Effect of Aging on Erythropoietin Secretion in Male Rats

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The secretion of erythropoietin (EPO) and testosterone in response to hypoxia in old (22–25 months), middle (mid-) aged (15–17 months), adult (6–7 months), and young (3 months) male rats was studied. Rats of different ages were bled by cardiac puncture before and subsequent to 8 h exposure to 12% O2. The metabolic clearance rate of EPO was determined by a single-injection method. The effects of orchidectomy and replacement of testosterone propionate on plasma EPO concentrations were also investigated. Analysis of the direct effects of testosterone on EPO release from kidney tissue was carried out in an in vitro study. Both basal and hypoxia-induced EPO levels were lower in old rats than in mid-aged, adult, and young rats (p < .01). Plasma testosterone levels decreased in response to hypoxia in all rats (p < .01 for young, adult, and mid-aged rats, and p < .05 for old rats). The old rats also had lower plasma testosterone levels following hypoxia when compared with other rats (p < .05). The metabolic clearance rate of EPO was not affected by age. Orchidectomy decreased rat plasma EPO concentration (p < .05). This decrease could be restored to intact levels after testosterone propionate replacement. Both 10⁻¹⁰ M (p < .05) and 10⁻⁹ M (p < .01) testosterone stimulate EPO release from kidney tissue in vitro. Our findings indicate that the basal levels of plasma EPO and testosterone are decreased, and the hypoxia-induced EPO is also diminished with aging in male rats. These data suggest that the mechanism of tolerance to hypoxia and the endocrine function of the kidneys in male rats during the aging process are testosterone-dependent.

Materials and Methods

Animals. — Old (22–25 months, n = 7), middle (mid-) aged (15–17 months, n = 12), adult (6–7 months, n = 9), and young (3 months, n = 7) male Sprague–Dawley rats were housed in a temperature-controlled room (22 ± 1 °C) with 14 h of artificial illumination daily (0600–2000). All animals were given food and water ad libitum.

Effects of aging on plasma EPO and testosterone levels. — Rats of different ages were anesthetized with ether and bled by cardiac puncture (5 ml/kg) within 3 min. The blood sample was mixed with 50 μl heparinized saline (100 IU/ml). The plasma was separated by centrifugation of the blood samples at 10,000 × g for 1 min to determine the level of EPO and testosterone.

Effects of hypoxia on plasma EPO and testosterone levels in rats of different ages. — Normobaric hypoxia was used as a stimulus for EPO production. Rats of different ages were exposed to an atmosphere low in oxygen (12% O2) in a plastic chamber. All rats were simultaneously exposed to
hypoxia for 8 h. Within 30 min following the end of hypoxic exposure, rats were bled by cardiac puncture under ether anesthesia for determination of plasma EPO and testosterone levels.

**MCR of rat EPO.** — The MCR of rat EPO was determined by a single-injection method as previously described with modification (Pu et al., 1987). Briefly, 500 μl blood samples were collected at 0, 5, 10, 30, 60, and 120 min following injection of 125I-labeled EPO with 0.2 × 10⁶ cpm in 1 ml saline solution per kg via a catheter inserted in the right jugular vein. Human EPO was iodinated with 125I (Amersham, Buckinghamshire, UK) using a modification of the chloramine-T method (R.Y. Wang et al., 1994). Labeled EPO from serum was immunoprecipitated as follows: each serum sample was reacted with excess amounts (1:100) of rabbit anti-EPO (RYW 55) for 48 h prior to an additional 48-h incubation with sheep anti-rabbit γ-globulin. The reaction of the serum sample with normal rabbit serum (1:100) served as a nonspecific control. After centrifugation at 2500 × g for 30 min, the radioactivity of the precipitate was counted in an automated gamma counter (1277 GammaMaster; Pharmacia, Turku, Finland). The total quantity of serum immunoprecipitable radioactivity (cpm/ml) versus time after injection.

**Effects of orchidectomy and testosterone replacement on rat plasma EPO concentration.** — Young male rats were orchidectomized (Orch) or sham Orch. Two weeks after orchidectomy, rats received a single injection subcutaneously daily for 1 week with either sesame oil or testosterone propionate (TP; 20 mg/kg/bw). The sham-Orch rats received sesame oil only. Rats were bled on the day following the final injections. Blood was collected by cardiac puncture from rats under ether anesthesia. The plasma was separated by centrifugation at 10,000 × g for 1 min for EPO determination.

