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## **Stress-Induced Changes in Tyrosine Hydroxylase Enzyme Activity and Adrenomedullin Levels in Rat Hypothalamus, Adrenal Medulla and Heart Tissues**

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**Abstract:** In this study, it was aimed to investigate the effects of chronic cold stress on adrenomedullin levels and tyrosine hydroxylase enzyme activities in some rat tissues. In this study, 12 female Sprague Dawley rats weighing 200 to 250 g were used. Rats were housed under diurnal lighting conditions (12-12 h) with free access to food and water. For this study, rats were divided into two groups, namely control group and cold stress treated group. Adrenomedullin levels were measured by high performance liquid chromatography. Tyrosine hydroxylase enzyme activities was spectrometrically measured. Tyrosine hydroxylase activity was found to be increased significantly ( $p < 0.05$ ) in adrenal medulla, hypothalamus and heart tissues depending on cold stress treatment. Adrenomedullin levels were determined to be decreased in the cold stress treatment group. The differences of between control and cold stress treatment groups were significant ( $p < 0.05$ ). The results suggested that adrenomedullin may play a possible role in adaptation to stress. Exposure to cold increases the synthesis and release of catecholamines. Tyrosine hydroxylase is the rate limiting step in the biosynthesis of catecholamines for this reason cold stress increased tyrosine hydroxylase enzyme activities. Further studies are needed to reveal correlation between adrenomedullin, tyrosine hydroxylase and cold stress.

**Key words:** Adrenomedullin, tyrosine hydroxylase, cold stress, rat

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### **INTRODUCTION**

An organism's ability to respond to the external and internal environments is explained in terms of the concept of biological stress. The causes of these responses are called stressors. They may be physical, chemical, emotional, or traumatic (Yürekli and Yarpuzlu, 2006). Exposure the extreme environments such as heat and cold is a form of stress to be endured by all organisms (Kayanama *et al.*, 1999). All living organisms respond to stress changes in the environment in various ways (Crousus, 2000). Catecholamines have an important role in maintaining biological homeostasis to stress (Yürekli and Yarpuzlu, 2006; Yüksel and Yürekli, 2003). Catecholamine biosynthesis starts from tyrosine and in the first step DOPA is synthesized from tyrosine and catalyzed by the enzyme Tyrosine Hydroxylase (TH). In rats this enzyme activity varies depending on cold stress, exercise and age (Yürekli and Yarpuzlu, 2006).

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Exposure to extreme environment is a form of stress to be competed by the organism. Recently animal models have been developed utilizing cold application to study the physiopathology mechanism and squeal of stress (Yüksel *et al.*, 2002). The physiological components of stress response to cold are metabolic, circulatory and hormonal. Different physical stressors show a somewhat specific neuroendocrine response profile (Yüksel *et al.*, 2002; Saban and Kvetnansky, 2001). Physical activity, psychological stress, drug treatment and generalized allergic reactions enhance the synthesis and release of catecholamines in organisms (Yürekli and Yarpuzlu, 2006).

Exposure to cold and the administration of anti hypertensive drugs are known to proceed the synthesis and release of catecholamines in the adrenal medulla (Fregly *et al.*, 1994). TH is the rate limiting step in the biosynthesis of catecholamines (TH; tyrosine-3-monooxygenase, EC: 1, 14, 16, 2). TH catalyses the formation of DOPA (dihydroxy-phenylalanine) from L-tyrosine (Tümer *et al.*, 1993).

Adrenomedullin (AdM) is produced as apart of a 185 amino acid pro-hormone, called proadrenomedullin which also contains on unique 20 amino acid residue in its N-terminus, exerting hypotensive action and named proadrenomedullin N-terminal 20 peptide (PAMP) (Kalman, 2002). AdM is a 52 (human) or 50 amino acid (rat) hypotensive peptide belonging to a peptide super family which includes Calcitonin Gene-Related Peptide (CGRP) and amylin (Mazzocchi *et al.*, 1999). Human AdM is a 52 amino acid peptide with a single internal disulphide band (Ueta *et al.*, 2000).

AdM has been reported to be present in a normal adrenal medulla, heart, lung and kidney as well as in plasma and urine (Kilinç *et al.*, 2003). AdM is an important circulating hormone participating in the regulation of blood pressure (Ishiyama *et al.*, 1993). AdM may function as a circulating hormone and an autocrine-paracrine mediators involved in the regulation of cardiovascular system-blood pressure and renal function (Ishiyama *et al.*, 1993).

