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Investigation of Some Antioxidant Enzymes Activities Depending on Adrenomedullin Treatment and Cold Stress in Rat Liver Tissue*

[Soğuk Stresi ve Adrenomedullin Uygulamasına Bağlı Olarak Sıçan Karaciğer Dokusunda Bazı Antioksidan Enzim Aktivitelerinin Araştırılması]

Nuran Cikcikoğlu Yıldırım[1],
Muhittin Yürekli[2],
Numan Yıldırım [1]

[1] Department of Environmental Engineering, Faculty of Engineering, Tunceli University, Tunceli, Turkey

[2] Science Education, Amasya University, Amasya, Turkey

Yazışma Adresi
[Correspondence Address]

Yrd.Doç.Dr. Nuran CIKCIKOĞLU YILDIRIM

Department of Environmental Engineering, Faculty of Engineering, Tunceli University, Tunceli, Turkey
Telephone
e-mail: nurancyildirim@tunceli.edu.tr

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ABSTRACT

Purpose: The aim of this study is to investigate the compensating effects of adrenomedullin on liver tissue after cold stress treatment.
Materials and Methods: Male Wistar rats were divided into four groups as control, adrenomedullin, cold stress and cold stress + adrenomedullin treated. Adrenomedullin was given by intraperitoneal injection (2 mg/g body weight) once a day for a week. For cold stress the rats were kept at 10°C for a week. Catalase, glutathione peroxidase and superoxide dismutase activities were measured in liver tissue.
Results: Catalase activity was decreased significantly as compared to control in adrenomedullin treated group (p<0.05). There was no significant difference in superoxide dismutase activity (p>0.05). Glutathione peroxidase activity was increased in adrenomedullin treated group but this was statistically insignificant (p>0.05). All enzyme activities were decreased by cold stress (p<0.05). In Adrenomedullin+cold stress group, only the glutathione peroxidase activity was significantly decreased (p<0.05).
Conclusion: It is concluded that cold stress induces decrease in catalase, superoxide dismutase and glutathione peroxidase activities in rat liver. Adrenomedullin treatment corrected only glutathione peroxidase activity while decreasing catalase and superoxide dismutase activities further. The discrepancy may be explained by different responses of antioxidant systems to oxidative stress. It can be concluded that oxidative factors may display unexpected effects on the regulation of glutathione peroxidase activities in the adrenonmedullin case.
Key words: adrenomedullin, cold stress, antioxidant enzymes, liver

ÖZET

Amaç: Bu çalışmanın amacı soğuk stresi uygulaması sonrası karaciğer dokusu üzerine Adrenomedullin'in telafi edici etkisinin araştırılmasıdır.
Materyal ve Metod: Erkek Wistar sıçanları kontrol, adrenomedullin, soğuk stresi, soğuk stresi+adrenomedullin uygulama grubu olarak dörde ayrılmıştır. Adrenomedullin bir hafta boyunca günde bir kez intraperitoneal enjeksiyon yolu ile verilmiştir. Soğuk stresi için sıçanlar bir hafta boyunca 10ºC'de muhafaza edilmiştir. Katalaz, glutatyon peroksidaz ve süperoksit dismutaz aktiviteleri karaciğer dokusunda ölçülmüştür.
Bulgular: Kontrolle karşılaştırıldığında, katalaz aktivitesi, adrenomedullin uygulama grubunda önemli düzeyde azalmıştır (p<0.05). Süperoksit dismutaz aktivitesinde önemli bir fark yoktur (p>0.05). Glutatyon peroksidaz aktivitesi Adrenomedullin uygulama grubunda artmıştır ama bu istatistiksel olarak önemsizdir (p>0.05). Bütün enzim aktiviteleri soğuk stresle azalmıştır (p<0.05). Adrenomedullin+soğuk stres grubunda, sadece glutatyon peroksidaz aktivitesi önemli düzeyde azalmıştır (p<0.05).
Sonuç: Soğuk stresin sıçan karaciğerinde, katalaz, süperoksit dismutaz ve glutatyon peroksidaz aktivitesinde azalmayı indüklediği sonucuna varılmıştır. Adrenomedullin uygulaması süperoksit dismutaz ve katalaz aktivitesini daha çok azaltırken, sadece glutatyon peroksidaz aktivitesini düzeltir. Bu uyuşmazlıklar, antioksidan sistemin oksidatif strese karşı farklı yanıtlarıyla açıklanabilir. Adrenomedullin söz konusu olduğunda, oksidatif faktörlerin glutatyon peroksidaz aktivitesi üzerinde beklenmeyen etkiler oluşturabileceği sonucuna varılabilir.
Anahtar Kelimeler: adrenomedullin, soğuk stresi, antioksidan enzimler, karaciğer

