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CONTENT OF MICROELEMENTS IN THE RAT PINEAL GLAND AT DIFFERENT AGES AND THE EFFECTS OF SELENIUM SUPPLEMENTATION

M. DEMAJO[1], OLGA JOZANOV-STANKOV[1], and IVANA ĐUJIĆ[2]

[1] *Vinča Institute of Nuclear Sciences,* 11001 Belgrade, Serbia and Montenegro
[2]*Institute of Chemistry, Technology and Metallurgy, Center of Chemistry,* 11000 Belgrade, Serbia and Montenegro

Abstract – The mammalian pineal gland regulates a number of important physiological processes. In this paper we report changes in the content of iron (Fe), zinc (Zn), copper (Cu), and selenium (Se) in the male rat pineal glands at 4, 5, 8, and 12 months of age. The effect of Se supplementation in drinking water on the content of pineal gland microelements was also studied. Selenium (Se)-dependent changes in pineal gland reported in this study suggest novel physicochemical and biochemical properties of Se, an important element essential in the antioxidative processes, yet known to influence a number of endocrine processes.

Key words: Pineal gland, rat, microelements, selenium supplementation

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INTRODUCTION

The pineal gland is a neuroendocrine organ with a multitarget regulative role. Numerous findings report its dominant role in processes such as coordination and synchronization of homeostasis and behavior (M i l i n e, 1980; R o m i j i n, 1978), ageing (R e i t e r *et al.*, 1995, 1997), maintenance of the circadian rhythm in mammals (L a a k s o *et al.*, 1988), regulation of reproductive functions (R e i t e r, 1981) and response to stress (M i l i n *et al.*, 1993, 1996). The latest research proposes an existence of precise temporal programme for growth, fertility, aging, and death in the "pineal complex" of the brain and further suggests the existence of an evolutionary-developmental role for the pineal complex during growth, fertility and aging. There is a certain set time when the pineal gland actively starts to deliver ageing and death "signals" to the body, thus accomplishing its genetically inscribed sequence (P i e r p a o l i and B u l i a n d, 2005).

The objective of our study was to monitor changes in the microelements of the pineal gland during different age periods with the assumption that that the pineal programme is associated with age-dependent changes of the "antioxidant defense system"-AODS (D j u j i c *et al.*, 1995; X i a *et al.*, 1992), specifically the content of selenium (Se), zinc (Zn), copper (Cu) and iron (Fe). Since selenium plays an important role in antioxidant defenses we further investigated a response to selenium supplementation in different age groups. There are suggestions that oxidative injury plays a role in normal ageing, schizophrenia, Parkinson's and Alzheimer's disease and the possible role of selenium is considered, although its significance and mechanisms of action in reducing the symptoms of the neurological diseases is not yet clear (B e n t o n , 2002). However, the reality of antioxidant treatment is complex in all biological levels, and cannot be measured or defined by a single parameter. The aim of this study was to determine whether the level of microelements in the pineal gland is modified by selenium intake, thus possibly influencing pineal gland activity, this being of interest as it has been shown that lower melatonin secretion is found in patients with Alzheimer's but not with Parkinson's disease (S a n d y k , 1997). Understanding the metal-coordination mechanism in selenium antioxidant activity will help us understand diseases caused by oxidative stress.

MATERIALS AND METHODS

Male rats of a Wistar strain bred at the Vinča Insti-

tute for over 50 years were used. Rats aged 3 months (160-225 g of body mass) were individually caged. At this age of 3 months, one group of animals started receiving pure brewery yeast dissolved in drinking water (Ycontrol animals), while the second group of animals received Se enriched brewery yeast in drinking water (SeY). In both cases, the pure brewery yeast and Se coupled to brewery yeast were given in drinking tap water as stabilized emulsions prepared by placing the yeast suspension in water in a laboratory ultrasound bath for better homogenization. The animals had free access to this drinking water emulsion. Consumption of SeY emulsion resulted in a mean daily intake of 0.5 μg Se/animal (J o - z a n o v – S t a n k o v *et al.*, 1998). The animals were fed rat pellets *ad lib*, obtained from the Subotica Veterinary Institute in Subotica, Serbia. According to the declaration of the supplier, the pellets also contained: 100 mg/kg Fe and Zn, 20 mg/kg Cu, and 0.1 mg/kg Se. Lights in the animal room were on from 7:00 to19:00 hrs, room temperature being 20 ± 2° C. Both groups of animals were subsequently sacrificed between 9:00 and 12:00 hrs by decapitation with a small animal guillotine at the ages of 4, 5, 8 and 12 months. Throughout the experiment, the animals continuously received Y or SeY until the last groups of animals were sacrificed at 12 months of age.

