Early postnatal cold imprinting influence on the adult rats’ adrenal medulla functional state

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Abstract. The article describes the cold imprinting modifying effect on the adrenomedullary cells functional state in adult animals. It has been shown that short-term cold exposure in early ontogenesis leads to an increase in norepinephrine reserves in the adult rats’ adrenal glands. With these animals test cooling, the phenylethanolamine-N-methyltransferase activity in the adrenal glands decreases, thereby preserving the hormone reserves, which is the thermogenesis dominant activator in the mammalian body.

1. Introduction

Adult animals long-term maintenance at low temperatures causes adaptation to the cold. An important role in these rearrangements is played by adrenocortical and sympathoadrenal systems hormones and mediators, which activation is well known when exposed to cold on the body.

The morphological equivalent of the developing state of cold resistance during long-term cooling is an increase in the mass of brown adipose tissue [1]. At the same time, brown adipose tissue is one of the most sensitive areas of non-shivering thermogenesis to the action of norepinephrine. This effector is important not only for the acute thermogenic process, but also for the control of cell proliferation, cell differentiation, and apoptosis [2].

Earlier, we showed that short-term cooling in early ontogeny (cold imprinting) significantly increases the resistance of adult animals to low temperatures [3]. This phenomenon is based on an increase in the adrenocortical and sympathoadrenal systems’ tone involved in the body adaptation processes to external conditions. It was found that, under thermal comfort conditions, the corticosterone content in the blood plasma and the norepinephrine excretion level with urine in adult rats subjected to short-term cooling in early ontogenesis were higher than in control animals by 70% and 40%, respectively [4].

Immediately after birth, chromaffin cells differentiate into morphologically distinguishable cells that accumulate adrenaline and norepinephrine in the rats’ adrenal glands immature medulla in the postnatal development first days. It is known that glucocorticoid hormones play an important role in the phenylethanolamine-N-methyltransferase (FNMT) activity initial expression and maintenance, an enzyme involved in the norepinephrine methylation and its conversion into adrenaline [5]. Thus, glucocorticoid hormones are involved in the adrenal chromaffin cells noradrenergic and adrenergic phenotypes development Glucocorticoid hormones action another phenotypic manifestation is an increase in the catecholamines synthesis, storage and secretion regulation [6].

This work aim was to study the adult animals adrenal medulla functional state subjected to short-term cooling in the postnatal ontogenesis early period, in the testing cold effects dynamics.
2. Materials and methods
The work was carried out on male rats of the Wistar population in compliance with the principles of humanity set forth in the European Convention for the Protection of Vertebrate Animals used for experiments or other scientific purposes (Strasbourg, 1986).

The rat pups of the experimental group daily, starting from the first day of life, for seven days, were subjected to cooling at a temperature of 2 ... 4°C for 15 minutes – «postnatal cold imprinting» [3]. The rat pups in the control group were not exposed to these or other influences in the early postnatal period. At the age of 2-2.5 months, rats of both groups were seated in individual cages and kept at a temperature of 20 ... 22°C with adjustable light mode: 12 h - light, 12 h - darkness. Rats received a standard diet of vivarium with free access to water. Control and experimental animals were adapted to cold, for which they were kept at a temperature of 4 ... 5°C for 1, 7, or 49 days. The lighting and feeding conditions were kept the same.

The mass of the interscapular brown adipose tissue (BAT) and the adrenal glands was determined. The catecholamines content was measured in adrenal glands - by the fluorometric method, using sorption of alumina according to Brockmann for their isolation; and also the phenylethanolamine-N-methyltransferase activity according to the method [7], using S-Adenosyl-L- [methyl-14C] methionine as a methyl groups donor.

Statistical processing of the results was carried out in the program Statistica 10.0. The Newman-Keuls test was used. The data are presented in the form $M \pm m$, where $M$ is the sample mean, $m$ is the standard error. The probability of validity of the null hypothesis was taken at a 5% significance level

3. Results
Table 1 shows the rats’ organs relative mass in the control and experimental groups in the cold exposure dynamics.

| Effect | ANIMAL GROUP |          |          | P       |
|--------|--------------|----------|----------|---------|
|        | Control      | Experimental |          |         |
|        | n | $M \pm m$ | n | $M \pm m$ |         |
| BAT    | 22°C | 185±13 | 11 | 204±9 | 8 |
|        | 4 ... 5°C: |          |          |         |
| - 7 day |          | 215±16 | 6 | 191±27 | 6 |
| -49 "-" |          | 340±21** | 6 | 258±11** | 6 <0.01 |
| ADRENAL GLANDS | 22°C | 15.0±0.3 | 11 | 19.1±1.0 | 8 <0.01 |
|        | 4 ... 5°C: |          |          |         |
| - 7 day |          | 18.4±0.9** | 6 | 18.0±1.1 | 6 |
| -49 "-" |          | 19.5±1.0** | 6 | 23.5±1.1* | 6 <0.05 |

Note: * - P<0.05; * * - P<0.01 compared to the rats’ organs mass in the same group animals, kept at 22°C

In control animals, after 7 weeks of exposure to cooling conditions, the relative mass of BAT increased by 84%, while in the experimental group of rats only by 26%.

The adrenal glands mass under the thermal comfort conditions was higher in the experimental animals and additionally increased by the experiment ended in both groups’ rats. This could be associated with hypertrophy and, accordingly, with both cortical and medulla hyperfunction.

