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ACTIVITY OF PITUITARY-ADRENAL AXIS IN RATS CHRONICALLY EXPOSED TO DIFFERENT STRESSORS

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Changes in plasma levels of adrenocorticotropic hormone (ACTH) and corticosterone (COTR) of adult male rats exposed to two chronic types of psychosocial stress: isolation and crowding, as well as physical stress i.e. forced swimming and combined psychosocial and physical stress i.e. isolation accompanied by forced swimming, both under basal conditions and in response to immobilization and cold as short-term stressors, were studied. Chronic isolation produced a significant elevation of basal plasma ACTH and CORT, but chronic isolation accompanied by forced swimming produced a significant elevation only in basal plasma ACTH, but not CORT, while chronic crowding and forced swimming did not elevate the basal plasma levels of these hormones. Chronically stressed animals exposed to immobilization or cold show a significant elevation of plasma CORT. No significant differences in plasma CORT between the four chronically stressed groups of rats were recorded. Novel acute immobilization and cold stress produce a markedly increased plasma ACTH in chronic crowding, forced swimming and isolation accompanied by forced swimming rats. These data suggest that chronic isolation seems to be a stronger stressor for rats compared to other chronic stressors.

Key words: ACTH, CORT, chronic physical stress, chronic psychosocial stress

INTRODUCTION

Although the pituitary-adrenal axis response to acute, short-lasting stimuli has been well documented (Djordjević *et al*., 2003) it is not quite clear how the overall function of this system is altered by exposure to a sustained stimulus. Several authors have clearly indicated that the pattern of neuroendocrine response is dependent on the applied stress stimulus (Pacak and Palkovits 2001; Goldstein *et al*., 1996; Kvetnansky *et al*., 2002). Plasma adrenocorticotropic hormone (ACTH) concentration elevates under the influence of various types of acute stressors applied. The most intense ACTH increase is provoked by 20 and 60 min heat exposure, as well as by overcrowding stress, being 15, 9 and 4 times as much as that of the respective controls (Djordjević *et al*., 2003). On the other hand, fasting and cold stress are weaker stressors, as compared to heat and

overcrowding, as they produce a 2,4 times increment of ACTH concentration. The pituitary response to acute stress is rapid and so is the return to the prestress level, except for corticosterone (CORT) (Kant *et al*., 1989). The greatest increase in CORT synthesis appears under the influence of environmental stressors, such as heat and cold. Chronic stress is considered to be one of the most important precipitating factors in cardiovascular and mental disorders. However, in recent years a large body of evidence has accumulated suggesting that physical exercise expressed positive effects on the cardiovascular and immune system, 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 (Kramer *et al*., 2000). On the other hand, van Praag *et al*. (1999) recorded a decrease in neurogenesis in rats or mice exposed to psychosocial stress, since social interactions are an important source of stress.

The objective of this study is to investigate changes in the activity of pituitary-adrenal axis in adult male rats exposed to a chronical psychosocial (21 days isolation; 21 days crowding) and chronical physical stress (21 days forced swimming) as well as to a chronical combination of these two stresses (isolation accompanied by forced swimming), both under basal conditions and in response to acute novel stressors, such as immobilization and cold. The activity of the pituitary-adrenal axis was judged based on the changes in plasma ACTH and CORT level.

MATERIALS AND METHODS

Male rats 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 7.00 h and 19.00 h. The rats were divided into five groups. The first (control group), consisted of four animals per cage. The second group involved the rats individually housed for 21 days. In the third group, 12 animals were housed per cage, these were the conditions of social overcrowding. 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 ^{o}C, throughout 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 the plasma ACTH and CORT 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 blood collection, carefully transferred to their cages into the cold chamber (4 ^{o}C) and the blood samples were collected 30, 60 and 120 min later. Plasma ACTH concentration was determined by chemiluminescent method using an IMMULITE automatic analyzer (DPC, Los Angeles, CA, U.S.A.) and the concentration of ACTH is expressed in pg/ml plasma. Plasma CORT content was measured

directly upon prior extraction using RIA commercial kits (ICN, Biochemicals, Costa Mesa, CA, U.S.A.) and the concentration of CORT is expressed in ng/ml plasma.