After sacrifice, the pineal glands were removed by opening the dorsal skull bones with dental pliers and surgical scissors. The pineal glands were cleaned of surrounding connective tissue and kept frozen at - 20° C until analyzed for Fe, Zn, Cu, and Se content. The content of microelements was measured in the pineal glands after a wet-ashing procedure as described by us previously (D j u j i c *et al.*, 1992, 1995a). Briefly, the tissues were wet-ashed with a mixture of nitric and perchloric acid and finally with hydrochloric acid in borosilicate test tubes placed in an aluminium block heater (Tecator). Selenium was determined by gaseous hydride generation atomic absorption spectrometry using a Perkin Elmer 5000 atomic absorption spectrometer equipped with an MHS-10 vapor-generator accessory. The content of Zn, Cu and Fe was measured by flame atomic absorption spectrometry.

It should be pointed out that because the pineal gland is very small in the rat, average mass being 1.61 mg and not changing during the time period studied, 4-8 glands were pooled. To obtain microelement content, three pooled samples with the same number of pineal glands for microelemnt content were measured for each point.

The results are expressed as nmol/gland of microelement content, obtained by dividing the total microelement content by the number of pineal glands pooled in each sample and finding the median of three repeated sample measurements. For small sample analysis, the non-parametric Kruskal-Wallis test was used to compare the Y and SeY groups and the time points within each group. The parametric coefficient of correlation was used to cross-compare the different microelement content relationships. In all statistical tests, the level of significance was set as $p<0.05$. The "Origin 5.0" graphic program was used to integrate the surface below the line connecting the values of microelement content during the whole time period the animals were investigated (between 4 and12 months of age).

RESULTS

Content of Fe in the pineal gland

In the Y group at the ages of 4, 5, 8, and 12 months of age, the content of Fe was 4.03, 5.91, 4.39, and 9.58 nmol/gland, respectively (Fig.1). There was a significant difference of Fe content ($P<0.05$) between all time points studied except between 5 and 8 months.

Animals from the SeY group showed lower Fe con-

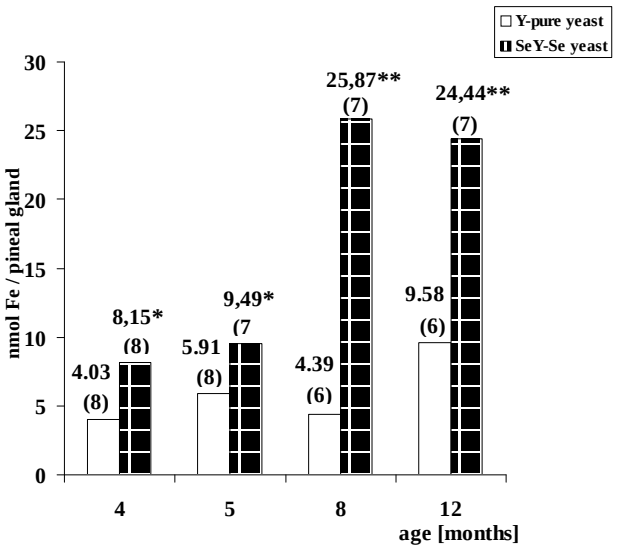

Fig. 1. Content of Fe in rat pineal glands at different ages. (n) Number of pineal glands pooled. Levels of significance between Y and SeY groups: $p<0.05$* and $p<0.02$**.

tent at 4 and 5 months of age (8.15 and 9.49 nmol/gland, respectively) than at 8 (25.87 nmol/gland) and 9 (24.44 nmol/gland) months. The differences of Fe content between age groups were significant ($p<0.05$), the significance being higher ($p<0.02$) between 8- and 12-month-old animals as compared to 4- and 5-month-old animals.

