Studies on Erythropoietic Action of Angiotensin II

By Kiku Nakao, Taku Shirakura, Masami Azuma and Tadashi Maekawa

Since the establishment of a concept that erythropoiesis is controlled by erythropoietin (Ep), search for the production site of Ep has been one of the major projects. From the results obtained from both clinical and experimental research carried out so far, the kidney has been regarded as the organ playing an important role in the production of Ep. However, the mechanism of the production is yet to be clarified.

It has been pointed out by Osnes that potent activity of Ep in plasma obtained from adrenalectomized mice following phlebotomy is usually associated with an increase in granularity of the juxtaglomerular (J-G) apparatus in the kidney. Hirashima and Takaku noted a similar relationship between granularity of the J-G apparatus and the activity of Ep in plasma of phlebotomized or phenylhydrazine-administered rats. They also found that both granularity of the J-G apparatus and the activity of Ep in plasma showed a decrease in hypertransfused polycythemic rats. From these results, it is thought that the J-G apparatus might be a site of Ep production.

On the other hand, evidence that renin is elaborated by the J-G apparatus has been presented. Angiotensin II is a synthetic thermostable octapeptide which causes contraction of the smooth muscle of systemic blood vessels and other tissues. The action of renin upon the substrate is believed to produce the decapeptide, Angiotensin I. A "concerting enzyme" in plasma splits off the two terminal amino acids, histidine and leucine, to produce the active vasoconstrictor, Angiotensin II. Therefore, the latter can be regarded as an indirect product of the J-G apparatus. Thus, we carried out experiments to study the nature of erythropoietic activity of Angiotensin II.

Materials and Methods

Female rabbits weighing 2.0 to 2.5 Kg. and female rats of Wistar strain weighing 120 to 150 Gm. were used throughout the present experiment. Routine blood counts, including reticulocyte counts, were first carried out to exclude hematologically abnormal rabbits. Twenty-three female rabbits were separated into 5 groups, each consisting of 4 or 5 rabbits. Treatment was as follows:

Group A: Two daily intravenous injections of Angiotensin II, 100 μg./Kg. body weight, diluted to 0.001 per cent with physiologic saline.
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Group B: Injections of similar amounts of physiologic saline to serve as a control for Group A.
Group C: Dropwise intravenous injection of the same amounts of Angiotensin II as Group A, extending over 30 minutes.
Group D: Dropwise intravenous injection of 25 μg. of Angiotensin II and 250 μg. of norepinephrine diluted into 10 ml. saline per Kg. of body weight.
Group E: Dropwise intravenous injection of similar amounts of physiologic saline to serve as a control for Groups C and D.

To measure erythropoietic activity of these treated rabbits, one microcurie of Fe⁵⁹Cl₃ (specific activity, 17.886 μc./μg.) was injected intravenously 24 hours after the termination of the treatment. One ml. of blood was withdrawn on the 6th, 24th, 48th, 72nd, 96th, and 120th hour of the radioiron injection to measure the radioactivity of the washed red cells with a well-type scintillation counter (Scientific Research Institute, Ltd., Tokyo Radiation Counter Model 100).

Five hypophysectomized rats, operated 7 days previously, were injected with Angiotensin II, with the same dose as Group A rabbits. Five hypophysectomized rats were similarly injected with saline to serve as the control. These rats were given 0.3 microcuries of Fe⁵⁹Cl₃ intravenously 24 hours after the administration of Angiotensin II and saline. Eighteen hours later, 1 ml. of blood was withdrawn from those rats by cardiac puncture to count the radioactivity. Percentages of the radioiron incorporated into peripheral blood of the rats were calculated by assuming their total blood volume to be 5 per cent of body weight.

To measure changes in the plasma erythropoietin level after treatment with Angiotensin II, another 24 rabbits were separated into 5 groups, each consisting of from 4 to 6 rabbits. They were treated as follows:

Group F: The same treatment as Group E.
Group G: The same treatment as Group C.
Group H: Received x-irradiation of 400 y (50 y/min. at 200 kv./25 ma., Filter Cu 1.0 mm. + Al 0.5 mm.) 24 hours prior to the dropwise intravenous injection of physiologic saline.
Group I: Received 400 y x-irradiation and dropwise intravenous injection of Angiotensin II, 100 μg./Kg. diluted to 0.001 per cent with physiologic saline.
Group J: Five hypertransfused polycythemic rabbits (hematocrit over 60 per cent) were similarly treated to Group C.

