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Thermogenesis and longevity in mammals. Thyroxin model of accelerated aging

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ABSTRACT

Development of experimental models of life span regulation is an important goal of modern gerontology. We proposed a thyroxin model of accelerated aging. Male Wistar rats at the age of 17 months received thyroxin in drinking water at a concentration of 6 mg/L for 2 months as a model of induced hyperthyroidism (IH). Administration of thyroxin resulted in a decrease in life span and a 2 °C increase in body temperature that was accompanied by a 2 fold increase in thyroxin level and a 40% increase in triiodothyronine in blood serum. Induced hyperthyroidism can be used as a model of accelerated aging. We also found that thyroxin administration acts as uncoupler of oxidative phosphorylation as treatment was accompanied by an increase in the generation of superoxide radicals by 50%. Antioxidant enzyme activity remained unchanged (glutathione peroxidase, glutathione reductase mitochondrial) or was reduced (glutathione-S-transferase by 1.7 times) as compared with the control. The activity of glucose-6-transferase was increased by 2.8 times as compared with control, and malate dehydrogenase activity in liver increased by 6.8 times. Induced hyperthyroidism in rats resulted in distinct epigenotype which was accompanied by a decrease in life span.

1. Introduction

Experimental modeling of life span (LS) is one of methodological approaches to study the mechanisms of aging (Bozhkov, 2001). Development of experimental models of LS regulation is a central goal of gerontology.

Caloric restriction (CR) is the most validated and widely used scientific model to study aging. The usage of this model in gerontology began in 1960–70s (Mc Cay et al., 1935; Weindruch and Walford, 1988). Insulin and somatotropic signaling is critically important not only in the control of aging and longevity under conditions of unlimited food supply but also in mediating the effects of CR on life span (Bonkowski et al., 2006). It was found that limiting caloric uptake altered the functioning of almost all metabolic processes examined (Timmers et al., 2011; Qiu et al., 2010; Merry, 2002), suggesting a cooperative effect and/or generalized restructuring of metabolism. This also suggests that CR induces adjustment of metabolism in a nonspecific manner. To explain this, CR was suggested to provide a selection of animals with specific epigenotype, which is associated with increased life span (Bozhkov and Nikitchenko, 2013).

CR results in a decrease in animal body temperature and thus may act as a factor of selection. The hypothermia can explain high "spontaneous" death level of the experimental animals after applying the CR and, as a consequence, the selection of potential long-livers. Furthermore, a well-defined relationship between the rate of growth retardation, thyroxin level in blood serum and LS increase has been reported (Bozhkov and Nikitchenko, 2013). On the other hand, the experimentally increasing level of thyroxin in the organism – hyperthyroidism induction – was shown to be accompanied by a reduction of life span (Ooka and Shinkai, 1986). A review of thyroid hormone relationship with human age is found in Aggarwal and Razvi (2013). Subclinical hypothyroidism appears to lead to a survival benefit in the elderly population; subclinical hyperthyroidism appears to be associated with higher mortality (Aggarwal and Razvi, 2013).

Thyroxin is known to be involved in the regulation of thermogenesis (Silva, 2003), hypo- or hyperthyroidism, that is manifested in a hyper - or hypothermia and may exert a cooperative effect on the metabolic processes, and as a consequence, on life span. The effect of thyroid hormone on life span was shown more than 50 years ago (Johnson et al., 1963; Denscka, 1974). A detailed study of the effect of chronic hyperthyroidism on the rat life span was reported by Hiroshi Ooka (Ooka and Shinkai, 1986). Here, we hypothesized the existence of a close relationship between thermogenesis and life span.

The free radical hypothesis of aging still remains one of the most reasonable heuristic hypotheses (Barja, 2004). We used the induced hyperthyroidism model of accelerated aging, to experimentally examine the free radical hypothesis of aging. In this work, we analyzed two experimental models of life span modulation: CR-induced increase of life span and experimental life span reduction by induced hyperthyroidism (IH). In these experimental models the thyroxin and triiodothyronine contents, the functional state of mitochondria, and measures

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of prooxidant and antioxidant systems in the liver and blood serum of animals were determined.

2. Materials and methods

2.1. Experimental facilities

The study was conducted on male Wistar rats maintained in standard vivarium conditions and carried out as per the guidelines of the European Convention for the Protection of the Vertebrata used for experimental and scientific aims (Council Directive, Strasburg, 1986).

The diet change in the experimental group of the one-month old rats from the standard diet to calorie restricted diets resulted in average 60% loss of body weight compared to control animals as described (Nikitin, 1960).

For the IH model, 17 month old male rats received thyroxin in drinking water in a concentration of 6 mg/L in 0.002 N NaOH for 2 months as described previously (Ooka and Shinkai, 1986). The control and experimental animals were fed equally. The body temperature of the rats was measured with a thermometer TW 2-193 2 Microtherma T Hand Held Thermometer (Braintree Scientific, Inc., USA). The body temperature was measured between 8 and 9 PM. Tissue sampling was carried out at the same time from 9 to 10 PM allowing to exclude the influence of circadian rhythms.

Rats were euthanized via decapitated under ether anesthesia.

2.2. Fractionation of liver cells

Cooled liver samples were processed by the press and homogenized with 100 mM of Tris–HCl buffer, pH 7.4, at 4 °C. Mitochondria, microsomes and the cytosolic fraction were obtained by differential centrifugation (Kamatch and Narayan, 1972).

