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Emotional Stress Induces Sex-Specific Sympatho-Adrenomedullary Responses in Lambs

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The aim of the study was to compare the effects of emotional stress measured by the plasma levels of catecholamines and cortisol in male and female lambs. Three-months-old lambs were stressed by 30 minutes of isolation from the herd. Stressed animals were separated individually in special room without acoustic and visual contact with each other, control animals stayed with the herd. Blood was taken by venipuncture (jugular vein) from all animals 10 min before the start of experiment and 10, 20, 30 and 60 minutes after the beginning of isolation. Plasma levels of cortisol and catecholamines-adrenaline, noradrenaline and dopamine were estimated by radioimmunoassay using commercial kits. Additionally, AUCs (areas under curve) were calculated for each parameter. The obtained results clearly showed significant differences in male (rams) and female (ewes) lambs responses to emotional stress. Plasma level of cortisol was much higher in females during the stress (P<0.01) than in males, similar pattern of stress response was observed in plasma dopamine level. In contrast, plasma levels of adrenaline and noradrenaline were significantly higher in males than in females (P<0.05). Therefore, the emotional stress induced sex-specific stress responses of sympathetic and adrenomedullary systems in growing lambs.

Key words: catecholamines, cortisol, stress, lamb, isolation

INTRODUCTION

The physiological response to stress includes activation of the central nervous system as well as the endocrine and immune systems. The systems interactions are possible through the activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis, and the secretion of numerous neurotransmitters, neuropeptides and hormones (Deiss et al., 2009, Caroprese et al., 2010). Increased activation of the HPA axis and sympathetic system as a results of stress leads to central and peripheral changes, which facilitate behavioural adaptation and redirection of energy to the nervous system, muscle and other organs.

Cortisol, glucocorticoid released from the adrenal cortex, and catecholamines synthesized in the

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sympathetic nervous system and adrenal medulla, are the main hormones involved into regulation of stress responses. Increased levels of glucocorticoids and catecholamines were observed in sheep as the effects of different stressors activity (Pierzchała-Koziec et al., 1999), hens (Pierzchała-Koziec et al., 1996) rats (Pierzchala and Vanloon, 1990), pigs (Peres et al., 2014).

However, most of the experiments were performed usually on male animals what may raise the question about the similarity of stressful response in male and female. Stackpole et al. (2003) proved the hypothesis that in sheep isolation and restraint stress decreases the responsiveness of the pituitary gland to exogenous GnRH and that these responses differ with sex and season. In humans, differences in the cold response between men and women were observed (Solianik et al., 2014). Also, cock and hen hormonal responses to stressors were different (Pierzchala et al., 1996).

Gregariousness is the most outstanding characteristic of sheep. A sheep needs to be with other sheep in order to be in a state of well-being. Sheep always try to maintain uninterrupted visual contact with at least one other sheep, and they flock close together at any sign of danger. Many experiments proved that sheep show endocrine, hematological and biochemical alterations (Mcnatty et al., 1972; Alexander et al., 1983; Parrott et al., 1987; Van Adrichem and Vogt, 1993; Carbajal and Orihuela, 2001; Caroprese et al., 2006). A marked increase in heart rate and respiration rate were observed when the animal was isolated from other sheep (Syme and Elphick, 1982; Carbajal and Orihuela, 2001).

It was also found that sheep stress reaction depends on age – younger individuals responses manifested by neuroendocrine system differ from that observed in older animals (Sapolsky, 1992). Nevertheless, in spite of extended research, the data about the catecholamines and glucocorticoids changes during stress reaction in growing lambs of both sexes are still not fully understood.

Therefore, as a part of study dealing with the estimation of animals stress responses, the aim of the experiment was to compare the changes of cortisol, adrenaline, noradrenaline and dopamine plasma levels induced by separation (isolation) of growing male (rams) and female (ewes) lambs from the herd.

MATERIALS AND METHODS

Experiment was carried out on 3-months-old lambs (n=24), rams and ewes, Polish Mountain strain, kept in standard conditions with free access to food and water. Animals were divided into control and experimental group stressed by 30 min of isolation from the herd. Stress was performed in the separate room without visual and acoustic contact.

