Functional state of the adrenocortical and sympathoadrenal systems in animals with early postnatal cold imprinting

V G Selyatitskaya and N A Palchikova

The Federal Research Center of Fundamental and Transnational Medicine, 2 str. Timakova, Novosibirsk, 630117, Russia

E-mail: labend@centercem.ru

Abstract. In the article presents information about changes in the functional state of the adrenocortical and sympathoadrenal systems in the dynamics of long-term adaptation to cold in adult rats that experienced short-term cooling in the first days of life. It is shown that the general principle of adaptive adjustment in these systems is to reserve their regulatory components, which ensures the maintenance of constant readiness of the body to respond to cold influences.

1. Introduction

Long-term maintenance of adult animals at low temperatures causes adaptation to cold. Adaptive changes in the body's thermoregulatory system include such functional modifications as a change in the capacity of effector systems, a decrease in the energy cost of maintaining temperature homeostasis, an increase in the physiological efficiency of heat generation, and a change in regulatory characteristics.

An important role in these rearrangements is played by hormones and mediators of the adrenocortical and sympathoadrenal systems, the activation of which is well known when exposed to cold on the body. In turn, the individual characteristics of the reactivity of the neuroendocrine system depend not only on hereditary factors but also on the conditions of its formation in early ontogenesis [4, 5]. We previously showed that the short-term cooling procedure in early ontogenesis significantly increases the resistance of adult animals to low temperatures [6]. It was suggested that short-term exposure to cold in early ontogenesis causes an adaptive modification of the program for endocrine regulation of temperature homeostasis, the features of which can be manifested in the process of adaptation to cold of adult animals.

The work presents the results of studying the functional state of adrenocortical (ACS) and sympathoadrenal (SAS) systems in the dynamics of long-term adaptation to cold of adult rats that experienced short-term exposure to cold in the first days of life.

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» [6]. 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 (30 males from each group) were adapted to cold, for which they were kept at a temperature of 4 ... 5°C for 7 weeks. The lighting and feeding conditions were kept the same.

In the dynamics of cooling, daily urine was collected individually from each animal. Progesterone and corticosterone were extracted from the urine with ethyl acetate, the organic solvent was evaporated, and the dry residue was dissolved in hormone determination buffer.

The content of corticosterone and progesterone in blood serum and urine was measured by the enzyme immunoassay, and catecholamines in the urine by the fluorimetric method.

Statistical processing of the results was carried out using the software package Statistica 10.0 (Statsoft, USA). The Student t-criterion was used to compare the values of the studied indicators in control and experimental rats; the Student t-criterion was used to evaluate the dynamics of indicators change. The data are presented in the form M ± m, where M is the sample mean, m is the standard error. The probability of the validity of the null hypothesis was taken at a 5% significance level.

3. Results

The functional state of the adrenocortical system was evaluated by the content of corticosterone and progesterone in the blood and the excretion of these hormones in the urine. As can be seen from table 1, the corticosterone content in the blood plasma of rats of the experimental group before the onset of cold exposures was higher than in control animals.

| Duration of cold exposure | Control group | Experienced group |
|---------------------------|---------------|-------------------|
| 0                         | 260±26        | 442±29 **         |
| 30 min                    | 727±75 **     | 638±87 *          |
| 5 h                       | 713±40 **     | 335±37 *##        |
| 24 hours                  | 620±29 **     | 525±81            |
| 7 days                    | 670±104 **    | 825±139 *         |
| 49 days                   | 537±35 **     | 407±43           |

Notes:
* - P<0.05, ** - P<0.01 compared with the level of the hormone in animals of the same group contained at 22°C.
# - P<0.05 compared with the same indicator in control rats

During the first days of cold exposure in adult rats of the control group, a high level of corticosterone in the blood was found. In rats of the experimental group, an increase in the level of the hormone in the blood was noted only 30 minutes after the onset of cold exposure. After 7 days of cold exposure, the level of corticosterone was increased in animals of both groups. After 49 days of cooling in the control animals, the corticosterone content in the blood remained high, and in the experimental rats, it did not differ from the initial value.

