REPEATED MATERNAL DEPRIVATION ALTERS BEHAVIORAL PATTERN AND ATTENUATES PROLACTIN RESPONSE TO MILD STRESSOR IN ADULT MALE WISTAR RATS

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Objective. To evaluate the impact of repeated neonatal mother deprivation (RMD) of male rats on the behavioral parameters and response of prolactin to mild stress stimuli in the adulthood.

Methods. Afer birth, the pups of Wistar Porton Olac rats were crossfostered and their number was adjusted to 8 per litter (4 males and 4 females). They were removed from the dam for 6 hours daily on postnatal day 6, 7, 8 and for 12 hours daily on postnatal day 12, 13, 15, 16 and placed to another cage lined with cotton wool at controlled temperature 37 °C. Body weight was estimated repeatedly from postnatal day 9 to 97. At 14 weeks of life the behavioral activity was measured in an open field on 2 occasions, 2 days apart. One week later the rats were exposed to 15 min novelty stress or to 3 min handling and decapitated 15 min after the initiation of both. Trunk blood was collected and plasma prolactin (PRL) was measured by radioimmunoassay.

Results. On postnatal day 15 the eye opening was found in 75 % of control pups and 73 % of pups with RMD. In the rats after RMD the body weight gain was significantly decreased from day 21 until the day 97. Vertical behavioral activity (rearing) was enhanced in RMD rats when measured on the first occasion. Horizontal behavioral activity did not significantly differ from the control group. Stress of novel environment elicited the activation of PRL secretion in untreated animals (19.3 1±4.6 ng/ml vs. 7.17±1.03 ng/ml, $P<0.05$), while no change was found in the rats after RMD (8.15±2.0 ng/ml vs. 4.35±0.48 ng/ml).

Conclusions. In the rats exposed to neonatal mother deprivation the lower emotionality was found. Significantly decreased body weight gain in these animals was probably due to the nutritional deprivation during the postnatal separation from the mother. The nonresponsiveness of lactotrophs to mild stressor in adult rats after RMD may have a negative impact on defense mechanisms to immune challenges.

Key words: Rats – Neonatal mother deprivation – Open field – Stress – Prolactin

It was repeatedly observed that the infantile experience affects various physiological and behavioral functions in adulthood. The first differences found in adulthood between the rats which were neonatally stimulated and nonstimulated controls were at the emotional level. Thus, Levine et al. (1966) described that adult rats after neonatal stimulation were more active when tested in the open field which was ascribed to decreased level of emotionality of these animals.

Among various stressors the maternal deprivation showed a profound impact on the neuroendocrine functions in the adulthood. Thus, repeated 15 min maternal deprivation for the first 3 weeks of life has been shown to reduce hypothalamic-pituitary-adrenocortical (HPA) response to restraint stress in the adulthood which was manifested by smaller ACTH and corticosterone increase and faster return to basal levels comparing to the controls (Meaney et al. 1989). These differences presumably resulted from the decreased CRH and AVP synthesis in parvocellular hypothalamic neurons of neonatally stressed rats (Viau et al. 1993). Postnatal handling increased the density of glucocor-

ticoid receptor and its mRNA in the hippocampus (O'Donnell et al. 1994) which are known to mediate the negative feedback effect of glucocorticoids on HPA axis (Jacobson and Sapolsky 1991).

On the other hand, postnatal maternal deprivation for several hours elicited reduced HPA response to restraint stress only at 7 weeks of life, while no more differences between neonatally stressed and control animals were found at 20 weeks (Ogawa et al. 1993).

Plotsky and Meaney (1992) reported that pups exposed to 3 hour periodic maternal separation showed enhanced CRF concentration in median eminence and CRF mRNA in hypothalamus after the restraint stress.

Although the effect of periodic neonatal handling or maternal separation on the HPA in the adulthood is well documented, there is no pertinent study on the effect of neonatal stress on the regulation of prolactin (PRL) in the adulthood. PRL aside from its function in the regulation of reproduction plays an important role in immunomodulation. Therefore it was of interest to study the effect of repeated maternal deprivation stress (RMD) on the PRL response to mild stressors in the adulthood.

