CALCIFICATION ADMINISTRATION DECREASES THYROID FUNCTIONING IN MIDDLE-AGED FEMALE RATS. B. Šošić-Jurjević, B. Filipović, M. Manojlović Stojanoski, and M. Sekulić. Sinisa Stanković Institute for Biological Research, 11000 Belgrade, Serbia.

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Most women take calcium (Ca) supplementation for the prevention or treatment of osteoporosis at or beyond the onset of the menopause (Singh et al., 2000). At the same time, this group is faced with an increased risk of thyroid disorders such as secondary and tertiary hypothyroidism (Schatz et al., 2002; Schindler, 2003). According to different studies, thyroid functioning decreases with advancing age, affecting the whole hypothalamo-pituitary-thyroid axis in both humans and rats (Robusch et al., 1987; Raymond et al., 1992). Rodents are considered to be useful models for thyroid studies, even though significant differences between rodent and human thyroid physiology have been reported (Poirier et al., 1999).

Implemented Ca treatment can affect thyroid functioning by influencing mechanisms that do not exclude each other. It can act indirectly, by influencing the regulatory pituitary TSH cells (Gillett et al., 1990; Bergenfelz et al., 1994), as well as by altering the level of peripheral conversion of T3 to T4 (Etting et al., 1986). Calcium can also influence activity of thyroid follicular cells directly, by modulating the TSH stimulatory effect (Gebescek et al., 1998; Vanvooren et al., 2000).

We previously demonstrated that chronic Ca administration to middle-aged female rats affects the immunohistochemical, histological, and morphometrical features of pituitary thyrotrophic cells and thyroid tissue (Sekulić et al., 1998; Šošić-Jurjević et al., 2005). As a continuation of these investigations, in the present study we examined the effects of Ca administration on serum levels of thyrotropin (TSH), total thyroxine (T4), and triiodothyronine (T3) hormones in the same middle-aged rat model.

Female Wistar rats (14-month-old) were maintained under standard laboratory conditions (22 ± 2 °C, 12-h light/dark periods) with free access to food and water. A group of animals (Ca; n = 6) received intramuscularly 28.55 mg/kg b.w. Ca-glubionate (Novartis, Nyon, Switzerland) once a day for two weeks except on Sundays. Age-matched controls (C; n = 6) received an equivalent volume of physiological saline. Experimental protocols were approved by the Local Animal Care Committee and conform to the recommendations given in the “Guide for the Care and Use of Laboratory Animals” (1996, National Academy Press, Washington D.C.). All animals were sacrificed 24 h after the last injection. Sera were separated from trunk blood after decapitation and stored at -70°C. To determine the serum concentration of TSH, total T4, and T3, ELISA assays were employed using a commercial human test kit (HUMAN, Wiesbaden, Germany). All serum samples were measured within the same assay in duplicate. The intra assay coefficient of variation was 5.0-13.8% for TSH, 9.8-8.7% for T4, and 13.2-7.7% for T3 (low and high sample levels). All results were expressed as the mean ± SD. Student’s t-test was used for comparative evaluation. The minimum level of significance was set at p<0.05.

The mean serum concentrations of TSH, T4, and T3 in the control and Ca-treated groups are summarized in Fig. 1 (a-c). Chronic Ca administration to middle-aged female rats brought about a decrease of all of the examined serum parameters in comparison with the vehicle-treated control values: (i) the serum level of TSH decreased by 30.6%, p<0.005 (Fig. 1a); (ii) the serum level of T4 decreased by 19%, p<0.025 (Fig. 1b); and (iii) the serum level of T3 decreased by 22%, p<0.05 (Fig. 1c).

These results clearly demonstrate that thyroid functioning decrease after chronic administration of Ca to middle-aged female animals.

The treatment promoted a striking reduction of serum TSH concentration. This is consistent with our previous findings that Ca supplementation brings about a decrease of the examined stereological parameters for pituitary TSH cells – the relative cellular volume and the relative percentage of thyrotropes within the pituitary unit volume in middle-aged female rats (Sekulić et al., 1998). Gillett et al., (1990) also found that acute and chronic hypercalcemia leads to a decrease of TSH secretion in males. It follows that, Ca treatment can affect thyroid functioning indirectly, by reducing level of its major stimulatory factor, pituitary TSH. In contrast to our findings, Kaljšnik et al. (1990) reported increased thyroid functioning to be accompanied by a decreased TSH concentration in adult rats. This discrepancy could be due to the different ages of the examined experimental animals.

Under our experimental conditions, a significant decrease of serum T4 and T3 was also detected. These results can be attributed to the above mentioned decrease of TSH, since the given hormone acts as a major positive factor in the regulation of thyroid functioning. Our previous morphometric research on thyroid gland structure showed that Ca administration significantly decreases volumetric density of the thyroid follicular cell epithelium, as well as its height and index of activation rate...
In vitro studies with FRTL-5 cells demonstrated that Ca does not affect the morphology of these cells, but acts directly when administered together with TSH by reducing the thyrotrophin stimulatory effect (Gaberšcek et al., 1998). Vanvooren et al. (2000) found that isoform VI of adenyl cyclase, the enzyme crucial for TSH-induced activation of thyroid follicular cells, is negatively modulated by Ca in human and dog thyroids.

The present study also showed that Ca supplementation decreases the serum concentration of total T\textsubscript{3} to a greater extent than the concentration of T\textsubscript{4}. This is consistent with the findings of Etling et al. (1986), who showed that dietary calcium inhibits the conversion of T\textsubscript{4} to T\textsubscript{3} in the serum, as well as in the liver and kidneys of rats.

It can be concluded that Ca administration exerts an adverse effect on thyroid functioning in middle-aged female rats, indirectly and/or directly. However, the mechanisms underlying the observed effect are still unclear.

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Fig. 1. Serum level of TSH (Fig. 1a), total T\textsubscript{4} (Fig. 1b), and T\textsubscript{3} (Fig. 1c) in control (C; n = 6) and Ca-treated (Ca; n = 6) middle-aged female rats. Results are means ± SD. * p<0.05, ** p<0.025, *** p<0.001.