There was a statistically significant difference of Fe content between the Y and SeY groups, the significance being lower at 4 and 5 months ($p<0.05$) than at 8 and 12 months ($p<0.02$).

The integrated values of surfaces below the Fe content lines as a function of age were 48.36 and 162.48 for the Y and SeY animals, respectively.

Content of Zn in the pineal gland

Zinc content in the Y group of rats was 2.57 nmol/gland at 4 months of age, rising to 3.04 nmol/gland at 5 months and falling to 2.57 and 2.22 nmol/gland at the ages of 8 and 12 months, respectively. The difference of Zn content was significant between all age groups ($p<0.05$) except between 4 and 8 months (Fig. 2).

In the SeY group, pineal gland Zn content increased slightly from 1.83 ng/ml at 4 months to 1.96 nmol/gland at 5 months, with a significant ($p<0.05$) rise to 2.55 and 2.59 nmol/gland at 8 and 12 months, respectively. The content of Zn in the SeY group did not significantly change between 8 and 12 months of age.

Comparing the Y and SeY groups, significant differences of Zn content were observed at 4, 5, and 12 month of age ($p<0.05$).

For the overall time period studied, the integrated surfaces between the Y group (20.78) and SeY group (18.94) did not show marked differences.

Content of Cu in the pineal gland

The content of Cu varied significantly between 4 and 12 months in the Y group of animals, as seen in Fig. 3. From 2.09 nmol/gland at 4 months of age, Cu content rose significantly ($p<0.02$) to 4.33 nmol/gland at 5 months, falling to 1.38 nmol/gland at 8 months and rising to 2.72 nmol/gland at 12 months ($p<0.02$). The difference of Cu content between the 4-, 8-, and 12-month groups was significant ($p<0.05$).

The group of animals receiving a selenium supplement (SeY) showed a rise in Cu content from 4 months of age (1.07 nmol/gland) to 2.42 nmol/gland at 5 months ($p<0.02$). There was a significant fall of pineal gland Cu content by the 8th month, and it remained low at 12 months of age. This difference was significant at a level of $p<0.02$ as compared to the Cu content at 4 and 5 months.

As demonstrated in Fig. 3, the Cu content was sig-

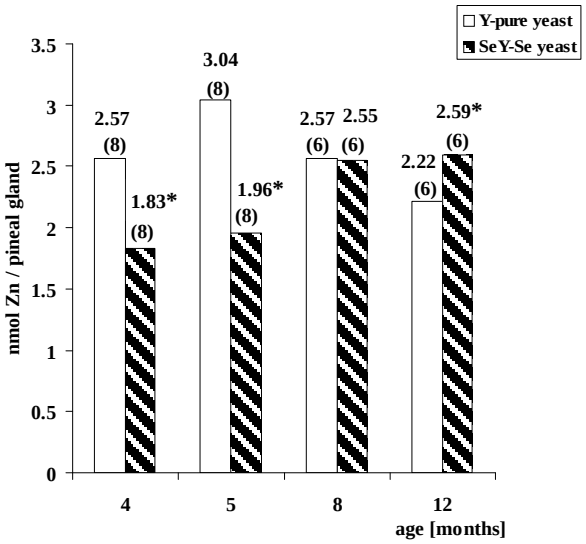

Fig. 2. Content of Zn in rat pineal glands at different ages. (n) Number of pineal glands pooled. Level of significance between Y and SeY groups: $p<0.05$*.