Blood was taken from the jugular vein from all lambs 5 times [ten minutes before (I) and 10 (II), 20 (III) and 30 min (IV) after start of stress, and 30 min (V) after the isolation was terminated]. Blood, taken on heparin, was centrifuged and plasma was stored at -80°C until cortisol and catecholamines (adrenaline, noradrenaline and dopamine) were estimated by radioimmunoassay using commercial tests from DIA Source, Belgium and LDN, Germany, respectively.

Additionally, areas under curve (AUC) for cortisol and each catecholamine were calculated. Protocol of animals manipulations was approved by I Local Ethics Committee.

STATISTICAL ANALYSIS

Results were statistically calculated using analysis of variance, and when significant effects were found ($P<0.05$), Student's t-test was used to locate significant differences between means (Statistica10).

RESULTS

Cortisol (Fig. 1A,B)

Plasma level of cortisol in control ewes (Fig. 1A) slightly varied from 66.5±1.8 pmol/ml at the beginning of experiment (-10 min,I) to 53.1±1.5 pmol/ml at the end of study (V, $P>0.05$). Isolation from the herd caused significant increase of the plasma level of cortisol after first 10 min of stress (from 70.4± 2.1 to 136.7±8.7 pmol/ml, $P<0.05$). Plasma cortisol level has grown with the stress time and after 20 min (III) reached the value 208.0±15.6, $P<0.01$), at the end of stress the level was even higher – 211.3±11.9 pmol/ml ($P<0.01$). Thirty minutes

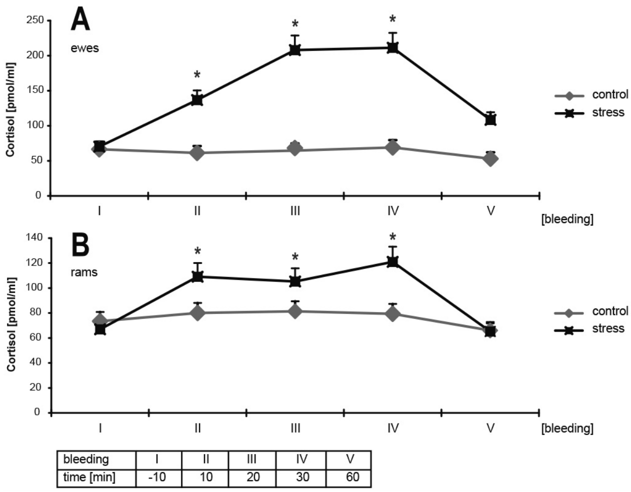

Fig. 1. Plasma cortisol level in control and stressed ewes (A) and rams (B), pmol/ml, X±SEM, *P<0.05–0.01 compare to bleeding at -10 min.

after terminating the stress, plasma cortisol level was much lower (108.1±5.9 pmol/ml, V) but still significantly higher than before isolation (P<0.05).

Plasma cortisol level in rams (Fig. 1B) from control group was not changed during 60 min of study, it slightly varied from 73.5±4.1 pmol/ml (-10 min, I) to 66.1±3.7 pmol/ml (60 min, P>0.05). Isolation stress increased the plasma cortisol level from 67.0±3.9 pmol/ml to 109.1±6.7 pmol/ml (P<0.05) after 10 min of stress (II), to 105.3±3.9 pmol/ml after 20 min and to 121.0±6.9 pmol/ml (P<0.01) at the end of stress (IV). The plasma cortisol level in stressed lambs was not different from that seen in control animals thirty minutes after termination of isolation.

Area under curve (AUC) of cortisol (Table 1) in stressed ewes was much higher than in control lambs, it was increased from 4410 to 10688 pmol/ml/60min (P<0.01). In contrast, AUC of cortisol in isolated rams was increased in much smaller extend, but still was statistically significantly elevated from 5330 to 6758 pmol/ml/60min (P<0.05).

Adrenaline (Fig. 2A,B)

Plasma level of adrenaline in control female lambs (Fig. 2A) slightly varied from 0.60±0.04 pmol/ml at the beginning of experiment (-10 min,I) to 0.65±0.02 pmol/ml at the end of study (V, P>0.05). Unexpectedly, isolation from the herd caused significant increase of the plasma adrenaline level only 30 min after terminating the stress from 0.64±0.04 pmol/ml (I blood collection) to 0.79±0.05 pmol/ml (V blood collection, P<0.05).