**Effects of testosterone on EPO release in vitro.** — To study the direct effects of testosterone on EPO, normal young rats were decapitated, and the kidneys were removed and decapsulated and cut into six equal pieces. One-sixth of the kidney was used for incubation. The kidney blocks were preincubated at 37 °C for 90 min before basal incubation for 30 min with Locke’s solution containing 10 mM glucose, 0.003% bacitracin, and 0.05% Hepes. Each kidney block was assigned to a flask containing 1 ml of the medium. The medium was then aerated with 95% O₂/5% CO₂ (R. Y. Wang et al., 1994). The kidney blocks were challenged with testosterone (10⁻¹⁰⁻¹⁰⁻⁶ M) for 30 min. At the end of the incubation, renal tissues were weighed and the media collected for EPO measurement by radioimmunoassay (RIA).

**RIA of EPO.** — The concentration of plasma EPO was measured by RIA kits provided by Incstar Corporation (Stillwater, MN) as previously described (Wang et al., 1995). The medium EPO levels were measured by a RIA using an antiserum (RYW 55) to EPO generated in our laboratory by immunizing rabbits with human recombinant EPO (R. Y. Wang et al., 1994). The sensitivity is measured at 0.62 mlU. Cross reactivities are less than 0.1% with human choriionic gonadotropin (hCG), human IgG, human albumin, and human γ-globulin. The intra- and interassay coefficients of variability (CV) were 1.8% (n = 3) and 6.5% (n = 8), respectively.

**RIA of testosterone.** — The concentration of plasma testosterone was determined by RIA as previously described using antitestosterone serum W8 (P.S. Wang et al., 1994). The sensitivity of testosterone RIA was 2 pg per assay tube. The intra- and interassay CV were 4.1% (n = 6) and 4.7% (n = 10), respectively.

**Statistical analysis.** — All data were expressed as mean ± SEM. Treatment means were tested for homogeneity with a two-way analysis of covariance (ANCOVA) (Steel and Torrie, 1980). The differences between the specific means were tested for significance by Scheffe’s multiple range test or Student’s t-test. A difference between two means was considered to be statistically significant when p < .05.

**RESULTS**

**Effects of aging on plasma EPO level.** — Figure 1 illustrates the levels of plasma EPO in rats of different ages and their response to hypoxia. The basal levels of plasma EPO in old rats were lower than those in young rats (p < .01). There were no differences in basal plasma EPO levels among young, adult, and mid-aged rats. The EPO levels were increased in all rats in response to hypoxia (p < .01). The plasma EPO levels following exposure to hypoxia were lower (p < .05) in old than in young rats.

**Figure 1.** Effect of hypoxia on plasma EPO in male rats with different ages. Rats were housed in a hypoxic box (12% O₂) for 8 h. + + p < .01 vs normoxia; * p < .05; **p < .01 vs young rats.
Effects of aging on plasma testosterone level. — The plasma testosterone levels in rats of different ages and their response to hypoxia are illustrated in Figure 2. The basal plasma testosterone levels change with age. Mid-aged and old rats had lower basal testosterone levels than young rats ($p < .01$). The plasma testosterone levels decreased in response to hypoxia in all rats ($p < .01$ for young, adult and mid-aged rats; $p < .05$ for old rats), but the old and mid-aged rats demonstrated lower plasma testosterone levels following hypoxia compared with young rats ($p < .01$).

Effects of aging on the MCR of EPO. — The mean MCR of EPO in rats following a single injection of $^{125}$I-labeled EPO ranged from 0.8 to 1.1 ml/min. There is no difference in the MCR of EPO in rats of different ages ($p = .30$).

Effects of orchidectomy and testosterone replacement on plasma EPO concentration. — Figure 3 illustrates the changes in EPO concentration subsequent to orchidectomy and the replacement of testosterone in young rats. Orchidectomy decreased rat plasma EPO concentration ($p < .05$). After TP replacement, the plasma EPO concentration of the Orch rats was increased to the intact levels and was different from the Orch rats with oil replacement ($p < .05$).

Effects of testosterone on EPO release in vitro. — The release of EPO in response to testosterone in vitro is shown in Figure 4. Both $10^{-10}$ M ($p < .05$) and $10^{-9}$ M ($p < .01$) of testosterone stimulate EPO release. The increase of medium EPO in response to testosterone appears dose-dependent.

Correlation between EPO and testosterone in rat plasma. — The plasma EPO and testosterone levels of individual rats from different age groups under normoxia were used for calculation of correlation. There is a positive correlation between the levels of EPO and testosterone in rat plasma (Figure 5).

