Effect of Exogenous Erythropoietin on Juxtaglomerular Cells[1] (34216)

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Although the kidney may not be the exclusive source of erythropoietin (ESF), it is a significant site for its formation in many species. The role of renal erytropoietin (REF) in ESF production has been considered analogous to that of renin in the renin–angiotensin–aldosterone system with REF representing an enzyme capable of converting a plasma protein substrate to ESF (1, 2). Hypertrophy and/or increased granularity of juxtaglomerular cells (JGC) following stimuli producing an increase of ESF have suggested that JGC may represent the site of REF formation (3–6). Unfortunately, these studies are complicated by alterations in blood volume and distensibility of renal vasculature, factors well recognized to affect juxtaglomerular cell granularity (JGG). Apparent dissociation of these effects was subsequently accomplished by utilizing hypoxia as a stimulus for ESF formation since it is unaccompanied by hypovolemia. Some have interpreted increases in JGG after hypoxia to indicate that JGC represent the renal site of REF (7), whereas others have been less certain in this regard (8, 9). The site of REF production has also been ascribed to medullary as well as cortical portions of the kidney (10, 11). Results of a fluorescent antibody study have localized REF in glomerular epithelium and/or endothelial cells (12).

Since exogenous administration of renin results in a decrease in JGG and renal content (13), apparently by the common hormonal control mechanism of feed-back inhibition it appeared pertinent to examine the effects of exogenous ESF on juxtaglomerular cell indices (JGI) of kidneys from normal animals as well as those subjected to hypoxia and other modalities recognized to induce an increase in JGG. Indices of the width of the zona glomerulosa of adrenal cortices (ZGI), a morphologic expression of aldosterone production, were also estimated because of its relationship to JGI in the renin–angiotensin–aldosterone system.

Materials and Methods. One hundred sixty-two male Sprague-Dawley rats weighing 250–300 gm were separated into groups as indicated in Table I.

Renovascular hypertension was induced by the constriction of one renal artery with a clip fashioned from silver ribbon.

Sodium depletion was produced by withdrawal of 12 ml of peritoneal fluid after intraperitoneal instillation of 25 ml of 5% glucose in water. These rats were maintained on a sodium-deficient diet (General Biochemicals Co., Chagrin Falls, Ohio) and deionized water as drinking fluid.

Rats subjected to hypoxia were placed in a relatively air-tight, large incubator box with a thermoregulator maintained at 25° for 8 hr daily for 2 weeks. Nitrogen was introduced into the inlet and the air–nitrogen mixture was continuously recirculated by means of a pump connected to inlet and outlet tubes. A Beckman oximeter was interposed between the pump and the outlet allowing for periodic monitoring of the oxygen content which was maintained at 10%. Preliminary studies revealed this schedule sufficient to induce an erythropoietic response as indicated by reticulocytosis, increase in hematocrit, and elevated plasma ESF. JGI were also increased.

Approximately half of the normal rats and half of those subjected to unilateral renal artery constriction, sodium depletion, or hy-

[1] Supported in part by U.S.P.H.S. Research Grants C-5195 and AM 12223.

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TABLE I. Effect of Exogenous ESF and Renin (R) on Body Weight (bw), Blood Pressure and Volume.

Group	No. rats	Gain body wt (g)	Blood pressure (mm Hg)		Blood vol (% bw)
			Pre	End	
Normal + DW[a]	19	+28	103 ± 12[b]	108 ± 10	6.4 ± .4
Normal + ESF	18	+26	102 ± 8	95 ± 14	7.0 ±1.0
Renal clip + DW	23	+25	102 ± 10	147 ± 14	5.8 ± .5
Renal clip + ESF	19	+22	105 ± 14	144 ± 13	6.1 ± .8
Na deplete + DW	12	+ 8	108 ± 12	106 ± 15	3.1 ± .9
Na deplete + ESF	17	+ 6	109 ± 10	100 ± 12	3.5 ± .7
Unilateral nephrect. + R	10	+ 5	93 ± 17	148 ± 14	7.2 ± .8
Hypoxia + DW	16	+16	106 ± 8	125 ± 12	9.9 ± .7
Hypoxia + ESF	16	+17	104 ± 11	122 ± 6	10.2 ± .8
Hypoxia + unilateral nephrect. + R	12	+ 4	106 ± 8	142 ± 10	9.6 ± .8

[a] DW = Distilled H_2O.
[b] Standard deviation.

poxia received daily subcutaneous injections of 4.3 U ESF (Connaught Medical Research Laboratories, Willowdale, Ontario, Canada) in 1 ml distilled water for 14 days. Preliminary studies indicated that this dose produced a maximum erythropoietic effect. The remainder, serving as controls, received 1 ml of distilled water only.