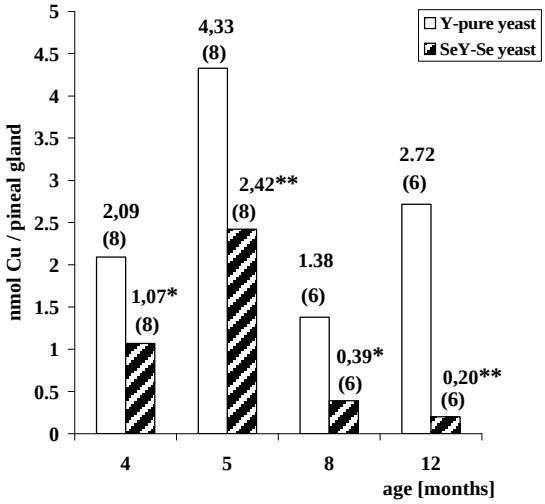

Fig. 3. Content of Cu in rat pineal glands at different ages. (n) Number of pineal glands pooled. Levels of significance between Y and SeY groups: $p<0.05$* and $p<0.02$**.

nificantly lower at all times in the SeY group of animals when compared to the Y group.

The time period-integrated surface for the Y group was 19.98, which was 2.81 times higher than the integrated surface for the SeY group (7.14).

Content of Se in the pineal gland

At the age of 4 months in the Y group of animals, pineal gland Se content was 1.058 nmol/gland, falling significantly ($p<0.02$) to 0.63, 0.58, and 0.43 nmol/gland at 5, 8, and 12 months, respectively (Fig. 4). Significant differences ($p<0.05$) were noted between the 5- and 8-month-old groups when compared to the 12-month-old group.

A similar pattern of fall in Se content is evident in the SeY group as compared with the Y group. From high Se content at 4 months (1.063 nmol/gland), Se content fell to 0.87 and 0.72 nmol/gland at 5 and 8 months ($p<0.05$) and further to 0.59 nmol/gland at 12 months ($p<0.02$). The level of significance between 5 and 8 months was $p<0.05$.

Except in the case of 4 months, where no significant difference of Se content was observed between the Y and SeY group, the value of this index at the remaining studied times was significantly higher ($p<0.05$) in the SeY group of animals than in the Y group.

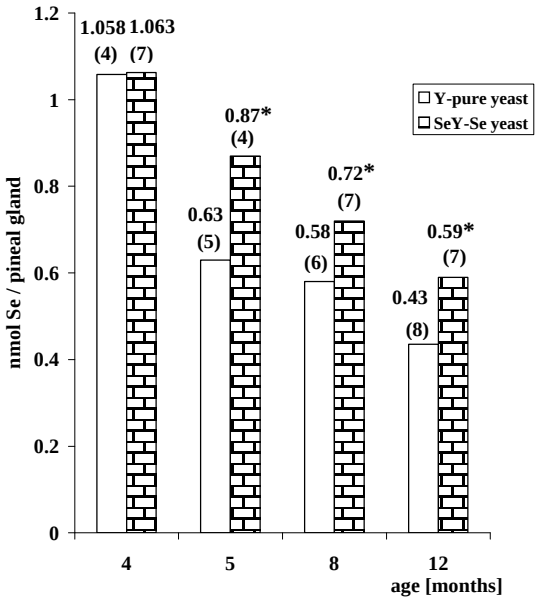

Fig. 4. Content of Se in rat pineal glands at different ages. (n) Number of pineal glands pooled. Level of significance between Y and SeY groups: $p<0.05$*

The integrated surface for the SeY group was 5.98, this being 1.28 times higher than for the Y group (4.68).

Correlation of the content of different microelements in the pineal gland between 4 and 12 months of age

In the Y group of animals, no correlation was observed between the different microelements. However, the animals whose water was supplemented with selenium (those of the SeY group) showed a significant ($p<0.05$) negative correlation between Fe and Se content, as well as between Zn and Se ($p<0.01$). In these animals, high ($p<0.001$) positive correlation was observed between pineal gland Fe and Zn content.

