NEUROENDOCRINE OR BEHAVIORAL EFFECTS OF ACUTE OR CHRONIC EMOTIONAL STRESS IN WISTAR KYOTO (WKY) AND SPONTANEOUSLY HYPERTENSIVE (SHR) RATS.

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Objective. Spontaneously hypertensive rats (SHR) selected from Wistar Kyoto (WKY) strain represent an animal model of human essential hypertension. This strain of rats is known by excessive neuroendocrine and cardiovascular responses under stress. The aim of the present study was: 1. To compare the reactivity of hypothalamic–pituitary–adrenocortical axis (HPA) to acute mild stress of handling between SHR and WKY rats, 2. to compare the behavioral activity of both strains under basal conditions and during chronic unpredictable emotional stress.

Methods. Seven to eight weeks old male SHR and WKY rats bred in the Physiological Institute, Academy of Sciences of he Czech Republic (Prague) were used. Acute stress was induced by 2-minute handling of the animals in their cage. Blood plasma was analyzed for ACTH and corticosterone (CORT) by specific radioimmunoassay. Chronic unpredictable stress lasted 20 days and consisted of random exposures to following interventions: Light on or off for 24 h, overcrowding i.e. pooling the rats from two cages into one (size 24 x 39 x 23 cm) for 24 h, isolation by placing a single rat into one cage for 24 h, new hierarchy by mixing 4 rats from two different cages for 24 h, limited access to food or water for 1 hour in one day between 3 and 6 p.m., inescapable foot shock (20 shocks, duration 5 s, intensity 10 mA, intershock interval 30 s), tilting the cages for 24 h. The sequence of individual stress exposures was the same in all rats. On day 6, 10 and 20, behavioral activity was measured using the elevated plus-maze in non-stressed control and stressed rats. The results were evaluated by non parametrical Kruskal -Wallis test followed by Man-Whitney U- test.

Results. The two-minute handling resulted in a significantly higher activation of HPA in the SHR than in the WKY rats (plasma ACTH: 350 ± 65 pg/ml for SHR vs. 97 ± 17 pg/ml for WKY $p<0.01$; plasma corticosterone: 2.8±1.4 mg/100ml for SHR vs. 0.7±0.06 mg/100ml for WKY $p<0.05$). In WKY rats no activation of HPA was observed. Elevated plus-maze anxiety test showed inverse behavioral pattern between SHR and WKY rats. In the first test of anxiety the number of open arm entries (OAE) as well as total mobility expressed as total arm entries of the SHR was lower than of the WKY rats ($p<0.01$) without any difference between stressed and non-stressed animals in either strain. It was gradually increasing in stressed and non-stressed SHR in subsequent sessions markedly exceeding the activity of WKY rats ($p<0.01$). Stressed WKY rats showed less OAE and total mobility than their controls ($p<0.01$).

Conclusions. Our results show enhanced neuroendocrine response to acute handling and enhanced anxiety in acute novelty stress in SHR comparing to WKY rats which suggests a common mechanisms for neuroendocrine and behavioral changes. These results further underline the lack of anxiety related behavior of SHR under chronic emotional stress.

Key words: SHR - WKY rats - Handling - ACTH- Corticosterone- Chronic emotional stress- Elevated plus-maze

The spontaneously hypertensive rats (SHR) were first described in the early 1960s. They were selected from Wistar Kyoto (WKY) strain and represented the animal model of human essential hypertension. Hypertension in these animals has been developed up to 8 weeks, and becomes fully manifested at about 12 to 13 weeks of life (Okamoto and Aoki 1963). Hypertensive state was associated with profound endocrine and cardiovascular changes. Thus, plasma noradrenaline and adrenaline have been significantly elevated in the SHR suggesting an important role of peripheral sympathetic activity and adrenal medulla in the development of hypertension in these rats (Bernton et al.1998). The renin-angiotensin (ANG) system is also of importance, and AT receptors have been proposed to participate in the enhanced endocrine response during stress (Johren et al. 2003).

