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EFFECTS OF ADRENALINE INDUCED STRESS AND FLUOCINOLONE-TREATMENT ON SOME ENDOCRINE GLANDS IN MALE WISTAR RATS

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SUMMARY. Synthetic glucocorticoids are widely used as antiinflammatory and antiallergic drugs. Since drugs contaning glucocorticoid are effectively absorbed, a prolonged glucocorticoid treatment leads to a surplus of endogen glucocorticoids. Long-term glucocorticoid treatments disrupt the endocrine system of the body causing hormonal and metabolic side effects. For this reason, it is necessary to specify the endocrino-metabolic effects of these drugs. Starting from the above findings and from the important physiological roles of glucocorticoids, we investigated the reactions of some endocrine glands after adrenaline-stress and fluocinolone treatment. For this purpose, it was important to compare the effects of high glucocorticoid level produced by stressors with the effects of high glucocorticoid level which occur during dermocorticoid treatment. In the present study adrenaline treatment was the source of glucocorticoid excess on the one hand, and fluocinolone on the other. In both cases, significant physiological changes were observed, the most sensitive gland in changes of glucocorticoid levels being the adrenal gland. Adrenaline stress caused atrophy of the cortical zone of adrenal gland. The fluocinolone-treatment determined hypertrophy of the cortical zone of the adrenal gland. In both cases we observed significant weight changes for the whole body and for each gland.

Keywords: adrenalin, adrenals, glucocorticoids, pancreas, thymus.

Introduction

Organisms survive by maintaining equilibrium with their environment. The stress system is critical to this homeostasis. Hormone secretion by the hypothalamic-pituitary-adrenocortical (HPA) axis is modulated by multiple factors which include the circadian rhytm, various types of stressors and glucocorticoids. It is also well demonstrated that multiple stimulatory agents can independently affect the function of the HPA axis (Kis *et al*., 2001b).

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These stimulatory agents include catecholamines, vasopressin and the corticotropin-releasing hormone (CRH). Stress induced activation of HPA axis is attended by release of CRH from paraventricular nucleus (NPV) of hypothalamus, followed by adrenocorticotropine (ACTH) and glucocorticoid release (Cole and Sawchenko, 2002). Among many possible neurotransmiters released in the PVN during exposure to various stressors, noradrenaline and adrenalin are thought to be the potent stimulators of CRH neurons in PVN (Kis and Crăciun, 2003a, 2003b).

Treatment with synthetic glucocorticoids e.g. dexamethasone or dermocorticosteroids, or repeated immobilization stress, decreases the total body weight gain of animals by disturbing the HPA axis function and accelerating the catabolism of the organism (Kis *et al*., 2001a, Gruver-Yates and Cidlowski, 2013, Noguchi, 2014).

Synthetic glucocorticoids are widely used as anti-inflammatory and anti-allergic drugs. Nevertheless, their administration may cause side effects related to the normal functioning of several organs. Glucocorticoids modulate the stress response at a molecular level by altering gene expression, transcription and translation, among other pathways. Glucocorticoids also modulate growth, reproductive and thyroid axes (O'Connon *et al.,* 2000, Kis *et al.,* 2001a, Kis and Crăciun, 2006).

Excessive glucocorticoid secretion or treatment, on the other hand, has been reported to have deleterious effects on the organism: it can induce tissue injury and even cell death (Craciun *et al.,* 1997, 1998, Kis and Crăciun, 2006). Madar and co-workers (1993) have studied the effect of excessive glucocorticoid levels generated through repeated formaldehyde stress or Fluocinolon acetonid-N (FC) treatment in rats. Formaldehyde was used as an endogenous glucocorticoid inducer, while FC was an exogenous source of the hormone. Both formaldehyde- and FC-treated groups showed significant metabolic disorders.

The exact mechanism of glucocorticoid-induced cell death is unknown, but several reports indicate that glucocorticoid-mediated generation of reactive oxygen species (ROS) occurs with the concomitant increase of calcium influx and morphological degeneration of the cell (Landfield and Eldridge, 1994, Viegas *et al*., 2008).