Progesterone is a precursor in the biosynthesis of corticosterone in the adrenal glands and is partially secreted into the blood, like other steroids. In conditions of increased synthesis of hormones in the adrenal cortex, its concentration in the blood also increases. Initially, the progesterone content in the blood of rats of the control group was 0.88±0.26 nmol / L, of the rats of the experimental group - 1.52±0.18 nmol/L. After 7 weeks of adaptation to cold, the blood progesterone content in the control animals increased to 2.56±0.38 nmol/L and to 2.90±0.33 nmol/L in rats of the experimental group (P<0.01).

Table 2 presents data on the daily excretion of non-metabolized corticosterone and progesterone with urine in rats of the control and experimental groups in the dynamics of cold exposure. The change in the values of corticosterone excretion in the dynamics of cold exposures was of a phase nature: the reaction
to cooling was most pronounced on the first day, decreased to the initial values on the third day, and on the 4th day of cooling, the second phase of an increase in the level of hormone excretion began.

**Table 2.** Daily excretion of corticosterone and progesterone with urine (nmol/day) in rats of the control and experimental groups in the dynamics of cold exposure.

| Duration of cold exposure | Control Group | Experienced group |
|--------------------------|---------------|-------------------|
|                          | Corticosterone | Progesterone       | Corticosterone | Progesterone |
|                          |               |                   |               |             |
| - 0 day                  | 0.90±0.08     | 0.034±0.002       | 0.99±0.07     | 0.043±0.002# |
| - 1 day                  | 2.20±0.41**   | 0.058±0.008*      | 3.07±0.53**   | 0.052±0.009  |
| - 2 '-'                 | 1.88±0.35*    | 0.050±0.007*      | 2.75±0.58**   | 0.063±0.007* |
| - 3 '-'                 | 1.38±0.31     | 0.067±0.011*      | 1.35±0.47     | 0.051±0.008  |
| - 4 '-'                 | 2.39±0.30**   | 0.092±0.014**     | 2.47±0.50*    | 0.070±0.007**|
| - 5 '-'                 | 2.09±0.29**   | 0.097±0.017**     | 2.96±0.64**   | 0.109±0.018**|
| -49 '-'                 | 3.61±0.25**   | 0.184±0.025**     | 4.31±0.40**   | 0.223±0.030**|

Notes:
* - P<0.05, * * - P<0.01 compared with hormone excretion in animals of the same group, kept at ±22oC.
# - P<0.05 compared with the same indicator in control rats

The excretion of progesterone in the first day increased to a lesser extent compared with corticosterone and starting from 4 days there was a correlation in the increased excretion of corticosterone and progesterone with urine in rats of both groups.

After 7 weeks of cooling, the excretion of progesterone was 5 times higher than the original, and corticosterone 4 times higher in rats of both groups.

The state of the sympathoadrenal system in the dynamics of adaptation to cold was evaluated by daily excretion of catecholamines in the urine. Adrenaline (A), found in urine, is primarily of the adrenal origin, noradrenaline (NA) is mainly secreted by the ends of the sympathetic nerves. Despite the fact that free catecholamines found in urine make up 4-5% of the total amount metabolized in the body, a change in their content in the urine reflects the general direction of changes in SAS. Under comfortable temperature conditions in rats of the experimental group, NA excretion was higher than in control rats (table 3), which indicates an increased tone of the mediator SAS link.

**Table 3.** The daily excretion of catecholamines with urine (mmol/day) in rats of the control and experimental groups in the dynamics of cold exposure.

| Duration of cold exposure | Control Group | Experienced group |
|--------------------------|---------------|-------------------|
|                          | Adrenaline    | Noradrenaline     | Adrenaline    | Noradrenaline |
|                          |               |                   |               |             |
| - 0 day                  | 2.83±0.24     | 1.59±0.19         | 3.25±0.42     | 2.22±0.16#   |
| - 1 day                  | 2.06±0.25     | 6.97±0.47**       | 3.42±0.69     | 8.08±1.09**  |
| - 2 '-'                 | 2.64±0.29     | 8.68±1.10**       | 2.60±0.39     | 7.98±0.47**  |
| - 3 '-'                 | 2.80±0.24     | 7.73±0.65**       | 2.94±0.38     | 9.22±1.14**  |
| - 40 '-'                | 3.29±0.63     | 11.51±0.70**      | 5.48±0.63*    | 9.32±0.85**  |