Table 2 presents data on the catecholamines content in the animals adrenal glands in the control and experimental groups in the cold exposure dynamics.

In experimental rats kept in comfortable temperature conditions, the norepinephrine content was increased, which correlates with the previously obtained results on its urinary excretion determination
in experimental animals [4].

After a day of exposure to cold, the adrenal glands in the control rats increased both adrenaline and norepinephrine content. After 7 weeks, the catecholamines content in the adrenal glands did not differ from the initial one.

In experimental animals, after cooling a day the catecholamines content in the adrenal glands decreased, and after 7 days, it returned to the initial level. After 7 weeks of cold exposure, the norepinephrine content in the adrenal glands did not differ from the initial one, and the adrenaline content was reduced.

**Table 2.** The catecholamines content in the rats' adrenal glands (μmol/g tissue) in the control and experimental groups in the cold exposure dynamics.

| Effect | ANIMAL GROUP | P     |
|--------|--------------|-------|
|        | Control      |       |
|        | 22°C         |       |
|        | 1            | 5.14±0.48 |       |
|        | 2            | 2.39±0.29 |       |
|        | 3            | 5.28±0.46 |       |
|        | 4            | 3.41±0.33 | 2-4 <0.05 |
|        | 4 ... 5°C:   |       |
| - 1 day| 7.82±1.08*   | 3.48±0.20* |       |
| - 7 day| 6.39±0.09*   | 3.21±0.32 |       |
| -49 °C | 5.94±0.54    | 2.19±0.59 |       |
|        | Experimental  |       |
|        | 22°C         |       |
|        | 1            | 3.64±0.49* | 1.97±0.28** | 1-3.2-4 <0.01 |
|        | 2            | 3.54±0.25 | 4.00±0.70 | 1-3 <0.05 |
|        | 3            | 3.36±0.51* | 3.38±0.40 | 1-3 <0.01 |
|        | 4            | 5.28±0.52 |       |
| Notes: | * - P<0.05, ** - P<0.01 compared with the hormone level in the same group animals contained at 22°C; in each group n = 8 |

Table 3 presents data on the phenylethanolamine-N-methyltransferase (FNMT) activity study in the rats' adrenal glands in the control and experimental groups in the cold exposure dynamics.

**Table 3.** The FNMT activity (imp/min x100 per 1 μg of protein) in the rats' adrenal glands in the control and experimental groups in the cold exposure dynamics.

| Effect | ANIMAL GROUP | P     |
|--------|--------------|-------|
|        | Control      |       |
|        | 22°C         |       |
|        | 18           | 1.84±0.08 |       |
|        | 4 ... 5°C:   |       |
| - 1 day| 7            | 2.03±0.05 | 1.35±0.07** | 5 <0.01 |
| -49 °C | 12           | 2.08±0.14 | 0.97±0.02** | 8 <0.01 |
| Note: | ** - P <0.01 in comparison with the enzyme activity in the same group animals kept at 22°C. |

 Initially, the FNMT activity in both groups' rats was the same. In control animals, when cooled during the day, or within 7 weeks, the enzyme activity in the adrenal glands did not change. In experimental rats, its activity began to decrease within a day after the cooling start, and after 7 weeks it was halved compared to the initial one.

**4. Discussion**

In rats of both groups, the relative weight of BAT increased 7 weeks after their maintenance under cooling conditions. However, BAT hypertrophy in experimental animals was less pronounced when adapting to cold than in control rats. It can be assumed that cold imprinting contributes to a reduction in the contribution of the thermogenesis of BAT to the overall maintenance of temperature homeostasis during cooling of adult animals.

It is known that during the early postnatal ontogenesis period when the body is especially sensitive to the environmental and endogenous factors' effects, epigenetic changes a complex can form, which determine the physiological systems further development trajectory [8]. In vitro experiments have
shown that FNMT regulation is sensitive to epigenetic modification, which may have implications for epinephrine biosynthesis [9].

It can be assumed that rat pups cooling during the chromaffin cells' differentiation, being a stress factor in the external environment, caused an increase in blood glucocorticoid hormones acting at the gene transcription level, which, in turn, led to a change in the adrenomedullary cells functional state in adult animals. The endocrine regulation peculiarities in NISAG rats - animals with hereditary stress-induced arterial hypertension obtained from the Wistar rat population as a selection result according to the blood pressure level against the emotional stress background - can serve as the relationship a similar example between the changes that occurred and fixed in the genotype with hormonal parameters. It was found that in these animals, under thermal comfort conditions, the corticosterone level in the blood was steadily increased, the adrenaline content and FNMT activity increased in the adrenal glands, the norepinephrine level was not reduced, which indicates an increase in sympathoadrenal activity [10].

5. Conclusion
The data obtained indicate that short-term cold exposure in early ontogenesis leads to an increase in norepinephrine reserves in the adult rats' adrenal glands.

During these animals test cooling, the reserve norepinephrine is quickly released into the bloodstream. At the same time, the activity of FNMT in the adrenal glands decreases, thus increasing the norepinephrine reserves, as a hormone more specific for cold effects. This situation persists after 7 weeks. The enzyme activity decreases, even more, the adrenaline content in the adrenal glands also decreases, and the norepinephrine reserves are restored.

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