HORMONE RESPONSE TO STRESS IN RAT STRAINS OF DIFFERENT SUSCEPTIBILITY TO IMMUNOLOGIC CHALLENGE.

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Objective. The aim of this study was to compare the changes in plasma levels of hormones involved in modulation of the immune system function after exposure to stress in two rat strains with different susceptibility to immunoantigens.

Methods. Adult rat males of Lewis (LEW) and Fischer 344 (FIS) strains were exposed to restrain stress for 2 hours and blood samples were collected during stress exposure. Other groups of animals were exposed to restrain stress for 2 hours and sacrificed 3 hours later for blood and organ collection. Corticosterone, testosterone, dehydroepiandrosterone, 17ß–estradiol and progesterone were estimated by radioimmunoassay, epinephrine and norepinephrine levels were determined by radioenzymatic method.

Results. The levels of plasma corticosterone and catecholamines were significantly higher during stress exposure in FIS as compared to LEW rats. Greater decrease of testosterone levels and higher levels of estradiol were noted after exposure to stress in LEW rats. Higher values of progesterone plasma levels were noted in FIS rats after stress.

Conclusions. These results demonstrated the differences in the response of catecholamines, adrenal and gonadal steroids after exposure to stress in LEW and FIS rats with lower levels of hormones with anti-inflammatory action in LEW rats.

Key Words : hormone levels, stress, rat strains,

It was repeatedly demonstrated that Lewis rats (LEW) are more susceptible to immunoantigen induced inflammatory processes as compared either to Fischer 344 rats (FIS) or Sprague Dawley rats (BERNARDINI et al.1996, LARIVIERE et al. 2006). Inbred female LEW rats develop an arthritis and acute febrile response after the administration of group A streptoccocal cell wall polysacharide (SCW LPS), or complete Freud´s adjuvant, or carrageenan, while in FIS rats the response is either attenuated or arthritis does not develop (TAYLOR et al. 2005; FECHO et al. 2006). LEW rats showed impaired plasma corticosterone and ACTH responses to SCW LPS as compared to FIS rats (STERNBERG et al. 1989). It was suggested that susceptibility to develop adjuvant arthritis in LEW rat is associated with the dysfunction of hypothalamic pituitary adrenal axis (HPA) and a defect in pituitary corticotrophin secretion could be among the causes of the hyporesponsiveness of HPA axis in Lewis rats (BERNARDINI et al. 1996). These differences in the HPA axis responsibility to acute stress or immunologic challenge have been the basis for numerous studies investigating strain differences in immunological and behavioral parameters (STERNBERG et al. 1992; ARMARIO et al. 1995; GOMEZ et al. 1998; DHABHAR et al. 1997).

However, different response to induce an inflammation in LEW and FIS rats could be affected not only by

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changes in HPA axis activity, but also by the plasma levels of other hormones with immunomodulatory effects and by their changes during the exposure to stressors (Miller et al. 2000). An alteration of norepinephrine content in lymphoid organs of LEW rats with exacerbated adjuvant induced arthritis (AA) was noted, supporting the role of noradrenergic innervation and catecholamine content in immune organs during inflammatory process (Lorton et al. 1997). Denervation of noradrenergic nerve fibers in lymphoid nodes (popliteal and inguinal) with 6-hydroxy-dopamine resulted in enhanced inflammation of AA in LEW rats suggesting the possibility, that adrenergic system in lymphoid nodes can modulate the severity of arthritis (Felten et al. 1992).

In human studies it was noted that several steroid hormones are involved in the regulation of immune response by estrogens as enhancers of at least the humoral immune activity as well as by androgens, progesterone and glucocorticoids as natural immune supressors (Castanetta et al. 1999; Morishita et al. 1999; Masi 2000; Elenkov and Chrousos 2002; Imrich 2002; Cutolo et al. 2006). The elevation of estrogens in synovial fluids of patients with rheumatoid arthritis was observed and also the alterations of plasma sex hormone levels and changes of estrogens/androgens ratio were found (Cutolo et al. 2004; Rovensky et al. 2005).