Eight ml. of plasma were obtained from each of these treated rabbits 3, 6, 12, 24 and 48 hours after the termination of the treatment. Erythropoietin activity of the plasma was bioassayed with starved rats by the method of Fried et al. with slight modifications. In this assay, female Wistar rats weighing 130 to 150 Gm. were used. Radioiron was injected intravenously to measure its 18-hour incorporation into peripheral red cells of recipient rats. Total blood volume of starved rats was assumed to be 5 per cent of body weight.

Renal blood flow rate was estimated by phenolsulfonphthalein (PSP) excretion method. Polyvinyl tubes were inserted into both ureters of the rabbits. The amount of urine excreted was maintained at from 5 to 8 ml./min. by dropwise intravenous injection of physiologic saline. After an intravenous injection of 0.2 ml. of 1.6 per cent PSP solution, urine was collected every 5 minutes. PSP content of each collection measured photometrically (Filter 550 μm.) was expressed as a per cent of the PSP administered.

RESULTS

1. Effects of Angiotensin II upon the Erythropoiesis of Recipient Rabbits. As shown in Table 1 and Figure 1, intravenous injection of Angiotensin II totaling 200 μg./Kg. into hypophysectomized rats or normal rabbits did not
Table 1.—Effect of Intravenous Injection of Angiotensin II on Fe\textsuperscript{59} Utilization by Erythrocytes in Hypophysectomized Rats

<table>
<thead>
<tr>
<th>Material Injected</th>
<th>Number of Rats</th>
<th>Fe\textsuperscript{59} Utilization Up to 18 Hours (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Angiotensin II in normal saline</td>
<td>5</td>
<td>4.97 ± 0.23</td>
</tr>
<tr>
<td>Normal saline</td>
<td>5</td>
<td>5.44 ± 0.30</td>
</tr>
</tbody>
</table>

Fig. 1.—Effects of Angiotensin II injection on erythropoiesis in rabbits.

elicit any increased erythropoiesis. On the contrary, intravenous injections given dropwise resulted in a significant acceleration of Fe\textsuperscript{59} utilization (Fig. 1). Similar result was obtained in 5 rabbits of Group D receiving dropwise intravenous injections of 25 μg./Kg. of Angiotensin II and norepinephrine. No acceleration of Fe\textsuperscript{59} utilization was observed in 5 control rabbits of Group E receiving a dropwise injection of normal saline extending over 30 minutes (Fig. 1).

2. Changes in Plasma Erythropoietin Level of Rabbits following Angiotensin II Administration. As shown in Table 2, only the polycythemic rabbits of Group J showed a significant increase in their plasma erythropoietin
Table 2.—Changes in the Levels of Plasma Erythropoietin Activity following the Completion of the Drop Instillation of Angiotensin II in Various Groups of Rabbits

<table>
<thead>
<tr>
<th>Group of Experimental Rabbits</th>
<th>Erythropoietin Activity before</th>
<th>3</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal rabbits no Angiotensin</td>
<td>6.2 ± 0.8</td>
<td>6.8 ± 1.0</td>
<td>6.6 ± 0.8</td>
<td>6.6 ± 1.0</td>
<td>5.9 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>Normal rabbits receiving Angiotensin</td>
<td>6.6 ± 0.9</td>
<td>5.8 ± 0.4</td>
<td>8.2 ± 4.1</td>
<td>7.3 ± 2.3</td>
<td>5.1 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>Irradiated rabbits no Angiotensin</td>
<td>5.1 ± 0.1</td>
<td>6.1 ± 0.8</td>
<td>5.3 ± 0.2</td>
<td>4.7 ± 0.3</td>
<td>4.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>Irradiated rabbits receiving Angiotensin</td>
<td>8.4 ± 3.2</td>
<td>8.7 ± 3.3</td>
<td>9.8 ± 2.9</td>
<td>8.2 ± 4.3</td>
<td>4.5 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Polycythemic rabbit receiving Angiotensin</td>
<td>5.9 ± 5.8</td>
<td>19.0* ± 6.1</td>
<td>19.4* ± 13.2</td>
<td>13.2* ± 0.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(M ± S.D.)

* Statistically significant (p < 0.05).

level after 6, 12, and 24 hours of Angiotensin II administration. No significant increase of plasma erythropoietin level was observed in other groups of rabbits.

3. Effects of Angiotensin II on Renal Blood Flow. A slight and transient depression of PSP excretion was observed in 4 normal rabbits immediately after an intravenous injection of 100 μg./Kg. of Angiotensin II. In 4 rabbits, which were administered with equal amounts of Angiotensin II by means of intravenous dripwise injection extending over 30 minutes, a marked decrease in PSP excretion ratio was observed and maintained throughout the period of dripwise injection, as shown in Figure 2.