SHR show more robust hypothalamic-pituitary-adrenocortical response to severe stress of immobilization (Sudo and Miki 1993) or ether inhalation (Hausler et al. 1983). They display marked cardiovascular hyperactivity to psychological open field stress (Van den Buuse et al. 2001), enhanced sympathetic nerve activity as well as higher increase in heart rate (HR) and blood pressure (BP) in mental stress caused by air-jet comparing to their WKY counterparts (Zhang and Thoren 1998; Knardahl and Hendley 1990). These rats also possess an impaired coping mechanisms to repeated stress of restraint (Stuart et al. 2000). Social stress is associated with repeated defeat and involves cardiovascular, neuroendocrine, as well as behavioral mechanisms. SHR have been characterized by differences in the social- and anxiety-related behavior and are often used as a model of attention- deficient-hyperactivity disorder (Sagvolden 2000).

The aim of this study was to compare the reactivity the HPA axis to acute mild stress of handling between SHR and Wistar Kyoto rats and also to compare the behavioral activity of both strains under basal conditions and during chronic unpredictable emotional stress.

Materials and Methods

Animals and stress models. Seven to eight weeks old male WKY and SHR bred in the Physiological Institute, Academy of Sciences of the Czech Republic (Prague) were used. Four rats per cage were housed in an animal room with a 12 h light/dark cycle, controlled humidity and temperature. They had free access to tap water and standard peleted diet. Acute stress was induced by a mild 2-minute handling of the animals in their cages. Immediately thereafter the rats were decapitatated, blood plasma was collected and stored at –35 °C. In another series the rats were subjected to a series of chronic unpredictable stressors for 20 days. The stress consisted of random exposures to following interventions: Light on or off for 24 h, overcrowding i.e. pooling the rats from 2 cages into 1 (size 24 x 39 x 23 cm) for 24 h, isolation by placing a single rat into one cage for 24 h, new hierarchy by mixing 4 rats from 2 different cages for 24 h, limited access to food or water for 1 hour in one day between 3 and 6 p.m., inescapable foot shock, (20 shocks, duration 5 s, intensity 10 mA, intershock interval 30 s), tilting the cages for 24 h. The sequence of individual stress exposures was in all rats the same. On the days 6 (water restriction stress), 10 (food restriction stress), and 20 (food restriction stress), and in groups of nonstressed control SHR and WKY rats the emotional state of the animals was tested using elevated plus maze. The test is based on the conflict between the exploratory drive of the rat and its fear of the open area. Increased open arm entries thus indicate less anxiety and reversed, increased closed arm entries indicate higher anxiety. Elevated plus-maze consists of four elevated arms (10 ´ 45 cm) in a cross-like position 50 cm above the floor. Two opposite arms are enclosed by 40 cm high side and end walls. Two opposite arms are without walls. At their intersection is a central square (10 ´ 10 cm). The rats were brought to the laboratory 1.5 h before the beginning of the test, to adapt to the milieu. When starting the test, the animals were placed on the central square area and the numbers of entries into the open and closed arms were recorded for 3 minutes. The experiments were performed in accordance with the national law of the Czech Republic on the use of laboratory animals # 167/1993.

Analysis. Plasma CORT was extracted with methylenechloride and analyzed by radioimmunassay using specific antibodies (Sigma Aldrich, Deisenhofen, Germany) and [1.2.4.7-^{3}H]-corticosterone (Amersham, Buckinghamshire, England). Free and bound hormones were separated on dextran coated charcoal. Plasma ACTH was estimated by radioimmunoassay using commercial kit supplied by Amersham Pharmacia Biotech (Czech Republic).

Statistical evaluation. Because of significant differences between SD of individual groups the results were evaluated by nonparametric Kruskal-Wallis test followed by Mann-Whitney U test for comparing the

mean ranks either between stressed and control groups or within the control and stressed rats at first maze test.

Results

Fig. 1 shows the plasma levels of ACTH and CORT as obtained in both SHR and WKY rats after two minute handling. This stressor caused a significant elevation of both ACTH and CORT in the SHR, while the levels of these hormones in WKY rats were not increased which apparently means that in the latter strain of rats the activation of the HPA axis did not occur.

