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# THYROID GLAND FUNCTION IN THE BELGRADE LABORATORY (b/b) RAT

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*The Belgrade laboratory (b/b) rat has a unique hereditary defect in iron metabolism, that induces profound anemia and hypoxia. Serum thyroid hormone levels in anemic b/b rats and non-anemic controls (+/+) were investigated using radio-immunoassay (RIA). Hematologic parameters and erythropoietin (Epo) concentrations were also determined in the anemic b/b and control rats. Our results confirmed the existence of severe anemia and extremely high Epo concentrations in the anemic b/b rats. The results for serum thyroid hormone concentrations imply the existence of thyroid gland hypofunction in the b/b rats. These preliminary results points to the b/b rat anemic syndrome as a unique model, which is not only interesting for the hematologist, but also for the endocrinologist.*

*Key words: thyroid hormones; anemia; hypoxia; Belgrade laboratory (b/b) rat.*

## INTRODUCTION

A major metabolic effect of thyroid hormones in humans and animals is stimulation of oxygenconsumption. Investigations of the response of thyroidgland function to hypoxia in experimental animals have brought conflicting results: from unaltered thyroid activity *(Mulvey et al., 1969)*, to decreased *(Galton et al., 1972)* or increased thyroidal activity *(LaRoche et al., 1967)*. Studies of thyroid function in humans exposed to acute environmental hypoxia revealed a significant increase in blood levels of total thyroxine and triiodothyronine *(Surks, 1966a)*, while hypobaric hypoxia induced an increase in thyroidal accumulation of $^{131}I$ *(Rawal et al., 1993)*. Later results of *Rawal et al. (1993)* indicate that the increased concentrations of circulating thyroid hormones in subjects exposed to hypoxia are at least partly caused by increased thyroidal activity.

The Belgrade laboratory (b/b) rat has a unique defect in iron metabolism due to a radiation-induced mutation designated by "b" *(Sladić-Simić et al., 1966)*. As a consequence of this mutation the Belgrade laboratory rat has severe microcytic anemia accompanied by hyperferremia *(Sladić-Simić et al., 1969)*. The

"b" mutation is inherited as an autosomal recessive trait. The recessive homozygous rats (b/b) are anemic, whereas the heterozygous (b/+) and dominant homozygous rats are phenotypically normal *(Sladić-Simić et al., 1966).* The state of chronic hypoxia caused by the anemia in (b/b) rats must have a profound effect on thyroidal gland function. Therefore, the following study was conducted to examine thyroidal hormone levels in the serum of the Belgrade laboratory (b/b) rat.

## MATERIAL AND METHODS

Body weight, hematocrit (Htc), hemoglobin (Hb) and red blood cell counts (RBC) were determined in a control (+/+) group of 5 rats and an experimental group of 10 anemic Belgrade laboratory (b/b) rats. RBC counts were made using a hemocytometer, Hb concentration was determined by the cyanmethemoglobin technique, and Htc was determined using the microhematocrit method. All values are represented as mean $\pm$ SD.

Serum triiodothyronine ($T_3$) and thyroxine ($T_4$) levels were determined in the control group of 5 normal phenotype Belgrade laboratory (+/+) rats and the experimental group of 10 eight-week-old anemic Belgrade laboratory (b/b) rats, using commercial RIA-kits (INEP-Zemun). The mean intra-assay coefficents of variation of duplicate samples were as follows: $T_3$ - 7.2% and $T_4$ - 4.2%. The statistical significance of differences between the means for the experimental and control group of rats was estimated by Student's t-test.

Serum erythropoietin (Epo) concentrations were determined in 3 phenotypically normal control (+/+) rats and 3 anemic Belgrade laboratory (b/b) rats, using a commercial enzymeimmunoassay kit (EPO-ELISA, Medac GmbH, Hamburg). Enzyme activity was determined at 405 nm by a programable ELISA-Reader (EAR 400 AT; SLT-Labinstruments, Overath). A linear standard curve was made automatically with the following Epo standards: O, 1.25, 2.5., 5, 10, 20, 40, and 80 U/L.The standards were of rat serum origin and previously calibrated by bioassay in polycythemic mice. Serum samples of the anemic b/b rats were diluted to fit within the range of the standard curve, and the results of Epo concentrations scaled up according to the degree of dilution.

## RESULTS

The results presented in Table 1. show that the body weight, Htc values and Hb concentrations were significantly lower in the anemic b/b rats ($p<0.01$). At the same time there was no significant difference in the RBC counts between the control group and anemic b/b rats. Our results for hematologic parameters confirmed of microcytic hypochromic anemia in the b/b rats, as reported previously *(Sladić-Simić et al., 1969; Pavlović-Kentera et al., 1989).*

Table 1. Body weight, hematocrit (Htc), hemoglobin (Hb) and red blood cell count (RBC) in the control (+/+) group of rats and anemic (b/b) rats.

| parameters | control group (5) | anemic b/b rats (10) | statistical significance |
|---|---|---|---|
| Body weight (g) | 144.00 ± 10.12 | 64.44 ± 14.26 | $p < 0.01$ |
| Htc (%) | 41.00 ± 1.00 | 20.22 ± 11.20 | $p < 0.01$ |
| Hb (g/dL) | 13.91 ± 0.57 | 3.57 ± 1.40 | $p < 0.01$ |
| RBC (x $10^3/mm^3$ | 5.38 ± 1.06 | 5.10 ± 2.67 | $p > 0.05$ |

legend: numbers inparenthesis represent the number of experimental animals

The results presented in Table 2. reveal a statistically significant difference ($p<0.01$) in the serum levels of triiodothyronine and thyroxine between the control and anemic b/b rats. The serum levels of both thyroid hormones were Lower in the anemic b/b rats, implying the existence of thyroid gland hypofunction in these rats.

