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Testosterone to 17β-estradiol Ratio in Rat Males with Dominant and Submissive Types of Behaviour

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Introduction. Every year more than 700. 000 people worldwide die because of assault [1]. Suicide is an important public health problem and major depressive disorders appeared to confer greater risk for suicide [12, 26]. Major depression is twice as frequent in women as in men [3, 4]. According to classical view of sexual differentiation of mammalian brain, sex differences in structure and functions of brain are provided by testosterone synthesized in the fetal and neonatal testis. It diffuses to male brain, where it is locally aromatized to estradiol and initiates the process of masculinization. Masculinization of brain is evidenced in adults by a capacity to express male-typical sexual behaviour and high level of aggression [17]. Estradiol is potent steroid of both gonadal and neuronal nature which has deep and long-term effects in brain, which develops [21]. Aromatase, key enzyme of androgen conversion to estrogens, controls many physiological and behavioural processes. In vertebrates the regulation of aromatase expression in brain is involved in modulation of sexual and aggressive behaviour in males [6]. Furthermore β-estradiol which is produced by testes inhibits the synthesis of androgens either by autocrine or paracrine ways [22]. It is believed that relative but no absolute levels of sex hormones play more important role in etiology of depression [20].

Objective was the investigation of testosterone and 17β-estradiol levels and their ratio in rat males with dominant and submissive types of behaviour.

Materials and Methods. Experiments were carried out on 76 Wistar rat males (three-, six- and twelve-month-old), bred and kept under standard vivarium conditions at the Kharkiv National Medical University. According to classification of age groups of laboratory animals [27], tree-month-old rats belong to II period (juvenile); six- and twelve-month-old rats belong to III – reproductive (to young reproductive and mature reproductive, respectively). The sensory contact model [10] with some modifications [16] was used to separate animals with alternative types of behaviour. According to this model, during 5 days rats were kept in individual cages to prevent an effect of group interaction. Then the males were placed in small cages divided into halves by perforated transparent partitions, permitting the sensory contact. After 2 days of sensory contact, testing commenced. The partition was removed for 10 min to allow agonistic interaction. Testing was performed 10 days during the second half of the day (14.00-16.00). According to results of testing, the animals were separated into 3 groups: dominant, balanced and submissive. Twenty hours after the last testing, the animals were decapitated.

Plasma testosterone and 17β-estradiol levels were determined by method of immune-enzymatic analysis using "Alkor-Bio" and ELISA kits.

Statistical analysis of the results was carried out by methods of nonparametric statistics using the package "Statistica 6.0". Nonparametric analogues of dispersion analysis – Kruskal-Wallis and median tests – were used to reveal the dependence of parameters on group. Mann-Whitney test and correlation analysis according to Spearman were used to compare groups in pairs.

Results. Blood plasma testosterone level was found to vary depending on the period of adulthood. Analysis of blood plasma testosterone level in rats of different age groups but the same behaviour type revealed the following predictability: in rat males independently on a type of behaviour the testosterone level was significantly increased in six-month-old rats versus three-month-old ones and decreased in twelve-month-old rats versus six-month-old ones. No differences were found between testosterone levels in three- and twelve-month-old rats with the same type of behaviour (**table 1**).

Table 1

Testosterone level (nmol/L) in rat blood plasma of different age groups with alternative types of behaviour

Age of animals	Number	Median Me	Quartile 25%; 75%
Submissive males			
Three-month-old	7	3,00	2,50; 4,40
Six-month-old	8	6,30*	3,90; 10,05
Twelve-month-old	8	2,70**	1,55; 4,05
Balanced males			
Three-month-old	9	7,30	6,60; 9,80
Six-month-old	9	17,80*	17,40; 21,30
Twelve-month-old	9	7,90**	7,30; 8,30
Dominant males			
Three-month-old	8	20,90	19,70; 30,60
Six-month-old	9	44,90*	38,70; 48,00
Twelve-month-old	8	16,05**	14,20; 19,40

Note: *- P<0. 05 versus three-month-old males; **-P<0. 05 versus six-month-old males.

