

Changes in cholesterol levels of rat adrenals, caused by methanandamide, depend on sex hormones

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Introduction

There are no doubts about participation of endocannabinoids in pituitary-adrenocortical system regulation. Endocannabinoids are specifically involved in the fast feedback implementation in\by/during stress conditions [1, 2]. Literature analysis, concerning bond between sex hormones and endocannabinoid system, is given in a review [3]. Endocannabinoid system and androgen secretion are tied connected by reciprocal feedback [3]. The effects of endocannabinoids in the endocrine glands have been less studied at the tissue and at the cell level. The mechanism of influence of endocannabinoids on steroidogenesis are insufficiently known studied. It has been ascertained that the main implementation phase of these effects is to receive connection of endocannabinoids with receptors of various kinds (CB₁ i CB₂). CB₁-receptors are expressed in the cells of the human adrenal glands [4]. Corticosteroid hormone formation is controlled by a complex system of regulators system, where the main place have estrogenic compounds play the main role [5,6].

It is known that in the adrenocorticotrophic hormone secreting pituitary is also expressed CB₁- receptors [7]. The CB₁-receptors expression in the pituitary is regulated by sex hormones in both male and female rats. The pituitary-adrenocortical system activation, caused by administration of the CB₁-receptors antagonist, depends on animal gender [8].

Methanandamide (being a metabolic stable derivative of endogenous anandamide and preserving its biological properties) influences the multidirectional effects on

steroidogenesis and apoptosis in the adrenal glands of male and female rats that was showed by our group [9,10]. It is known that in the steroid hormones producing glands cholesterol, apart from participating in membrane structures construction, is a precursor by which hormones are synthesized. This defined our research aim – to estimate methanandamide influence on the levels of cholesterol in the cells of adrenal glands of animals.

Materials and methods

Keeping and using of laboratory animals during experiment was satisfied by Universal Norms on Bioethics[11]. The permission on experimentation was obtained by Committee on Bioethics of State Institution “V.P. Komisarenko Institute of Endocrinology and Metabolism, Natl. Acad. of Med. Sci. of Ukraine”.

Experiments were conducted on sexually mature male and female rats of Wistar line. Weight of the animals was 140-200 g before the experiment. Such experimental groups have been used: intact animals; orchiectomized and ovariectomized rats, injected with solvent (refined sunflower oil); orchiectomized and ovariectomized rats, injected with dissolved in refined sunflower oil estradiol. Spaying in females and testectomy in males rats were taken under ether anesthesia. Rats were used in experiments 8 weeks after castration. Rats were intramuscularly injected with estradiol benzoate solution (Koch-Light, Great Britain) in dose 100 mcg for about 3 days. Hormone injection and slaughter of animals were effected at one and the same time of the day (in the morning). Animals, injected with estradiol benzoate solution or with solvent, were slaughtered by decapitation in a day after the last introduction of medicament. The adrenal glands were cleaned from adipose and connective tissues, weighted, cut into slices with thickness of $\approx 0,5$ mm thick with a blade. The last ones were incubated for 3 hours at $37.0\text{ }^{\circ}\text{C}$ continuously shaking in one ml of nutrient medium RPMI-1640 (20 mmol/L HEPES and L-glutamine) (Sigma, the USA) contained 5% bovine serum (Sigma, the USA) contained 5% bovine serum (Sigma, the USA), in the presents of R(+)-methanandamide in ethanol solution of final concentration of 10^{-8} - 10^{-6} mol/l. The check sample was added with an appropriated amount of ethanol. The tissue slices were homogenized in 0,05 mol/L tris-HCl (pH 7,4) buffer for 10% homogenate production. The quantitative

content of total cholesterol was determined using a reagent set C 7510 (Pointe Scientific, the USA). The method involves hydrolysis of cholesterol esters with cholesterol estherase, oxidation of cholesterol with cholesterol oxidase and next cholesterol quantification formed with H₂O₂.

The total cholesterol was expressed as mcg / mg of tissue. Statistical analysis of the results was performed using Student's t-test and Wilcoxon-Mann-Whitney U-test. $p < 0.05$ was considered a statistically significant difference.