Table 2. Serum $T_3$ and $T_4$ levels (nmol/L) of the control (+/+) and anemic (b/b) groups ofrats.

| hormone (nmol/L) | control group (5) | anemic b/b rats (10) | statistical significance |
|---|---|---|---|
| $T_3$ | 0.76 ± 0.26 | 0.42 ± 0.16 | $p < 0.01$ |
| $T_4$ | 42.42 ± 3.38 | 27.61 ± 5.65 | $p < 0.01$ |

legend: numbers in parenthesis represent the number of experimental animals

The results presented in Table 3.are show the extremely high serum Epo concentrations in the anmic b/b rats.

Table 3. Serum erythropoietin (Epo) concentrations in some control (+/+) and anemic (b/b) rats.

| Group of rats | No. | Epo (U/L) |
|---|---|---|
| controls (+/+) | 1. | 3.9 |
| | 2. | 2.0 |
| | 3. | 3.4 |
| anemic (bb) | 1. | 28,957 |
| | 2. | 35,790 |
| | 3. | 39,545 |

## DISCUSSION

The results presented here imply the existence of thyroid gland hypofunction in the anemic b/b rats, probably, owing to severe anemic hypoxia. Essentially, these results are in agreement with the findings of decreased thyroid gland activity in environmental and hypobaric hypoxia *(Surks, 1966a; Surks, 1966b; Galton, 1972; Verela et al., 1982; Sawhney & Malhotra, 1990).* The fall in the plasma $T_3$ and $T_4$ concentrations during acclimatization to severe hypoxia was considered by *Connors & Martin (1982)* to be the result of a block of thyroid gland

secretion, rather than a fall in TSH level. The decline in thyroid hormone levels under hypoxic stress has been ascribed to an adaptation to low oxygen availability *(Sawhney & Malhotra, 1990).* This would be a reasonable explanation of the decreased levels of thyroid hormones in b/b rats found here, since the b/b rats have severe life-long anemic hypoxia *(Rolović et al., 1991),* due to the exceptionally low level of Hb in circulation. However, an additional factor might affect thyroid gland function in the b/b rats, aparat from the severe anemic hypoxia. This might be an intracellular iron deficiency, which was well documented in b/b rats for reticulocytes *(Edwards, 1980; Bowen & Morgan, 1987; Garrick et al., 1993),* as well as committed *(Pavlović-Kentera et al., 1989; Stojanović et al., 1990)* and pluripotent hematopoietic progenitors *(Ivanović et al., 1995 Ivanović & Milenković, 1996).* If it also exists in the thyrocytes, the intracellular iron deficiency could result in low activity of the iron containing thyroid peroxidases, followed by a decreased rate of thyroid hormone synthesis.

On the other hand, there are a number of reports demonstrating unchanged *(Gunga et al., 1994)* or even increased *(Rawal et al., 1994, Wright, 1979)* thyroid gland function as a response to hypoxia. The apparent discrepancy in previously listed reports and our results probably could be explained by the different experimental conditions and regimens causing hypoxia.

The most interesting phenomenon, related to the adaptation to hypoxia is the relationship between thyroid hormones and Epo production. Tissue hypoxia is the primary stimulus for Epo production *(Jelkmann and Metzen, 1996).* A hypoxia-inducible factor (HIF-1) has been identified that binds to the hypoxia-responsive enhancer in the 3'-flanking sequence of the erythropoietin gene *(Wang and Semenza,1995).* Although *in vitro* findings demonstrated that $T_3$ and $T_4$ stimulate hypoxia-induced Epo production, *Gunga et al., 1990* concluded that thyroid hormones, *in vivo*, seem to play only a minor role in the regulation of Epo production under conditions of mild hypobaric-hypoxia. Our results for serum Epo concentration obtained by ELISA confirmed the enormously high Epo levels found using biological assay and RIA in b/b rats *(Pavlović-Kentera et al., 1989; Biljanović-Paunović et al., 1992).* In the light of these findings and the decreased levels of thyroid hormones, some questions could be raised: first, is the decreased serum thyroid hormones level in b/b rats correlated with the severity of anemia, and second, does the registered phenomenon reflect the intracellular iron deficiency? These questions probably could be resolved by thyroid hormone determination in b/b rats treated with hemin, which leads to iron supplementation without correction of anemia *(Ivanović et al., 1995). In vitro*, iron repletion by hemin inhibited Epo production in serum-deprived hepatoma cells *(Kling et al., 1996).*

The results of this investigation point to the b/b rat anemic syndrome as a unique model, not only interesting for the hematologist, but also for the endocrinologist.

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## FUNKCIJA TIROIDNE ŽLEZDE KOD BEOGRADSKOG LABORATORIJSKOG (b/b) PACOVA

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SADRŽAJ

Beogradski laboratorijski (b/b) pacov poseduje urođeni i jedinstven defekt u metabolizmu gvožđa, koji dovodi do izražene anemijei hipoksije. Istraživanje je vršeno kod anemičnih b/b pacova i kontrolnih životinja i ispitivana je koncentracija hormona tiroidne žlezde u krvnom serumu korišćenjem radioimunološke (RIA) metode. Kod anemičnih b/b pacova i kontrolnih životinja takođe su određivani hematološki parametri i koncentracija eritropoetina (Epo). Naši rezultati potvrđuju postojanje izražene anemije i izuzetno visoke koncentracije Epo kod anemičnih b/b pacova. Rezultati nivoa tiroidnih hormona u krvnom serumu anemičnih b/b pacova ukazuju na hipofunkciju tiroidne žlezde kod b/b pacova. Dobijeni rezultati upućuju da je anemični b/b pacov intresantan eksperimentalni model, kako za hematologe tako i za endokrinologe.