Notes:
* - P<0.05, * * - P<0.01 compared with hormone excretion in animals of the same group, contained at 22°C.
# - P<0.05 compared with the same indicator in control rats

In both control and experimental animals, from the first day of cold exposure, the excretion of NA sharply increased. After 40 days of cooling, in experimental animals, the excretion of NA was increased 4 times as compared with the initial one, and 7 times in control rats.

**4. Discussion**

In rats of the experimental group kept at a comfortable temperature, the plasma corticosterone level was 1.7 times higher than in control animals, while the excretion of non-metabolized hormone did not differ
in animals of the two groups. The progesterone content in the blood plasma of rats of the experimental group was also 1.7 times higher, but the hormone excretion was higher.

In the dynamics of cold exposure during the first day in adult rats of the control group, a high level of corticosterone in the blood was maintained, reflecting the intense stress response of the ACS. In the experimental group of rats, an increase in the content of corticosterone in the blood was observed only 30 minutes after the onset of cold exposure. After 5 hours, the hormone content decreased compared to the initial level, which is typical for a short-term stress response of the body as a phase following an increase in the level of the hormone in the blood. From a comparison of the data, it follows that in the first three days in rats of both groups a stress reaction developed with the predominant release of the active glucocorticoid hormone, corticosterone, into the blood. This is reflected in the increased excretion of non-metabolized hormone in the urine. The excretion of progesterone increased to a lesser extent, which is probably due to an unsteady balance between an increase in the synthesis of this hormone, that is, activation of the early stages of steroidogenesis, and its further use as a precursor in the production of corticosterone. Starting from 4 days, a balance was established between the increase in activity of the early and late stages of steroidogenesis.

The results obtained suggest that in rats of the experimental group, corticosterone in the blood plasma is predominantly in the inactive form associated with proteins, that is, the body has a “reserve” of the hormone in case of emergency, which determines the constant willingness to respond to changes in external conditions. In these animals, the activity of the early stages of steroid synthesis is also increased, as can be seen from the increased levels of progesterone in the blood and urine.

On the first day of cold stress, an increase in blood corticosterone level in experimental rats was less pronounced than in control animals and was observed only in the first 30 minutes of cooling. At the same time, the daily excretion of the hormone with urine was higher than the initial level, i.e., the experimental animals in the first day reacted to cold stress with a more intense, but short-term reaction. Short-term cold exposures in early ontogenesis also led to an increase in the activity of the mediator SAS in rats of the experimental group, however, in response to cold exposures, urinary excretion of urine in rats of the experimental group increased to a lesser extent than in control animals.

This suggests that in adult animals that underwent short-term cold exposures in early ontogenesis, more cold-specific stress response units are included in response to cooling more quickly. Since the increase in the level of glucocorticoids in the blood is a non-specific component of any stress reaction and its energy value is high, this type of regulation of the corticosteroid function of the adrenal cortex, as in experimental animals, is more beneficial for the formation of specific mechanisms to maintain increased resistance to cold.

Consequently, changes in the functional state of the adrenocortical and sympathoadrenal systems in adult rats subjected to cooling in the first days of life are interrelated and support each other. It is shown that the general principle of adaptive adjustment in these systems is to reserve their regulatory components, which ensures the maintenance of constant readiness of the body to respond to cold influences.

5. Conclusion
Our results allow us to say that short-term exposure to cold in early ontogenesis causes an adaptive modification of the program for endocrine regulation of temperature homeostasis. It consists in the creation and maintenance of constant response reserves from the two main hormonal systems involved in the adaptation of the body to external conditions - SAS and ACS, as well as a coordinated and mutually supportive reaction to cooling. It is characterized by a decrease in the intensity and time of development of a nonspecific stress response, and the rapid appearance of a specific SAS reaction. At the level of the whole organism, this is expressed in greater resistance of animals to extreme cooling.

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