Arch. Biol. Sci., Belgrade, 58 (3), 161-164, 2006.

EFFECT OF COLD AND HEAT STRESS ON RAT ADRENAL, SERUM AND LIVER ASCORBIC ACID CONCENTRATION

JELENA DJORDJEVIĆ, S. DJURAŠEVIĆ, TAMARA VUČKOVIĆ, N. JASNIĆ, and GORDANA CVIJIĆ

Institute of Physiology and Biochemistry, Faculty of Biology, University of Belgrade, 11000 Belgrade, Serbia

Abstract - Changes in ascorbic acid (AA) concentration were examined in the adrenals, serum and liver of Wistar rats exposed to cold (6 ºC) and heat stress (38 ºC) for 60 min. The exposure of animals to cold stress for 60 min did not change concentration of AA in the serum, adrenals and liver as compared to controls maintained at room temperature. After a 60 min heat exposure the concentration of AA in the adrenals decreased (***$p<0.001$), in the liver remained unchanged whereas it significantly increased in the serum (***$p<0.001$) in respect to control values.

Key words*:* Adrenals, ascorbic acid, cold stress; heat stress, liver, serum.

UDC 616:59

INTRODUCTION

Body tissues differ in the ascorbic acid (AA) concentrations. However, tissues of high metabolic need, such as the corpus luteum in the female, the testis in the male and the adrenal glands, generally have higher concentrations of this vitamin. Knockout genes preventing AA synthesis in pigs have demonstrated a poor skeletal and collagen formation and poor antioxidant protection (M a h a n *et al.*, 2004). Ascorbic acid is thought to have a role in the regulation of corticosteroid synthesis in the adrenal glands (F i n n and J o h n s , 1980). The data obtained with bovine adrenocortical cells *in vitro* favor its role as an antioxidant that especially regulates aldosterone synthesis, possibly by protecting the cytochrome $P450_{11\beta}$ from lipid peroxidation. Vitamin C may also act as a part of an auxiliary electron transport system for the last step of aldosterone synthesis (R e d m a n n *et al.*, 1995; B a h r *et al.*, 1996). According to B j o r k h e m *et al.* (1978) the adrenal ascorbate might be of some importance for the capacity to convert cholesterol into pregnenolone. Besides, stress and the associated rise in ACTH may reduce the level of AA in pigs adrenals, but the animals rapidly recovers to its resting state once the stressor agent is removed (M a h a n *et al.*, 2004). A r a d *et al.* (1985) found a significant increase in AA level in plasma of rats and rabbits after hypoxia and the following asphyxia. The observed changes in plasma AA levels are obviously mediated through ACTH and the adrenal gland is the major source of ascorbate efflux into the circulation during oxygen deprivation.

Bearing in mind the facts mentioned above, we examined the ascorbic acid turnover e.g. the concentration of AA in the serum, adrenals and liver of rats exposed to physical – environmental stressors, cold and heat.

MATERIAL AND METHODS

The male rats of Wistar strain (*Rattus norvegicus*), 60-90 days old, weighing 180-220 g, were used for the experiments. The animals were acclimated to 22 ±1ºC, kept at a 12:12h light-dark cycle and given commercial rat food and tap water *ad libitum*. The rats were divided into three groups, each consisting of six animals. The first group represented intact controls. The rats from the second and third group were exposed to the extreme ambient temperatures 6 ºC for 60 min and 38 ºC for 60 min, respectively, in temperature controlled chambers. The animals were decapitated immediately after the stress exposure. Blood was collected from the trunk and serum frozen for ascorbate determination, while the adrenals and liver were quickly excised at +4 °C, weighed and stored at -20 °C prior to AA concentration measurement. The AA was determined by the method of Roe and K u e t h e r (1943) based on the oxidation of ascorbic acid to

dehydroascorbic acid (DHAA) which is irreversibly transformed to 2,3-diketogulonic acid (DKA). DKA is coupled with 2,4-dinitrophenylhydrazine and the resulting derivative is treated with H_2SO_4 to produce color which is measured on LKB Micro Plate Reader, on 540 nm.

The values are expressed as μg/mg tissue (the adrenals or liver) or mg/l serum. Anova one way test was used to detect the differences among the groups. The values are presented as means ± S.E. of six animals and the level of significance was set at $p<0.05$.

RESULTS

The exposure of the animals to cold stress for 60 min did not change concentration of the ascorbic acid in the serum, adrenals and liver as compared to the controls maintained at room temperature. After a 60 min heat exposure the concentration of ascorbic acid in the adrenals decreased (***$p<0.001$), in the liver remained unchanged, whereas it significantly increased in the serum (***$p<0.001$), in respect to control values.