Materials and Methods

Animals: The pups of Wistar Porton Olac rats of specific pathogen free colony bred at the Institute of Experimental Endocrinology Slovak Academy of Sciences (Bratislava) were used. The animals were housed in a room with controlled temperature, humidity, 12 h light/dark cycle, having free access to pelleted diet and water. On the day of birth the litters were crossfostered, the pups being removed from their biological mothers and placed with another lactating female, each litter being adjusted to 4 males and 4 females. Five litters were submitted to repeated mother deprivation (RMD) and 4 litters served as controls. RMD consisted of transferring the pups to another cage lined with cotton wool at controlled temperature 37 °C on the postnatal day 6, 7 and 8 for 6 hours and on the postnatal day 12, 13, 15 and 16 for 12 hours. On day 21 the animals were weaned. From the day 47 the males and females were housed separately, seven animals per cage. Only male rats were used in this study. Body weight was estimated in both groups in 10 day intervals.

Open-field tests: At 14 weeks of life open-field test was performed in a square apparatus having the floor divided into 12 squares 10x10 cm. The tests were performed between 9:00 and 11:00 a.m. The animals were placed into a center of the field and the number of squares entered as well as the rearing were measured for 2 min on 2 occasions, 2 days apart.

Stress exposures: One week following the open-field tests the rats were exposed to novelty or handling stress. Novelty stress consisted of transferring the rats in their cages into an adjacent room with having the lids open. After 15 min the trunk blood was collected by decapitation. Handling stress consisted of 3 min touching the animals by hand in their home cages, then they were left closed and in minute 15 from starting the handling the rats were decapitated.

Plasma PRL assay: Plasma PRL was measured by radioimmunoassay using the double antibody technique. Specific rPRL materials was kindly provided by National Hormone and Pituitary Program Ogden BioServices Co. The incubation was performed at room temperature for 24 hours followed by overnight precipitation at +4 °C. The between assay variance was 5.6 %. The results were expressed in term of rPRL-RP-3 standards.

Statistical evaluation: The results were evaluated by one way ANOVA followed by Dunn's test for rPRL or Dunnett's test for body weigh. For behavioral parameters the nonparametric Mann-Whitney's test was used.

Results

The results presented are the means of 2 separate experiments. The suckling pups of both groups started opening their eyes on the 15th postnatal day (75 % of the controls and 73 % of rats with RMD).

Body weight was measured from the postnatal day 9 to 97 and showed a significant decrease in the growth rate of rats after RMD. This became significant on day 21 and persisted until to the end of the observation period (Fig. 1).

Fig. 2 shows open-field behavioral activity. In rats after RMD the vertical activity (rearing) was the same on both occasions. However, control rats showed significantly lower frequency of rearing at the first exposure ($P<0.01$), while the tendency to enhanced horizontal activity (ambulating) in the group after RMD did not significantly differ from controls.

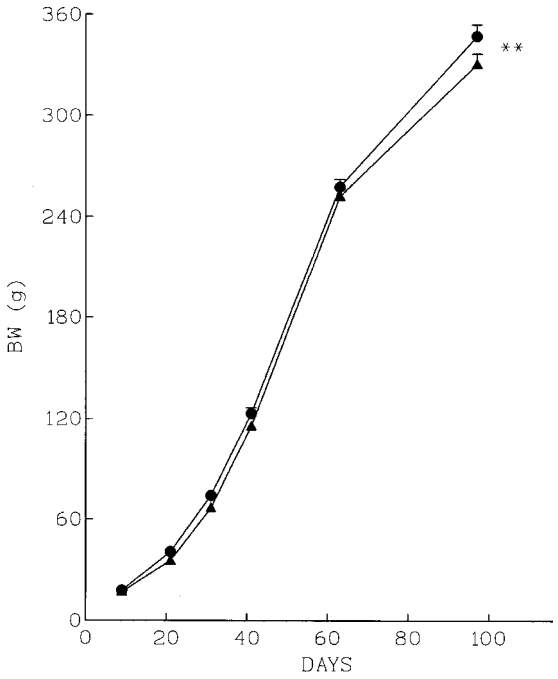

Fig. 1.
Body weight (mean±S.E.) of rats after RMD (full triangles) and control rats (full circles) measured on postnatal day 9, 21, 31, 41, 63 and 97.
**** - P<0.01 between the control and RMD group**

As expected, the exposure to novel environment elicited a mild but clear activation of PRL in untreated group (Fig. 3, left panel). The animals after RMD did not show any activation of PRL to this stressor (Fig. 3, right panel). Handling for 3 min did not stimulate PRL release under these experimental conditions in either group.