Plasma level of adrenaline in control rams was not significantly changed during the whole experiment and varied from 0.52±0.06 pmol/ml (I) to 0.55±0.07 pmol/ml (V blood collection). Quite different profile of adrenaline changes during isolation stress was observed in rams (Fig. 2B). Stress revealed a significant growth of plasma adrenaline after 10 min [increase from 0.38±0.03 pmol/ml (I) to 0.79±0.04 pmol/ml (II), P<0.01]. During the time of isolation, decrease of this high adrenaline level was noticed, however at the end of experiment (V blood collection) plasma level of catecholamine was still significantly elevated (0.61±0.02 pmol/ml, P<0.05).

Area under curve (AUC) of adrenaline (Table 1) in stressed ewes was not different from that calculated in control animals; it was 41.7 pmol/ml/60min and 45.2 pmol/ml/60min, respective-

TABLE 1. Area under curve (AUC) for cortisol, adrenaline, noradrenaline and dopamine in control and stressed ewes and rams (pmol/ml/60min, X±SEM, * P<0.05–0.01 in comparison between control and stressed lambs).

PARAMETERS [pmol/ml/60min]	EWES		RAMS	
	control	stress	control	stress
Adrenaline	41.7 ± 1.2	45.2 ± 1.1	37.3 ± 2.3	44.1 ± 2.8*
Noradrenaline	106.5 ± 6.7	117.9 ± 3.7*	97.1 ± 6.7	171.1 ± 9.9*
Dopamine	17.7 ± 1.2	47.0 ± 2.9*	129.5 ± 8.6	121.9 ± 7.6
Cortisol	4410 ± 310	10688 ± 863*	5330 ± 470	6758 ± 510*

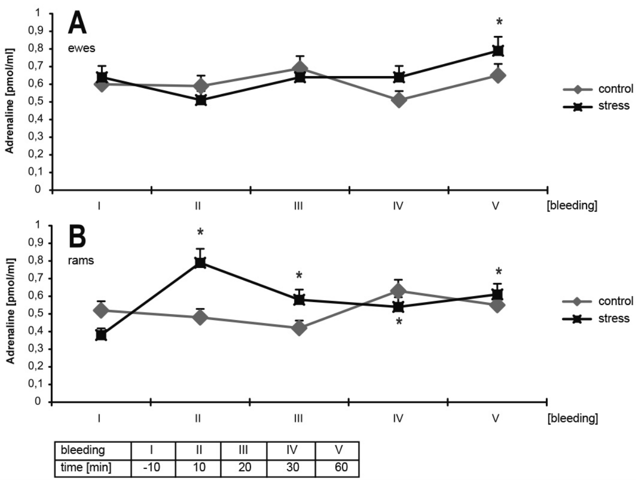

Fig. 2. Plasma adrenaline level in control and stressed ewes (A) and rams (B), pmol/ml, X±SEM, *P<0.05–0.01 compare to bleeding at -10 min.

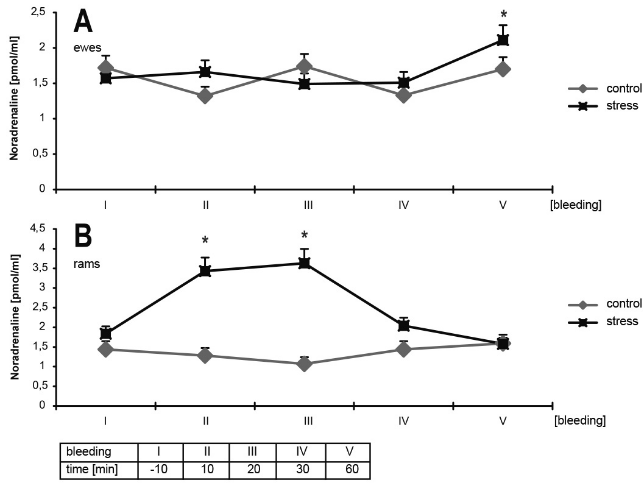

Fig. 3. Plasma noradrenaline level in control and stressed ewes (A) and rams (B), pmol/ml, X±SEM, *P<0.05–0.01 compare to bleeding at -10 min.

ly. On the other hand, AUC in control rams was calculated at the level of 37.3 pmol/ml/60min and was significantly (P<0.05) increased in isolated animals to 44.1 pmol/ml/60min.