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

RESULTS

The effects of acute immobilization (2h) on plasma ACTH and CORT levels in the control and in rats previously exposed to four different chronic stressors are depicted in Fig. 1 and Fig. 2. Basal plasma ACTH levels were significantly elevated in the chronic isolation group and in the exposed to combined stress, while basal plasma CORT levels were significantly elevated in chronic isolation group and to a lesser extent in chronic overcrowding, forced swimming and combined stress groups. Immobilization elicited a conspicuous increase of plasma ACTH in the controls, chronical overcrowding, forced swimming and isolation accompanied by swimming groups, but somewhat lower increase was recorded in the chronic isolated group. The highest levels of ACTH were found 15 and 30 min after the onset of immobilization and reached about a 30-fold increase in the control and chronical overcrowding, forced swimming and combined stress groups, while ACTH was increased about 12-fold in the chronic isolation group. Immobilization

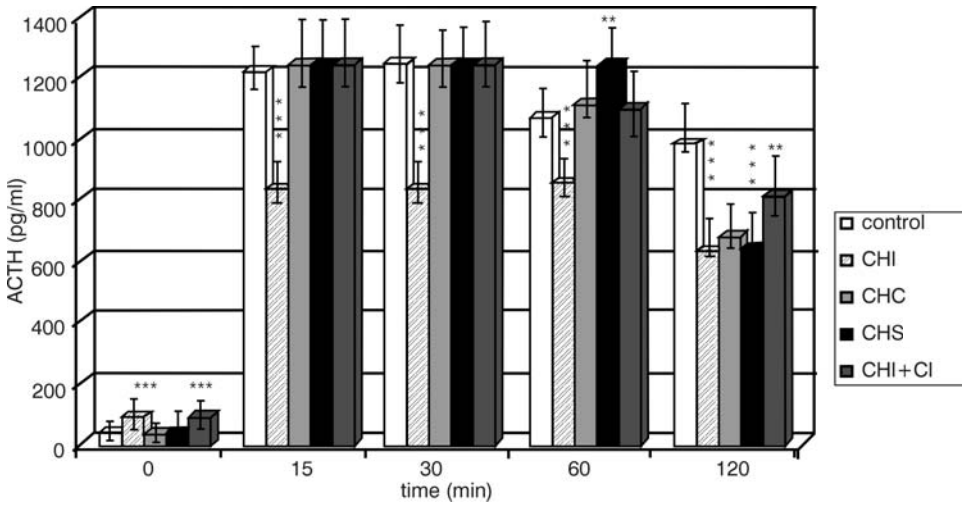

Figure 1. The effects of acute immobilization on plasma level of adrenocorticotropic hormone (ACTH) (pg/ml) in the controls, rats exposed to chronic isolation (CHI), chronic overcrowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

