Effect of Neonatal Handling in Rats with Hereditary Stress-Induced Arterial Hypertension (NISAG Rats)

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NISAG rats were subjected to handling on days 1-21 after birth. Blood pressure and plasma corticosterone concentration were measured in 6-month-old handled and control NISAG rats at rest and under stress conditions. Animal behavior was studied in the open-field test. Handling had no effect on body weight and relative weights of the heart and adrenal glands. In rats subjected to handling, changes in blood pressure and adrenal cortex produced by acute emotional stress (30-min restriction) were less pronounced than in control animals. Handled rats demonstrated less fear in a new environment and exhibited high exploratory activity in the open-field test. Our findings suggest that neonatal handling reduces stress reactivity and decreases the severity of hypertension in adult NISAG rats.

Key Words: *handling; arterial hypertension; stress; behavior*

Published data show that simple procedure of handling in rats (taking in hands and separation from mother for a short time) during early postnatal ontogeny leads to permanent changes in a variety of physiological functions [3-5]. It primarily concerns the systems involved in stress reactions of the organism. Various characteristics of the stress response undergo considerable changes (behavioral activity, hormonal reactions, and functions of target organs). Increasing interest in this problem is due to better understanding of the mechanism underlying changes produced by stimulation during the early ontogeny [6,7,9,10].

Our previous studies showed that modulation of gene expression regulated by glucocorticoids underlies the development of permanent changes after early stimulation [8]. It is interesting to study the effect of handling in rats with hereditary stress-induced arterial hypertension (NISAG rats). We hypothesized that the decrease in stress reactivity after neonatal handling can reduce the severity of hypertension in NISAG rats.

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MATERIALS AND METHODS

Experiments were performed on male NISAG rats [4]. The animals were kept in a vivarium of the Institute of Cytology and Genetics under standard conditions. They had free access to water and food. Handling was produced by placing the rats in an organic glass box (10×10×10 cm) with sawdust on the floor for 10 min. Rat pups were subjected to handling on days 1-21 after birth. Intact NISAG rats served as the control. The rats were isolated from mothers and divided by sex after handling.

Further tests were performed with handled and control rats at the age of 6 months. The animals were narcotized with ether to exclude the influence of psychological stress. Basal blood pressure (BP) was measured by the tail-cuff method.

Rat behavior was studied in the open field (OF) after 3 days. This procedure included three 6-min tests within 3 days. Behavioral activity was recorded on a semiautomatic system [2]. The rats were placed on a brightly illuminated area (140×70 cm) surrounded by walls and divided into 10×10-cm squares. The area included 12 central squares. We recorded locomotor activity (LA), *i.e.* number of crossed central and peri-

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pheral squares, number and duration of grooming acts, number of vertical rearing postures in central and peripheral region, time spent in the central region, latency of the first movement, and defecation rate (number of fecal boluses). The index of LA was recorded at 1-min intervals and after completing the test.

The rats were exposed to emotional stress 1 day after the last test. The animals were placed into narrow cylindrical wire-mesh boxes for 30 min (restriction). BP was measured in awake rats 30 min after restriction. The blood (0.5 ml) was taken from the end of the tail tip.

The rats were then returned to home cages for 1 week and then decapitated. Blood samples were taken after decapitation. We measured body weight and weights of the heart and adrenal glands. Blood corticosterone concentration was estimated by the method of competitive binding.

The results were analyzed by Student' *t* test for small samples.

RESULTS

Basal BP in rats handled during in the neonatal period was higher than in control animals (statistically insignificant) (Table 1). However, after stress BP in handled rats was lower than in control animals.

In handled rats plasma corticosterone concentration after 30-min stress was much lower than in control animals. Corticosterone content in handled and control rats increased by 186 and 372 ng/ml, respectively, compared to the baseline level. The difference between poststress and basal levels became less pronounced not only due to reduction of plasma corticosterone concentration after stress, but also due to higher basal corticosterone content in handled animals (statistically insignificant).

BP underwent similar changes. Handled rats were characterized by low poststress, but high basal level of BP. After stress BP in handled and control rats increased by 12 and 48 mm Hg, respectively. Therefore, non-handled animals exhibited low reactivity to acute stress. However, the initial state of these rats can be designated as "permanent stress". Our conclusion was confirmed by increased basal levels of BP and blood corticosterone concentration. Similar results were obtained when studying expression of hormone-dependent genes [3]. For example, activity of hydrocortisone-induced tyrosine aminotransferase in the liver of rats subjected to handling was much lower than in control animals. By contrast, basal enzyme activity in handled rats was high. These data explain the phenomenon of reduced inducibility of enzymes regulated by glucocorticoids. In handled rats the degree of stress-produced changes in physiological indexes was lower than in control animals. However, handling had no effect on body weight and weights of the heart and adrenal glands.

The OF behavior of handled rats differed from that of control animals (Table 1). LA of handled rats was lower than in the control. These differences primarily concerned LA in the peripheral region of OF and number of vertical rearing postures (specific form of rat behavior). LA of adult rats subjected to neonatal handling decreased most significantly over the 1st min of test I. These differences became less pronounced and statistically insignificant during repeated tests on days 2 and 3.

Handled rats displayed higher activity in the center of OF compared to control animals; vertical rearing postures in the center of OF were absent in control animals, but were sometimes displayed by handled rats.

