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Research Paper

Serum Melatonin Levels in Animal Model of Andropause Undergoing Testosterone Propionate Replacement Therapy

Biomedical

KEYWORDS : melatonin, testosterone propionate, andropause, experimental orchiectomy

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ABSTRACT

Introduction: The pineal gland, called epiphysis as well, is a small endocrine structure, situated in the middle brain. Its main hormone is N-acetyl-5-methoxytriptamine or more often called melatonin. A set of studies show that the levels of melatonin lower with the age. There is discrepant data about the collaboration between the pineal gland and the gonad function. Aim: The aim of the current experiment is to study the dynamics in the values of serum testosterone and melatonin during replacement therapy with testosterone propionate in dose 4 and 8 mg/kg body mass (b. m.). Material and Method: Orchiectomized and aged male rats were used, in a condition of acute and chronic treatment (15 days, 15 weeks). The levels of serum testosterone and melatonin were prosecuted. Results: The supplementation with testosterone increased its levels with significance at the higher dose. Increased serum levels of testosterone were observed in the aged male rats as well, as a result of the application of its propionate salt. Significance resulted from the chronic use of dose 8 mg/kg b. m. The acute and chronic application of testosterone propionate on aged male rats in 4mg/kg b. m. dose decreases significantly the levels of serum melatonin (p=0.041, p=0,019). At the higher dose this effect is not significant. Conclusions: Testosterone propionate, applied on rats with androgen deficiency in dose 8 mg/kg b. m. reestablishes the physiological levels of total testosterone. Testosterone propionate has a bi- directional effect over the level of melatonin. Data about its increase in aged male rats treated with testosterone propionate is established.

Introduction:

The pineal gland secretes melatonin during the night time, and it goes in the circulation of the vertebrates,. This night secretion determines the endogen circadial rhythms and influences a number of physiological processes. The biosynthesis and the secretion of melatonin decrease with the age, as its levels are significantly low even in the middle age and this tendency continues during the whole period of aging.

Some studies show a correlation between the function of the pineal gland and the serum levels of the sex hormones. The plasma melatonin decreases with the age, but it can be increased when there is hypogonadism. The therapy of hypogonadism could restore its initial levels, but it is not clear enough whether this is casuistic or regularity.

Aim:

The aim of the present study is to explore the dynamics in the levels of serum testosterone and melatonin during replacement therapy with testosterone propionate at a dose of 4 and 8 mg/kg body mass (b. m.) on aged male rats in acute and chronic trial.

Materials and methods:

140 male Wistar rats were used, with weight from 270 to 380 grams. The design of the experiment is approved from the Bulgarian Food Safety Agency (License №1/19.03.2012) and decision of the Ethic committee at MU-Plovdiv with record №/25.07.2012. The animals are divided in groups (table 1).

Table 1. **Groups - description**

Group	Legend	Description
1	CYC	Control group, young castrated, chronic treated animals
2	CSC	Control sham operated group, young chronic treated animals
3	YC4	Young, chronic treated animals with 4 mg/ kg b.w Testosterone propionate
4	YC8	Young, chronic treated animals with 8 mg/ kg b.w Testosterone propionate
5	COC	Control group, old, chronic treated animals
6	OC4	Old, chronic treated animals with 4 mg/ kg b.w Testosterone propionate
7	OC8	Old, chronic treated animals with 8 mg/ kg b.w Testosterone propionate
8	CYA	Control group, young castrated, acute treated animals
9	CSA	Control sham operated group, young acute treated animals
10	YA4	Young, acute treated animals with 4 mg/ kg b.w Testosterone propionate
11	YA8	Young, acute treated animals with 8 mg/ kg b.w Testosterone propionate
12	OA4	Old, acute treated animals with 4 mg/ kg b.w Testosterone propionate
13	OA8	Old, acute treated animals with 8 mg/ kg b.w Testosterone propionate
14	COA	Control group, old, acute treated animals

The young animals in the experimental study are aged 6 months and with average weight 275 5,1 grams. The old rats are aged above 3 years with average weight 376 6,2 grams. After castration under general anesthesia or sham operation and acclimatization of 14 days have been carried out, the rats were injected i.m. (gluteal muscle) once a week as follows (table 2).

Table 2. **Experimental design**

Group	Legend	N	Treatment	Duaration
1	CYC	10	0,5 ml Oleum helianti	15 weeks
2	CSC	10	0,5 ml Oleum helianti	15 weeks
3	YC4	10	4 mg/ kg b.w Testosterone propionate	15 weeks

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4	YC8	10	8 mg/ kg b.w Testosterone propionate	15 weeks
5	COC	10	0,5 ml Oleum helianti	15 weeks
6	OC4	10	4 mg/ kg b.w Testosterone propionate	15 weeks
7	OC8	10	8 mg/ kg b.w Testosterone propionate	15 weeks
8	CYA	10	0,5 ml Oleum helianti	15 days
9	CSA	10	0,5 ml Oleum helianti	15 days
10	YA4	10	4 mg/ kg b.w Testosterone propionate	15 days
11	YA8	10	8 mg/ kg b.w Testosterone propionate	15 days
12	OA4	10	4 mg/ kg b.w Testosterone propionate	15 days
13	OA8	10	8 mg/ kg b.w Testosterone propionate	15 days
14	COA	10	0,5 ml Oleum helianti	15 days

During the experiment all the animals were bred at standard laboratory conditions. Air temperature 24±1 №air humidity 65 ± 5%, free water and food access.

Blood collection was gathered through decapitation under ether narcosis under glass ring filled with diethyl ether vapor for 60 seconds. The received samples were sent immediately to the clinical laboratory of MU-Plovdiv. Total testosterone and melatonin were tested trough ELISA with analyzer SIRIO- microplate reader, SEAC, ITALY.