Introduction

The stress system coordinates the adaptive response of the organism to real or perceived stressors (1). Activation of the stress system leads to behavioral and peripheral changes to improve the ability of the organism to adjust homeostasis and increase its chances for survival (2). The physiological components of stress response to cold are metabolic, circulatory and hormonal (3). Long-term cold exposure increases in mitochondrial volume density, capillary diameter, aerobic enzyme activity and tissue oxygen consumption (4).

Stressful conditions leads to the formation of excessive free radicals which are major internal threat to cellular homeostasis of aerobic organisms. Envionmental stress has been demonstrated to cause an increase in the oxidative stress, an imbalance in the antioxidant status. The elevation of endogenous corticosterone due to the stress response has been reported to accelerate the generation of free radicals. Free radicals inhibited the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GP_X). This might be attributed to the utilization of these antioxidants to alleviate free radical induced oxidative stress (5).

Mammalian cells have developed antioxidant defense systems to prevent oxidative damage and to allow survival in an aerobic environment. These systems consist of nonenzymatic antioxidants with low molecular weights (vitamins A and E, betacarotene, uric acid) and of enzymes such as SOD, CAT, GPx, and glutathione reductase (GR) (6). Cells can respond to oxidants with CAT, SOD and GPX enzymes (5).

Adrenomedullin (AdM) is a 52-amino acid vasodilating and natriuretic peptide, originally isolated in human pheochromocytoma (7). AdM, a potent vasodilator peptide having a wide range of biological actions such as reduction of oxidative stress and inhibition of endothelial cell apoptosis, was originally isolated from pheochromocytoma cells (8,9).

Through its antioxidative effect it can protect organs from damage induced by high blood pressure, ischemia and aging (9). The antioxidative properties of AdM have been reported recently. Through its antioxidative effect, AdM can protect organs from damage induced by stressors (10). It is suggested that endogenous AdM possesses a protective action against the vascular response to injury, possibly through the inhibition of oxidative stress production. (11). AdM directly inhibits oxidative stress so that AdM might be a negative feedback substance against reactive oxygen species (ROS) -induced organ damages. (12).

The main goal of the present study was to investigate the effects of AdM administration on some antioxidant enzyme activities in rat liver exposured to cold stress. The activities of SOD, CAT and, GPx were determined in liver of Wistar rats.

Materials and Methods

Treatment of Animals

Rats were obtained from Experimental Animal Research Facility, Inonu University. Twenty four male Wistar albino rats (8 months old, 190–240 g) were housed individually under diurnal lighting conditions (12–12 h) with free access to drinking water and a standard pellet diet. The environment in the animal rooms was maintained at a temperature of 22 ± 2 °C, relative humidity of 45 ± 5%. The rats were divided into the following four experimental groups: control group (n = 6), adrenomedullin group (AdM) (n = 6), cold stress group (n = 6) and cold stress+AdM group (AdM+ cold stress) (n = 6). The AdM-treated groups received an i.p. injection of AdM (2 mg/g body weight) for 1 week. In cold stress treatment group, animals were exposed +10 °C cold during a week. In cold stress + AdM treatment groups, both animals were exposed +10 °C cold during a week and were given AdM intraperitoneally at a single dose of 2 mg/g body weight for a week.

