before propranolol</td>
<td>108 ± 11.8 (n = 4)</td>
<td>766 ± 129</td>
<td>-0.96 ± 1.4</td>
</tr>
<tr>
<td>After propranolol</td>
<td>110 ± 6.65 (n = 4)</td>
<td>916 ± 295</td>
<td>0.98 ± 0.15</td>
</tr>
<tr>
<td>Before atropine</td>
<td>107 ± 6.67 (n = 9)</td>
<td>691 ± 118</td>
<td>-1.2 ± 2.8</td>
</tr>
<tr>
<td>After atropine</td>
<td>105 ± 9.65 (n = 9)</td>
<td>457 ± 83.2</td>
<td>-1.45 ± 3.69</td>
</tr>
<tr>
<td>Before atropine</td>
<td>108 ± 8.6 (n = 5)</td>
<td>771 ± 41.1</td>
<td>-0.46 ± 2.76</td>
</tr>
<tr>
<td>After atropine</td>
<td>102 ± 12.6 (n = 5)</td>
<td>429 ± 99</td>
<td>0.09 ± 3.1</td>
</tr>
<tr>
<td>After atropine + propranolol</td>
<td>106 ± 12.7 (n = 5)</td>
<td>445 ± 87.6</td>
<td>-1.09 ± 1.02</td>
</tr>
</tbody>
</table>

* The mean and standard deviations of the resting systolic pressures (mm Hg) and resting pulse intervals (RPI, msec) prior to infusion of angiotensin II and the mean changes in systolic pressure during infusions of different doses of angiotensin when the pressor action of angiotensin was antagonized by sodium nitroprusside. In experiments designed to test the effects of propranolol on the angiotensin-induced tachycardia, the doses of angiotensin infused ranged from 1.1 to 40 μg/min. In the two other experimental groups, the doses of angiotensin were 1.1-11 μg/min.

sodium nitroprusside so that there was no change in systolic pressure (Table 1), then infusions of angiotensin caused a tachycardia (Fig. 1). Furthermore, this decrease in pulse interval was dose-dependent (Fig. 2). In 17 control experiments, infusions of sodium nitroprusside did not alter the pulse interval when the hypertensive action of sodium nitroprusside was prevented by intravenous infusion of phenylephrine.

The Effect of Sympathetic or Parasympathetic Blockade and Combined Sympathetic and Parasympathetic Blockade on the Dose-Dependent Tachycardia Produced by Angiotensin When its Pressor Response was Antagonized

In four sheep, β-adrenergic blockade with propranolol caused a small increase in resting pulse interval (Table 1) but had no effect on the dose-dependent decrease in pulse interval caused by angiotensin II (Figs. 1 and 3). The effect of atropine on the dose-dependent tachycardia caused by angiotensin when its pressor effect was prevented was studied in four sheep on nine occasions (Table 1). In these experiments, angiotensin produced a progressive fall in pulse interval over the dose range 1.1-11 μg/min. The maximum reduction in pulse interval was 42.3 ± 10.1%. After atropine, angiotensin no longer reduced pulse interval to the same extent. The maximum fall in pulse interval after atropine was only 20.2 ± 10.6%. This reduction in the fall in pulse interval after atropine was significant for all doses of angiotensin (P < 0.02).

In three sheep on five occasions we compared the effects of atropine and the combination of atropine and propranolol on the cardiac response to non-pressor infusions of angiotensin II (Fig. 4, Table 1). After administration of either atropine alone, or atropine and propranolol, angiotensin failed to produce any significant change in pulse interval. There was no significant difference between the degree to which atropine suppressed the response to angiotensin and the degree to which atropine and propranolol suppressed the chronotropic effect of angiotensin (Fig. 4).

Effect of the Pressor Response to Angiotensin on the Dose-Dependent Tachycardia

In six sheep, the pressor action of angiotensin was not antagonized by concomitant intravenous infusion of sodium nitroprusside, and the dose-dependent tachycardia seen with intravenous angiotensin was not present (Fig. 5). A variable degree of cardiac slowing was seen. However, in 10 sheep, the amount of cardiac slowing seen with intravenous infusions of pressor doses of angiotensin was less than that seen with pressor infusions of phenylephrine (Fig. 6).