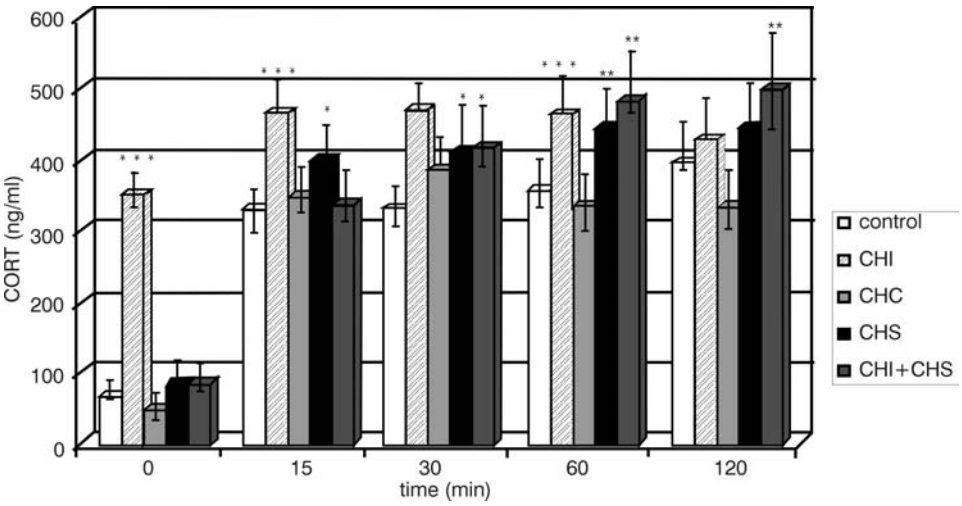

Figure 2. The effects of acute immobilization on plasma level of corticosterone (CORT) (ng/ml) in the controls, rats exposed to chronic isolation (CHI), chronic overcrowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

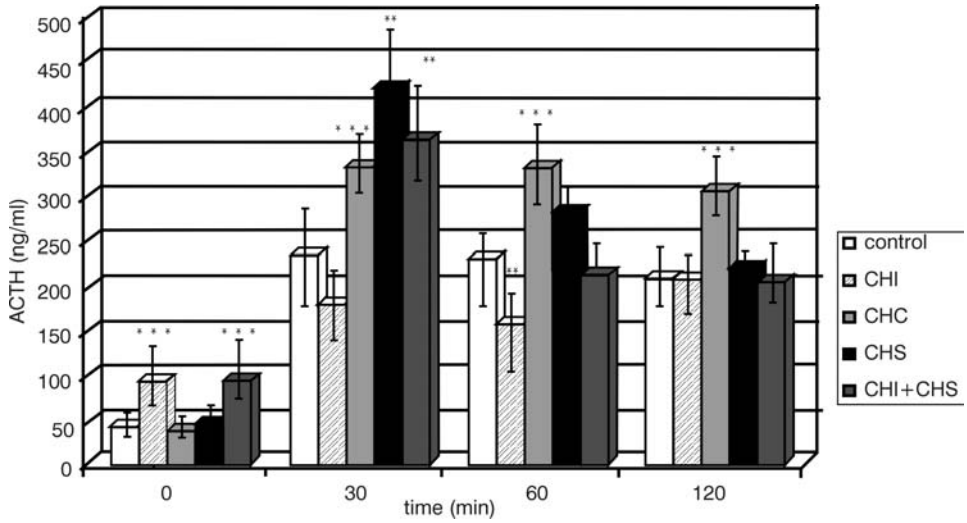

Figure 3. The effects of acute cold stress on plasma level of adrenocorticotropic hormone (ACTH) (pg/ml) in the controls, rats exposed to chronic isolation (CHI), chronic overcrowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

expressed a similar effect on CORT level in all investigated groups and its concentration was significantly elevated throughout the entire period of immobilization.

