Release of Adrenal Catecholamines by Angiotensin II

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ABSTRACT

Several recent investigations have presented indirect evidence that angiotensin stimulates the release of adrenal medullary catecholamines. The following work was undertaken in an attempt to show in vivo angiotensin stimulation of the adrenal medulla by analysis of plasma catecholamines. Mongrel dogs, anesthetized with pentobarbital, were infused for 10 min with doses of 0.025, 0.05, and 0.1 μg per kg per min of angiotensin or injected with single doses of 0.5, 1.0 and 2.0 μg per kg. Blood samples were taken from the inferior vena cava (above the outflow from the adrenal veins) at the peak of the pressor responses to single doses or during and after the infusions. The samples were fluorometrically analyzed for epinephrine and norepinephrine. It was found that angiotensin produced an increase in circulating catecholamines. Epinephrine was significantly increased by infusions of 0.05 and 0.1 μg per kg per min and all three injected doses. Plasma norepinephrine was significantly increased also except with the 0.5 μg per kg injected dose of angiotensin. Angiotensin infusion of 0.025 μg per kg per min did not cause a detectable change in circulating catecholamine levels. In the infusion experiments, norepinephrine was more transiently increased than epinephrine. These results show that various pressor doses of angiotensin stimulate release of adrenal catecholamines.

ADDITIONAL KEY WORDS venous plasma catecholamines angiotensin infusions and injections arterial blood pressure heart rate adrenal medulla anesthetized dogs

 Probably the first indirect evidence that angiotensin stimulates the release of endogenous catecholamines was presented by Renson et al.1 in 1959. They reported that angiotensin caused a contraction of the nictitating membrane in cats pretreated with cocaine and that this contraction could be blocked with an α-adrenergic blocking agent, phenoxybenzamine. Haas and Goldblatt2 and Kaneko et al.3 showed that in dogs infused with DMPP (1, 1 dimethyl-4-phenylpiperazinium iodide), a ganglion stimulating agent, there was a potentiation of angiotensin pressor responses. Kaneko et al. abolished this increased angiotensin pressor response after DMPP sensitization by adrenalectomy or by the administration of phentolamine. A potent stimulation of adrenal catecholamine release with intra-arterial injections of angiotensin in the cat was reported by Feldberg and Lewis.4 All of these investigations show indirectly that angiotensin stimulates the release of catecholamines from the adrenal medulla.

The following experiments were undertaken to show this adrenal medullary stimulation in the dog, by demonstrating an increase in circulating catecholamine levels. Experiments using single injections of angiotensin were carried out to determine if this adrenal stimulation was immediate. Experiments with infusions were performed to elucidate what effect the continued presence of angiotensin in the blood had with respect to medullary
stimulation. It was felt these experiments might assist in clarifying the in vivo catecholamine contributions to angiotensin responses.

**Methods**

Experiments were carried out on 34 mongrel dogs of either sex with weights ranging from 7.1 to 19 kg. The animals were anesthetized with pentobarbital Na, 30 mg per kg, given intravenously. A cannula for blood sampling was inserted through the left femoral vein into the inferior vena cava to the level of the Xiphoid process so that the tip of the cannula would be situated well above the outflow from the adrenal veins; all analyses of circulating catecholamines were done on blood drawn from the inferior vena cava. The right femoral vein was cannulated for the administration of synthetic angiotensin (Hypertension, CIBA®) by single injections and infusions. A cannulated right cephalic vein served for volume replacement. Blood pressure was recorded via a cannulated right femoral artery attached to a Statham transducer and Grass polygraph. Heart rate was also recorded by means of a Grass tachograph.

For the infusion experiments, doses of 0.025, 0.05, and 0.10 μg per kg per min of angiotensin were infused for 10 min with a Harvard infusion pump. A 35-ml control blood sample was taken before the administration of angiotensin. At 2 min into the infusions and 5 and 15 min after the end of the infusions, 35-ml blood samples were withdrawn. All samples were taken in about 30 sec with a 50-ml syringe. Simultaneous with blood sampling the 35-ml volume was replaced with 35 ml of physiological saline or packed blood cells resuspended in saline. The blood samples were quickly placed in chilled tubes containing 1.5 mg of heparin and centrifuged at 3,000 rpm for 15 min at 4°C. The plasma was then removed from the packed cells, protein was precipitated and the plasma catecholamines were absorbed on alumina according to the method of Von Euler and Lishajko. Following elution with 0.2 N HAc, the samples were analyzed for norepinephrine and epinephrine on a Technicon AutoAnalyzer according to the method of Robinson and Watts. The injection experiments were carried out in an identical manner except that the doses of angiotensin were 0.5, 1.0, and 2.0 μg per kg and the blood samples were withdrawn at the peak of the pressor responses or about 1 min after injection.

Statistical analysis for significance of data was done using Student's t test.

**Results**

The results of the infusion experiments are illustrated in Figure 1. The values for the 0.05 μg per kg per min dose represent 6 dogs and the 0.10 μg per kg per min, 7 dogs. The infusion of 0.025 μg per kg per min produced no change in circulating catecholamines so it is not included in the figure. None of the infusions caused changes in heart rate. The infusion of 0.05 μg per kg per min increased mean arterial blood pressure about 10 mm Hg and had significantly (P<0.05) increased norepinephrine levels within 2 min after the beginning of the infusion. At the end of the infusion period and 5 and 15 min post-infusion, norepinephrine levels were not significantly different from the control values. Epinephrine, however, was significantly increased (P<0.05) throughout the 10-min infusion. Epinephrine had decreased to control levels by 5 min after the end of infusions and was stable as shown by the level found at 15 min postinfusion.