In recent studies elsewere we have reported that short-term and long-term epicutaneous applications of halogenated glucocorticosteroids in pregnant rats induced changes in thymus oxidative status of dams and newborn animals (Kis, 2010, 2012a, 2012b).

In our experiments we followed changes in bodyweight as well as the weight of some glands, histological aspects of rat thymus and adrenals during adrenaline (ADR) and FC treatment. We used adrenalin and Fluocinolon-N ointment, as endogenous and exogenous sources of elevated glucocorticoid levels.

Materials and methods

Experiments were carried out in male Wistar rats. Animals were kept under standardized bioclimatic conditions and fed on common rat chow, with water ad libitum.

Commercial Fluocinolone-acetonid-N ointment containing 25 mg Fluocinolon-acetonid-N/100 g excipient was applied topically to the skin at 2 cm2 for five consecutive days, by smearing 50 mg ointment/100 g b.w on the inguinal region, the daily dose of ointment being equal to 12.5 µg/100 g b.w. Epinephrine-treated animals were injected subcutaneously with 42 µl/100 g b.w. of Adrenaline solution.

Animals were divided into the following groups: K-control group-untreated animals, FC-Fluocinolone-N-treated animals and ADR- adrenaline-treated group.

After 16 hours of fasting and 24 hours following the cessation of treatments, the treated animals together with controls were sacrificed by exsanguination.

Body, pancreas, adrenals and thymus weights of male rats were measured with an accuracy of 0.00001 g immediately after excision. Data are presented as mean ± standard error of the mean. The comparison between the three groups was performed using Student t test for unpaired data.

The thymus and adrenal gland were fixed in Bouin liquid and afterwards processed in view of being embedded in paraffin. The fragments were sectioned at the Reichart microtome with a tickness of 7 µ. The staining of glands was carried out with the method of Hurduc and co-workers (Muresan *et al*., 1974). Histological preparations obtained were examined under an IORC4 optical microscope. Photos were taken using 10x and 20x objectives with an Olympus digital camera.

Results and discussion

The results presented in Figure 1 show that in our experiment FC treatment and ADR stress caused a progressive inhibition of total body weight gain in animals compared to the controls. Body weight loss in the ADR-treated group was lower than in the FC-treated one, indicating that ADR induces a milder stress than the direct cortisol treatment.

The fluocinolone treatment caused a significant weight loss in contrast with the control group (Fig. 2). However, significant weight loss in the FC group was observed as compared to the ADR treated animals. The relative weight of the pancreas in FC treated animals showed a slight increase. This result is in agreement with the literature, according to which short-term dexamethasone administration induces increased pancreatic rat beta-cell proliferation (Rafacho *et al*., 2009, 2010, 2011)

In the adrenaline treated group the weight of the gland showed a significant reduction compared with the control as well as in the FC treated groups (Fig. 3). GC excess induced by different stressors causes decreased b-cell insulin production and

insulin resistance; furthermore, it reduced effectiveness of insulin in suppressing hepatic glucose production and in increasing glucose uptake in muscle and fatty tissue (Andrews and Walker, 1999). Persons who have a diminished b-cell function or are low-insulin responders are predisposed to develop diabetes during glucocorticoid excess (Henriksen *et al*., 1997; Wajngot *et al*., 1992).