DISCUSSION

It was previously shown by electron probe X-ray microanalysis (H u m b e r t and P e v e t, 1996) that the rat pineal gland contains a whole range of elements: S, Ca, Al, Si, P, Fe, Na, Mg, Cl, K, Ti, Cr, Mn, Ni, Cu, and Zn. In this study we demonstrated an age-dependent change in microelements content in rat pineal gland and have shown their association with the selenium supplementation treatment (Figs. 1, 2, 3 and 4). We have analysed 4, 5, 8, and 12 months age groups and our results are similar to observations reported by H u m b e r t and P e v e t (1996). Thus, our study has shown a significant increase of Fe content in 12-month-old rats. However, contrary to the above authors, we found significant variations of Zn content at different ages investigated in the present study (Fig. 2). In our SeY group, where the animals were given water supplemented with Se, Fe content was significantly higher than in the Y group at all ages studied, and this difference increased with age. Statistical analysis of integrated surfaces of the variables for age and pineal gland Fe content are compared, the SeY group of animals are found to have a 3.36 times higher than the control Y group). This observation is in agreement with our previous report (D j u j i ć *et al.*, 1995) in which we demonstrated that Se supplementation for a month also increased Fe content in the forebrain and hypothalamus of rats aged 4 months. In the same study, reported minor change of Zn content in the brain of Se-supplemented rats as compared to the control group. Further, the authors have shown a dramatic rise of Zn content in the pituitary gland of Se-supplemented group. In our present report,

Se supplementation induced an increase in Zn content in the pineal gland at the ages of 8 and 12 months. In the forementioned paper (D j u j i ć *et al.,* 1995), it was demonstrated that Se supplementation increased Cu content in the forebrain, hindbrain, and pituitary while the same nutrition treatment was associated with a fall of Cu content in the hypothalamus. In the present study, we demonstrated that Se supplementation has a similar effect on the pineal gland Cu content, as previously demonstrated for the hypothalamus. These observations suggest that selenium modifies biochemical characteristics and probably physiological functions of different brain structures in a spatial and timely manner. An interesting observation from our present study, further demonstrates that Se supplementation increases Se content in the pineal gland, as it does in different brain structures (D j u j i ć *et al.,* 1995). The preferential retention of selenium in the brain suggests that it plays important roles in antioxidant mechanisms. Moreover, our present data suggest that one-month of Se supplementation might not be sufficient for the induction of biochemical changes in the pineal gland. As our results demonstrate (Fig.4), the supplemented Se is first distributed to other tissues of the rat body and only later deposited in significant amounts in the pineal gland itself.

Selenium is an integral part of glutathione peroxidase (GSHPxs) and phospholipid glutathione peroxidase (PLGSHPx), which are especially significant for nervous and brain cells, and is a component of other seleno-enzymes and seleno-proteins, active in the conversion of hydrogen peroxide and lipid peroxide produced during oxidative stress in the cells (Dj u j i c *et al.,* 1995). The gradual fall of Se with progressing age in both, SeY and Y groups of animals described in this study (Fig. 4) suggests that selenium plays a role in normal aging. Selenium may be depleted in the pineal gland while its secretory product melatonin executes its role in scavenging free radicals formed in the organism during aging, doing so especially by promoting activity of the antioxidative enzyme glutathione peroxidase (R e i t e r *et al.,* 1995). This may explain the initial depletion of Se content with increasing age observed in the SeY group of animals. However, Se supplementation did maintain a higher Se content in the pineal glands of rats at the different ages studied compared to control group of animals (Fig. 4).

The hormone melatonin is mainly secreted by the pineal gland and has strong antioxidant properties (stronger than those of vitamin E) and an oncostatic action (T o u i t o u, 2005). As an highly efficient scavanger of both the hydroxyl (T a n *et al.,* 1993) and peroxyl radicals (P i e r i *et al.,* 1994) melatonin has been suggested as an important agent associated with a delay of ageing process, although its precise role in the mechanism of ageing remains to be determined (K a r a s e k and R e i t e r, 2002). It has been reported that melatonin can modulate Zn turnover. Plasma levels of melatonin and Zn decline with advancing age. The addition of melatonin restores the altered Zn turnover in aged mice (M o c h e g i a n i *et al.,* 1994). As shown in this study, Zn content in the pineal gland was relatively stable in different age periods in the control groups (Fig. 2). However, in Se-supplemented group (SeY), our data suggest that selenium could restore the Zn “pool” in the pineal gland at the ages of 8 and 12 months. This observation is important since Zn is required as a catalytic, structural and regulatory ion for enzymes, proteins and transcription factors, and is thus a key trace element in many homeostatic mechanisms of the body, including immune responses. Low zinc ion bioavailability results in limited immunoresistance to infection in ageing (F e r e n c i k and E b r i n g e r, 2003), the Zn however mediating an immuno-reconstituting effect by melatonin secreted by the pineal gland as suggested by M o c h e g i a n i *et al.,* 1994. Our demonstration of a negative correlation between Se and Zn content in the in the SeY group of animals but positive correlation between Zn and Fe apparently corroborates the results of D e m m e l *et al.* (1982), who found different correlations between microelements in the human pineal gland. According to the mentioned authors, this is related to the specific roles played by microelements in the physiology and biochemistry of the pineal gland.

