A Study of Hypertension
The Actions of Synthetic Angiotensin II on
Adrenal and Myocardial Catecholamines

MINORU KAWABATA

Several recent investigations have presented indirect evidence that angiotensin stimulates the release of adrenal medullary catecholamines. The following work was undertaken to elucidate the angiotensin action on the adrenal medulla and effect of myocardial catecholamines through observations of the changes in the adrenal and myocardial catecholamines caused in the rat by angiotensin injection.

An injection of 0.5 μg/kg body weight of angiotensin into the coccyeal vein brought about a significant decrease in adrenal noradrenaline after 3 minutes and also a significant decrease in adrenal adrenaline after 10 minutes. On the other hand, myocardial noradrenaline rose significantly. These results show that angiotensin is an effective stimulant for adrenal medullary catecholamines, and also angiotensin causes an increase in myocardial catecholamine, or noradrenaline, in particular.

Many factors are believed to have a hand in the development of hypertension and their exploration is naturally quite difficult. Much effort has been made clinically as well as experimentally to elucidate the hypertensive mechanism of the endocrine and neurotic origins and a great progress has been attained in the knowledge of hypertension. Among these factors associated with the development of hypertension, it is the renal and endocrine factors that have attracted general attention for a long time.

As to the renal factor, TIEGERSTEDT et al. first extracted renin as a hypertensive substance out of the excised kidney of a rabbit in 1898. Since then, the renin-angiotensin system was studied by BRAUN-MENENDEZ et al. and PAGE et al., the structural formula of angiotensin II was revealed by PEART and SKEGGS et al., and then its synthesis was accomplished by BUMPS et al.

The adrenal gland, as an endocrine factor, has also been noticed for a long time. DAVIS et al. disclosed that the renin-angiotensin system plays the leading part among the endocrine factors of hypertension. Then there arose a conception that the physiological action of the renin-angiotensin system is that of an aldosterone-stimulating hormone, and they were collectively termed as the “renin-angiotensin-aldosterone system.”

Due to the hypertensive action of Catecholamine (to be abbreviated as CA), certain relationship of the adrenal medulla with hypertension also came to be suspected. The action of angiotensin as a humoral factor on the adrenal gland was also brought to light, and there succeeded reports by many workers that angiotensin is a prominent stimulant for CA to be freed from the adrenal gland. In 1940, BRAUN-MENENDEZ et al. first demonstrated that angiotensin stimulates the secretion of adrenaline (to be abbreviated as A) in the adrenal medulla, and since then, the stimulative action of angiotensin on the adrenal

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Table I Effect of Angiotensin on Tissue Catecholamine Levels in Rat

<table>
<thead>
<tr>
<th>Organs</th>
<th>Tissue Catecholamines Content&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control</th>
<th>after Angiotensin injection&lt;sup&gt;b&lt;/sup&gt;</th>
<th>3 min</th>
<th>10 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>0.12 ± 0.007</td>
<td>0.056 ± 0.02</td>
<td>(p &lt; 0.003)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.096 ± 0.04</td>
<td>0.073 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>A 0.96 ± 0.36</td>
<td>0.70 ± 0.27</td>
<td>(p &lt; 0.003)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44 ± 0.39</td>
<td>0.70 ± 0.41</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>0.79 ± 0.52</td>
<td>1.27 ± 0.75</td>
<td>(p &lt; 0.003)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.93 ± 1.0</td>
<td>2.08 ± 0.71</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>A 0.063 ± 0.01</td>
<td>0.076 ± 0.03</td>
<td>(p &lt; 0.003)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.07 ± 0.03</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>: γ/mg wet tissue.  
<sup>b</sup>: 0.5 μg/kg injected into the coccyeal vein.  
<sup>c</sup>: Compared with control data.  
All value are means ± standard error of eight to ten animals.