Therefore the aim of our observations was to study the changes in plasma levels of catecholamines, corticosterone, estrogens, androgens and progesterone in LEW and FIS rat strains after the exposure to stress to understand whether these hormones could participate on the difference of the sensitivity to immunological challenge of these animals.

Methods

Rat males of Lewis (LEW) and Fischer 344 (FIS) strains, body mass 250-300 g were used. The animals were housed 4- per cage under controlled conditions (light on between 6AM to 6PM, temperature 24±2 °C) with tap water and chow pellets *ad libitum*. The Ethical Committee of the Institute approved the study design.

In the first experiment an indwelling cannula was inserted into the tail artery under sodium barbital anesthesia one day before the observation. On the next day the animals (8 rats per group) were exposed to restrain stress (by immobilization to a board) for 2 hours (Kvetnansky and Mikulaj 1970). Blood samples were collected from the cannula immediately before and 20 and 120 minutes after the beginning of exposure to stressor. The levels of catecholamines and corticosterone were estimated in plasma (for methods used see bellow)..

In the second experiment the rats were exposed to restrain stress for 2 hours and sacrificed 3 hours later for blood and organ collection, while control rats were not exposed to restrain stress. Plasma was separated by centrifugation at 4 °C immediately after the blood collection and stored at -20 °C until analyzed. Epinephrine (E) and norepinephrine (NE) were determined by radioenzymatic method (Kvetnansky and Mikulaj 1970). Corticosterone (CS), testosterone (TE), dehydroepiandrosterone (DHEA), 17ß-estradiol (ES) and progesterone (PGS) were determined by commercial kits from Immunotech (Marseille, France).

Results were expressed as mean values ± S.E. of means. Statistical significance was estimated with the aid of Student t-test.

Results

Basal levels of CS, E and NE in plasma were lower in LEW rats (Table 1). The elevation of plasma CS and catecholamine levels was significantly higher at all time intervals during the exposure to stress in FIS rats as compared to LEW rats (Table 1). These results demonstrate the differences in the response of FIS and LEW rats to immobilization stress suggesting the hyporeactivity of hypothalamo-pituitary-adrenal axis and sympatoergic systems in LEW rats.

The determination of the levels of steroid hormones, which are involved in the modulation of immune system activity, showed that basal levels of plasma TE were higher in LEW rats as compared to FIS rats (Fig. 1). After exposure to restrain stress, the plasma levels of TE were decreased in both LEW and FIS rats, however, greater decrease of plasma TE was noted after exposure to stress in LEW rats in comparison to FIS rats (Fig.1). Plasma levels of DHEA were similar in LEW and FIS rats before and after the stress (Table 2). The basal plasma levels of estradiol were not significantly different in FIS and LEW rats (Table 2). However, the differences in FIS and LEW rats were noted after the exposure to stress, because a significant decrease of estradiol level was noted only in FIS rat strain. Therefore during stress exposure of LEW rats, the levels of estradiol, a hormone with stimulatory action on immune response, are higher as compared to FIS rats. There were no differences in basal plasma levels of progest-

Table 1. Changes of plasma corticosterone (CS µg/100ml), epinephrine (E, pg/ml) and norepinephrine (NE, pg/ml) during the restrain stress in Fischer 344 (FIS) and Lewis (LEW) rat

	C FIS	C LEW	STRESS 20 min FIS	STRESS 20 min LEW	STRESS 120 min FIS	STRESS 120 min LEW
CS	10.8 ± 2.0	3.9 ± 1.0*	39.9 ± 3.2	21.3 ± 0.7*	28.3 ± 2.7	17.0 ± 1.8*
E	85 ± 9	24 ± 6*	2050 ± 650	450 ± 50*	1100 ± 250	330 ± 52*
NE	450 ± 32	310 ± 30*	1150 ± 100	710 ± 51*	1910 ± 300	800 ± 120*

C non stressed controls. * FIS to LEW p< 0,05.

Table 2. Plasma hormone levels after exposure of Fischer 344 (FIS) and Lewis (LEW) rats to restrain stress.