The fact that Zn is an antagonist to Cu absorption (Dj u j i c *et al.,* 1995), can explain the low content of Cu in the Y and SeY groups of rats (Figs. 2 and 3). The causal relationship between Zn and Cu is most evident in the Se-supplemented (SeY) rats at the age of 12 months, when Zn content rises and Cu content in the pineal gland falls to its lowest level.

Since Cu is essential for normal Fe metabolism, it may be that it is used up in the SeY group of rats to ensure the high content of Fe in all the age groups studied (Figs. 1 and 3). It should be pointed out that Fe^{3+} is the favored species in mammalian cells (S k o v, 1987); when sensitized, it is reduced to the Fe^{2+} complex. An excess of Fe in the tissues catalyzes the overproduction of hydrox-

yl radicals (K i e f f e r, 1993), and this excess of Fe is detected in aged organisms and in humans suffering from Parkinson's disease. This may also explain the significant negative correlation we found between Fe and Se content in the pineal glands of rats of the SeY group, Se being used up to scavange hydroxyl radicals catalyzed by Fe (Figs. 1 and 4). Since Cu, Zn, and Se are linked together in cytosolic defense against reactive oxygen and nitrogen species (K l o t z *et al.*, 2003), higher Se content in the pineal glands of Se-supplemented (SeY) rats compared to the control group (Fig. 4) may have a beneficial effect in reducing the negative effects resulting from increased hydoxyl radical formation in the pineal gland during the ageing process. Moreover, Cu, Zn and Fe are known to be essential for normal nervous system development and function and their imbalances, either excess or deficiency, can result in neuronal apoptosis (L e v e n s o n, 2005). Our study suggests that a certain balance of these essential metals in the rat pineal gland is much needed for a number of endocrine processes, most notably, those involved in the aging process. We speculate that the proper balance of Se, Cu, Zn and Fe is needed for the normal functioning of the immune system as also previously suggested by C a l d e r and K e w (2002), could have an important role in viral suppression, and might be implicated in delaying the aging process. During the last few years, the biochemistry and pharmacology of selenium-based compounds are subjects of intense interests as potential therapeutic agents in the treatment of several diseases. We strongly believe that the results from our study will add novel information to this attractive field of research, especially emphasizing the interaction of microelements and pineal gland function.

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САДРЖАЈ МИКРОЕЛЕМЕНАТА У ПИНЕАЛНОЈ ЖЛЕЗДИ ПАЦОВА И ЕФЕКТИ СУПЛЕМЕНТАЦИЈЕ СЕЛЕНОМ

М. ДЕМАЈО[1], ОЛГА ЈОЗАНОВ-СТАНКОВ[1] и ИВАНА ЂУЈИЋ[2]

[1]*Институт за нуклеарне науке «Винча»,* 11001 Београд, Србија и Црна Гора; [2]*Институт за хемију, технологију и металургију, Центар за хемију,* 11000 Београд, Србија и Црна Гора

Пинеална жлезда сисара регулише бројне физиолошке процесе. У овом раду смо приказали промене у садржају Fe, Zn, Cu и Se у пинеалним жлездама пацова старих 4, 5, 8 и 12 месеци. Такође је изучаван садржај микроелемената у пинеалној жлезди након суплементације селеном у води за пиће. Показано је да суплементација селеном доводи до промена садржаја микроелемената у пинеалној жлезди пацова. Селен (Se)-зависне промене у пинеалној жлезди приказане у овом раду указују на нове физиохемијске и биохемијске особине Se, важног микроелемента, неопходног у антиоксидативним процесима, а за којег се зна да утичу на бројне ендокрине процесе.