Experimental Design

At the end of the experiment, the rats were anaesthetized by intraperitoneal injection of ketamine/xylazine hydrochloride (20/2 mg kg−1 b.w.) and the liver was removed immediately. Liver tissue was homogenized (PCV Kinematica Status Homogenizator) in ice-cold phosphate buffered saline (pH 7.4). The homogenate was sonified with an ultrasonifier (Bronson sonifier 450) by six cycles (20 s sonications and 40 s pause on ice). The homogenate was centrifuged (15 000 *g*, 10 min, 4 °C) and the supernatant was subjected to enzyme assays immediately.

The animal experiments were performed in accordance with the guidelines for animal research from the National Institute of Health and were approved by the Committee of Animal research at Inonu University, Malatya, Turkey (2007/51).

Enzyme Assays

The activity of CAT was determined spectrophotometrically. CAT activity was measured at 37 °C by following the rate of disappearance of hydrogen peroxide (H_2O_2) at 240 nm ($\varepsilon 240 = 40\ M^{-1}\ cm^{-1}$) (13). One unit of catalase activity is defined as the amount of enzyme catalysing the degradation of 1 μmol of H_2O_2 per min at 37 °C and the specific activity corresponding to transformation of substrate (in μmol) (H_2O_2) per min per mg protein.

The activity of SOD was determined by using The Cayman Chemical Superoxide Dismutase Assay kit (catalog No. 706002). The Cayman Chemical Superoxide Dismutase Assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

The activity of GP_X was determined by using The Cayman Chemical Glutathione Peroxidase Assay Kit (catalog No. 703102). GPx activity was determined in a coupled assay with glutathione reductase by measuring the rate of NADPH oxidation at 340 nm using H_2O_2 as the substrate.

Statistical analysis

SPSS 13.0 statistical software package program was used (SPSS Inc., Chicago, IL, USA). Kruskal-Wallis H test was applied for statistical comparison of groups, followed by analysis with Bonferroni-corrected Mann-Whitney test to analyse the different groups. Probability values of 0.05 or less were considered statistically significant. The results were presented as median and min-max values.

Results

The liver CAT enzyme activity was significantly decreased in AdM treated group when compared to control (p<0.05) (Table 1). CAT enzyme activity was reduced depending on cold stress in liver tissue when compared to control (p<0.05). In AdM + cold stress group, CAT enzyme activity was decreased when compared to control but this decrease was statistically insignificant (p>0.05).

In all groups, there was no differences found statistically in liver SOD enzyme activity when compared to control (p>0.05) (Table 1). SOD enzyme activity was decreased depending on cold stress in liver tissue when compared to control. In AdM + cold stress group SOD enzyme activity was decreased when compared to control.

Liver GP_X enzyme activity was increased in AdM treated group when compared to control this increase was statistically insignificant (p>0.05) (Table 1). GP_X enzyme activity was decreased depending on cold stress in liver tissue when compared to control (p<0.05) (Table 1). In AdM + cold stress group GP_X enzyme activity was decreased when compared to control (p<0.05). The present study reveals antioxidant property of AdM against the cold stress.

Discussion

In this study we have aimed to investigate the compensating effects of AdM on some antioxidant enzymes in liver tissue. For this purpose, the activities of antioxidant enzymes such as CAT, SOD and GPx, were determined in liver. To our knowledge there is no previous study about the effect of AdM on the oxidative damage induced by cold stress.

Previous study have implicated the effects of cold stress on antioxidant enzyme activities and it was determined that cold stress can disrupt the balance in an oxidant/antioxidant system and cause oxidative damage to several tissues by altering the enzymatic and non-enzymatic antioxidant status, protein oxidation and lipid peroxidation (2).