Discussion

Recent evidence suggests that angiotensin may have two opposing effects on heart rate [one mediated through its hypertensive action, which results in baroreceptor stimulation (Lumbers et al., 1979) and reflex slowing of the heart; the second through a central action which inhibits vagal discharge (Scroop and Lowe, 1969; Lumbers et al.,...]

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If angiotensin does have these two opposing effects on heart rate, then when the hypertensive action of angiotensin is antagonized it should be possible to show that intravenous infusions of angiotensin increase the heart rates of animals in which resting cardiac vagal tone is high. In the experiments described, the hypertensive action of angiotensin was antagonized by concomitant infusions of sodium nitroprusside and, in these experiments, increasing doses of angiotensin caused a progressive fall in pulse interval, i.e., a progressive increase in heart rate (Figs. 1-5).

In control experiments, sodium nitroprusside had no effect on heart rate independent of its effects on blood pressure. Systolic pressure levels were used to monitor the balance between the hypertensive actions of angiotensin and the hypotensive effects of sodium nitroprusside because Smyth et al. (1969) have shown that the relationship between systolic pressure and pulse interval is a good index of baroreflex control of heart rate. Also, Arndt et al. (1977) have shown that baroreceptor discharge is related to the level of systolic pressure but not to changes in heart rate or pulse pressure.

It is unlikely that any changes in central venous pressure occurring during combined infusions of angiotensin and sodium nitroprusside would produce a tachycardia that was related to the dose of angiotensin infused. This was confirmed by three experiments which showed that the dose-dependent tachycardia was not abolished when changes in central venous pressure were offset by attaching a saline reservoir open to atmosphere to a catheter.
When the pressor action of angiotensin was not prevented (○), there was no progressive fall in pulse interval. This contrasts with the dose-dependent fall in pulse interval seen in the same six sheep (●) when the pressor action of angiotensin was antagonized by a concomitant infusion of sodium nitroprusside. Bars represent SE of mean. Ordinate: percent fall in pulse interval; abscissa: dose of angiotensin (μg/min, logarithmic scale).

There are three possible ways in which angiotensin could cause a dose-dependent tachycardia. First, it could cause activation of cardiac sympathetic nerves (Nishith et al., 1962) or release of adrenal medullary catecholamines (Feldberg and Lewis, 1964). Second, angiotensin could have a direct chronotropic effect on the heart, and, third, angiotensin could cause a dose-dependent central inhibition of cardiac vagal tone.

Since β-adrenergic blockade with propranolol did not abolish the dose-dependent tachycardia caused by angiotensin (Figs. 1 and 3), this tachycardia was not due to increases in cardiac sympathetic nerve activity or to release of adrenal medullary hormones by angiotensin. Furthermore, atropine greatly reduced the fall in pulse interval (Fig. 4) caused by angiotensin, and this would not have occurred if angiotensin’s action had been solely or principally through activation of “sympathoadrenal” mechanisms.

After atropine was administered, the resting pulse interval fell (Table 1) and the ability of angiotensin to produce a tachycardia was also decreased. Since it can be shown that isoproterenol can decrease pulse interval in sheep to 250 msec (unpublished observations), the reduction in the efficacy of angiotensin II after atropine could be due to the associated impairment of vagal control of the heart produced by intravenous infusion of atropine.

Angiotensin did produce a small tachycardia after atropine. However, when experiments were carried out in three of the animals (Fig. 4) to see whether this residual tachycardia was due to an action of angiotensin on sympathetic mechanisms, it was found that there was no difference between the ability of atropine alone and that of atropine and propranolol in combination to suppress the angiotensin-induced tachycardia. Therefore, it is not possible to conclude from our results that angiotensin has even a minor effect on heart rate through “sympathoadrenal mechanisms.”

