

SYMPATHO-ADRENOMEDULLARY SYSTEM RESPONSES TO VARIOUS CHRONIC STRESS SITUATIONS

Ljubica Gavrilović, Slađana Dronjak

*Institute of Nuclear Sciences »Vinča«, Laboratory of Molecular Biology and Endocrinology,
Belgrade, Serbia and Montenegro*

Summary: We studied the effects of four different chronic stressors: isolation, crowding, forced swimming and isolation followed by forced swimming, on the level of plasma noradrenaline (NA) and adrenaline (A), both under basal conditions and in response to acute immobilization and cold as additional stressors. None of these chronic stressors changed basal plasma NA and A concentrations. When chronically isolated rats are exposed to immobilization they show significant elevation of plasma NA and A, but cold stress significantly increases only NA level and not the concentration of A. When animals exposed to chronic crowding, forced swimming and isolation plus forced swimming are exposed to immobilization plasma catecholamine also increases, but less in comparison to the chronically isolated rats. Based on these results, it may be concluded that chronic isolation seems to be a stronger stressor for animals than other chronic stressors. Chronic forced swimming stress and crowding seem to be the weakest stressors, if measured by the activity of sympatho-adrenomedullary system. However, daily short-term swimming stress seems to attenuate the effect of chronic isolation on the activity of the sympatho-adrenomedullary system.

Key words: plasma catecholamines, chronic psychosocial stress, chronic physical stress, additional stress

Introduction

Catecholamines are important mediators of stress response. Activation of the sympatho-adrenomedullary system is one of early responses to stress. Studies, using a wide variety of stressors, have clearly indicated that the pattern of neuroendocrine response is dependent upon the stress applied (1–3). Immobilization of experimental animals was shown to produce a rapid increase in secretion of noradrenaline (NA) and adrenaline (A), while cold stress induced somewhat lower elevation of NA level, comparing to immobilization, but did not affect A secretion (4). Prolonged or repeated stress is associated with the development of cardiovascular, mental, gastrointestinal disorders and cancer. However, in recent years, a

large body of evidence was accumulated suggesting that physical exercise expressed positive effects on cardiovascular and immune systems, as well as on the brain. It has been found that voluntary exercise increased neurogenesis in the dentate gyrus of the rat and mice hippocampus (5). On the other hand, Van Praag et al. (6) recorded a decrease in neurogenesis in rats or mice exposed to psychosocial stress, since social interactions are an important source of stress. Individual housing of rats, frequently termed »isolation stress«, represents very strong psychosocial stress. However, restriction of the environmental space itself acts as a kind of stress (7). Studies on the effect of population density demonstrated body weight gain inhibition, reduction of food intake, atrophy of thymus in experimental animals exposed to either isolated or overpopulated environments (8). Physical stress involves both physical and emotional components, while psychosocial stress does not involve a physical component.

Taking into account the above studies, the aim of the present work was to investigate changes in the activity of sympatho-adrenomedullary system in adult rat males exposed to chronic psychosocial (21 days

Address for correspondence:

Slađana Dronjak, Ph.D.
Lab. Mol. Biol. Endocrinol.
Institute of Nuclear Sciences »Vinča«
P.O.B. 522
11000 Belgrade
Serbia & Montenegro
Tel./fax: 00381112455561
e-mail: sladj@vin.bg.ac.yu

isolation and 21 days crowding) and chronic physical (21 days forced swimming) stress, as well as to a chronic combination of isolation and forced swimming, both under basal conditions and in response to additional stressors – immobilization or cold. The activity of the sympatho-adrenomedullary system was judged *via* changes in plasma NA and A level.

Materials and Methods

Rat males of Wistar strain weighing 300–360 g were used. They were offered water and food *ad libitum*. The light schedule in the room was reversed, with lights on between 7.00h and 19.00h. The rats were divided into five groups. The first, naive control group, consisted of four animals per cage. The second group involved rats individually housed for 21 days. In the third group, 12 animals were housed per cage, i.e. these were conditions of social crowding. The fourth group was exposed to long-term forced swimming stress. The animals were housed four per cage and submitted to forced swimming every day for 15 min in water heated to 32 °C, during the 21-day-period. The rats of the fifth group were individually housed and exposed to forced swimming under the same conditions as those of the fourth group. On the day before blood sampling, a cannula was inserted into the tail artery under pentobarbital (40 mg/kg i.p) anaesthesia. This allowed plasma NA and A level estimation without additional stressing of the animals during manipulations. After the baseline blood collection, the rats were immobilized or exposed to cold stress. Blood was collected 15, 30, 60 and 120 min after the onset of immobilization. The animals exposed to cold for 2 h were initially kept at room temperature and after the baseline blood collection carefully transferred within their home cages into the cold chamber (4 °C), and the blood samples were collected 30, 60 and 120 min later. Plasma catecholamines were assayed by a modification of the radioenzymatic method described previously (9). Catecholamines present in plasma aliquots were converted into their labeled O-methylated derivatives by S-[3H]adenosylmethionine and lypophilized catechol-O-methyltransferase isolated from rat liver. The O-methylated derivatives of the amines were then extracted along with unlabeled carrier compounds, separated by thin-layer chromatography, eluted and reacted with periodate.