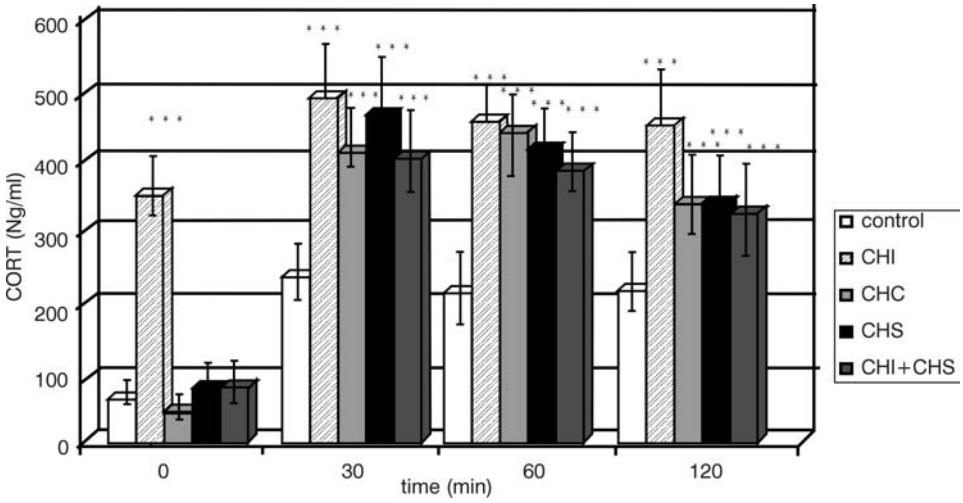

Figure 4. The effects of acute cold stress on plasma level of corticosterone (CORT) (ng/ml) in the controls, rats exposed to chronic isolation (CHI), chronic overcrowding (CHC), chronic forced swimming (CHS) and chronic isolation accompanied by forced swimming (CHI+CHS). The values are means ±SEM of 6-8 animals. Statistical significance *p<0.05, **p<0.01 and ***p<0.001 as compared to the control

Plasma levels of ACTH and CORT in the control, and four different chronically stressed rats after acute cold stress (4°C for 2h) are represented in Fig. 3 and Fig. 4. Exposure to cold produced a significant increase in ACTH level in the chronic overcrowding group, somewhat less pronounced increase in the control and chronic forced swimming and isolation accompanied by forced swimming groups, but surprisingly low elevation in the chronically isolated group. The cold produced a remarkable increase in plasma CORT concentration of all chronically stressed animals as compared to the control.

DISCUSSION

Observations of chronically stressed rats have indicated that the psychological aspects of stressful situations are important in determining the magnitude of the physiological response. Recently it has been reported that physical and emotional stress differed with regard to long-term effects on behaviour because the former resulted in the inactivity in a small open field and the latter led to hyperactivity of experimental animals (Pijlman *et al*., 2002). Somewhat later, the same authors found that physical stress induced a long-term

decrease of both preference for saccharin and open field activity but emotional stress showed an increase in open field activity and a slight increase in saccharine preference. It has been concluded that response to a novel stress depends on stress modality (Pijlman *et al*., 2003). Based on these data, the aim of the present study was to investigate the effects of chronic psychosocial, physical and combined psychosocial and physical stress followed by the action of novel stressors on the activity of the pituitary-adrenal axis. For this purpose, we measured plasma ACTH and CORT levels in adult male rats exposed to chronic overcrowding, isolation, forced swimming and the combination of both isolation and forced swimming. The rats were also exposed to short-term action of two novel heterotypic stressors; immobilization and cold. Because of combining physical and emotional stress, immobilization is considered to represent the strongest stress, while cold stress is assumed to be a mild stress. The data show that the four chronic stressors applied in the present work expressed different effects on the level of plasma ACTH and CORT in rats. In rats exposed to chronic isolation, as well as in those exposed to chronic combined stress, the basal plasma ACTH level was increased approximately two times, as compared to other groups. At the same time, basal plasma CORT content was enhanced several fold in the chronic isolation group, while only a slight increase was observed in the group of rats exposed to chronic overcrowding and forced swimming, as well as in the group exposed to the chronic isolation plus forced swimming. These results could be to some degree connected to the data published by Miachon *et al*. (1993) who showed that the 13-week-isolation resulted in an increase in ACTH and a decrease in CORT levels. The explanation for the differences between our results and the data of Miachon *et al*. (1993) could be related to the differences in the duration of the isolation. We decided to apply the isolation stress for 21 days, because several authors suggested that this period of stress was sufficiently long to induce long-lasting behavioural sequel associated with reproducible neurochemical and immunological modifications (Mangiavacchi *et al*., 2001; Popovic *et al*., 2000; Wood *et al*., 2003).