		CONTROL FIS	CONTROL LEW	STRESS FIS	STRESS LEW
DHEA	ng/ml	0.21 ± 0.02	0.20 ± 0.04	0.22 ± 0.01	0.23 ± 0.01
ES	pg/ml	62 ± 8	64 ± 6	37 ± 2 *+	48 ± 6
CS	µg/dl	4.38 ± 1.30	1.19 ± 0.50*	7.80 ± 0.60+	5.50 ± 0.90+

Stress – restrain stress for 2 hours, Abbrevations of hormones DHEA—dehydroepiandrosterone, ES—17beta- estradiol, CS — corticosterone * FIS to LEW p<0.05, + Control to Stress p<0.05.

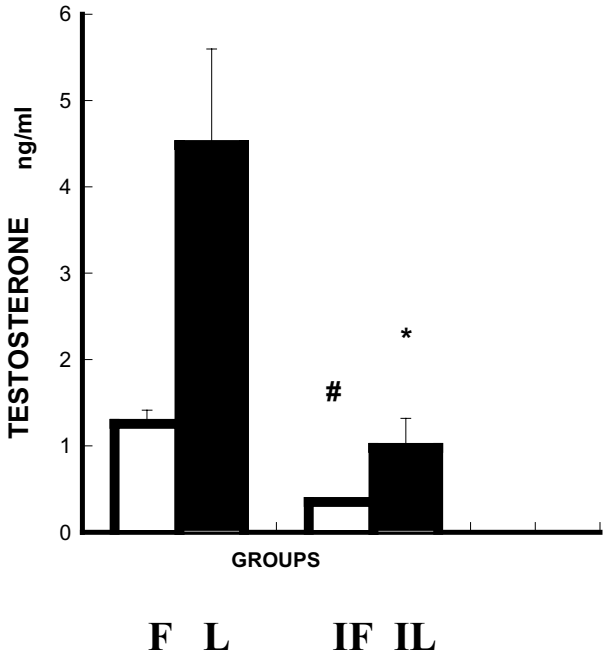

Fig. 1 Changes of plasma testosterone in Fischer 344 (F) and Lewis (L) rats after exposure to restrain stress F and L non stressed control, IF and IL after restrain stress. * = Lewis rats, controls and after stress p< 0.05; # = Fischer rats, controls and after stress p< 0.05.

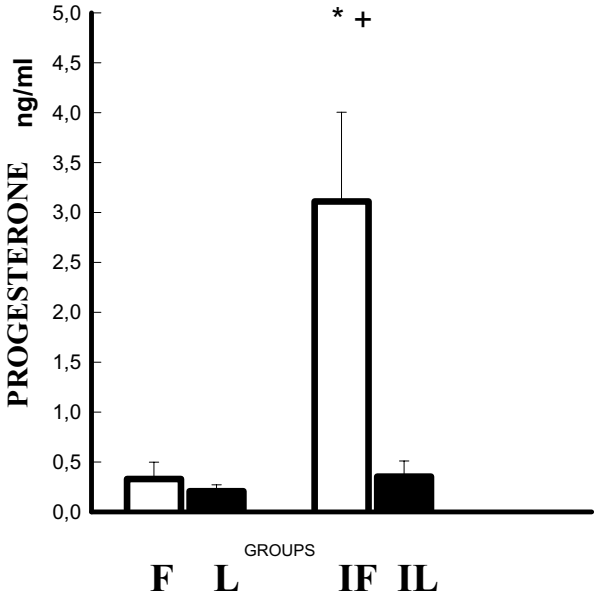

FIG. 2. Plasma progesterone levels after exposure of Fischer 344 and Lewis rats to restrain stress. F and L non stressed controls, IF – Fischer rats and IL – Lewis rats exposed to stress. * Fischer rats – controls to stressed rats – p<0.05; + IF to IL – p<0.05.

erone in intact LEW and FIS rats. After exposure to stress, the increases of plasma progesterone were noted in FIS rats, while no significant changes were noted in LEW rats (Fig. 2). Similarly, as in the first experiment, higher plasma levels of CS were noted in FIS rats even three hours after stress in comparison to LEW rats (Table 2), thus supporting a lower susceptibility of FIS rats to immunoantigens.