Statistical significance of the differences between treated groups and the control one was evaluated by one-way ANOVA test.

Results

As shown in *Figure 1* and *2*, 21 days exposure of adult rat males to four different stressors did not influence the basal plasma NA and A. However, exposure to short-term immobilization (2h) produced an increase in plasma NA and A level in all investigated groups. In the

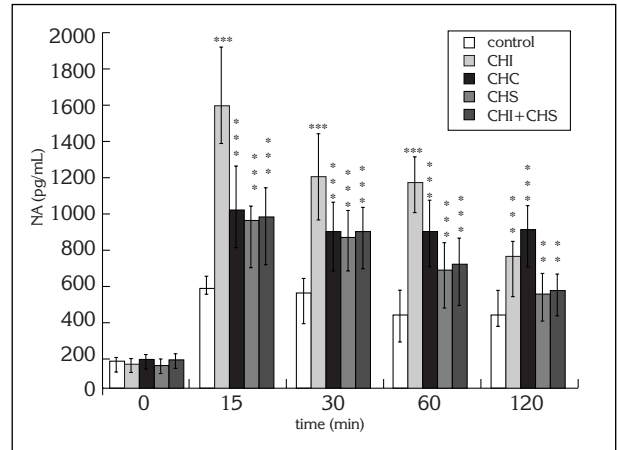


Figure 1 The effects of acute immobilization on plasma level of noradrenaline (NA) (pg/mL) in the controls, rats exposed to chronic isolation (CHI), chronic crowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS).

The values are means \pm SEM of 6–8 animals.

Statistical significance * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to the control group.

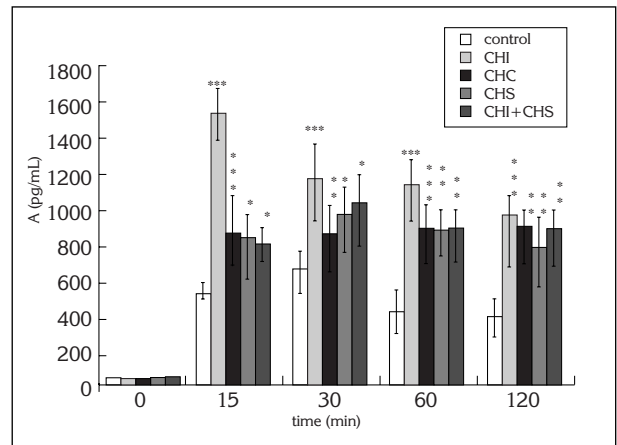


Figure 2 The effects of acute immobilization on plasma level of adrenaline (A) (pg/mL) in the controls, rats exposed to chronic isolation (CHI), chronic crowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS).

The values are means \pm SEM of 6–8 animals.

Statistical significance * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to the control group.

chronic isolation group, plasma NA and A levels were elevated at all experimental time points during immobilization. In chronic crowding, forced swimming and isolation followed by swimming groups, immobilization also resulted in increased plasma levels of NA and A. This increase was higher than that found in the controls exposed to immobilization only, but lower than in the chronically isolated group additionally subjected to immobilization.

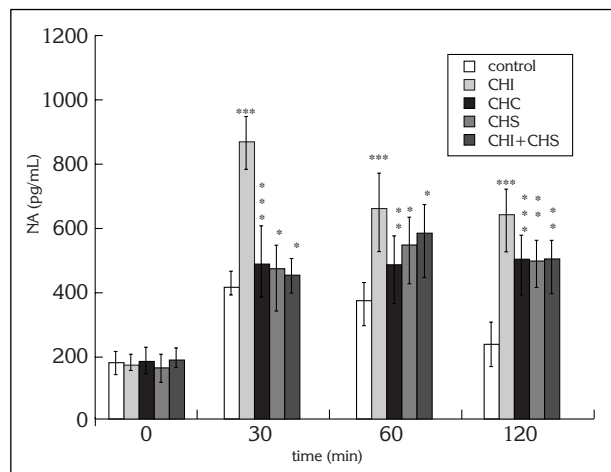


Figure 3 The effects of acute cold stress on plasma level of noradrenaline (NA) (pg/mL) in the controls, rats exposed to chronic isolation (CHI), chronic crowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means \pm SEM of 6–8 animals. Statistical significance * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to the control group.