DISCUSSION

Stress is always accompanied with an increased ascorbate demand because of its increased consumption and/or decreased synthesis and uptake (B a n h e g y i *et al.*, 1998). While species such as rodents produce ascorbic acid via hepatic synthesis (G r o l l m a n and L e h n i n g e r, 1957), primates, guinea pigs and fruit-eating bats must acquire this vitamin through dietary intake as they lack L-gulonolactone oxidase, the terminal enzyme in the L-ascorbic acid biosynthetic pathway. These animals survive because membrane transport processes allow dietary ascorbate to be absorbed as chime moves through the small intestine (R o s e , 1991). Regardless of its acquisition route, the ascorbic acid is accumulated in adrenocortical cells by membrane transporters against a large concentration gradient even in the animals in which ascorbate is not a dietary requirement as synthesis occurs only in the liver (P a l l u d a n and W e g g e r, 1984).

Our present results show that exposure of rats to extreme environmental temperature of 38 ºC for 60 min caused a significant decrease in the adrenal ascorbate concentration accompanied by its increase in circulation. Obviously, the source of the ascorbate efflux into the cir-

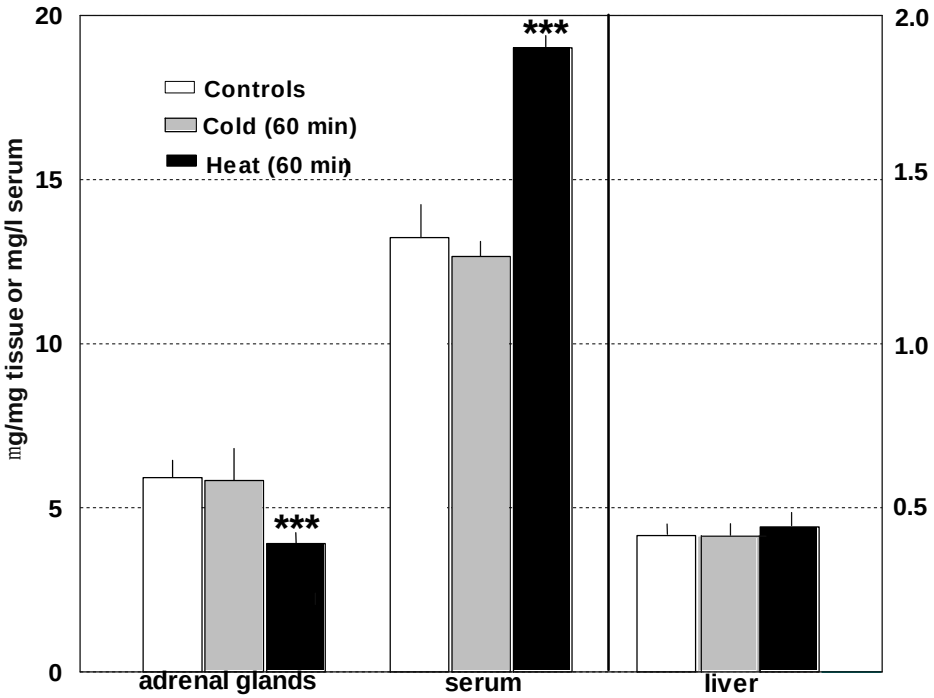

Fig 1. The effect of 60 min exposure of rats to cold (6 ºC) and heat (38 ºC) stress on ascorbate content of liver, serum and adrenal gland. The values represent the means ± SE of six animals and are expressed in μg/mg tissue (the adrenals or liver) or mg/l serum; ***$p<0.001$.

culation are adrenals since there were no changes in AA content of the liver, which is the site of AA synthesis in rats. Besides, A r a d *et al.* (1985) showed that adrenalectomy performed 24h before exposure of rats to stress abolished the rise of AA in blood. Our findings are in agreement with S a y e r s and S a y e r s (1972) who have shown the AA concentration decrement under stress when adrenal cortical hormone activity is high. The ascorbic acid is involved in the process of steroidogenesis since the adrenal cortex contains high levels of vitamin C which are depleted upon adrenocorticotropic hormone (ACTH) stimulation of the gland (L a h i r i and L l o y d , 1962; L a n e y *et al.*, 1990). However, some data suggesting that differing plasma concentrations of AA regulate *in vivo* steroidogenesis by altering the activity of the membrane-bound enzyme adenylate cyclase (D o u l a s *et al.*, 1987).

In spite of the fact that heat stress affected changes of AA in serum and adrenals, the same duration of cold exposure did not alter the concentration of AA in the liver, serum and adrenals. It is not surprising since we have already shown that heat is the stronger stressor judging by the fact that it provokes a six fold greater plasma ACTH increment as compared to cold stress (D j o r d j e v i ć *et al.*, 2003).

In conclusion, present results demonstrate that the exposure of rats to heat stress caused the ascorbic acid turnover e.g. the increase in the amount of ascorbate in blood due to the depletion of adrenals content, without *de novo* hepatic synthesis and support previous findings that heat is the stronger stressor in comparison to cold.

Acknowledgements - This paper is supported by the Serbian Ministry of Science and Environmental Protection, Grant No 143050. Experiments are performed with assistance of the 4th grade students of Molecular Biology and Physiology, Faculty of Biology, generations 2004/2005 and 2005/2006.