Approximately equal numbers of hypoxic rats and rats kept at room air which were unilaterally nephrectomized 1 week prior to the start of the experiment received subcutaneous injections of 20 U renin (Nutritional Biochemical Co., Cleveland, Ohio) in 1 ml distilled water three times daily for 14 days. Controls received distilled water only.

Blood pressure, determined by a microphonic technique; hematocrit, estimated by a microcapillary method; and reticulocyte counts of peripheral blood were performed prior to and at the conclusion of the experiments. Total blood volume was estimated in six members of each group prior to sacrifice at 14 days by determining the dilution of RISA^{131}I (Abbott Laboratories, North Chicago, Ill.) in a 10-min postinjection sample of 1 ml of blood.

Two milliliters of plasma obtained from blood at time of sacrifice was injected intraperitoneally for 2 consecutive days into young Sprague-Dawley rats previously starved for 24 hr for assay of erythropoietic activity using the ^{59}Fe red blood cell incorporation method as performed by Cooper and Nocenti (14).

Portions of kidneys, spleen, liver, heart, and bone marrow were obtained from each animal at sacrifice. These were fixed in Helly's fluid, processed in the conventional manner, and stained with hematoxylin and eosin. Sections of kidney were also stained for estimation of JGI according to the method of the Hartrofts (15). Adrenals were fixed in 10% formalin and frozen sections were stained with oil red O for estimation of width of the zona glomerulosa which was expressed as percentage of cortex (ZGI) (16). Aliquots of kidneys from at least six animals of each group were also fixed immediately in 1% osmium tetroxide, buffered with veronal to pH 7.4, dehydrated, and embedded in Maraglas. Ultrastructural characteristics of JGC in ultrathin sections were studied with a Philips EM 200 electron microscope.

Results. As indicated in Table I, all animals gained weight. This was least evident in those subjected to sodium depletion or renin administration.

Blood pressure was significantly elevated in those subjected to unilateral renal artery

TABLE II. Effect of Exogenous ESF and Renin (R) on Reticulocytes (Retic), Hematocrit (HCT), and Plasma ESF.

Group	Retic (%)		HCT (%)		Plasma ESF (^{59}Fe uptake)
	Pre	End	Pre	End	
Normal + DW	.8 ± .3[b]	1.1 ± .9	47 ± 1.8	47 ± 1.2	3 ± 1.8
Normal + ESF	.6 ± .5	2.8 ± .8[c]	48 ± .9	51 ± 2.2[c]	6 ± 2.0[c]
Renal clip + DW	.9 ± .2	1.0 ± .5	47 ± 2.1	46 ± 1.8	4 ± 1.3
Renal clip + ESF	.8 ± .4	3.7 ± 1.1[c]	46 ± 1.9	51 ± 2.0[c]	7 ± 1.9[c]
Na deplete + DW	.8 ± .3	.9 ± .3	47 ± 1.6	48 ± 2.1	4 ± .9
Na deplete + ESF	.6 ± .4	2.2 ± .4[c]	47 ± 1.8	49 ± 1.7	5 ± 1.6
Unilateral nephrect. + R	.6 ± .4	1.0 ± .6	47 ± 2.4	46 ± 2.0	4 ± 2.8
Hypoxia + DW	1.2 ± .6	3.7 ± 1.5	46 ± 1.5	52 ± 2.2	8 ± .9
Hypoxia + ESF	.9 ± .5	4.6 ± 2.2	48 ± 2.3	53 ± 1.9	10 ± 2.3[c]
Hypoxia + unilateral nephrect. + R	.6 ± .4	3.2 ± .9	47 ± 2.1	51 ± 2.7	7 ± 1.7

[a] DW = Distilled H_2O.
[b] Standard deviation.
[c] $p = <.01$ compared to controls (DW).

constriction, exogenous renin administration, or hypoxia. Rats receiving ESF had blood pressures similar to their controls whether the level of pressure was normal or increased.

Blood volumes were significantly decreased in all sodium-depleted animals and significantly increased in all subjected to hypoxia.

Reticulocytes or hematocrits or both were significantly greater than their controls in all groups receiving ESF except those subjected to hypoxia (Table II). All hypoxic animals showed an increase in reticulocytes and hematocrits when compared to normal animals.

Uptake of ^{59}Fe was increased in assay animals receiving plasma from all hypoxic and ESF-treated rats except those subjected to sodium depletion.

Administration of ESF failed to significantly influence JGI or ZGI in any group studied whereas exogenous renin caused a significant decrease in JGI and increase of ZGI in hypoxic rats and those maintained in room air (Table III).