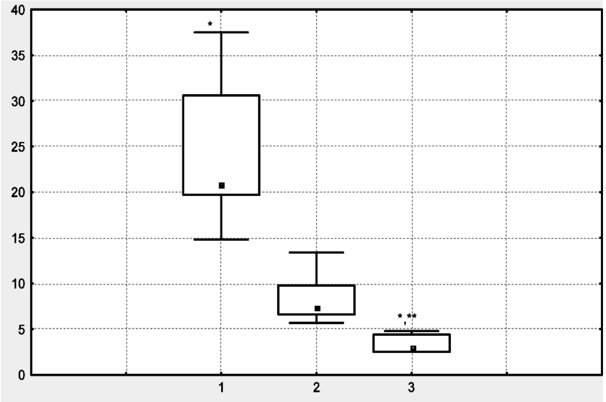

Figure 1. Testosterone levels (nmol/L) in blood plasma of three-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of behaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

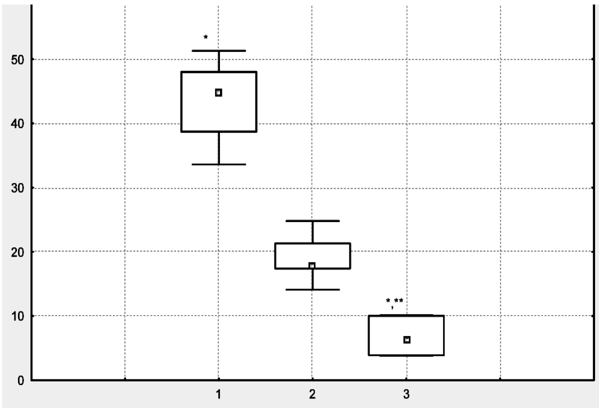

Figure 2. Testosterone levels (nmol/L) in blood plasma of six-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of behaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

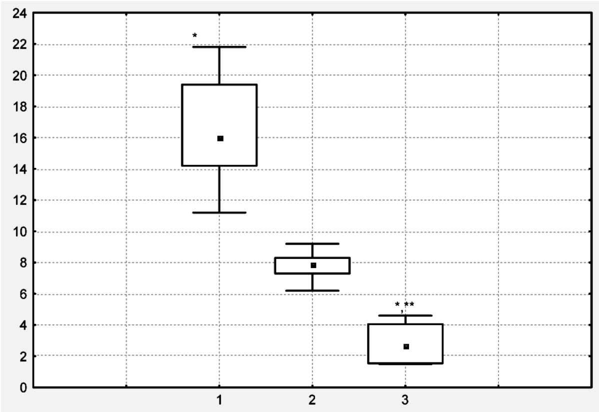

Figure 3. Testosterone levels (nmol/L) in blood plasma of twelve-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of behaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

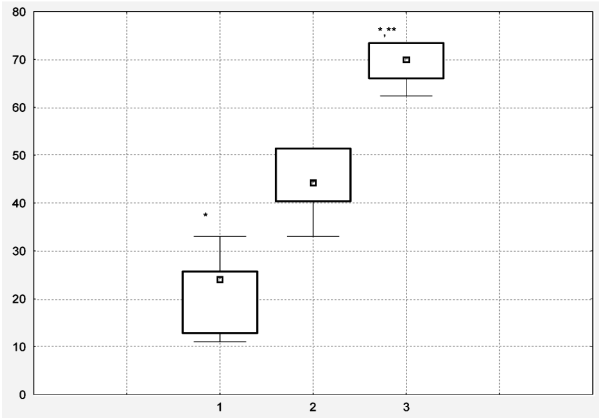

Figure 4. 17β-estradiol levels (pmol/L) in blood plasma of three-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of behaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

