Effect of Sex Differences on Extrarenal Erythropoietin Production[1] (35555)

WALTER FRIED, WILLIAM H. KNOSPE, AND FRANK E. TROBAUGH, JR.
(Introduced by Paul Heller)

Section of Hematology, Rush Presbyterian St. Luke's Hospital and Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois 60612

Erythropoietin (Ep) is produced primarily by the kidneys (1, 2). However, extrarenal sites of Ep production exist (3–5) and are responsive to stimulation by anemia, hypoxia, and cobalt (6, 7). Although the plasma Ep level of anephric rats is only a fraction of that attained by rats with intact kidneys after exposure to comparable stimuli, significant elevations of the plasma Ep titers occur in anephric rats when exposed to intense hypoxia (6).

Injection of pharmacologic doses of testosterone propionate into female rats three times weekly for 2 weeks enhances the ability of their kidneys to produce Ep in response to hypoxia (7), but does not detectably alter the production of Ep by their extrarenal sites (7). Male mice have been shown to attain higher plasma Ep levels in response to hypoxia than do identically stimulated females (8). This is presumed to result from chronic exposure of the kidneys to the elevated endogenous androgen levels of males. The following experiments were performed to ascertain whether sex differences in the rate of extrarenal Ep production also exist.

Methods. Adult male and female Sprague-Dawley rats, weighing 250 to 350 g, were used. Hypoxia was produced by placing rats into an airtight chamber connected to a vacuum pump and fitted with a valve capable of maintaining the desired vacuum. Plasma Ep levels were assayed in transfusion-induced-polycythemic mice by the method of De Gowin *et al.* (9) Results are expressed as the mean percentage 72-hr RBC ^{59}Fe uptake of 7–10 assay mice ± 1 standard error of the mean. Bilateral nephrectomy was performed on rats through a ventral incision while they were under ether anesthesia. Operations were completed within 15 to 30 min of exposure to hypoxia. The animals were completely awake at the time they were placed into the hypoxic atmosphere.

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TABLE I. Plasma Ep Levels in Unoperated Hypoxic Male and Female Rats.

Hypoxia (atm)	Plasma assayed (ml)	Plasma Ep[a] activity	
		Males	Females
0.5	0.5	11.5 ± 1.2 (7)[b]	4.8 ± 0.4 (7)[b]
0.47	0.4	20.1 ± 2.3 (7)	10.7 ± 0.9 (7)
0.43	0.3	14.0 ± 1.7 (10)	9.8 ± 0.9 (7)
0.4	0.25	16.0 ± 1.4 (7)	19.4 ± 1.5 (8)

[a] Expressed as mean % RBC ^{59}Fe uptake of assay mice ± 1 SE.

[b] Number of assay mice given in parentheses.

Groups of 5 unoperated female rats, five unoperated male rats, 5 nephrectomized female rats and 5 nephrectomized male rats were exposed simultaneously to either 0.5, 0.47, 0.43, or 0.4 atm for 8 hr. Within 15 min after return to ambient pressure the rats were exsanguinated and the plasma from each group was pooled and stored at —5° until assayed for Ep. All plasmas were assayed simultaneously.

Results. The plasma Ep activity of hypoxic unoperated male and female rats is shown in Table I.

Ep activity of males reached a maximum level on exposure to 0.47 atm and did not increase on exposure to more intense hypoxia. Ep activity in the plasma of female rats, on the other hand was less than that of males when exposed to 0.5, 0.47, and 0.43 atm but reached a level equal to the maximum at-

TABLE II. Plasma Ep Levels in Nephrectomized Hypoxic Male and Female Rats.

Hypoxia (atm)	Plasma assayed (ml)	Plasma Ep[a] activity	
		Males	Females
0.5	1.0	6.0 ± 0.5 (7)[b]	0.9 ± 0.8 (6)[b]
0.47	1.0	8.9 ± 0.8 (7)	1.6 ± 0.2 (8)
0.43	1.0	7.8 ± 0.6 (8)	2.1 ± 0.3 (7)
0.40	1.0	7.4 ± 0.5 (6)	6.7 ± 0.6 (8)

[a] Expressed as mean % RBC ^{59}Fe uptake of assay mice ± 1 SE.