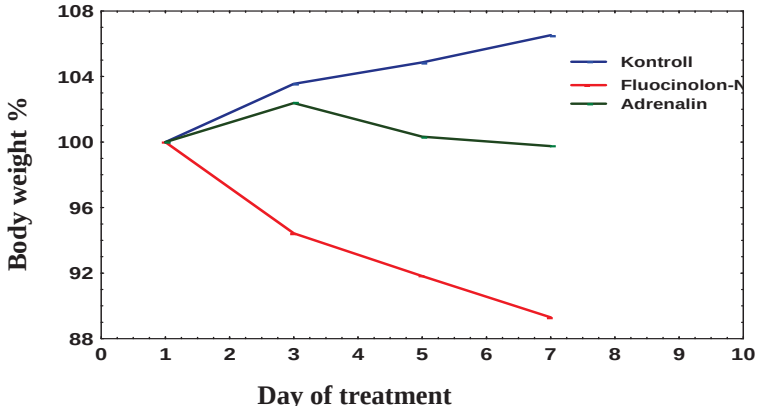
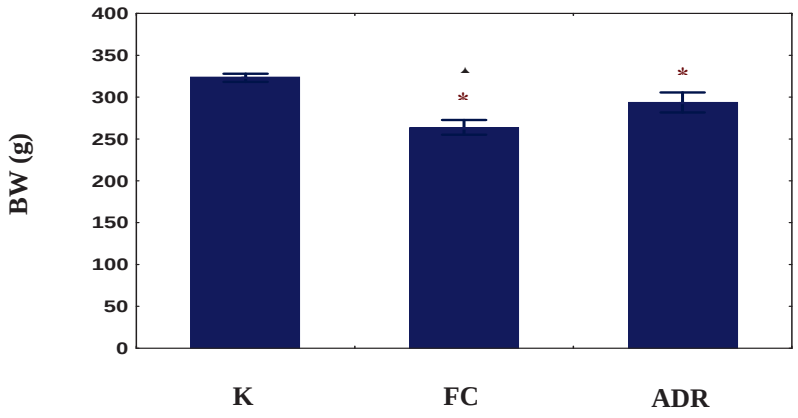
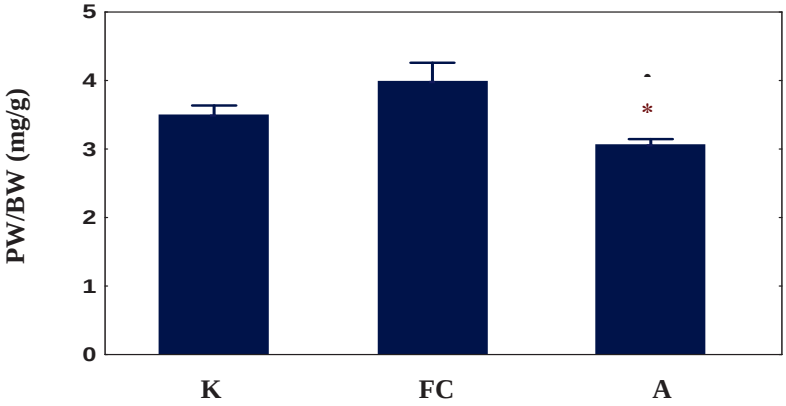


Figure 1. Evolution of the body weight.



* significant differences from the C-group ($p < 0.004$), ♠ significant differences from the ADR-group ($p<0.007$)

Figure 2. Body weight (BW) on the last day of treatment.



* significant differences from the C-group (p< 0.04), • significant differences from the FC group (p< 0.02)

Figure 3. Relative weight of the pancreas (PW).

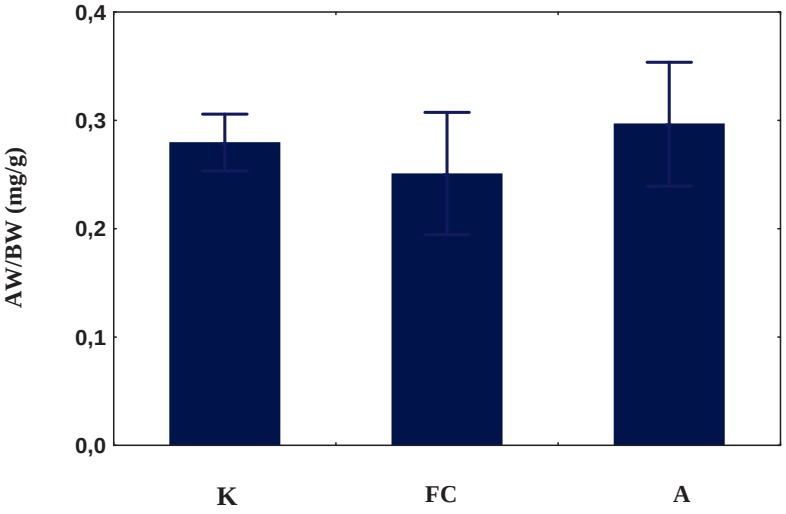
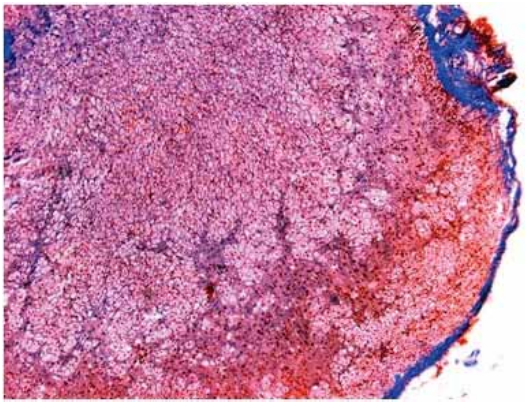