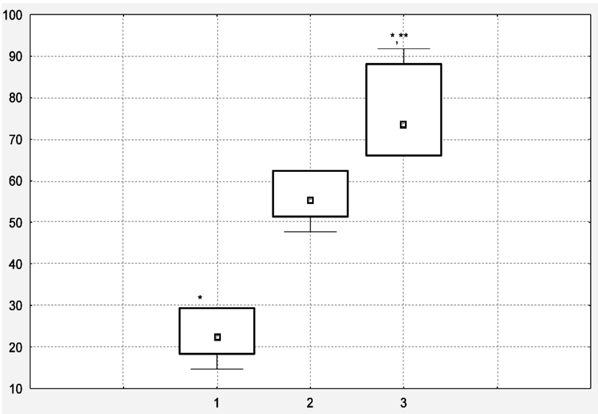

Figure 5. 17β-estradiol levels (pmol/L) in blood plasma of six-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of bechaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

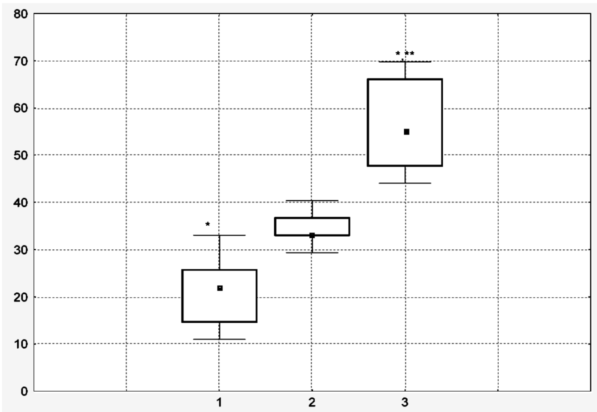

Figure 6. 17β-estradiol levels (pmol/L) in blood plasma of twelve-month-old rat males with dominant (1), balanced (2) and submissive (3) types of behaviour (Me [25 %; 75 %], min and max).

Note: *-P < 0. 05 versus the rats with balanced type of behaviour; **-P < 0. 05 versus the rats with dominant type of behaviour.

In all investigated age groups the testosterone level was revealed to depend on behaviour type. It was increased in dominant rat males versus both balanced and submissive ones. Testosterone level in balanced rats was higher than in submissive males but lower than in dominant ones. The most considerable differences were observed in six-month-old rats (**Figures 1-3**).

In contrast to testosterone the level of blood plasma 17β-estradiol was revealed to decrease in dominant rat males versus both submissive and balanced ones (**Figures 4-6**). The level of 17β-estradiol in balanced rats was higher than in dominant males but lower than in submissive ones.

Statistically significant negative correlation between testosterone and 17β-estradiol levels was revealed in all investigated age groups regardless of the behaviour type (**table 2**).

Table 2

Correlation between testosterone and 17β-estradiol levels in blood plasma of rat males with different types of behaviour

Age / Type of behaviour	Three-month-old	Six-month-old	Twelve-month-old
Balanced	-0.95*	-0.88*	-0.69*
Submissive	-0.98*	-0.92*	-0.96*
Dominant	-0.92*	-0.92*	-0.99*

Note: * – P < 0. 05 Correlation is statistically significant.

Statistically significant differences of testosterone/17β-estradiol ratio were found between males with alternative types of behaviour (**table 3**). The highest ratio of testosterone to 17β-estradiol was found in dominant rat males.

Discussion. The revealed correlation between testosterone level and type of behaviour, the highest level of testosterone in dominant adulthood males are associated with the role of testosterone in processes of sexual differentiation and development of brain [17]. Testosterone is the principal male sex steroid which is involved in the regulation of aggression both in animals, and in humans [25]. In sensitive period of early fetal and perinatal development testicular androgens influence neuroendocrine system and mechachisms of brain providing gender-specific behaviour [8]. During embryonal period the influence of testosterone on hypothalamus predetermines the character of gonadotropin secretion in adults and therefore the specific proportion between male and female sex hormones. In perinatal period androgens facilitate the formation of neuronal networks which are expressed by aggressive behaviour in adults [2]. In adulthood testosterone is suggested to contribute modulation of neuronal ways which regulate aggression [14].

There are evidences of relationship between circulating testosterone and aggression, for example in pubertic period when testosterone and aggression levels increase parallely in most mammalian species [9]. In adolescent men the aggression correlates with increased level of testosterone in blood plasma [19]. There is positive correlation between testosterone level and criminal behaviour [24].