[b] Number of assay mice given in parentheses.

tained by male rats after exposure to 0.4 atm.

Table II shows the plasma Ep activity of hypoxic anephric male and female rats. It should be noted that whereas 0.25 to 0.5 ml of plama was assayed in the groups described in Table I, 1.0 ml of plasma was assayed from all groups reported in Table II. Plasma Ep activity in anephric male rats reached peak activity on exposure to 0.47 atm and did not increase after exposure to more intense hypoxia. On the other hand, the plasma Ep activity of anephric females was less than that of comparably hypoxic anephric males at all levels of hypoxia studied except after exposure to 0.4 atm. On exposure to 0.4 atm, the plasma Ep activity of anephric males and females reached comparable levels. The maximum plasma Ep activity of anephric rats of both sexes was about 1/10 that attained by unoperated hypoxic rats.

Discussion. Since anephric patients and some of those with chronic renal disease depend on extrarenal sites of Ep production for maintenance of their plasma Ep level and consequently of their rate of erythropoiesis (10, 11), an understanding of the factors controlling extrarenal Ep production has obvious practical value.

Previous studies have shown that injection of testosterone proprionate in doses capable of increasing renal Ep production has no detectable effect on extrarenal Ep production. Yet, the data reported here indicate that extrarenal Ep production of adult male rats is greater than that of adult female rats in response to most hypoxic stimuli. We suggest the following possible interpretations of this data: (i) extrarenal sites of Ep production are responsive to prolonged exposure to elevated androgen levels, as occurs in males, but not to short-term intermittent doses of testosterone, even when given in massive amounts. In support of this concept is the report of Mirand *et al.* (11) that extrarenal Ep production was increased in an anephric patient after androgen therapy; (ii) naturally occurring androgenic steroids other than testosterone increase Ep production from extrarenal sites or; (iii) the increased production of Ep from extrarenal sites of males compared to females results from causes unrelated to differences in androgenic steroid levels. These various possibilities are currently under study.

The present data indicate also that anephric females are capable of increasing their plasma Ep activity to levels comparable to maximum levels observed in hypoxic anephric males. However, more intense hypoxia is required to achieve these levels in females than in males.

Renal sites of Ep production in males are also more sensitive to lesser intensity of hypoxia than are those of females; yet those of females are capable of achieving comparable peak plasma Ep levels when sufficiently stimulated.

Summary. Evidence is presented to show that extrarenal as well as renal sites of Ep production are more sensitive to hypoxia in male than in female rats. However, on exposure to very intense hypoxia, the peak plasma Ep levels of both anephric and intact female rats are comparable to those of males.

1. Jacobson, L. O., Goldwasser, E., Fried, W., and Plzak, L., Nature (London) **179**, 633 (1957).

2. Naets, J. P., Nature (London) **181**, 1134 (1958).

3. Erslev, A. J., Arch. Intern. Med. **101**, 407 (1958).

4. Mirand, E. A., and Prentice, T. C., Proc. Soc. Exp. Biol. Med. **96**, 49 (1957).

5. Gallagher, N. I., McCarthy, J. M., Hact, K. T., and Lange, R. D., Blood **14**, 662 (1959).

6. Fried, W., Kilbridge, T., Krantz, S., McDonald, T. P., and Lange, R. D., J. Lab. Clin. Med. **73**, 244 (1969).

7. Fried, W., and Kilbridge, T., J. Lab. Clin. Med.

74, 623 (1969).

8. Fried, W., and Gurney, C. W., J. Lab. Clin. Med. **67**, 420 (1966).

9. DeGowin, R. L., Hofstra, D., and Burney, C. W., Proc. Soc. Exp. Biol. Med. **110**, 48 (1962).

10. Naets, J. P., and Wittek, M., Blood **31**, 249 (1968).

11. Mirand, E. A., Murphy, G. P., Steeves, J. M., Groenwald, J. M., and De Klerk, J. N., J. Lab. Clin. Med. **73**, 121 (1969).

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