Previous studies showed that LA of rats in the OF test has a bicomponent motivational structure [1]. On the one hand, LA is associated with the feeling of fear in a new environment and represents a locomotor active avoidance response (searching for exit). On the other hand, LA is related to the exploratory motivation. The motivation of fear prevails over the first minutes of test I. Then motivational structure changes and exploratory activity appear. High LA in the first minutes of test I and entire LA in test I reflect more pronounced fear reaction. These data suggest that handled rats demonstrate less fear in a new environment in the OF test. In test I, LA of handled rats was much lower compared to control animals. These differences were less pronounced and became significant in further tests. NISAG rats demonstrated vertical rearing postures in the center of OF, which reflects less fear and strong exploratory motivation. This form of behavior is a typical manifestation of exploratory activity observed only in handled rats. It should be emphasized that standing on hindlimbs with forelimbs leaning on the wall of the cage reflects the reaction of avoidance and is dictated by fear (especially in test I). As differentiated from handled rats, this form of behavior was typical of control animals.

Studies of the OF behavior indicate that neonatal handling decreases sensitivity of NISAG rats to emotional stress factors. These data are consistent with less pronounced changes in BP and plasma corticosterone concentration in handled NISAG rats in response to 30-min restriction. Therefore, handling in early postnatal ontogeny produces a positive effect on the development of stress-reactivity systems in rats with stress-induced arterial hypertension. Moreover, this procedure reduces animal reaction to acute emotional stress. Changes in basal plasma corticosterone level should not be considered as a result of "permanent stress". This state is difficult to explain from the tradi-

TABLE 1. Effect of Neonatal Handling on Physiological Characteristics of Hypertensive NISAG Rats ($M \pm m$)

Index		Handling (n)	Control (n)
BP, mm Hg	basal	177.1±4.0 (10)	167.3±7.0 (19)
	poststress	189.7±4.0 (10)**	215.4±5.0 (19)
Plasma corticosterone concentration, ng/μg			
	basal	18.3±3.0 (10)	11.5±2.0 (19)
	poststress	36.9±2.0 (10) ***	60.2±4.0 (19)
Body weight, g	239.7±5.0 (10)	229.5±9.0 (19)	
Weight of heart, g/100 g		0.36±0.01 (10)	0.36±0.01 (19)
Weight of adrenal glands, g/100 g		19.2±0.4 (10)	18.9±3.0 (19)
LA	1st minute of test I	40±4 (19)**	63±5 (10)
	2nd minute of test I	28±4 (19)*	44±6 (10)
	1st minute of test II	22±3 (19)**	42±5 (10)
	1st minute (total activity in 3 tests)	75±6 (19)***	124±10 (10)
	peripheral activity in test I	164±18 (19)*	230±22 (10)
	total peripheral activity in 3 tests	271±30 (19)*	394±47 (10)
Number of vertical rearing postures in test I			
	periphery	12±2 (19)*	20±3 (10)
	center	1.26±0.52 (19)*	0
Time of standing on hindlimbs in test I, sec			
	periphery	36±4 (19)*	70±12 (10)
	center	3.32±1.37* (19)	0
Total number of vertical rearing postures in 3 tests			
	periphery	18±3 (19)*	30±4 (10)
	center	1.32±0.51* (19)	0
Total time of standing on hindlimbs in the center (3 tests), sec		3.53±1.36* (19)	0

Note. *$p<0.05$, **$p<0.01$, and ***$p<0.001$ compared to the control.

tional notion about physiology of the stress reaction. It is more likely that that handling affects the system for regulation of hypophyseal-adrenal function. The resulting changes are manifested in the increased baseline level and reduced reaction to acute emotional stress.

REFERENCES

1. A. L. Markel, Yu. K. Galaktionov, and V. M. Efimov, *Zh. Vyssh. Nervn. Deyat.*, **38**, No. 5, 855-863 (1988).
2. A. L. Markel and R. A. Khusainov, *Ibid.*, **26**, No. 2, 1314-1318 (1976).
3. R. I. Salganik, I. M. Gryaznova, A. L. Markel, *et al.*, *Dokl. Akad. Nauk SSSR*, No. 245, 473-476 (1979).
4. A. L. Markel, L. N. Maslova, G. T. Shishkina, *et al.*, *Development of the Hypertensive Phenotype: Basic and Clinical Studies. Handbook of Hypertension*, Eds. R. McCarty *et al.*, Amsterdam (1999), pp. 493-526.
5. M. J. Meaney, J. B. Mitchell, D. H. Aitken, *et al.*, *Psychoneuroendocrinology*, **16**, No. 1-3, 85-103 (1991).
6. N. E. Ordyan, S. G. Pivina, V. V. Rakitskaya, and V. G. Shalyapina, *Steroids*, **66**, No. 12, 883-888 (2001).
7. A. Papaioannou, U. Dafni, F. Alikaridis, *et al.*, *Neuroscience*, **114**, No. 1, 195-206 (2002).
8. R. I. Salganik, I. M. Gryaznova, A. L. Markel, *et al.*, *Experientia*, **36**, 43-44 (1988).
9. J. W. Smythe, C. M. McCormick, and M. J. Meaney, *Brain Res. Bull.*, **40**, No. 3, 195-199 (1996).
10. M. Vallee, W. Mayo, F. Dellu, *et al.*, *J. Neurosci.*, **17**, No. 7, 2626-2636 (1997).