Discussion

The results of present experiments showed that there are strain related differences in catecholamine response to restrain stress in FIS and LEW rats with higher concentrations of E and NE in FIS rat strain. The elevated plasma catecholamines in FIS rats support the studies demonstrating the anti-inflammatory effects of sympathetic nervous system (Basbaum. and Levine 1991). The reduced response of catecholamines in LEW rats to stress might serve as one of the factors responsible for the increased sensitivity of immune system to respond to immunologic challenge and to develop the adjuvant arthritis

The results also showed lower levels of CS in LEW rats which is in agreement with previous observations on the hypofunction of hypothalamic-pituitary-adrenal axis and lower responses of CS to lipopolysacharide (Grota et al. 1997), morphine administration (Baumann et al. 2000) and to stress (Moncek et al. 2001) in this strain.

Gonadal and adrenal steroids have been shown to influence immune processes (Castagnetta et al. 1999, Masi et al. 1999; Cutolo and Wilder 2000; Chrousos 2001). It was proposed that estrogens stimulate immune responses whereas androgens suppress inflammatory reaction (Castanetta et al. 2003). Elevated estradiol levels and lower testosterone concentrations were described in plasma and synovial fluids from knee joints of patients with rheumatoid arthritis (Rovensky et al. 2005). In present observations we have noted that there are no significant differences in basal estradiol plasma levels in LEW and FIS rats. However, a marked decrease of plasma ES was observed after the exposure to stress only in FIS rat strain, the changes in estradiol plasma levels of LEW rats after stress were not significant. This suggests that estrogens could participate in higher sensitivity of LEW rats to immunological challenge especially during stress conditions.

Surprisingly elevated concentration of TE was noted in male LEW rats in our experiment. Plasma testosterone concentrations showed decrease after exposure to stress in both strains, however, the decrease in LEW rats was deeper as compared to FIS rats. Therefore the values of estrogens to testosterone ratio were higher in LEW rats. The higher values of estrogen to testosterone plasma and synovial fluid concentrations were described in patients with rheumatoid arthritis (Cutolo et al. 2002; Rovensky at al. 2005) which is in agreement with pro inflammatory action of estrogens.

The results of our experiments showed for the first time that there are differences in the plasma progesterone concentrations in LEW and FIS after exposure to stress. Elevated levels of plasma progesterone were noted in stressed FIS rats only and no changes in LEW rats were observed. The increases of progesterone and corticosterone plasma levels were described in adult Wistar male rats after exposure to footshock or sleep deprivation (Andersen et al. 2004). An elevation of plasma progesterone was noted in male rats after application of restrain stress (Romeo et al. 2005). These authors also found that the stress induced corticosterone and progesterone responses are greater and more prologed in prepubertal animals as compared to adults. Wirth et al. (2007) demonstrated a positive correlation of salivary progesterone levels with salivary cortisol in human subjects. These findings suggest that progesterone is released from adrenals along with glucocorticoids due to general activation and can serve as an additional negative feedback mechanism to down regulate the stress response. The differences in progesterone levels in FIS and LEW rats suggest that also progesterone could be partially responsible for resistance of FIS strain rats to immunologically induced inflammation, because progesterone and androgens, besides corticosterone, suppress the activity of immune system (VanVollenhover and McGuir 1994; Cuchacovich et.al. 1991; Borel et al. 1999).

In conclusions the results of our experiments demonstrated the differences in the response of corticosterone, epinephrine, norepinephrine, and gonadal steroids to stress in Lewis and Fischer 344 rats with lower levels of hormones with anti-inflammatory action in LEW rat strain.

Acknowledgements

We wish to express sincerely gratitude to Mrs. E. Visztova for her precise technical assistance. This study was supported by grants APVT 21-0086002 and also by grant from Ministry of Health of the Slovak Republic.

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