The conversion of androgens into estrogens in the brain is the key mechanism by which testosterone regulates many physiological and behavioural processes throughout the animal life [17]. Neonatal aromatization of testosterone into 17β-estradiol is the major factor responsible for development of adult aggression between males [7]. In adult males the aggression also depends on circulating testicular hormones [15] and may be mostly, if not entirely, mediated through activation of estrogen receptors (ER), with enhancement of aggression via ERα activation and inhibition via ERβ activation [15, 18]. It should be noted that in the postnatal and adult brain regions the content of 17β-estradiol considerably decreases. However the endogenous content of 17α-estradiol and estrone in the postnatal and adult neocortex, hippocampus, hypothalamus, and cerebellum of both sexes is significantly elevated compared with 17β-estradiol. 17α-Estradiol is not involved in expression of aggression. It has strong neuroprotective activity [5, 11], is able to induce short-latency effects on spatial memory through influences on hippocampal synaptic plasticity [13]. The synthesis of 17α-estradiol in brain does not depend on gonadal steroids. Gonadectomy and adrenalectomy does not influence 17α-estradiol content [23]. Very low levels of 17β-estradiol in adult brain regions and adrenals [23] testify to testicular origin of blood plasma 17β-estradiol. This suggestion is confirmed by revealed negative correlation between testosterone and 17β-estradiol levels since production of estrogens in testis inhibits the synthesis of androgens.

Table 3

Testosterone/17β-estradiol ratio in rat males with different types of behaviour

Age / Type of behaviour	Three-month-old		Six-month-old		Twelve-month-old	
	Median Me	Quartile 25 %; 75 %	Median Me	Quartile 25 %; 75 %	Median Me	Quartile 25 %; 75 %
Submissive	43.01*,**	34.05; 66.59	41.40*,**	44.39; 152.09	49.03*,**	23.57; 85.61
Dominant	844.11*	766.53; 2510.22	2008.05*	1318.12; 2794.12	759.07*	552.53; 1321.53
Balanced	165.75	128.43; 242.69	346.37	278.80; 414.48	233.05	215.20; 251.21

Note: * – P < 0,05 versus the rats with balanced type of behaviour; ** – P < 0,05 versus the rats with dominant type of behaviour.

Conclusion. Considerable difference between testosterone/ 17β-estradiol ratio in males with dominant and submissive types of behaviour may be used to determine the predisposition of organism to aggression or depression.

Prospectives of further research. The research of testosterone and 17β-estradiol and their ratio in men with different predisposition to aggression will be carried out.

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СПІВВІДНОШЕННЯ ТЕСТОСТЕРОНУ ТА 17β-естрадіолу У САМЦІВ ЩУРІВ З ДОМІНАНТНИМ ТА СУБМІСИВНИМ ТИПАМИ ПОВЕДІНКИ

Попова Л. Д., Васильєва І. М., Богданова Т. В.

Резюме. Було досліджено рівень тестостерону та 17β-естрадіолу у плазмі крові самців щурів з домінантним та субмісивним типами поведінки. Виявлено залежність рівня тестостерону від типу поведінки. У межах кожної вікової групи рівень тестостерону був найвищим у домінантних щурів. У межах одного типу поведінки найвищій рівень тестостерону спостерігався у шестимісячних щурів. Рівень 17β-естрадіолу був нижчим у домінантних порівняно із субмісивними щурами**.** У самців щурів усіх вікових груп та типів поведінки виявлено тісний негативний кореляційний зв'язок між рівнями 17β-естрадіолу та тестостерону. Було виявлено значну різницю між домінантними та субмісивними щурами у співвідношенні рівней тестостерону до 17β-естрадіолу**.** Визначення цього співвідношення може бути використано для визначення схильності організму до агресії або депресії.

Ключові слова: тестостерон, 17β-естрадіол, домінантні і субмісивні самці щурів.

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СООТНОШЕНИЕ ТЕСТОСТЕРОНА И 17β-эстрадиола У САМЦОВ КРЫС С ДОМИНАНТНЫМ И СУБМИССИВНЫМ ТИПОМ ПОВЕДЕНИЯ

Попова Л. Д., Васильева И. М. Богданова Т. В.