Figure 4. Relative weight of the adrenal gland (AW)

The relative weight of the adrenal gland in the FC group showed a slight decrease compared with the control group (Fig. 4). In the ADR treated group we observed an increase of the gland weight compared with the control one.

These are confirmed with the results of microscopical examination (Fig. 5).

a.



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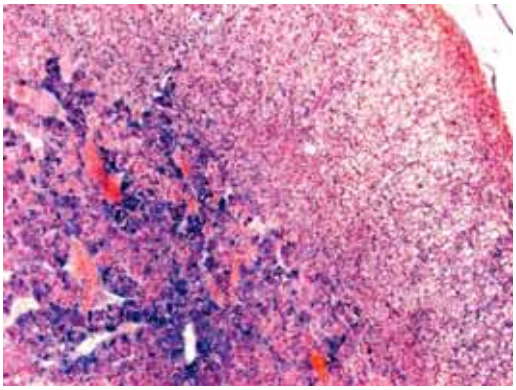


c.

Figure 5. Histological aspects of adrenal gland (20x).: a) normal aspect, b) hypertrophy of cortical zone in ADR stressed animals, c) atrophy of cortical zone in fluocinolone-treated rats

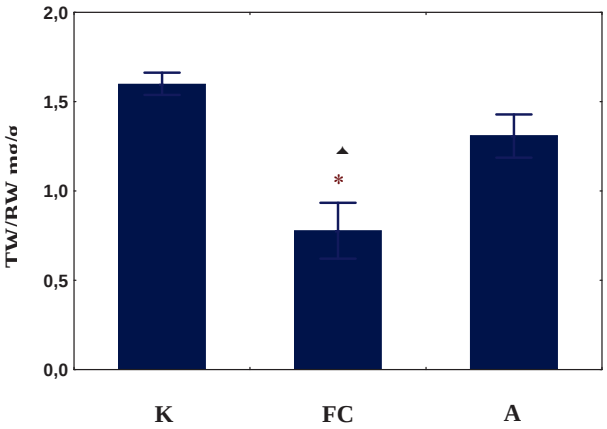
Results of our microscopical examination confirm these changes. The adrenals of animals treated with FC are atrofiated (Fig. 5b), whereas we observed hipertrophy of the gland cortex in animals treated with ADR drenaline (Fig. 5c).

Glucocorticoid administration results in a negative feedback effect via glucocorticoid receptors in the anterior hypothalamus, which, in turn, suppresses the production of corticotrophin-releasing hormone and the release of POMC/ACTH from adenohypophysis. The prolonged suppression of adrenocorticotropine levels leads to atrophy of the adrenal cortex and secondary adrenal insufficiency (Kis and Crăciun, 2003a, 2003b).

Glucocorticoids mediate these effects via both DNA binding-dependent and DNA binding-independent mechanisms (Schaöcke *et al.,* 2002, Anacker *et al.*, 2013).