Noradrenaline (Fig. 3A,B)

Blood collection procedure did not have significant impact on the plasma level of noradrenaline (Fig. 3A) in control female lambs. It was quite stable during the experiments, starting from 1.72±0.07 pmol/ml (I) and finishing with 1.70±0.08 pmol/ml (60 min of experiment, V blood collection). Similarly to adrenaline, isolation affected the plasma level of noradrenaline only 30 min after terminating the stress; it was increased from the value of 1.57±0.04 pmol/ml (I collection) to 2.11±0.05 pmol/ml (V collection, 60 min, P<0.01).

Plasma noradrenaline level (Fig. 3B) in rams of control group also did not show significant response to blood drawing, however tendency of slight decrease was noticed between I and III blood collection from 1.44±0.04 pmol/ml to 1.07±0.07 pmol/ml, respectively. The concentrations of noradrenaline in the blood taken during IV and V collections were similar to the level observed in the first collection. In contrast, isolation caused increase of the plasma noradrenaline level after 10 min from 1.84±0.09 pmol/ml before stress to 3.43±0.17 pmol/ml (P<0.01), and after 20 min of stress to 3.63±0.09 pmol/ml (P<0.01). At the time of stress termination, plasma noradrenaline level was significantly higher than measured in control animals but showed declining tendency compare to the level present in the plasma of isolated lambs during first blood collection.

Area under curve (AUC) of noradrenaline (Table 1) in stressed female lambs was increased during the whole experiment compare to control animals (15%, P<0.05), in male lambs the AUC was higher after stress by 76% (P<0.01).

Dopamine (Fig. 4A,B)

Plasma dopamine level (Fig. 4A) in control ewes did not show significant changes during the experiment (the level varied from 0.29±0.05 pmol/ml at the beginning to 0.25±0.09 pmol/ml at the end). Twenty min of isolation (III blood collection) strongly stimulated release of dopamine into the blood what caused increase of its level to the value of 0.79±0.09 pmol/ml (P<0.01). Contrary to previous expectations, dopamine level was growing until the end of experiment when reached the value of 0.97±0.10 pmol/ml (P<0.01).

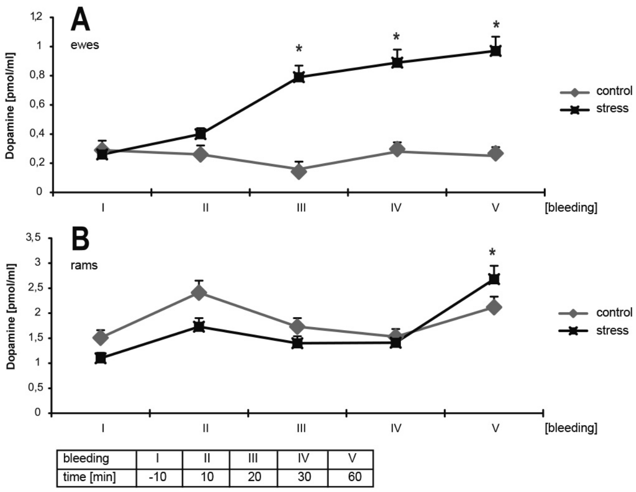

Fig. 4. Plasma dopamine level in control and stressed ewes (A) and rams (B), pmol/ml, X±SEM, *P<0.05–0.01 compare to bleeding at -10 min.

Analysis of dopamine in the blood plasma of control rams (Fig. 4B) showed significantly higher level than in ewes [mean value 1.86 (rams) vs 0.25 pmol/ml (ewes) P<0.01]. Also, the effect of stress on the dopamine level was seen as late as at 60 min of experiment, what was manifested by an increase from the 1.10±0.09 pmol/ml to 2.68±0.11 pmol/ml (P<0.01).

Area under curve (AUC) of dopamine (Table 1) in stressed female lambs was increased from the level of 17.7 pmol/ml/60min to 47.0 pmol/ml/60min (P<0.01). In contrast, AUC in male lambs were similar in control (129.5 pmol/ml/60min) and stressed animals (121.9 pmol/ml/60min).

DISCUSSION

All organisms have adaptations mechanisms to deal with stress. During the stress situation the sympatho-adrenomedullary system is one of the components of the primary systems for maintaining or reinstating homeostasis (Fontes et al., 2014; Yawata et al., 2015). The elevation of plasma catecholamines levels regulates heart rate, blood pressure, hormones release, immunological responses in order to speed adaptation to novel conditions. On the other hand, stress-induced cortisol secretion has many additional effects aiming to control mobilization of energy resources during the initial and prolonged stress (Belda et al., 2015).