Резюме. Было исследовано уровень тестостерона и 17β-эстрадиола в плазме крови самцов крыс с доминантным и субмиссивным типами поведения. Выявлена зависимость уровня тестостерона от типа поведения. В пределах каждой возрастной группы уровень тестостерона был самым высоким у доминантных крыс. В пределах одного типа поведения самый высокий уровень тестостерона наблюдался у шестимесячных крыс. Уровень 17β-эстрадиола был ниже у доминантных по сравнению с субмиссивными крысами. У самцов крыс всех возрастных групп и типов поведения выявлена тесная отрицательная корреляционная связь между уровнями 17β-эстрадиола и тестостерона. Было выявлено значительную разницу между доминантными и субмиссивными крысами в соотношении уровней тестостерона к 17β-эстрадиолу. Определение этого соотношения может быть использовано для определения склонности организма к агрессии или депрессии.

Ключевые слова: тестостерон, 17β-эстрадиол, доминантные и субмиссивные самцы крыс.

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Testosterone to 17β-estradiol Ratio in Rat Males with Dominant and Submissive Types of Behaviour

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Abstract. Aggression and suicide are significant problems of modern society. The investigation of features of neurohumoral status in animals predisposed to dominant or submissive types of behaviour and the identification of peripheral markers of these features are very important to prevent the development of aggression and depression in humans. Testosterone is involved in regulation of aggression both in animals and in humans. The conversion of testosterone into estradiol is a key mechanism of many physiological and behavioural processes regulation by testosterone.

The objective of the study was to investigate plasma testosterone and 17β-estradiol levels and their ratio in rat males with dominant and submissive types of behaviour.

Experiments were carried out on 76 Wistar rat males (three-, six- and twelve-month-old). According to classification of age groups of laboratory animals, tree-month-old rats belong to II period (juvenile); six- and twelve-month-old rats belong to III – reproductive (to young reproductive and mature reproductive, respectively). The animals were separated according to the sensory contact model with some modifications into 3 groups: dominant, balanced and submissive ones. Plasma testosterone and 17β-estradiol levels were determined by method of immune-enzymatic analysis using “Alkor-Bio” and ELISA kits. Statistical analysis of the results was carried out by methods of nonparametric statistics using the package “Statistica 6. 0”. Nonparametric analogues of dispersion analysis – Kruskal-Wallis and median tests – were used to reveal the dependence of parameters on group. Mann-Whitney test and correlation analysis according to Spearman were used to compare groups in pairs.

Blood plasma testosterone level was found to vary depending on the period of adulthood. Analysis of blood plasma testosterone level in rats of different age groups but the same behaviour type revealed the following predictability: in rat males independently on a type of behaviour the testosterone level was significantly increased in six-month-old rats versus three-month-old ones and decreased in twelve-month-old rats versus six-month-old ones. No differences were found between testosterone levels in three- and twelve-month-old rats with the same type of behaviour. In all investigated age groups the testosterone level was revealed to depend on behaviour type. It was increased in dominant rat males versus both balanced and submissive ones. Testosterone level in balanced rats was higher than in submissive males but lower than in dominant ones. The most considerable differences were observed in six-month-old rats.

The blood plasma 17β-estradiol was revealed to decrease in dominant rat males versus both submissive and balanced ones. The level of 17β-estradiol in balanced rats was higher than in dominant males but lower than in submissive ones. Statistically significant negative correlation between testosterone and 17β-estradiol levels was revealed in all investigated age groups regardless of the behaviour type.

Statistically significant differences of testosterone/17β-estradiol ratio were found between males with alternative types of behaviour. The highest ratio of testosterone to 17β-estradiol was found in dominant rat males. Considerable difference between testosterone/17β-estradiol ratio in males with dominant and submissive types of behaviour may be used to determine the predisposition of organism to aggression or depression.

The research of testosterone and 17β-estradiol and their ratio in men with different predisposition to aggression will be carried out.

Keywords: testosterone, 17β-estradiol, dominant and submissive rat males.

Рецензент – проф. Дубінін С. І.

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