The hypertrophy of the cortex under ADR treatment suggests increases of glucocorticoids synthesis. ADR treatment as a stressor leads to glucocorticoid secretion through the induction of sympathoadrenal system, which is followed by the activation of hypothalamo - pituitary - adrenal axis (Kis and Crăciun, 2003a, 2003b). Noradrenalin microinjection in NPV causes elevation in plasma corticosteron levels (Cole, 2002, Harris *et al.,* 2001, Leibowitz, 1998).

The relative thymus weight was significantly reduced in the FC group (Fig. 6). During ADR treatment we observed a slight decrease of the thymus weight.

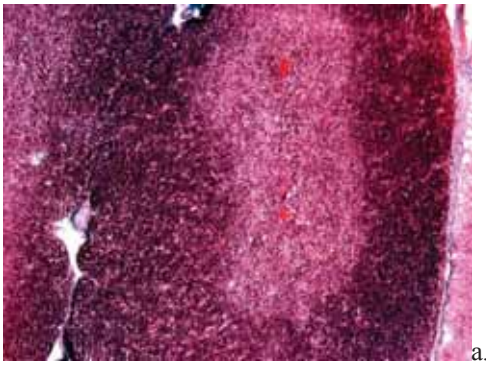


* significant differences from the K-group ($p < 0.02$), ♠ significant differences from the ADR-group ($p < 0.03$)

Figure 6. Relative weight of the thymus

This result is in agreement with the literature according to which stress-induced thymic involution is characterised by reduction in thymus size caused by acute loss of cortical thymocites and reduced output of native T cells to the periphery (Crăciun *et al.,* 1997, 1998, Wang *et al.*, 1994).

Microscopic studies confirm these results. FC treatment caused the atrophy of the thymus, while thymus structure in the animals treated with adrenaline did not differ from the control group (Fig. 7).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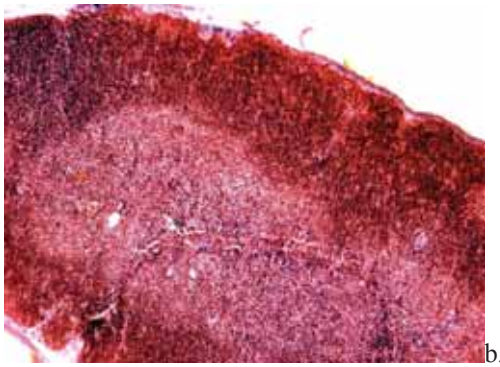
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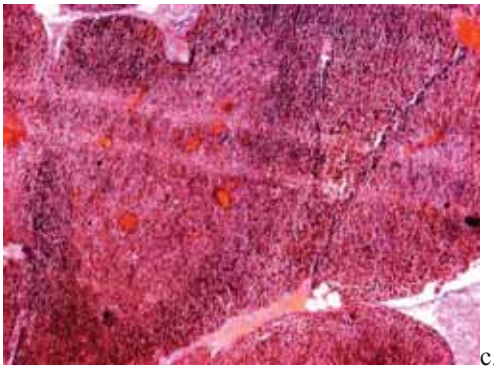
c.

Figure 7. Histological aspects of thymus (10x): a) normal structure, b) relatively normal aspect of thymus in ADR group, c) moderately alterated in the FC group, slightly rarefied cortical zone of the thymus lobules.

Acute stress-induced thymic atrophy is a complication of many environmental stressors as well in which transient reduction in thymus function persists until the physiological stressor is removed (Gruver and Sempowsky, 2008).

Conclusions

1. In summary, we can conclude that glucocorticoids can lead to tissue injury even at relatively low doses.
2. Milder organ atrophies were observed in the thymus and pancreas of the ADR treated group.
3. Severe adrenal hypertrophies were observed in the ADR treated group.
4. More severe atrophies were observed in the thymus and adrenals of the FC-treated group.
5. The response to elevated glucocorticoid levels was tissue dependent, thymus and adrenals being the most susceptible to injury.