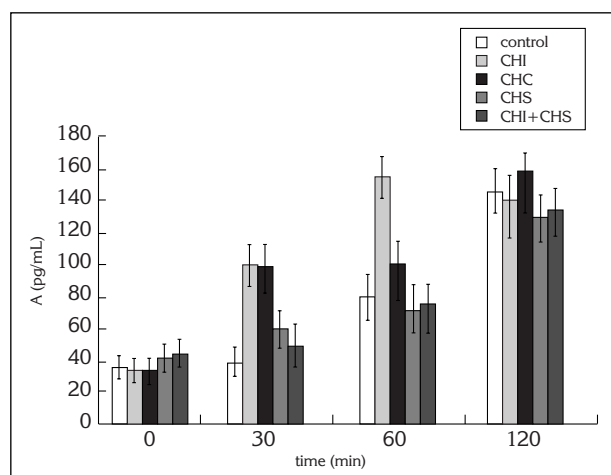


Figure 4 The effects of acute cold stress on plasma level of adrenaline (A) (pg/mL) in the controls, rats exposed to chronic isolation (CHI), chronic crowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means \pm SEM of 6–8 animals. Statistical significance * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ as compared to the control group.

From Figure 3 and 4 it can be seen that acute exposure to cold stress (2h) resulted in an increase of NA levels in all experimental groups, the most pronounced effect being recorded in the chronic isolation group. Concentration of A in all groups of animals after exposure to cold stress was increased, but this increase was not statistically significant. This slight elevation could be the result of transferring the animals into a cold chamber and other manipulations.

Discussion

In the present study, we showed that four 21-day-long stressors with different characteristics applied in the present work expressed different effects on the level of plasma NA and A. During chronic physical and psychosocial and combined stress, the basal plasma catecholamine levels remained unchanged. It seems that only acute stress markedly activated the sympatho-adrenomedullary system and recovery after long-term stress. This is in agreement with the findings of Pol et al. (10) who reported that chronic exposure to immobilization stress did not alter NA in frontal cortex, hippocampus or hypothalamus, as judged by its content measured approximately 20 h after the last exposure to immobilization, but 2-h-immobilization resulted in a significant decrease of NA level in these brain structures.

Exposure of rats to long-term psychosocial, physical and combined stress and then to immobilization for 2 h led to the activation of sympatho-adrenomedullary systems. The highest elevation of plasma NA and A was observed in chronic isolation rats, somewhat lower increase was found in chronic crowding, chronic forced swimming and combined group rats, and the least one in control rats. Sgofio et al. (11) suggested that social defeat induced a much greater elevation in NA and A content, indicating a higher involvement of the sympatho-adrenomedullary system. We found that an additional stressor, such as immobilization, led to the most conspicuous activation of sympatho-adrenomedullary system in the group exposed to chronic isolation. Interestingly, chronic forced swimming and chronic isolation plus forced swimming rats responded to immobilization as a heterotypic additional stressor by less significant activation of the sympatho-adrenomedullary system, comparing to chronic isolation group. It seems that forced swimming (15 min daily for 21 days) combined with long-term isolation attenuated the enhanced activity of sympatho-adrenomedullary system elicited by the action of novel stress. The data of Haller and Halasz (12) showed that when isolated rats were daily exposed to short-term defeats, the anxiogenic effect of isolation was completely abolished. They wondered whether a mild daily stressor would abolish the neurochemical effects of isolation. Our results suggested that 15-min-swimming for 21 days activated the sympatho-adrenomedullary system in chronic isolation rats to a lesser extent when exposed to novel stressors than in rats exposed to chronic isolation only.

Short-term exposure to cold of the rats that suffered chronic isolation, crowding, forced swimming or the combination of the two kinds of stress, led to a pronounced activation of the sympathoneural, but not the adrenomedullary system. These findings could be related to the data of Vollmer et al. (13) who found that the concentration of NA was significantly

increased during 24 h of cold stress (4 °C), while the level of A remained unchanged.

The present study demonstrates that, compared to cold stress, immobilization produces greater increase in plasma NA and A levels in chronically stressed rats. Therefore, it has been concluded that the nature of the new acute stressor has a role in the development of enhanced activity of the sympatho-adrenomedullary system.

Based on the results obtained throughout the present study, it seems that repeated short-term forced swimming attenuates the effect of long-term isolation on the activity of sympatho-adrenomedullary system. Radak et al. (14) investigated effects, of immobilization, a single bout of exercise, and immobiliza-

tion followed by exercise, on oxidative damage of macromolecules in the hippocampus of rat brain. They found that the oxidative damages of lipids, proteins and nuclear DNA were significantly increased in immobilization group, while no increase was observed after a single bout of exercise and immobilization followed by exercise. It appears that short-term daily swimming expressed a protective effect against the effects of long-term isolation. Furthermore, it can be concluded that individual housing of rats seems to act as a stronger psychosocial stressor than crowding conditions.