When the animals of both strains were exposed to the elevated plus-maze anxiety test, the nonstressed control SHR showed inverse behavioral pattern as compared to the WKY rats in all 3 sessions measured (Fig. 2). In the first session of anxiety test, the numbers of open arm entries (OAE), and total mobility representing the sum of open and closed arm entries, were significantly lower in the SHR than in the WKY rats ($p<0.01$). In successive tests the mobility was enhanced in the SHR compared to their WKY counterparts ($p<0.01$ for the third session). The opposite behavioral activity of the two strains was amplified by the repeated stress exposures: In the first session of anxiety test i.e. after 6 days of repeated stress exposure, no differences between non stressed and stressed WKY were visible. In following tests WKY stressed rats displayed more anxious behavior as indicated by lower number of OAE and as well as lower total mobility (control WKY vs. stress WKY - $p<0.01$; stress WKY vs. stress SHR - $p< 0.01$). The SHR did not respond to this type of chronic emotional stress in any session measured; the numbers of OAE as well as total mobility increased gradually in the same way in both stressed and non-stressed SHR.

Discussion

Our results showed enhanced reactivity of HPA axis in the SHR to the acute mild stimulus such as the touching the animal by hand thus indicating higher susceptibility of central nervous system of these rats. It has been shown that SHR possess higher number of ACTH-immunoreactive cells and also higher ACTH concentration in adenopituitaries than WKY rats (Hausler et al. 1984). Therefore the enhanced availability of ACTH in the adenopituitary may be a contributing factor to a more pronounced stress response. However, our finding is not in line with previous results by Sudo and Miki (1993) who showed reduced response of SHR to psychological stress such as the restriction to small space or introduction of new rats into the cage. Since both protocols involved emotional stress, the difference is difficult to interpret. One possible explanation might be the difference in the duration of stress stimuli.

We also observed opposite response of these two strains of rats in anxiety test using elevated plus-maze. In the first session the SHR spent more time immobile, that could have occurred due to impaired habituation of these rats to the laboratory environment and also due to the acute novelty stress exhibited by the testing apparatus which caused higher anxiety. In contrast, the higher mobility of WKY rats indicated lower level of anxiety and emotional stress when exposed to the testing apparatus for the first time. Our results showed that SHR of this substrain are more sensitive to a short acute social stress as measured by neuroendocrine response to short handling or behavioral activity in new environment comparing to the WKY rats. There are numeral reports indicating that SHR are less fearful than Lewis rats in the open field, elevated plus maze, elevated T maze, or black and white box (Berton et al. 1998; Ramos et al. 2002). Under our conditions we found comparable results when exposed the animals to the test apparatus repeatedly.

The fact that intact and 6-times stressed animals behaved in the same way demonstrated the lack of effect of repeated stress exposures on the anxiety related behavior in both SHR and WKY rats. However, in subsequent sessions the opposite behavioral pattern of these two strains became more profound: Gradually increased mobility of SHR indicated higher degree of impulsivity. It can be interpreted so that the rats tested in the identical apparatus remembered the new environment, habituated to the procedure and were able to express their exploratory activity. Surprisingly, chronic unpredictable stress did not induce any changes towards the anxiety-related behavior in SHR as was the case in the normotensive WKY rats. This difference cannot be accounted for by the effect of the last previous stress, since the sequence of stress exposures was the same in all rats. In the stress responsiveness the developmental phase of the hypertension may be of importance: Maslova et al. (2002) have shown an anxiogenic effect of repeated unpredictable stress in adult rats with inherited stress-induced arterial hypertension, when the stress stimuli were applied in the pre-pubertal time period. Similarly to our observation, stress of repeated