The endogenous antioxidant system may counteract the ROS and reduce the oxidative stress with the enzymatic antioxidants SOD, CAT, GP_X and SOD accelerates the conversion of superoxide to hydrogen peroxide while CAT or GP_X hydrogen peroxide to water (14). Depletion in the activation of enzymatic or nonenzymatic can

Table 1. The effect of AdM treaetment and cold stress on CAT, SOD and, GSH-Px activity in rat liver tissue

Groups	CAT (U/mg prot.) Median (min-max)	SOD (Unit) Median (min-max)	GSH-Px (Unit) Median (min-max)
Control	297,22 (291,66-302,77)	30,14 (28,41-40,71)	135 (134,90-140,70)
AdM	270[a] (169,44-281,48)	36,72[a] (26,14-36,72)	131,1[c] (126-173)
Stress	226,85[c] (214,25-230,18)	29,15[a] (25,45-35,13)	120,9[a] (105-142,6)
Stress+AdM	253,88[b] (245,55-310,92)	31,09[a] (30,14-33,93)	108[b] (97-120,90)
#KW - P	15.53 - 0.001*	2.23 - 0.525 (NS)	16.59 - 0.001*

Results were presented as median and min-max values. for six replicate experiments, #KW (Chi-Square): Kruskal Wallis, *Result is significant according to the non-parametric Kruskal–Wallis analysis of variance p<0.05, NS= No significant.

CAT activity; [a]p<0.05 compared to control group, [b]p<0.05 compared to stress group, [c]p<0.05 compared to control group by using Mann-Whitney U Test

GSH-Px activity; [a]p<0.05 compared to control group, [b]p<0.05 compared to stress and control group, [c]p<0.05 compared to stress+AdM group by using Mann-Whitney U Test

be owed to an enhanced radical production during stress conditions. Addition to this physiological response to stress is to activation of hypothalamic-pituitary-adrenal axis and subsequently release of corticosteron from the adrenal cortex into the blood stream (15). The elevation of endogeneous corticosterone due to the stress response has been reported to accelerate the generation of free radicals (16). In the present study confirmed the fact that the free radicals inhibited the activities of SOD, CAT and GP_X.

Endogenous AdM has been shown to possess a protective action against the cardiovascular damage and vascular response to injury possibly through the inhibition of oxidative stress production in mice (11, 17). The antioxidant effect of adrenomedullin on angiotensin II-induced reactive oxygen species generation in vascular smooth muscle cells was investigated and suggested that AdM plays a protective role as an endogenous antioxidant in Ang II-induced vascular injury (18). It was suggested that AdM might have the endogenous antioxidant potential to protect against ROS-induced podocyte injury (19). The effect of AdM on rats exposed to lead was investigated and found that AdM may have protective or compensating effects in lead toxicity (10).

Exogenous administration of AdM or its gene delivery has been reported to protect tissues and cells from multiple types of damage both in vitro and in vivo (20).

It was suggest that AdM participates in the regulation of cellular redox status via reduced glutathione (GSH) synthesis. They have reported increased GSH levels after 4 hours of AdM treatment. GSH is the most abundant low molecular weight thiol found in mammalian cells and it participates in scavenging of free radicals (21).

In conclusion, this study examined whether AdM has compensating effect on some antioxidant enzymes in liver tissue. In our study, CAT activity was increased in stres +AdM group compared to AdM or stress group. This result indicated that AdM has compensating effect on CAT enzyme activity. AdM caused a significant increase in GP_X enzyme activity and a decrease catalase enzyme activities when compared to control but no differences was found statistically in SOD enzyme activity. It was also found that CAT, SOD and GP_X enzyme activities decreased with cold stress in rat liver tissue. When the results are taken together it can be suggested that AdM might have the endogenous antioxidant potential to protect against ROS and compensating effects on some antioxidant enzymes like CAT, SOD and GP_X enzymes in liver tissue. Moreover, further detailed studies are required to understand the molecular mechanisms underlying the benefical effects of AdM and to explore the optimum dosage and duration of AdM treatment to obtain better results in cold stress condition. There is some controversy between SOD-CAT and GPx activities in the present study. The discrepancy may be explained by different responses of anti oxidant systems to oxidative stress. Superoxide dismutase, glutathione peroxidase and catalase form the first-line of defense against ROS in affected tissues. SOD converts superoxide anion to H_2O_2, which is then detoxified to water by CAT. There is general agreement, that there are alterations of enzyme activitise due to increased free-radical production. It can be concluded that oxidative factors may display unexpected effects on the regulation of GPx activity in the adrenonmedullin case and additional studies are needed to understand underlying mechanism.

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