Discussion

Our results showed that RMD of male rats in the preweaning period was associated with the decrease of body weight gain which became significant in postnatal day 21 and persisted until the adulthood. This finding is not in line with the results by VALEE et al. (1993) who found an increase in body weight in the animals after exposure to daily 15 min handling for the first 21 postnatal days. However, in our experimental set up, the pups were separated from the dam for 6 and 12 hours; this manipulation represents a stronger intervention than 15 min handling and is most probably associated also with severe nutritional deprivation. Similarly, in neonatally undernourished rats (14 pups per litter) FICKOVA (1978) described reduced body weight and lower mass of epididymal fat tissue in the adulthood. It is reasonable to assume that permanent neonatal food restriction and repeated neonatal mother deprivation for several hours may have similar metabolic effects.

In the open field test, the higher rearing activity of animals after RMD measured on the first day of exposure shows a lessened emotionality of these animals. This may reflect the fact that these animals are less anxious and can better cope with novel situations than control rats. However, this interpretation is not supported by the ambulatory activity, since the frequency of square crossing was not significantly higher in the animals after RMD. In contrast, however, in the experiments by VAN HORSTEN et al. (1993) male rats after neonatal maternal deprivation for 2 hours showed ambulatory hyperactivity. The discrepancy between theirs and ours results may be due to the fact that our control animals were not completely undisturbed, since they were periodically handled when measuring the body weight and thus their threshold of sensitivity might have been higher due to certain degree of continuous adaptation.

Neonatal maternal separation for more than one hour appears to be a qualitatively different intervention from brief neonatal handling when tested by several endocrine responses in the adulthood. Thus, prolonged maternal separation was shown to suppress growth hormone secretion, while brief handling resulted in an increase of that (KUHN et al., 1990). The rats after a single 24 hour neonatal maternal separation exhibited as adults enhanced basal hypothalamic-pituitary-adrenocortical activity, enhanced dopamine responsiveness as well as higher expression of tyrosine hydroxylase in substantia nigra and normal basal levels of PRL (ROTS et al., 1996). In our experiments basal PRL remained unaffected by RMD as well. Stress of handling did not elicit any activation of PRL in either group, most probably because the animals were better adapted to handling in the course of the whole experiment. In contrast, the stress of novel environment activated PRL release in control rats and RMD prevented this effect. However, the mechanism underlying the PRL nonresponsiveness after RMD remains to be elucidates. One possible explanation

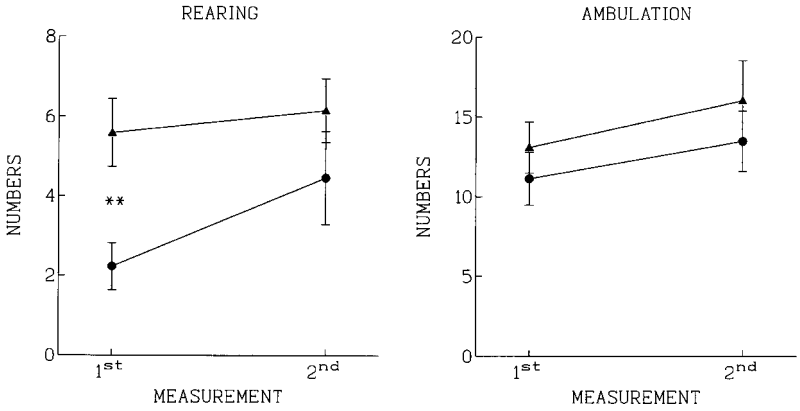

Fig. 2.
Open-field activity of rats (mean±S.E.) after RMD (full triangles) and control rats (full circles) measured on 2 occasions 48 hours apart.
**** - P<0.01 between the control and RMD group**