COMMENT

Several investigators have advocated that erythropoietin might be produced or activated either in, or in association with, the J-G apparatus of the kidney. However, more convincing evidence has been reported that renin is produced in the J-G apparatus. Fisher et al. reported that the administration of Angiotensin II to hypophysectomized and hypophyssectomized-adrenalectomized rats resulted in increased erythropoiesis without significant change in oxygen consumption. It was, however, reported by Bilsel et al. that no significant increase in erythropoietic activity was observed after the injection of Angiotensin II in amounts similar to those used by Fisher. In our experiments, similar results to those of Bilsel et al. were obtained in hypophysectomized rats as well as in normal intact rabbits. On the other hand, in
rabbits receiving the dropwise intravenous injection of Angiotensin II solution over a 30-minute period, acceleration of Fe\textsuperscript{55} utilization was observed. It is noteworthy that the total amount of Angiotensin II administered to the latter group of rabbits was one-half that given to the former. Moreover, a significant increase in erythropoiesis was observed in the rabbits receiving 25 \( \mu \)g of Angiotensin II and norepinephrine by dropwise intravenous injection. These results indicate that erythropoiesis-stimulating activity of Angiotensin II does not depend on its dosage but on the method of administration.

It is suggested that the increased erythropoiesis observed in the rabbits administered Angiotensin II by intravenous drip is due to the stimulating effect of erythropoietin. From a significant elevation of Ep activity found in the plasma of Angiotensin administered polycythemic rabbits, it is presumed that consumption of Ep may have prevented the detection of increased Ep activity in normal rabbits following the dropwise injection of Angiotensin II. Recently, Fisher et al.\textsuperscript{13} have reported that irradiation of the kidney prevents the rise in plasma erythropoietin levels commonly seen after an injection of cobalt. Absence of significant increase in Ep activity in plasma of irradiated rabbits following dropwise intravenous injection of Angiotensin II could signify that x-irradiation had prevented elaboration of Ep from the kidney.

It is of importance to note that a marked reduction of PSP excretion was
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maintained throughout the period of the dropwise intravenous injection of Angiotensin II. The sustained decrease in renal blood flow could be a cause of increased erythropoietin production.\(^6\)\(^8\)

**Summary**

The effects of synthetic Angiotensin II on erythropoiesis were investigated.

1. Two daily intravenous injections of Angiotensin II, 100 \(\mu\)g./Kg. of body weight, revealed no acceleration of Fe\(^{59}\) incorporation into erythrocytes of either normal rabbits or hypophysectomized rats.

2. When given by intravenous drip, 100 \(\mu\)g./Kg. of Angiotensin II significantly accelerated the radioiron incorporation.

3. The renal blood flow was markedly reduced throughout the period of the dropwise injection; the same effect was transitory after single intravenous injection. The elevation of plasma erythropoietin activity was observed in hypertransfused polycythemic rabbits following the dropwise injection of Angiotensin II.

From these results, it is concluded that erythropoietic activity of Angiotensin II results from an increased erythropoietin production. The increased production may have been induced by a renal ischemia through administration of Angiotensin II.

**SUMMARIO IN INTERLINGUA**

Esseva investigatc le effectos de synthetic Angiotensina II super le erythropoiese.

1. Duc injectiones intravenose per die de Angiotensina II (100 \(\mu\)g per kg de peso corporee) revelava nulle acceleration del incorporation de Fe\(^{59}\) ad in le erythrocytos de conilio normal o de ratti hypophysectomizate.

2. Quando administrate per inguttation intravenose, 100 \(\mu\)g per kg de peso corporee de Angiotensina II accelerava de maniera significative le incorporation de ferro radioactive.

3. Le fluxo le sangtine renal esseva reducite niarcatemente durante le integre periodo del injection guttatori. Le mesme effecto esseva transitori post injectiones intravenose individual. Le elevation del activitate de erythropoietina plasmatic esseva observate in hypertransfusionate conillos polycytheemic post le injection guttatori de Angiotensina II.

A base de iste resultatos le conclusion es formulata que le activitate erythropoietic de Angiotensina II resulta ab un augmento in le production de erythropoietin. Le augmentat\(\grave{e}\) productior es possiblemente induce per un ischemia renal causate per le administratior de Angiotensina II.

**ACKNOWLEDGMENT**

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**REFERENCES**


2. Hirashima, K., and Takaku, F.: Experimental studies on erythropoietin. II. The relationship between juxta-

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