Results and Discussion

Incubation of the adrenal glands of intact males and females tissue slices with methanandamide caused multidirectional effects on changes in cholesterol composition: cholesterol levels in males decreased by all tested concentrations of methanandamide, cholesterol levels in females - elevated at a concentration 10^{-7} - 10^{-6} mol/L (see Table.). This is the difference of the methanandamide effect in the intensity of steroidogenesis and DNA fragmentation in the adrenal glands in male and female rats, that has been demonstrated previously [9, 10], can be mediated by changes in cholesterol composition. Methanandamide in vitro had no effect on cholesterol levels in rats after ovariectomy, and only at high concentrations acted on rats after orchiectomy, which confirms the opinion about the sex hormones' role in effects of implementation of endocannabinoids [3].

As a result of a three-day administration of estradiol, methanandamide effect on cholesterol levels in castrated females, but not in males after orchiectomy, recovered. It is known that changes in estrogen levels during the estrous cycle in female rats and the menstrual cycle in women influence the level of mRNA expression of CB₁ receptors and the activity of enzymes responsible for the synthesis and metabolism of endocannabinoids in various types of cells [3].

Castration of animals is caused by elevated levels of cholesterol in males and females, but these changes are difficult to interpret, as castrated animals have received oil. Estradiol injection to castrated males does not change the content of cholesterol

content in the tissue, and after ovariectomy in females, cholesterol decreased and approached the baseline.

The Cholesterol content in the steroid-producing glands is determined by the balance between its use in steroidogenesis and fluxing to the cell. Cholesterol may be synthesized *de novo* and flux the cell from blood plasma as part of lipoproteins structure. Today, the greatest importance is attached to inflow of cholesterol in the adrenal glands of rats after high density lipoprotein (HDL) with a scavenger receptor binding. High doses of estrogen dramatically enhance the expression of the receptor on the plasma membrane adrenocorticocytes [12]. This effect of estrogen is mediated by adrenocorticotrophic hormone [13]. Perhaps sex hormones participate in CB₁-receptors expression regulation of many organs. It is shown that the castration of male rats reduces the density of CB₁-receptors in the salivary gland, and testosterone recovers the amount of receptors in this tissue [14]. Lack of effect of methanandamide on castrated male rats may be due to the density of CB₁-receptors in adrenal tissue decrease. The bond between endocannabinoid system and cholesterol homeostasis is unclear yet, but the activation of this system enhances the accumulation of cholesterol in macrophages [15].

Thus, the data obtained by our group received data afford to suggest that in the tissue of the adrenal cortex endocannabinoid system may modulate adrenocorticocytes function by changing the content of cholesterol in cells. This effect is associated with the gender of the animals.

Table. The Content of total cholesterol (mcg / mg of tissue) in the tissue of the adrenal glands of rats after incubation of tissue slices *in vitro* with different methanandamide concentrations (M±m, n=6-8).

Animal groups	Check sample	Methanandamide concentration		
		10 ⁻⁸ mol/L	10 ⁻⁷ mol/ L	10 ⁻⁶ mol/ L
Male rats				
Intact	17,3 ± 0,26	13,0 ± 1,24*	13,7 ± 1,51*	13,3 ± 1,40*
Orchiectomized rats injected with solvent (refined sunflower oil)	35,0 ± 2,87 ^{&}	39,1 ± 2,84 ^{&}	31,8 ± 2,79 ^{&}	26,6 ± 4,22 ^{#, &}

Orchiectomized rats injected with estradiol (100 mcg/animal)	34,4 ± 5,39	36,3 ± 3,92	35,9 ± 3,35	36,1 ± 6,63
Female rats				
Intact	15,8 ± 3,79	17,8 ± 3,15	34,9 ± 2,66*	30,2 ± 2,40*
Orchiectomized rats injected with solvent (refined sunflower oil)	37,9 ± 2,79 ^{&}	33,3 ± 3,13 ^{&}	36,9 ± 3,53	35,1 ± 2,65
Orchiectomized rats injected with estradiol (100 mcg/animal)	24,7 ± 5,14 ⁺	24,1 ± 5,80	39,9 ± 4,97 [#]	52,5 ± 5,58 ^{*,+}

Notes: * - $p < 0.05$ – compared to the control sample without methanandamide (Student's t-test); # - $p < 0.05$ - compared to the control sample without methanandamide (Wilcoxon-Mann-Whitney U-test); + - $p < 0.05$ - compared to the ovariectomized animals (Student's t-test); & - $p < 0.05$ – compared to the intact animals (Student's t-test).

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