Table II Effect of Angiotensin on Tissue Catecholamine Levels in Reserpine Pre-treated Rat

<table>
<thead>
<tr>
<th>Organs</th>
<th>Tissue Catecholamines Content&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Control</th>
<th>Reserpin&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Reserpin + Angiotensin</th>
<th>3 min</th>
<th>10 min</th>
<th>20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal glands</td>
<td>NA 0.12 ± 0.07</td>
<td>0.029 ± 0.009</td>
<td>0.018 ± 0.003</td>
<td>0.043 ± 0.007</td>
<td>0.047 ± 0.005</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>A 0.96 ± 0.36</td>
<td>0.26 ± 0.08</td>
<td>0.16 ± 0.05</td>
<td>0.264 ± 0.02</td>
<td>0.29 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>NA 0.79 ± 0.52</td>
<td>0.53 ± 0.06</td>
<td>0.53 ± 0.09</td>
<td>0.54 ± 0.06</td>
<td>0.77 ± 0.12</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>A 0.063 ± 0.01</td>
<td>0.015 ± 0.004</td>
<td>0.015 ± 0.004</td>
<td>0.013 ± 0.003</td>
<td>0.014 ± 0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>: γ/mg wet tissue.  
<sup>b</sup>: 0.1 mg/kg injected intraperitoneally.  
All value are means ± standard error of eight to ten animals.

Medulla was repeatedly demonstrated in Vivo as well as in vitro. (Renson et al<sup>8</sup> Pearch et al<sup>9</sup> Robinson<sup>10</sup>).  
On the other hand, attention was drawn in recent years to another factor, the sympathetic nerve, related to the maintenance of hypertension in conjunction with the humoral factor, and studies were directed to the angiotensin action on the central nervous system and peripheral sympathetic nerves. Bickerton et al<sup>11</sup> showed that angiotensin has the hypertensive action through the stimulation of the central nervous system, Lewis et al<sup>12</sup> reported the stimulation of the sympathetic ganglion, and Benelli et al<sup>13</sup> reported the stimulation of the sympathetic nerve ending which is involved in the liberation of the nerve-delivered substance.  

In relation to the sympathetic nervous system, Krasney<sup>14</sup> revealed that the angiotensin action on the cardiovascular has positive inotropic and chronotropic effects and that this results from the sympathetic innervation of the heart and the endogenous noradrenaline (to be abbreviated as NA) levels. However, it has been difficult to demonstrate consistent experimental effects of angiotensin on myocardial NA levels. Zimmerman et al<sup>15</sup> did not make any reference to the effect that the liberation of the myocardial NA is facilitated by angiotensin, and Buckely<sup>16</sup> reported that no change was brought about in the myocardial NA levels by the angiotensin infusion. On the contrary, an increase in the myocardial NA levels were recognized by Westfall et al<sup>17</sup> Sakai<sup>18</sup> his coworker, noted an increase.

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in blood CA resulting from the adrenal stimulation, and Raab et al. reported that a tissue with the postganglionic sympathetic nerve, including the heart, takes up circulating CA.

On the basis of these facts above mentioned, the present study was undertaken to elucidate the angiotensin action on the adrenal medulla and the effect of myocardial CA through observations of the changes in the adrenal and myocardial CA caused in the rat by angiotensin injection.

METHODS

(a) Animal used
Rats of both sexes, weighing 140 to 150g were used in the experiment.

(b) Method of Angiotensin II and reserpine administration.
1) Rats administered with angiotensin II alone
The animals were grouped according to the time lapsed after the injection of 0.5µg/kg body weight of angiotensin II into their coccgeal vein, as they were sacrificed at 3, 10, and 20 minutes later.
2) Rats administered with reserpine beforehand.
Reserpine was injected into the abdominal cavity of the animal at a dose level of 0.1mg/kg body weight a day for the preceding one week without interruption.
3) Angiotensin II administration to the rats given reserpine previously.
Twenty-four hours after the preliminary administration of reserpine, 0.5µg/kg body weight of angiotensin II was injected into the animal’s coccgeal vein. The rats were classified into three groups as they were killed at 3, 10, and 20 minutes after the injection.
4) On the control groups, distilled water of the same amount as angiotensin II was administered in the same manner. Each group consisted of 8 to 10 rats.