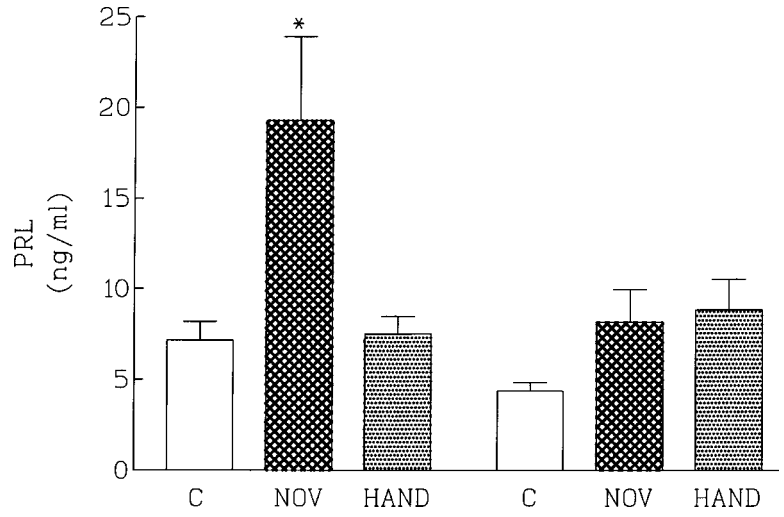

Fig. 3.
Left panel: plasma PRL levels (mean±S.E. of 8-10 animals) in nonstressed rats (C), after novelty stress (NOV) and after 3 minute handling (HAND) in control, untreated group.
Right panel: PRL levels after the same interventions in animals after PMD.
*** - P<0.05 .**

can be the enhanced dopaminergic inhibition of PRL secretion which is the major pathway for the regulation of PRL secretion. Perinatal stress has been shown to increase dopamine content in the mediobasal hypothalamus (Reznikov and Nosenko 1996). Such enhanced dopaminergic tone along with higher susceptibility of lactotrophs to dopamine may result in PRL nonresponsiveness to mild stimuli in rats after RMD. Since PRL plays an important role in immunomodulation (Berczi 1992), its deficit may negatively affect the immune functions of the organism. This hypothesis correlates with the fact that rats after prolonged neonatal maternal deprivation showed suppressed immune responses as measured by plaque forming cell response (Von Hoersten et al. 1993).

In conclusion, our results confirmed the lessened emotionality of rats after RMD. We found suppressed PRL response to mild stressor in these adult rats which may, in part, account for the worsening of the immune responses in rats after neonatal stress.

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BOOK REVIEW

HORMONES AND GROWTH FACTORS IN DEVELOPMENT AND NEOPLASIA

Editors: **Robert B. Dickson** (Washington, DC) and **David S. Salomon** (Bethesda), 461 pages, , Lst 80.95
John Wiley & Sons, Chichester 1998

This book brings a comprehensive and valuable broad discussion of several hot topics concerning the role of hormones and growth factors in tumorigenesis and malignant progression in general as well as in the etiology of particular tumors of male and female reproductive tract and mammary gland. Each of twenty three chapters has been written by the experts well known in the field. The topics discussed cover the role of growth factors and hormones in the development of invertebrates and amphibians with special aspects to Drosophila and estrogen control of Xenopus laevis egg yolk mRNA synthesis and degradation. Next section deals with the role of growth factors and hormones in mammalian development including such topics as cellular interactions mediated by tyrosine kinase receptors during development and the role of Erb B, estrogen and progesterone receptors and their ligands. The third part brings the aspects on postnatal development of reproductive tracts and mammary gland in the adults with the description of the role of prolactin, insulin-like growth factors and their binding proteins and finaly on the role of signal networks in the mammary gland. The last section deals with the growth factor-hormonal interactions in tumorigenesis and malignant progression. This includes the role of sex steroids in carcinogenesis, IGF-I receptors in normal and abnormal growth, mutations of tyrosine kinases receptors and special role of estrogens and growth factors in the carcinogenesis of reproductive tract. Special attention is paid to the hormonal and growth factor regulation of prostate cancer and, finaly, to some novel genes participating in the development of neoplasia in human reproductive tissues neoplasia such as WNT and Int-3 genes.

The references bring extensive list of papers up to 1996 and the instructive level of all chapters is being kept high by sophisticated schemes and figures. This book will atract the attention not only of biologists and research workers, but also of several clinicians who like to know more about the etiology and pathophysiology of malignant tumors.

Pavel Langer