Infusions of 0.10 μg per kg per min raised mean blood pressure about 25 mm Hg. With this larger infusion dose, norepinephrine was increased significantly (P<0.05) at 2 min and at 10 min. Within 5 min after termination of the infusion, this elevated plasma norepinephrine had rapidly decreased and at 5 and 15 min postinfusion was not significantly different from control. Epinephrine was markedly increased by this 0.10 μg per kg per min dose at 2 and 10 min (P<0.001). Even at 15 min after the termination of the infusion the epinephrine level was still significantly elevated (P<0.02). Compared with norepinephrine, plasma epinephrine decreased much more slowly after the infusion was terminated. Although not shown on the graph, plasma catecholamines were at control levels 45 min postinfusion. The increases in epinephrine produced by the 0.10 μg per kg per min infusion were significantly higher than the increases with the 0.05 μg per kg per min dose (P<0.05).

The increases in plasma catecholamines at the peak of angiotensin pressor responses with single doses of 0.5, 1.0, and 2.0 μg per kg are shown in Figure 2. The results represented are from three groups of 6 dogs each with...
two observations on each dog. Plasma catecholamines in blood samples taken 45 min after the last dose of angiotensin are indicated as postdose controls. Postdose control catecholamine levels were similar to the initial or predose controls. This finding tends to eliminate the possibility of prolonged angiotensin stimulation or the contribution of hemorrhagic stimulation of the sympatho-adrenal system to the observed increases in plasma catecholamine produced by angiotensin.

The 0.5 μg per kg dose increased mean blood pressure about 40 mm Hg and decreased heart rate approximately 10 beats per min. No change in circulating norepinephrine resulted with this injected dose. Epinephrine, however, was increased greatly (P<0.001).

The blood pressure was increased about 60 mm Hg and heart rate decreased 30 beats per min by the 1.0 μg per kg dose of angiotensin. Norepinephrine increased significantly (P<0.01) with this dose and epinephrine was markedly increased (P<0.001). These changes in epinephrine and norepinephrine were both significantly different (P<0.01) from the results of the 0.50 μg per kg dose.

The angiotensin dose of 2.0 μg per kg produced changes of about 100 mm Hg in mean blood pressure and 70 beats per min in heart rate. With this high dose, norepinephrine and epinephrine increased significantly (P<0.001). The increased catecholamine levels with the 2.0 μg per kg dose were not different from those found with the 1.0 μg per kg dose.

**Discussion**

It has been reported that adrenalectomy in rats decreased the action of angiotensin and that direct perfusion of rat adrenal glands with angiotensin caused an increase in catecholamine output. Benelli et al. also noted that the pressor responses to angiotensin in cats immediately following adrenalectomy were 7 to 23% less than initial angiotensin controls. The extreme potency of this adrenal stimulation by angiotensin was demonstrated by Robinson using the isolated perfused dog adrenals.

The results presented here have demonstrated that doses of angiotensin producing
moderate pressor responses caused increases in circulating catecholamines. Epinephrine was increased more than norepinephrine. It was observed that injections of 1.0 and 2.0 μg per kg of angiotensin produced a 4- to 5-fold increase in circulating epinephrine levels and about a 2-fold increase in norepinephrine. The smallest dose injected, 0.5 μg per kg, caused about a 3-fold increase in plasma epinephrine but no significant change in norepinephrine. The infusions of 0.10 and 0.05 μg per kg per min produced a 2- to 3-fold increase in epinephrine and a significant increase in plasma norepinephrine. These findings are consistent with adrenal medullary stimulation.

In general the increased levels of norepinephrine were much more transient than epinephrine. After the initial significant rise at 2 min with the 0.05 μg per kg per min infusion, plasma norepinephrine decreased to control, even though epinephrine release was still being stimulated in response to the infusion. This difference in the time course of elevated catecholamines was also seen with the 0.1 μg per kg per min dose; circulating norepinephrine levels had decreased to control by 5 min postinfusion while epinephrine was still elevated significantly at 15 min. This more rapid disappearance of norepinephrine from the circulation might also explain the fact that plasma norepinephrine was not increased by 0.5 μg per kg injections. The very transient nature of these increased norepinephrine levels could well be a result of preferential tissue uptake and binding of norepinephrine from the circulation.10-12

The prolonged significant increase in epinephrine at 15 min after terminating the 0.1 μg per kg per min infusion and throughout the 0.05 μg per kg per min infusion could be due to several things. As mentioned above, the specific tissue uptake and binding for epinephrine may be less than that for norepi-
nephrine. If this is true, an elevated plasma epinephrine might be expected to persist. Continued epinephrine release could also be stimulated by amounts of circulating angiotensin remaining after the infusion. However, the relatively short half-life of angiotensin in the circulation and the fact that no change was observed in plasma epinephrine in these experiments with the 0.025 μg per kg per min infusion make this unlikely. Khairallah et al. found large amounts of radioactivity in kidneys, adrenals, and uteri of rats 3 to 5 min after infusing tritiated angiotensin. Electrophoretic analysis proved that this radioactive substance was angiotensin. Thirty minutes postinfusion the radioactivity was high in the brain and had remained high in the kidneys and adrenal glands. Electrophoretically, however, this substance was different from angiotensin. The prolonged epinephrine response observed here could be due to continued stimulation of the adrenals by the relatively large amounts of angiotensin accumulated in the glands. Actually this continued epinephrine response is probably a result of some combination of the mechanisms discussed.

References
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