Immobilization produced a significant increase of plasma ACTH content in chronical overcrowding, forced swimming and exposition to combined stress. A somewhat lower increase of plasma ACTH in content chronically isolated animals was recorded. Plasma CORT level in chronic isolation, chronic overcrowding, chronic forced swimming and during the combination of these stress factors was also increased and no significant differences were found between these four groups. This is in accordance with the data of Moura and Moares (1994) and Rittenhouse *et al*. (2002) who reported that forced swimming resulted in an increased plasma ACTH and CORT levels. Our results clearly indicated immobilization-related activation of pituitary-adrenal axis. Short-term exposure to cold of the rats that suffered chronic overcrowding, isolation, forced swimming and isolation accompanied by forced swimming, led to a pronounced activation of the pituitary-adrenocortical axis. The exposure of all four groups of rats in our experiments to acute cold was followed by significantly elevated levels of CORT. Cold produced an increase in plasma CORT content in unstressed controls, as well, but this increase was lower compared to that observed in chronically

stressed rats. This is in agreement with the findings of Hashiguchi *et al*. (1997) who suggested that CORT is especially sensitive to the action of a new stress. The novel cold stress provoked the highest elevation of plasma ACTH level in chronic forced swimming (about 11-fold) and chronic crowding (about 10-fold), lower increase in chronic combined stress (about 5-fold) and the lowest in chronic isolation (about 2-fold) group. These results show that although chronic isolation resulted in a significant elevation of the basal plasma ACTH level, exposure to novel stressor produced the lowest increase of this hormone content in comparison with values recorded in chronic crowding, chronic forced swimming and chronic isolation plus forced swimming rats. The present study showed that immobilization produced a higher increase in plasma ACTH and CORT levels in chronically stressed rats comparing to cold stress. Therefore, it is obvious that the response of the animals additionally exposed to short-term action of additional stressors depended on the type of stressor.

The obtained results show that although chronic isolation significantly elevated the basal plasma ACTH level, exposure to novel stressors (immobilization or cold) produced the lowest increase in plasma ACTH in comparison with that observed in the other examined chronically stressed groups. Based on these results, it may be concluded that chronic isolation as a psychosocial stress acts as a stronger stressor than chronic forced swimming stress. Our results are consistent with the data of Endo *et al*. (2001; 2002) who found that long-term exposure to psychological, but not physical stress, caused a significant elevation of body temperature, probably due to an increase of sympathetic tone. Furthermore, it can be concluded that individual housing of rats acts as a stronger psychosocial stressor than crowding conditions.

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AKTIVNOST HIPOFIZNO-ADRENALNE OSOVINE KOD PACOVA HRONIČNO IZLAGANIH RAZLIČITIM STRESORIMA

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SADRŽAJ

Ispitivane su promene u koncentraciji adrenokortikotropnog hormona (ACTH) i kortikosterona (CORT) u plazmi kod mužjaka pacova izlaganih dvema vrstama hroničnog psihosocijalnog stresa: izolaciji i agregaciji, kao i hroničnom

fizičkom stresu: plivanju i hroničnom kombinovanom psihosocialnom i fizičkom stresu: izolacija sa plivanjem, u bazalnim uslovima i posle izlaganja novom kratkotrajnom (2 časa) stresu imobilizacije i hladnoće. Hronična izolacija dovodi do značajnog povećanja bazalne vrednosti ACTH i CORT u plazmi, dok hronična izolacija sa plivanjem dovodi do značajnog povećanja samo bazalne vrednosti ACTH ali ne i CORT. Stres hroničnе agregacije i hroničnog plivanja ne dovodi do povećanja bazalnih vrednosti ovih hormona. Kada se hronično stresirane životinje izlože imobilizaciji i stresu hladnoće, povećava se značajno nivo CORT u plazmi ali nisu uočene značajne razlike u povećanju CORT između ispitivane četiri grupe pacova. Imobilizacija i stres hladnoće značajnije povećavaju nivo ACTH u krvnoj plazmi pacova izloženih hroničnoj agregaciji, plivanju i kombinovanoj izolaciji i plivanju u odnosu na pacove izložene hroničnoj izolaciji. Izneti podaci ukazuju da hronična izolacija predstavlja jači stresor za pacove u odnosu na ostale ispitivane stresore.