Acknowledgement – This work was supported by the Ministry of Science, Technology and Development of Serbia, grant No. 1953.

ODGOVOR SIMPATO-ADRENOMEDULARNOG SISTEMA U RAZLIČITIM HRONIČNIM STRESNIM SITUACIJAMA

Ljubica Gavrilović, Slađana Dronjak

Institut za nuklearne nauke Vinča,
Laboratorija za molekularnu biologiju i endokrinologiju, Beograd

Kratak sadržaj: Ispitivano je dejstvo četiri različita hronična stresora: izolacija, agregacija, plivanje i kombinovana izolacija sa plivanjem, na nivo noradrenalina (NA) i adrenalina (A) u plazmi, u bazalnim uslovima i posle akutne imobilizacije i stresa hladnoće kao novih stresora. Nijedan od četiri hronična stresora ne menja bazalnu koncentraciju NA i A u plazmi. Kada se hronično izolovani pacovi izlože imobilizaciji oni pokazuju značajno povećanje NA i A u plazmi, dok stres hladnoće značajno povećava samo nivo NA ali ne i A. Kada se životinje hronično izlagane agregaciji, plivanju i kombinovanoj izolaciji i plivanju izlože imobilizaciji, takođe se značajno povećava nivo kateholamina u plazmi ali manje u poređenju sa hronično izolovanim pacovima. Na osnovu ovih rezultata može se zaključiti da hronična izolacija predstavlja jači stresor za životinje u odnosu na druge ispitivane hronične stresore. Hronična agregacija i plivanje su slabiji stresori sudeći po aktivnosti simpato-adrenomedularnog sistema. Međutim, izgleda da svakodnevno kratkotrajno plivanje umanjuje dejstvo hronične izolacije na aktivnost simpato-adrenomedularnog sistema.

Ključne reči: kateholamini u plazmi, hronični psihosocijalni stres, hronični fizički stres, dodatni stres

References

1. Pacak K, Palkovits M, Kopin IJ, Goldstein DS. Stress induced release in the hypothalamic paraventricular nucleus and pituitary-adrenocortical and sympatho-adrenal activity: *In vivo* microdialysis studies. *Front in Neuroendoc* 1995; 16: 89–150.
2. Pacak K, Palkovits M. Stressor specificity of central neuroendocrine responses: implications for stress-related disorders. *Endocrine Reviews* 2001; 22: 502–48.
3. Jezova D, Skultetyova I. Neuroendocrine response in stress-specific activation by individual stressors. *Arch Physiol Biochem* 1997; 105: 233–5.
4. Dronjak S, Kvetnansky R, Jezova D, Varagić V. Effect of immobilization and cold stress on sympatho-adrenal system in rats exposed to long-term cold. *Jugoslov Med Biochem* 2001; 20: 217–22.
5. Kramer JM, Plowey ED, Beatty A, Little HR, Waldrop TG. Hypothalamus, hypertension and exercise. *Brain Res Bull* 2000; 53: 77–85.
6. Van Praag H, Kempermann G, Gage FH. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nat Neurosci* 1999; 2: 266–270.
7. Mizumo Y, Kimura F. Attenuated stress response of hippocampal acetylcholine release and adrenocortical secretion in aged rats. *Neurosci Lett* 1997; 222: 49–52.
8. Kaminaga A, Shinorniya T. *Japan Journal Dermatology* 1997; 107: 615–22.
9. Peuler JD, Johnson GA. Simultaneous single isotope radioenzymatic assay of plasma norepinephrine, epinephrine and dopamine. *Life Sci* 1977; 21: 625–36.

10. Pol O, Campmany L, Gil M, Armario A. Behavioral and neurochemical changes in response to acute stressors: influence of previous chronic exposure to immobilization. *Pharmacol Biochem Behav* 1992; 42: 407–12.
11. Sgoifo A, De Boer S, Haller J, Koolhaas J. Individual differences in plasma catecholamine and corticosterone stress responses of wild-type rats: relationship with aggression. *Physiology&Behavior* 1996; 60: 1403–7.
12. Haller J, Halasz J. Mild social stress abolishes the effects of isolation on anxiety and chlordiazepoxide reactivity. *Psychopharmacology* 1999; 144: 311–5.
13. Volmerr RR, Baruchin A, Kolibal SS, Corey SP, Stricker EM, Kaplan BB. Selective activation of norepinephrine and epinephrine secreting chromaffin cells in rat adrenal medulla. *Am J Physiol* 1992; 263: 716–21.
14. Radak Z, Sasvari M, Nykas C, Kaneko T, Tahara S, Ohno H, et al. Single bout of exercise eliminates the immobilization-induced oxidative stress in rat brain. *Neurochem Int* 2001; 39: 33–8.

Accepted: November 18, 2005

Received: December 28, 2005