Short term isolation stress caused significant increase of plasma cortisol level in male and female lambs as early as 10 minutes of isolation. However, the cortisol level in females was higher by 94%, in males the increase of glucocorticoid level was only 62% (P<0.01). The plasma cortisol level was growing with the time of stress reaching the maximum after 30 min, but in ewes the level was increased almost by 200%, in contrast, in rams the cortisol was increased by 81%. The adrenal response to stress in males ended with the stress termination, in contrast, ewes had still elevated cortisol level at 60 min of experiment. Interestingly, in spite of fact that AUC value in isolated females was much higher (10668 pmol/ml/60min) than in stressed rams (6758 pmol/ml/60min), this parameter was lower in control females compare to the value observed in males.

These results indicate that glucocorticoid released from the adrenal served as a stimulator of glucose metabolism and on the other hand as an immunosuppressive agent during stress reaction. Glucocorticoids may modulate immune responses in many ways, including gene expression, transcription, translation, post-translational processing, protein secretion and cell proliferation (O'Connor et al., 2000).

It is known that even acute stress changed the activity of HPA axis in sheep and this effect can last for many days. Probably, this action is connected with different activity of glucocorticoid receptors. Stress and post-stress activities of glucocorticoids may be regulated by two types of receptors. The high-affinity (mineralocorticoid receptor, MR) called type 1, is involved into non-stress circadian changes of glucocorticoids level and is primarily activated (Dekloet et al., 1998; O'Connor et al., 2000). The glucocorticoid receptor (type 2, low-affinity GR) is activated during stress and have inhibitory or stimulatory effects on different physiological systems. Moreover, glucocorticoid affinity for the GR receptor is only 10% that of MR. In the last decade, a great interest has been paid to the long-term consequences of a single exposure to stress in various animal models aiming understanding mechanisms underlying such phenomenon (Belda et al., 2015).

It seems reasonable, that receptors sensitization appears to be a serious reason to explain enhanced responsiveness to stimulus such as trauma or emotional stress. The findings of the present experiment suggest an involvement of the two types of glucocorticoid receptors into regulation of lambs response to isolation manifested by elevated cortisol plasma level. It is also possible that in females the receptors activity was slower and prolonged allowing to keep the cortisol at higher level even after terminating the isolation.

On the other hand, the principal role of glucocorticoids during the stress response is thought to be restraint of the effectors of the stress response (O'Connor et al., 2000). Thus, it may be suggested that prolonged and elevated cortisol level in female stressed lambs affected adrenaline and noradrenaline outflow postponing and reducing their response to isolation. Unexpectedly, as the effect of such interaction the adrenaline and noradrenaline levels in female plasma were increased very late, at the end of experiment. In contrast, males reaction was seen as early as 10 min of stress by increased level of both catecholamines. These results are unlike to others stating that the stress-induced peak of plasma catecholamines concentration is achieved within 1-5 min (Pierzchala and Vanloon, 1990; Belda et al., 2015). This stronger stress reaction in rams was additionally confirmed by AUC for adrenaline and noradrenaline which were significantly larger than in ewes. The highly variable nature of plasma noradrenaline levels in sheep was found by Stackpole et al. (2003) during exposure to restraint and isolation what might be accounted for the lack of a significant rise in norepinephrine during stress in their study.

Completely opposite changes during isolation were found in dopamine levels. Plasma dopamine level in control ewes was more than 5-times lower than in rams (0.29 vs. 1.51 pmol/ml, $P<0.01$) but isolation caused almost 4-times stronger elevation of this catecholamine in ewes than in male lambs. Also, the time of response in ewes lasted from 20 to 60 minutes of experiment, in contrast, the increased dopamine level in rams was observed at the end (V blood collection). These discrepancies in dopamine responses were confirmed by AUC which did not change in stressed rams. These findings may suggest that dopamine in rams served as substrate to synthesis of adrenaline and noradrenaline; their levels were elevated almost during the whole isolation paralelly with declining cortisol level.

Taking under consideration that the reactivity of physiological systems to different stressors depends on animal species, age, environment and sex, results of the present experiment indicate that in spite of many data, there is a strong evidence that the interaction between glucocorticoids and catecholamines depends on the animal gender.

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