Authors are grateful to Ms. Jelena Brońić for language editing of the manuscript.

REFERENCES

Arad, .I, Sidi, A., and *Shohami, E.* (1985). Effect of acute hypoxia on ascorbate content of plasma, cerebral cortex and adrenal gland. *J. Neurochem.* **45**, 766-769.

Bánhegyi, G., Braun, L., Csala, M., Puskás, F., Somogyi, A., Kardon, T., and *Mandl, J.* (1998). Ascorbate and Environmental Stress. *Ann. N.Y. Acad. Sci.* **851**: 292-303.

Bahr, V., Mobius, K., Redmann, A., and *Oelkers, W.* (1996). Ascorbate and alpha-tocopherol depletion inhibit aldosterone stimulation by sodium deficiency in the guinea pig. *Endocr. Res.* **22**:595-600.

Bjorkhem, I, Kallner, A., and *Karlmar, K.E.* (1978). Effects of ascorbic acid deficiency on adrenal mitochondrial hydroxylations in guinea pigs. *J. Lipid Res.* **19**:695-704.

Djordjevic, J., Cvijic, G., and *Davidovic, V.* (2003). Different activation of ACTH and corticosterone release in response to various stressors in rats. *Phys. Res.* **52**: 67-72.

Doulas N.L., Constantopoulos, A. and *Litsios, B.* (1987). Effect of ascorbic acid on guinea pig adrenal adenylate cyclase activity and plasma cortisol. *J. Nutr.* **117**:1108-1114.

Finn, F.M., and *Johns, P.A.* (1980). Ascorbic acid transport by isolated bovine adrenal cortical cells. *Endocrinology*, **106**:811-817.

Grollman, A.P., and *Lehninger, A.L.* (1957). Enzymic synthesis of L-ascorbic acid in different animal species. *Arch. Biochem. Biophys.* **69**:458–467.

Lahiri, S., and *Lloyd, B.B.* (1962). The efect of stress and corticotrophin on the concentration of Vitamin C in blood and tissues of the rat. *Biochem. J.* **84**:478-483.

Laney, P.H. Levy, J.A., and *Kipp, D.E.* (1990). Plasma cortisol and adrenal ascorbic acid levels after ACTH treatment with a high intake of ascorbic acid in the guinea pig. *Ann. Nutr. Metab.* **4**:85-92.

Mahan, D.C., Ching, S., and *Dabrowski, K.* (2004). Developmental aspects and factors influencing the synthesis and status of ascorbic acid in the pig. *Ann. Rev. Nutr.* **24**: 79-103.

Palludan, B., and *Wegger, I.* (1984). Plasma ascorbic acid in calves. In: *Ascorbic Acid in Domestic Animals*. (Eds. I. Wegger, F. Tagwerker and J. Moustgaard), 131-138. Royal Danish Agricultural Society, Copenhagen, Denmark.

Redmann, A., Mobius, K., Hiller, H.H., Oelkers, W., and *Bahr, V.* (1995). Ascorbate depletion prevents aldosterone stimulation by sodium deficiency in the guinea pig. *Eur. J. Endocrinol.* **133**:499-506.

Roe, J.H., and *Kuether, C.A.* (1943). The determination of ascorbic acid in whole blood and urine through the 2,4-dinitrophenylhydrazine derivate of dehydroascorbic acid. *J. Biol. Chem.* **147**:399-407.

Rose, R.C. (1991). Intestinal transport of water solubile vitamins. In: *Handbook of physiology: The gastrointestinal system.* Vol IV, section 6 (Ed S.G.Shultz), 421-436. American Physiology Society, Bethesda.

Sayers, G., and *Sayers, M. A.* (1947). Regulation of pituitary adrenocorticotropic activity during the response of rat to acute stress. *Endocrinology*, **40**:265-273.

ЕФЕКАТ СТРЕСА ХЛАДНОЋЕ И ТОПЛОТЕ НА КОНЦЕНТРАЦИЈУ АСКОРБИНСКЕ КИСЕЛИНЕ У НАДБУБРЕЖНИМ ЖЛЕЗДАМА, СЕРУМУ И ЈЕТРИ ПАЦОВА

ЈЕЛЕНА ЂОРЂЕВИЋ, С. ЂУРАШЕВИЋ, ТАМАРА ВУЧКОВИЋ, Н. ЈАСНИЋ и ГОРДАНА ЦВИЈИЋ.

Институт за физиологију и биохемију, Биолошки факултет, Универзитет у Београду, 11000 Београд, Србија

У овом раду испитиван је промет, односно концентрација аскорбинске киселине у надбубрежним жлездама, серуму и јетри пацова излаганих стресу снижене (6 ºC) и повишене (38 ºC) температуре у трајању од 60 мин. Добијени резултати показују да је стрес повишене температуре довео до пражњења депоа аскорбинске киселине у надбубрежним жлездама и њеног повећања у циркулацији, за разлику од стреса хладноће који није утицао на промену концентрације аскорбинске киселине ни у једном од испитиваних ткива.