The ultrastructural appearance of JGC was unaffected by ESF or renin administration. Sections of spleen revealed increased foci of erythropoiesis in all hypoxic animals and in those that received ESF while maintained in room air. Vasculitis was noted at the hepatic hilus in 25% of rats receiving renin or subjected to unilateral renal artery constriction.

Discussion. Increased ESF, JGI, and blood volume observed in hypoxic rats have been interpreted as evidence relating the source of

TABLE III. Juxtaglomerular (JGI) and Zona Glomerulosa (ZGI) Indices after Distilled Water (DW), Erythropoietin (ESF), and Renin (R) Administration in Normal, Hypertensive, Sodium-Depleted, Unilaterally Nephrectomized, and Hypoxic Rats.

	JGI	ZGI
Normal + DW	30 ± 10[a]	8 ± 2
Normal + ESF	30 ± 12	9 ± 2
Renal clip + DW	27 ± 18/ 8 ± 4[b]	12 ± 1[c]
Renal clip + ESF	25 ± 12/10 ± 6[c]	12 ± 1[c]
Na deplete + DW	48 ± 11[c]	16 ± 3[c]
Na deplete + ESF	52 ± 10[c]	15 ± 4[c]
Unilat. nephrect. + DW	28 ± 12	8 ± 1
Unilat. nephrect. + R	8 ± 4[c]	11 ± 1[c]
Hypoxia + DW	44 ± 12[c]	10 ± 1
Hypoxia + ESF	48 ± 14[c]	10 ± 3[c]
Hypoxia + R	16 ± 6[c]	12 ± 3[c]

[a] Standard deviation.
[b] Numerator = clipped kidney; denominator = unclipped kidney.
[c] $p = <.01$ compared to normal.

REF to JGC (7), particularly since the converse effect on JGI is usually encountered in the hypervolemic state. Yet, effective substitution doses of ESF failed to influence JGI of hypoxic rats or those subjected to other situations in which it was increased by appropriate stimuli or was normal. This experience is contrary to the effect of other hormonal substances on their cellular sites of origin and represents indirect evidence that JGC are not concerned with REF formation. The failure of exogenous erythropoietin to provoke a greater reticulocytosis or increase in hematocrit in hypoxic animals than that noted in their controls suggests, as noted by others (17), that a maximum response to erythropoietin is not necessarily related to its maximum dose. The lack of effect of exogenous ESF on plasma ESF and hematocrit in sodium-depleted animals is unclear.

Exogenous renin, on the other hand, effectively reduced the elevated JGI but did not inhibit the increase in plasma ESF induced by hypoxia. Also, the level of JGI after renin administration in hypoxic rats was similar to that found in renin-treated, unilaterally nephrectomized animals kept at room air even though these latter had normal plasma ESF levels. These findings provide further evidence dissociating JGI from ESF production (19). Elevations of ESF observed by others (10) after renal artery constriction or ligation would appear to be related to the more severe degree of luminal compression apparently required for ESF production than that utilized in this study to produce renovascular hypertension.

The increased JGI, ZGI, and elevated blood pressure observed in hypoxic rats is more consonant with increased renal renin formation and secretion than increased ESF production. No angiotensin-like effect of ESF has been observed in this study or by others using different techniques (18). ESF was not increased after unilateral renal artery constriction, a modality producing an increase of endogenous renin and a relative increase of JGI in the clipped kidney. These interpretations do not mitigate against the presence of REF since ESF was elevated in hypoxic animals. Yet, they do suggest that the source of REF may occur at renal sites other than JGC. Alternatively, hypoxia may stimulate extrarenal ESF generation which is unrelated to its effect on JGC or other portions of the kidney.

The lack of qualitative ultrastructural differences in JGC in the various situations studied suggests that any physiologic changes associated with variations of JGI are quantitative rather than qualitative at least insofar as can be discerned by the electron microscopic technique employed.

Summary. Exogenous erythropoietin (ESF) failed to influence indices of juxtaglomerular granules (JGI) or width of zona glomerulosa (ZGI) in normal rats or those subjected to hypoxia, unilateral renal artery constriction, or sodium depletion, stimuli associated with increased JGI and ZGI. Administration of renin, on the other hand, decreased JGI without reducing plasma ESF levels. These findings are interpreted as indirect evidence that the source of renal erythropoietin is not related to juxtaglomerular granules (JGG). Further, findings suggest that the increased JGI induced by hypoxia may be related to an increase in renal renin. Ultrastructural examination of JGG revealed their similar appearance in all situations suggesting that changes in JGI associated with various physiologic phenomena are most likely quantitative rather than qualitative.

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Received May 26, 1969. P.S.E.B.M., 1969, Vol. 132.