DISCUSSION

There are four important findings from the present study. First, the concentration of plasma EPO and the response of
plasma EPO to hypoxia are decreased with age in male rats. Second, plasma testosterone is decreased in response to hypoxia. Third, the MCR is not altered with age in male rats. Fourth, a positive correlation can be found between plasma EPO and testosterone levels.

Previously, we found that the basal levels of plasma EPO increased with age in Ovx rats (Wang et al., 1995). A decreased MCR of EPO in the aged rats may therefore account for the increased EPO level, although this had never been tested. The present data indicate that the basal plasma EPO levels decrease with age in normal male rats, while the MCR is not appreciably altered with aging. According to our results, the change in plasma EPO levels with age cannot be explained by MCR. The change of secretion rate of EPO is suggested as a factor that effects the decrease of plasma EPO levels with age.

The secretion of testosterone has been reported to decrease with age based on measurements of peripheral and spermatic venous blood (Lewis et al., 1976; Serio et al., 1979). A decreased testosterone secretion has been shown to be due in part to impaired secretion of gonadotropins and in part to a primary testicular insufficiency (Hammar, 1985). The decreased plasma EPO level is at least in part due to the lower levels of plasma testosterone in old male rats. Compared with young or adult rats, where the level of plasma testosterone shows a decrease in mid-aged rats (Figure 2), the reduction of plasma EPO concentration did not reach a level of significance (Figure 1). The reason is not known but may be due to an existence of testosterone threshold in male rats for inducing hyposecretion of EPO. It is for this reason that a stimulatory effect of testosterone on EPO formation has been established. The present data indicate that orchidectomy causes a decrease in plasma EPO that can be returned to the intact level after replacement with TP. The plasma EPO levels of both anemic and nonanemic patients with intact kidneys has been shown to increase after androgen therapy (Rishpon-Meyerstein et al., 1968; Alexanian, 1969). A direct dose-dependent stimulatory effect of testosterone on EPO release from kidney tissue is also confirmed. The direct stimulatory effect of testosterone on the production of EPO in kidneys has been reported as well (Malgor and Fisher, 1970).

In the present study, we have established a simple and valid in vitro system to investigate EPO release from rat kidney tissues. Using this methodology, the mechanisms for EPO secretion can be further investigated. A positive correlation between EPO levels and testosterone levels in rat plasma is also verified in our study. It may still be possible that testosterone, in addition to its action on the kidneys to stimulate EPO production, may also act synergistically with EPO during red blood cell maturation (Udupa and Lipschitz, 1984). Experiments to test the effect of orchidectomy on the plasma EPO in response to hypoxia in young and old rats may lend additional support for the testosterone-dependent effect.

Reduced O$_2$ supply due to inspiratory hypoxia is a well-known stimulus for EPO production (Bauer, 1991). The effects of inspiratory hypoxia on the EPO system depend decisively upon the O$_2$ concentration and the duration of exposure to the hypoxic environment. An exposure interval of 5.5 h in a hypobaric chamber leads to a marked plasma EPO elevation in humans (Eckardt et al., 1989). In our study, plasma EPO was markedly increased in all rats when exposed to an atmosphere with 12% O$_2$ for 8 h. Based on our data, 12% O$_2$ hypoxia for 8 h is a strong stimulus for EPO production in rats. The plasma EPO level after hypoxia is lower in old than in young rats. In our previous study (Wang et al., 1995), the increase of plasma EPO after hemorrhage in both old and mid-aged Ovx rats was less than that in adult rats. A decrease in the reserve capacity of the kidneys to produce EPO in response to stimulation is indicated in old rats as compared with young or adult rats.

Depression of plasma testosterone concentration has been found in men with chronic obstructive airway disease, and the degree of testosterone depression was related to the severity of arterial hypoxia (Semple et al., 1980). An increase in plasma testosterone after long-term oxygen therapy has also been noted in male patents with respiratory failure (Aasebo et al., 1993). Our experimental results further confirm that plasma testosterone is attenuated in all rats subjected to hypoxia. Furthermore, the level of plasma testosterone is significantly lowered in old rats compared to younger rats following hypoxia. The underlying mechanism by which hypoxia reduces plasma testosterone levels is presently unknown and warrants further elucidation.

In summary, our findings indicate that the basal levels of plasma EPO and testosterone are decreased, and hypoxia-induced EPO is also diminished with aging in male rats. These data suggest that the changes in tolerance to hypoxia and the reduced endocrine function with respect to EPO production in the kidneys of male rats during the aging process are testosterone-dependent.

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REFERENCES


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