(c) Measurement of tissue CA (The Imaizumi’s method)
When 3, 10, and 20 minutes lapsed after the injection, the animals were decapitated and their heart and adrenal glands were removed. The organs were let dry on filter paper and measured for the weight. Then the organs were homogenized in 95% ethanol acid, the homogenates being absorbed and extracted by means of column chromatography. NA and A were determined according to Ethylene Diamine method.

By the way, Hypertensin-Ciba was used as the synthetic angiotensin II and Serpasil-Ciba as the reserpine.

Statistical analysis for the significance of data was performed by the student “t” test.

RESULTS
(a) Changes in adrenal CA after the independent administration of angiotensin II
As shown in Table I and Figure 1, at 3 minute after the angiotensin II injection, NA levels were significantly decreased compared with the control levels. (p < 0.003)

When 10 or 20 minutes lapsed, however, the difference from the control levels was no more significant. That is, the NA levels reach the minimum after 3 minutes, and then nearly recover the levels comparable to the controls in 10 and 20 minutes.

As to A, no significant was noted 3 minutes
after the injection, but the value decreased significantly after 10 minutes, \( (P < 0.003) \) as shown in Table I and Figure 2.

(b) Changes in myocardial CA after the independent administration of angiotensin II.

At 3 minutes after the injection, no significant difference was noted in the NA levels between the angiotensin-treated and the control groups. The value rose significantly in 10 and 20 minutes, as shown in Table I and Figure 3. \( (P < 0.003) \)

The changes in A remained insignificant in comparison with the control at either of the time for measurement, as shown in Table I and Figure 4.

(c) Changes in adrenal CA following the angiotensin II injection on the rat pre-treated with reserpine.

In the group that had had a prior administrations of reserpine alone, a distinctly significant decrease was observed in NA compared with the control.

In the group that had received reserpine in advance and angiotensin II afterwards, the NA levels were significantly reduced after 3, 10, and 20 minutes in comparison with the controls. \( (P < 0.003) \) But no significant difference was demonstrated when the value was compared with that for the group pre-treated with reserpine, as shown in Table II.

As to the changes in A, the group given reserpine, beforehand showed a significantly value than the control. \( (P < 0.003) \) In the group that had had the additional angiotensin II injection, the reduction in A was significant as against the control, but the value tended to be higher than that for the reserpine pre-treated groups, though the difference was not significant, as shown in Table II.

(d) Changes in myocardial CA after the administration of angiotensin II in the rat pre-treated with reserpine.

NA in the rat with the preliminary reserpine showed no significant difference in contrast to the control. After the additional injection of angiotensin II, the changes in NA remained insignificant as against the control until 20 minutes later when the Value was enhanced as compared with that for the reserpine pre-treated group and those at 3 and 10 minutes after the injection, as shown in Table II. \( (P < 0.003) \)

As for A, the value for the group with the preliminary reserpine was significantly lower than that for the control. \( (P < 0.003) \) After the additional injection of Angiotensin II, the decrease in A remained in a significant degree at 3, 10, and 20 minutes after the injection. But there was no significant difference demonstrated in the A value between the group with reserpine alone and the group with reserpine plus Angiotensin II, as shown in Table II.