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REFERENCES

Anacker, C., Cattaneo, A., Musaelyn, K., Zunszain, A.P., Horowitz, M., Motteni, R., Luoni, A., Calabrese, F., Tausey, K., Gemarelli, M., Thuret, S., Price, J., Uher, R., Riva, M. A., Parinate, M.C. (2013) Role for the kinase SGK1 in stress, depression, and glucocorticoid effects on hippocampal neurogenesis, *Proc. Natl. Acad. Sci*., **110 (21),** 8708–8713.

Andrews, R.C., Walker, B.R. (1999) Glucocorticoids and insulin resistance: old hormones, new targets. *Clin. Sci*., **96**, 513– 523.

Cole R.L, Sawchenko P.E. (2002) Neurotransmitter regulation of cellular activation and neuropeptide gene expession in paraventricular nucleus of the hypothalamus, *J. Neurosci*., **22(3):**959-969.

Crăciun, C., Madar, I., Tarba, C., Frăţilă, S., Ardelean, A., Crăciun, V., Ilyes, I. (1998) Correlation betwen ultrastructural thymus modification, thymolisis, thymus and blood-serum lipids in response to epicutaneously applied dermacorticosteroids in pubertal rats, in *Cur. Probl. Tech. Cel. Mol. Biol.,* Crăciun, C., Ardelean, A., (eds.), Risoprint, Cluj-Napoca, 218-235.

Crăciun, C., Ardelean, A., Madar, I., Tarba, C., Şildan, N., Crăciun, V., Fărcaş,T. (1997) Ultrastructural studies of the secondary effets induced at the level of thymus by topic application of Fluocinolon-acetonid-N in prepubertal rats, in *Cur. Probl. Tech. Cel. Mol. Biol.,* Crăciun, C., Ardelean, A., (eds.), Mirton, Timişoara, 176-186.

Gruver, L.A., Sempowsky G.D. (2008) Cytokines, leptin, and stress-induced thymic atrophy, *J. Leukoc. Biol*., **84,** 915–923.

Gruver-Yates, L.A., Cidlowski, J.A. (2013) Tissue-specific actions of glucocorticoids on apoptosis: A double-edged sword, *Cells*, **2**, 202-223.

Harris, R.B.S., Tiffany D.M., Simpson, J., Redmann, S.M., Youngblood, B.D., Ryan, D.H. (2002) Weight loss in rats exposed to repeated acute restraint stress is independent of energy or leptin status. *Am. J. Physiol. Regulatory Integrative Comp. Physiol.*, **282**, R77–R88.

Henriksen, J.E., Alford, F., Ward, G.M., Beck-Nielsen, H. (1997) Risk and mechanism of dexamethasone-induced deterioration of glucose tolerance in non-diabetic first-degree relatives of NIDDM patients, *Diabetologia*, **40,** 1439–1448.

Kis, E. (2010) Fluocinolon therapy induced oxidative stress effects on pregnant and neonatal rat thymus, In *Annals of RSCB*, Ardelean, A., Crăciun, C. (eds.) Ed. Risoprint **15 (2),** 130-134.

Kis, E. (2012a) Fluocinolon therapy induced oxidative stress on pregnant rat adrenal, pancreas and liver, In *Annals of RSCB*, Ardelean, A., Crăciun, C. (eds.) Ed. Risoprint, **17 (1),** 149-153.

Kis, E. (2012b) Glucocorticoid excess and fetal development in white Wistar rats. In *Annals of RSCB*, Ardelean, A., Crăciun, C. (eds.) Ed. Risoprint, **17 (2),** 82-85.

Kis, E., Crăciun, C. (2003a) Atenuarea modificărilor structurale şi metabolice induse de excesul glucocorticoidic prin administrarea de propranolol, *Studia UBB, Biologia*, **48 (1),** 67-79.

Kis, E., Crăciun, C. (2003b) Atenuarea modificărilor gravimetrice induse de tratamentul cu Fluocinolon- acetonid N prin administrarea de propranolol, *Studia UBB, Biologia,,* **48 (2),** 83-88.