These results in the conscious sheep differ from those obtained in the anesthetized vagotomized mongrel dog (Lumbers et al., 1979) in which it was possible to show that, after vagotomy, bolus injec-

Figure 6 The relation between systolic pressure (mm Hg) and pulse interval (msec) in 10 sheep when systolic pressure was increased by infusion of phenylephrine (●) and by infusion of angiotensin (○). Bars represent ± SE of mean.
tions of angiotensin caused a small increase in heart rate that was abolished by propranolol (Lumbers et al., 1979).

There is no evidence that angiotensin has a significant direct chronotropic effect (Koch Weser, 1964; James, 1965). Furthermore, Ismay et al. (1979) could not show any decrease in pulse interval when bolus doses of (2.5–25 μg) of angiotensin were injected intravenously into the sheep treated with atropine and propranolol.

If infusions of angiotensin did have any direct effect on heart rate, then pulse interval would have fallen when angiotensin was infused into sheep treated with atropine and propranolol. This did not happen. There was no significant change from resting pulse interval even when the largest doses of angiotensin were infused into the sheep treated with atropine and propranolol (Fig. 4). Since there was no direct chronotropic effect of angiotensin, it can be concluded that angiotensin failed to increase the heart rate of atropine-treated sheep because there was no effective vagal control of heart rate in these animals. Thus the degree of vagal withdrawal seen when angiotensin is infused will depend on the degree of vagal influence on heart rate prior to administration of angiotensin, as well as the doses of angiotensin given to the animal.

A second way in which the dose-dependent decrease in pulse interval produced by angiotensin was antagonized was through the pressor action of angiotensin. Since angiotensin has no direct effect on baroreceptor discharge (McCubbin et al., 1957; Lumbers et al., 1979), an increased systolic pressure acting through baroreceptor mechanisms antagonizes the vagal inhibitory action of angiotensin on the central nervous system.

In 1969, Scroop and Lowe claimed that angiotensin exerted a central inhibitory action on the vagus causing a rise in cardiac output which in part accounted for the centrally mediated component of the hypertensive action of angiotensin. They suggested that angiotensin enters the central nervous system through the area postrema (Joy and Lowe, 1970). If this is the case, then there might be a delay in the time course of the onset of the angiotensin-induced tachycardia proportional to the time taken by the drug to enter and exert an effect within the medulla. It is not possible to define the length of this delay in the present series of experiments because accurate recordings of pulse interval changes caused by angiotensin were not made until 3 minutes or more after the pressor action of angiotensin had been balanced by the concomitant sodium nitroprusside infusions. However, examination of the data presented by Ismay et al. (1979) does show that the loss of baroreflex cardiac slowing due to central inhibition of vagal tone is delayed relative to the pressor action of angiotensin. A similar conclusion can be drawn from the original studies of Smyth et al. (1969) who studied cardiac baroreflex sensitivity in humans and used angiotensin to raise arterial pressure. They found that, initially, there was a reflex bradycardia as systolic pressure rose, but at the peak of the pressor response there was a rapid decrease in pulse interval and loss of linear relation between systolic pressure and pulse interval. These findings suggest there is a considerable delay in time between the onset of the hypertensive action of angiotensin and the central inhibitory action of angiotensin on the vagus. However, the actual length of this delay cannot be quantified accurately, and may depend on brain blood flow and the concentrations of angiotensin used to increase arterial pressure.

It can be concluded from the results of the experiments described here that angiotensin can increase heart rate when the baroreceptor contribution to control of heart rate is prevented. This accounts for the lesser degree of cardiac slowing seen when angiotensin is used to raise arterial pressure (Fig. 6, and Wasserstrum and Herd, 1977). Moreover in sodium-deficient states where maintenance of a "normal" blood pressure becomes dependent upon angiotensin (Davis et al., 1974), and in other conditions where angiotensin levels are high and blood pressure is normal (e.g., pregnancy, Robertson et al., 1971) the central stimulatory effect of angiotensin on the heart will not be opposed by an increase in baroreceptor discharge. In these situations, heart rate may depend upon the level of endogenous angiotensin. Furthermore, the prevailing cardiac baroreflex response to changes in arterial pressure also are likely to be modified by angiotensin II, as suggested by Severs and Summy-Long (1977).

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