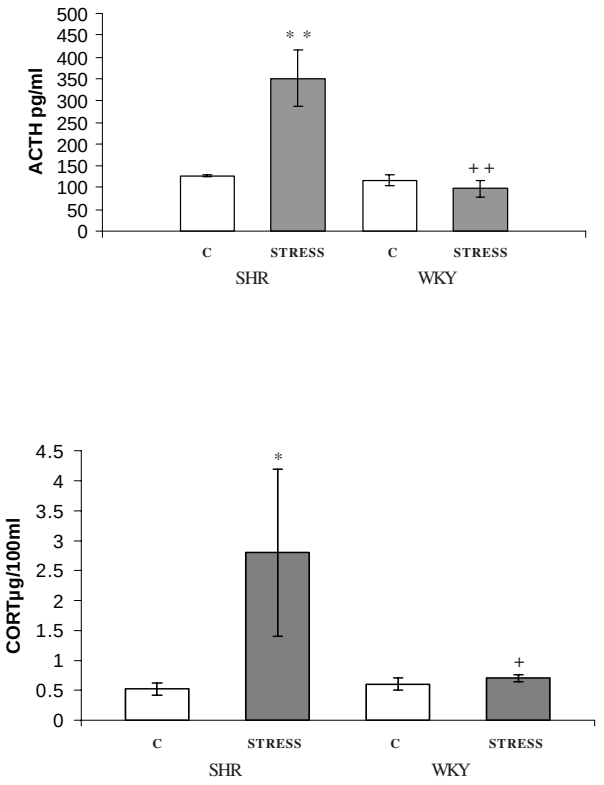

Fig. 1 Plasma levels of ACTH and corticosterone (CORT) in response to 2 minute handling in SHR and WKY rats. 7 rats per group were used. Significance: *p<0.05; **p<0.01 between control and stressed rats of the same strain; +p<0.05; ++p<0.01 between stressed SHR and WKY rats.

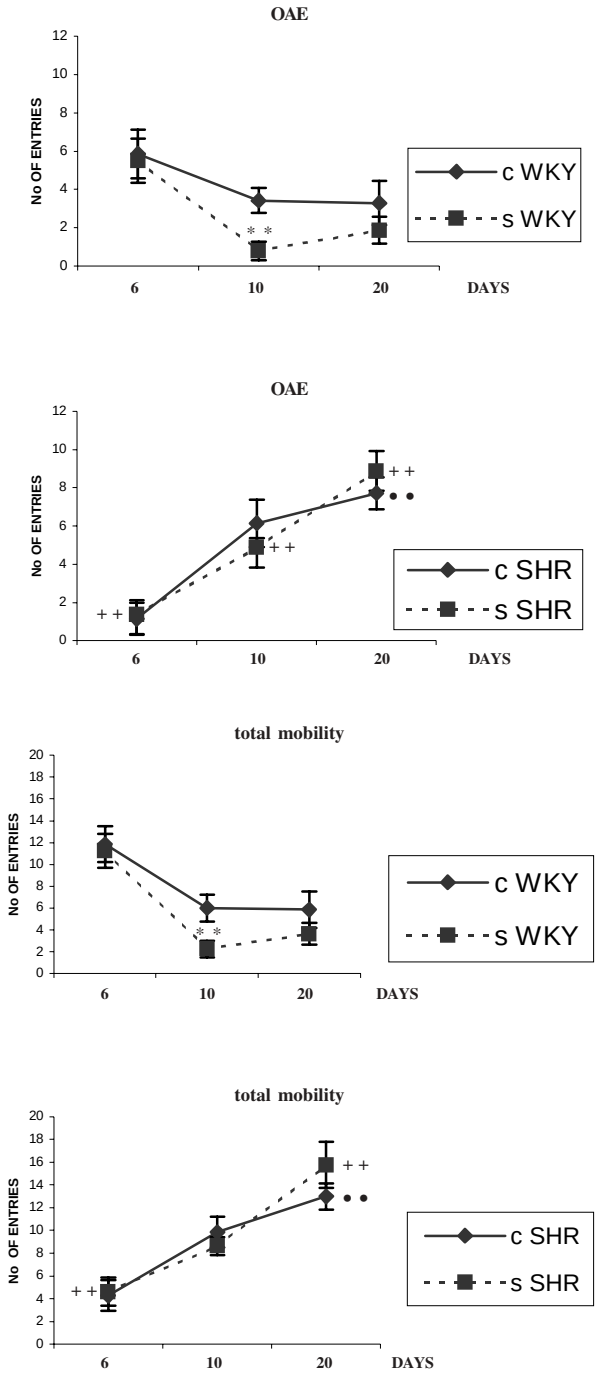

Fig. 2 Numbers of OAE and total mobility (sum of all entries into open and closed arms) in control and stressed SHR (cSHR, sSHR) and WKY rats (cWKY, sWKY) recorded on elevated plus-maze on days 6, 10, and 20 of stress. 7 to 8 rats per group were used. Significance: ++p<0.01 between the strains in given time-intervals, **p<0.01 between stressed and nonstressed WKY rats, ••p<0.01 between the first and third measurements in the SHR

defeat increased anxiety-related behavior in adult Lewis rats, but was ineffective in the SHR (BERTON et al. 1998). Taken together, our results showed enhanced neuroendocrine response to acute handling and enhanced anxiety in acute novelty stress in the SHR comparing to the WKY rats suggesting a common mechanisms triggering neuroendocrine and behavioral changes. These results further underline lack of anxiety related behavior of the SHR under chronic emotional stress.

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