**DISCUSSION**

Since BRAUN-MENENDEZ et al.\textsuperscript{2} reported in 1940 that angiotensin stimulates the secretion of adrenal A, many workers have put forth their findings on the relationship between angiotensin and the adrenal medulla. RENSON et al.\textsuperscript{8} found that when angiotensin was given to a cat which had been treated with cocain beforehand, its nictating membrane contracted, and they regarded that this was due to the by phenoxylbenzamine, \( \alpha \)-adrenergic blocking agent. KANEKO et al.\textsuperscript{21}
demonstrated that in the dog infused with DMPP, ganglion stimulant, the hypertensive reaction of angiotensin was enhanced, whereas in the dog that had adrenalectomy or treatment with phentolamine, the sensitization with KMPP did not bring about an increase in the hypertensive reaction of angiotensin. Feldberg et al.\textsuperscript{22} maintained that the angiotensin injection into the abdominal artery was an effective stimulant for CA in the adrenal medulla, Cessson et al.\textsuperscript{23} showed a reduction in the angiotensin action in an adrenalectomized rat and an increase in the CA liberation as a result of adrenal infusion with angiotensin, and Pearch\textsuperscript{9} and Sakai\textsuperscript{18} also demonstrated an increase of CA in blood through an angiotensin injection.

On the other hand, Vogt\textsuperscript{24} reported that a certain level of angiotensin was required to effectuate the stimulation of the adrenal medulla. Robinson\textsuperscript{10} observed in his experiment on the dog that by means of adrenal infusion, CA was liberated even with any amount of a low concentration, while Sakura\textsuperscript{25} detected no increase in the secretion of CA and its metabolic products in urine of a rabbit when the animal was intravenously infused with 0.04 µg/kg body weight/min of angiotensin for consecutive two hours, nor could find CA effuse from a section of the adrenal medulla of a cow immersed in angiotensin of 5 × 10\textsuperscript{-3} M. Similar reports have been brought forward by Paar et al.,\textsuperscript{26} who were unable to demonstrate on the dog any changes in CA and VMA in urine and CA in the adrenal tissue when 0.4 µg/kg body weight of angiotensin was injected into the abdominal cavity of a rat, no significant difference was demonstrated in the CA level 2 and 10 minutes later in comparison with the control.

In the present experiment on the adrenal gland of the rat, an injection of 0.5 µg/kg body weight of angiotensin into the coccyleal vein brought about a significant decrease in NA after 3 minutes and also a significant decrease in A after 10 minutes. This may suggest that angiotensin stimulates the adrenal medulla to liberate CA into blood, resulting in the decrease of CA contained in the adrenal tissue.

In the adrenal gland of the rat pre-treated with reserpine, both NA and A markedly decreased by a significant difference. As widely known, reserpine is a drug that causes a distinct reduction in the CA levels in the brain, heart and adrenal glands. Carlsson et al.\textsuperscript{28} observed that a large dose of reserpine (5 mg/kg body weight) almost completely evacuated CA from the adrenal glands, and Kroneberg et al.\textsuperscript{29} discovered that 90% of A disappeared at the introduction of 2 mg/kg of reserpine.

In the present study, too, adrenal NA was reduced by 75% and adrenal A by 70% as compared with the control when the rat had received 0.1 mg/kg a day of reserpine by abdominal injections for a whole week.

The angiotensin injection on the reserpine treated rat, however, failed to produce a significant difference in adrenal NA and A at 3 minutes after the injection as against the group treated with reserpine alone, significant difference might appear neither 10 nor 20 minutes later. This might be interpreted as that there is no CA to be liberated by angiotensin, or that the action of angiotensin is diminished when NA in the tissue is drastically decreased by a large dose of reserpine, just as is the case with tyramine. But no definite conclusion can be drawn from the results of the present experiment.

In our study on the rat's heart, myocardial NA was significantly increased at 10 and 20 minutes after the injection of angiotensin alone in comparison with the control.

On the other hand, no significant change was demonstrated in myocardial A at 3 and 10 minutes after the injection as compared with the control. Westfall et al.\textsuperscript{17} reported a similar result to ours that injections of angiotensin at dose levels of 0.3, 1.0, and 3.0 µg/kg body weight brought about a significant increase in myocardial CA after 2 minutes. On the contrary, Buckely\textsuperscript{16} maintained that an angiotensin infusion for an hour at a dose level of 1.0 µg/kg/min did not affect myocardial CA.