The statistic analyzes were done with IBM SPSS 22.0 (Statistical Package for Social Science) for Windows 8. For each index was calculated the average value (Mean) and standard error (SEM). In all analyzes, differences with $p<0.05$ were determined as statistically significant. At normal distribution the values were juxtaposed through Independent Samples T-test. The tables and figures were built with Microsoft Office 2013. MS Word №MS Excel. applications were used.

Results:

The orchiectomy significantly lowered the levels of serum cholesterol at the 15 days trial and insignificantly at the chronic one (fig.1). The supplementation with testosterone raised its levels with significance at the higher dose. A raise in the serum levels of testosterone was observed at the aged male rats too, a result from the application of its propionate salt. Significance was received at the chronic use of dose 8 mg /kg b. m. (fig.2).

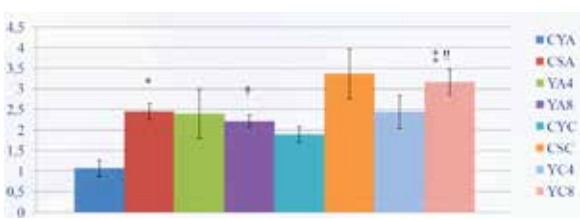

Fig. 1. Changes in the values of total testosterone (ng/ml) - young castrated animals: * - P=0,001 towards CYC; † - P=0,001 towards CYC; ‡ - P=0,02 towards CSC; № P=0,004 towards YA8.

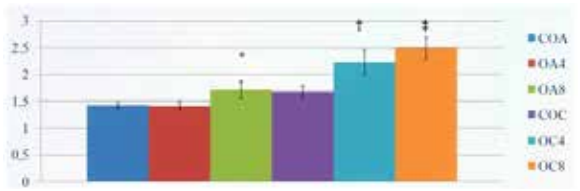

Fig. 2 . Changes in the values of total testosterone (ng/ml) – old animals: *- P=0,009 towards OC8; † - P=0,007 towards OA4; ‡ - P=0,007 towards COC.

There were no significant differences between the castrated and the simulative operated animals at the acute and the chronic trials. At 15 days treatment the application of testosterone propionate in dose 4 and 8 mg/kg b. m. lowered insignificantly the level of serum melatonin towards the orchiectomized control. In comparison with the simulative operated both doses didn't change significantly the levels of this index. Insignificant increase was established at higher dose.

There was no authoritative difference between the two control groups at the chronic trial. In comparison between the castrated and simulative operated groups the administration of testosterone propionate in both doses raised insignificantly the serum melatonin.

When juxtaposed the chronic and the acute groups didn't show any significant differences between them.

The acute and the chronic use of testosterone propionate at aged male rats in a dose 4 mg/kg b. m. significantly lowered the values of serum melatonin ($p=0.041$, $p=0.019$) (Fig. 3). At the higher dose this effect was insignificant. When juxtaposed the chronic and the acute treated groups didn't show authoritative differences between them. There is a tendency of reduction of the levels of melatonin at the chronic application of the low dose and its increase at high one.

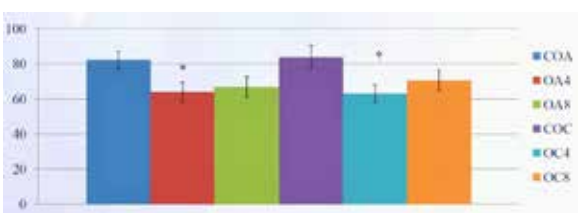

Fig. 3. Changes in the values of serum melatonin (pg/ml) - old animals * - P=0,041 towards COA; f - P=0,019 towards COC.

Discussion:

The pineal gland, also known as epiphysis, is a small endocrine structure situated in the middle brain. Its main hormone is N-acetyl -5-methoxitriptamin, more often called melatonin. It is secreted circadialy according to the dark and light cycle. During the night its secretion reaches maximum, while the light exposition of the retina leads to its rapid decrease. A number of studies show that the level of melatonin lowers with the age[w]. The data about the interaction between the pineal gland and the gonad function is contradictory[w]. Some studies show that the function of the pineal gland depends by the level of sex hormones, because the plasma levels of melatonin decrease with the age[w].

Luboshitzky et al. (1996) establish that the secretion of melatonin is stronger at men with GTRH. The same author proves that TRT lowers the levels of melatonin down to the normal values in such patients. At a clinic study of aged men with testosterone deficiency Rajmil and al (1997) determine high plasma levels of melatonin compared with controls as in regard of the day, as well as the night secretion. A 3 months treatment with

testosterone lowers both the day and the night levels of melatonin.

In current experimental study a similar correlation between the plasma level of melatonin and testosterone therapy is established. The orchiectiomy leads to insignificant increase of melatonin at the acute treated animals. At the aged male rats high serum levels of melatonin were also seen, while the treated groups were though insignificantly with lower values. The possible explanation of this fact is that testosterone induces the metabolism of indolamins (melatonin, serotonin etc.) in the liver. Another possible explanation is based upon the fact that the gonadectomy in rats increases the number of the receptors in the epiphysis. It is established that these receptors stimulate the melatonin synthesis. The present study confirms the already described in the literature changes in the values of melatonin at experimental models.

Conclusions:

1. Applied on rats with androgen deficiency, testosterone propionate in a dose 8 mg/kg b. m. recovers the physiological levels of the total testosterone.
2. Testosterone propionate has a bidirectional effect over the level of melatonin. There is data established for its increase in aged male rats, treated with testosterone propionate.

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