Kis, E., Crăciun C. (2006) *Efecte secundare ale unor glucocoticoizi topici*, Ed. Risoprint, Cluj-Napoca.

Kis, E., Crăciun, C., Paşca, C., Sandu, V.D., Crăciun, V., Madar, I. (2001a) Comparative studies of the ulrastructure of somatotrope, gonadotrope and corticotrope cells in mature rats treated with topical dermocorticoids, *Studia UBB, Biologia*, **56(1),** 99-110.

Kis, E., Sandu, V.D., Paşca, C., Crăciun, C. (2001b) Efectul antiglucocorticoid al propranololului, *Studia UBB, Biologia*, **56 (2),** 83-93.

Landfield, P.W., Eldridge I.C. (1994) The glucocorticoid hyphotesis of age related hippocampal neurodegeneration: role of dysregulated neuronal calcium, *Ann. NY Acad. Sci.*, **746,** 308-321.

Leibowitz, S.F., Diaz, S., Tempel, D. (1998) Norepinephrine in the paraventricular nucleus stimulates corticosterone release, *Brain Res.*, **496,** 219-227.

Madar, I., Şildan, N., Frecuş, G. (1993) Studiul comparativ al efectului stresului şi tratamentului cu fluocinolon-acetonid asupra unor parametri endocrino-metabolici la şobolani Wistar tineri, *St. şi cerc. biol., Seria Biol. Anim*., **t. 45, 1,** 53-59.

Mureşan, E., Gaboreanu, M., Bogdan, A.T., Baba, A.I. (1974) *Tehnici de histologie normală şi patologică*, Ed. Ceres, Bucureşti.

Noguchi K.K. (2014) Glucocorticoid induced cerebellar toxicity in the developing neonate: implications for glucocorticoid therapy during bronchopulmonary dysplasia, *Cells,* **3,** 36-52.

O'Connor, T.M., O'Halloran, D.J., Shanan, F. (2000) The stress response and the hypothalamic-pituitary adrenal axis: from molecule to melancholia, *Q.J.Med.,* **93,** 323-333.

Rafacho A., Marroquí L., Taboga S.R., Abrantes J.L., Silveira L.R., Boschero A.C., Carneiro E.M., Bosqueiro J.R., Nadal A., Quesada I. (2010) Glucocorticoids in vivo induce both insulin hypersecretion and enhanced glucose sensitivity of stimulus-secretion coupling in isolated rat islets, *Endocrinol.*, **151,** 85 – 95.

Rafacho, A., Cestari T.M., Taboga, S.R., Boschero A.C., Bosqueiro J.R. (2009) High doses of dexamethasone induce increased beta-cell proliferation in pancreatic rat islets, *Am. J .Physiol. Endocrinol. Metab*, **296 ,** E681 – E689.

Rafacho, A., Abrantes J.L.F., Ribeiro, D.L., Paula, F.M., Pinto, M.E., Boschero, A.C., Bosqueiro, J.R. (2011) Morphofunctional alterations in endocrine pancreas of short- and long-term dexamethasone-treated Rats, *Horm. Metab. Res.,* **43,** 275 – 281.

Sapolsky R.M., Romero L.M., Munck A.U. (2000) How do glucocorticoids influence stress responses? Integraing, permissive, supressive, stimulatory and preparative actions, *Endocrine Reviews,* **21(1),** 55-89.

Viegas, L.R., Hoijmana, E., Beatod, M., Pecci, A. (2008) Mechanisms involved in tissue-specific apopotosis regulated by glucocorticoids, *J. Steroid Biochem. & Mol. Biol.*, **109,** 273–278.

Wajngot, A., Giacca, A., Grill, V., Vranic, M., Efendic, S. (1992) The diabetogenic effects of glucocorticoids are more pronounced in low- than in high-insulin responders, *Proc. Natl. Acad. Sci. USA.,* **89,** 6035–6039.

Wang, S.D., Huang, K.J., Lin, Y.S., Lei, H.Y. (1994) Sepsis-induced apoptosis of the thymocytes in mice, *J. Immunol.,* **152,** 5014 –5021.