The increase in myocardial NA from the angiotensin injection encountered in our experiment might be attributed, as observed by Bickerton et al.\textsuperscript{30} and Smoller et al.\textsuperscript{31} to the enhanced synthesis of myocardial NA resulting from the stimulation of the central nervous system by angiotensin, or to the uptake of CA freed from the adrenal glands, or to both of the two. Pearch et al.\textsuperscript{32} reported that an angiotensin infusion for 10 minutes at a dose level of 0.1 µg/kg body weight caused an increase in blood CA, thus resulting in the increased myocardial uptake of CA by circulation.

As to the mechanism for the myocardial uptake, Raab et al.\textsuperscript{19} issued a report as already mentioned. Iversen\textsuperscript{33} demonstrated that the myocardium specifically takes up the circulating
CA and that A has less affinity to the myocardium than NA. These results are in agreement with ours in which no increase was noted in myocardial A.

In the experiment on the heart of the rat treated with reserpine beforehand, there was no significant decrease in NA and a significant decrease in A in the myocardium. Although the reserpine pre-treatment should have caused a decrease in myocardial CA as is the case with adrenal CA, the decrease in myocardial NA was not detected because the turnover rate of myocardial NA is about 12 hours as Brodie\textsuperscript{34} reported, which is higher than the rate of 7 to 10 days for adrenal NA, and yet the CA determination in the present study was carried out 24 hours after the reserpine pre-treatment. So these may be the reasons why reserpine did not affect the NA levels. The decrease in myocardial A, however, may be explained as that myocardial A is not synthesized within the tissue but almost entirely derived from the adrenal glands, so it is directly affected by the reserpine action.

The angiotensin injection on the rat pre-treated with reserpine brought about no significant changes in myocardial NA until its increase reached a significant level at 20 minutes after the injection as compared with the rat that had the reserpine pre-treatment only.

In myocardial A, no the other hand, no significant difference was revealed at 3, 10, and 20 minutes after the injection between the rat treated with reserpine plus angiotensin and the one with reserpine alone.

The significant increase in myocardial NA occurring 20 minutes later may be understood in the light of such findings that, ever after disappearance of myocardial CA from the reserpine administration, a very small amount of NA is kept to be taken up by the tissue without being interfered with by reserpine, as reported by Kopin et al\textsuperscript{35} and that reserpine has no effect on the earlier CA uptake by the myocardium as shown in the experimental infusion of the rat's heart by Iversen et al\textsuperscript{36}. Besides, Bhagat et al\textsuperscript{37} held that the sustenance of myocardial CA is not wholly dependent on the uptake of CA of the adrenal origin, and Kopin et al\textsuperscript{38} asserted that 80% of myocardial NA is synthesized by the tissue itself and the remaining 20% is taken up from circulating amines, some of which is derived from the adrenal glands. But these reports do not necessarily contradict a supposition that the CA uptake by the myocardium is also stimulated under a condition where blood CA reaches a high concentration as a result of the liberation of adrenal CA by the angiotensin administration.

On the basis of the foregoing experimental results and various literatures in this regard, it was inferred that angiotensin stimulates the adrenal medulla to free its CA into blood, and promotes the synthesis and uptake of myocardial NA.

**Conclusions**

Deliberation on the experimental results on the rat so far discussed may yield the following conclusions.

1. Angiotensin stimulates the adrenal medulla and causes CA contained in the tissue to decrease. This shows that angiotensin is an effective stimulant for CA in the adrenal medulla.
2. Angiotensin causes an increase in myocardial CA, or NA, in particular. It is speculated that is due to the enhanced uptake of circulating CA or to the promotion of NA synthesis by the myocardium as a result of the angiotensin action.
3. Angiotensin increases blood CA by stimulating the adrenal medulla and reinforces the contractile force of the heart by increasing myocardial CA. Thus, it may be justified regard angiotensin as one of the factors in development of essential hypertension.

**Acknowledgement**

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**REFERENCES**