Liver submitochondrial particles (LSP) were obtained by the sonification of mitochondrial suspensions in an ultrasonic disperser UZDN-A (Ukraine) (10 mg mitochondrial protein/mL of suspension) according to the scheme: 15 s of sonification — followed by 15 sec brake/cooling on ice, repeated three times. The obtained suspension was centrifuged for 10 min at 10,000 ×*g*, and the supernatant was collected and centrifuged again for 30 min at 60,000 ×*g*. The resulting pellet was resuspended in 100 mM Tris–HCl-buffer, pH 7.4 and used to determine the rate of generation of O^{2-}.

To obtain serum, the blood was collected in a sterile dry tube and allowed to clot for 30 min at 4 °C. Then, the blood was centrifuged for 15 min at 1000 ×*g*, and the resulting supernatant, designated serum was collected and stored.

3. Analytical methods

3.1. Thyroxin and triiodothyronine concentration

Thyroxin and triiodothyronine concentration in serum were determined by radioimmunoassay using standard reagent kits "Total T4 RIA" and "Total T3 RIA" production Immunotech (Czech Republic).

Mitochondrial swelling was recorded by changing the optical density in a thermostated (37 °C) cuvette with constant agitation in a spectrophotometer Specord UV VIS (Germany) at 610 nm in incubation solution of following composition: 10 mM Tris–HCl, pH 7.4, 0.25 M sucrose, 5 mM KH_2PO_4, 5 mM rotenone, 2 mM succinate and 25 mM $CaSO_4$. The lipid hydroperoxide (HPL) content in the liver microsomes and mitochondria was determined according to the Ohkawa method (Ohkawa et al., 1979). The HPL level in serum was determined as previously described (Asakawa and Matsushita, 1980). The absorption spectrum of the colored product was recorded on a double-beam spectrophotometer Specord UV VIS (Germany), measuring the difference in extinction at 535 and 520 nm (Massie et al., 1983). The HPL level was expressed in equivalent amounts of MDA using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\cdot cm^{-1}$.

3.2. The rate of generation of the superoxide anion (O^{2-})

The rate of generation of the superoxide anion (O^{2-}) in submicroscopic particles was measured by adrenochrome formation from the adrenalin in a solution containing 100 mM Tris–HCl buffer, pH 7.4, 5 mM succinate, 5×10^{-4} M adrenalin and 1 mg antimycin A (per 1 mL of reaction medium). The rate of O_2^- generation was calculated from the molar extinction coefficient for adrenochrome $4.02 \times 10^3\ M^{-1}\cdot cm^{-1}$ (Misra and Fridovich, 1972) considering that for the oxidization of 1 M of adrenalin 1.4 M superoxide radical is required (Gus'kova et al., 1980).

3.3. The rate of generation of O_2^- in microsomes

The rate of generation of O_2^- in microsome suspension at NADPH oxidation was determined by the formation of adrenochrome (at 480 nm) from the adrenalin in a medium solution containing 0.15 M Na^+, K^+-phosphate buffer, pH 7.8, 0.4 mM EDTA, 0.1 mM NADPH, 0.5 mM adrenaline, and 250 mg/mL microsomal protein. The rate of O_2^- generation was calculated as described above.

3.4. The respiration rate and mitochondrial oxidative phosphorylation

The respiration rate and mitochondrial oxidative phosphorylation were determined by a closed Clark oxygen electrode (Skulachev, 1969) in a thermostatic cuvette (30 °C) in a reaction medium of the following composition: 10 mM Tris–HCl, pH 7.4, 150 mM sucrose, 75 mM KCl, 10 mM KH_2PO_4, 2 mM $MgCl_2$, and 0.6–1.2 mg protein per 1 mL. The substrates of oxidation were added at the following concentrations: succinate, α-glycerophosphate and β-oxybutyrate — 10 mM. The concentration of ADP added was dependent on the type of oxidizable substrate and was 100, 200 and 400 μM, respectively. Additionally depending on the type of measured activity rotenone — 1 μM, EGTA — 1 or 2 pM, and BSA — 1 mg/mL were added to the polarographic cuvette. The rates of mitochondrial respiration in metabolic states 2 and 3 on Chance (V_2 and V_3) and respiratory control (RC, V_3/V_2) were assessed on curves of oxygen consumption.

3.5. Glutathione peroxidase activity (GP, EC 1.11.1.9)

Glutathione peroxidase activity (GP, EC 1.11.1.9) was measured in cytosolic fractions, liver mitochondria and blood serum spectrophotometrically at 340 nm according to the method reported by Paglia and Valentine (1967) in solution containing 50 mM K^+, Na^+-phosphate buffer (pH 7.4) containing 1 mM EDTA, 0.15 mM NADPH, 1 U/mL yeast glutathione reductase (GR), 0.2% Triton X-100 and 3 mM Na sodium azide to inhibit catalase activity. Then, 1.2 mM cumene hydroperoxide and 0.4 mM hydrogen peroxide were added. Incubation temperature was 37 °C. The activity was expressed as nmol NADPH/min per mg of protein or mL of serum considering a molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\cdot cm^{-1}$.

3.6. Glutathione-S-transferase activity (GT EC 2.5.1.18)

Glutathione-S-transferase activity (GT EC 2.5.1.18) was measured in the liver cytosol and mitochondria spectrophotometrically at 340 nm (Younes et al., 1980) in a solution containing 0.1 M K^+-phosphate buffer, pH 6.5, 1 mM 1-chloro-2,4-dinitrobenzene, 5 mM GSH, and 0.2% Triton X-100. The incubation temperature was 37 °C. The activity was calculated using a molar extinction coefficient of $9.6 \times 10^3\ M^{-1}\cdot cm^{-1}$.

3.7. The activity of glutathione reductase (GR EC 1.6.4.2)

The activity of glutathione reductase (GR EC 1.6.4.2) in the liver homogenates and mitochondria was measured spectrophotometrically as a decrease of NADPH (Carlberg and Mannerviek, 1975) in a solution