During stress the organism, must maximize its defense mechanisms, Thus the run or fight response is initiated by the activation of the sympathetic nervous system and the organism needs to stay in the activated state until the threat ceases. As the stress condition ceases; the organism needs to transit to the rest phase. Part of the response to the rest phase is maintained by the decrease in Corticotropin Releasing Hormone (CRH) levels, nitric oxide and AdM which is assumed to have both paracrine and autocrine effect (Yürekli and Yarpuzlu, 2006).

Recent studies have implicated AdM in the pathogenesis of some diseases, including; Behçet's disease, enuresis, some renal diseases, varicocele, pre-eclampsia, eye diseases and some psychological problems (Yürekli and Yarpuzlu, 2006).

In the present study, the effects of cold stress on AdM levels and tyrosine hydroxylase activities were investigated in some rat tissues to clarify the correlation between the cold stress with AdM and TH.

## **MATERIALS AND METHODS**

This research Project was conducted from 2001 (starting date) to 2003 (Ending date). My research Project was fully sponsored by İnönü University Scientific Research Projects Unit with Grant No. 2003/57.

In this study, twelve female Sprague-Dawley rats (200-250) were housed individually under diurnal lighting conditions (12-12 h) with free access to food and water. For this study, rats were divided into two groups, namely the control group (n = 6) and group receiving cold stress (n = 6). In the first group rats exposed to 22°C. The second group exposed to cold

stress were maintained in a 8°C room for a week (Yüksel and Yürekli, 2003). The rats were anesthetized by an intraperitoneal injection of urethane (1 mg kg<sup>-1</sup>). After surgery their adrenal glands, hearts, hypothalamus were removed and stored at -40°C. Then the tissues were weighed. After weighing, the tissues were homogenized to be contained as 100 mg tissue/150 µL pH 7.2 mM phosphate buffer. Total protein quantities were detected at 240 nm wavelength using Bovine Serum Albumin (BSA) as Standard (Bradford, 1976).

After homogenization 250 µL of homogenate was separated from each tissue and the homogenates were centrifuged at 13,000 g for 10 min at 4°C and 100 µL supernatant was separated for AdM analyses. For the analysis of AdM a HPLC linear gradient was applied and (Cecil 1100) 250×4.6 mm<sup>2</sup> and 5 µm long filling material C18 reverse phase Supelco filled chromatography column was used. The samples were applied in 1% trifluoroacetic acid in 60% acetonitrile (A) and 1% trifluoroacetic acid (B) using 60% A and 40% B solution with a double pump. The assays were done at a wavelength of 220 nm and standard rat AdM obtained from Phoenix Pharmaceuticals was used.

TH activity was measured using spectrophotometric assay, with modified Reinhard *et al.* (1986) TH activity was determined by monitoring the formation of L-dopa. Determination of TH enzyme activity was as follows: 25 µL homogenate was analyzed at pH 7.0 in the presence of 6-MPH4 (DL-6 Methyl-5, 6, 7, 8-tetrahydropterine; DL-2-amino-4-hydroxy-6-methyl-5, 6, 7, 8-tetrahydropteridine, Sigma M4758) and [3, 5, <sup>3</sup>H] tyrosine (100 µM, 1 µCi) (Sigma) in a total volume of 50 µL for 15 min at 37°C. For the calculation of enzyme activities total protein quantities were used and for each 25 µL homogenate, 0.02 mg of protein was accounted and the following formula was used:

$$\text{Specific activity} = \frac{(\text{OD-sample}) - (\text{OD-blind})}{(\text{OD-std}) \times (0.33 \text{ h}) \times 0.02 \text{ mg protein}} = \text{nmol/mg protein/saat}$$

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.

Data were analyzed using statistical software programme (SPSS 12.0 for Windows, Japan Inc., Tokyo, Japan). Data were compared with normal controls using one-way analysis of variance. To determine if there were any significant differences among groups, comparisons of differences between groups was done by the least significant difference (protected t) tests. Significance was set at 95% confidence limit. Results were shown as Mean±SD.

## RESULTS

In this study, the effects of cold stress on tyrosine hydroxylase enzyme activity and AdM levels were measured in some rat tissues.

AdM amounts of control group were measured as 296.68± 3.92 pmol mL<sup>-1</sup> in heart, 694.07±1.9 pmol mL<sup>-1</sup> in adrenal medulla, 276.16±1.72 pmol mL<sup>-1</sup> in hypothalamus. AdM amounts of cold stress application group were measured as 187.33±2.11 pmol mL<sup>-1</sup> in heart, 673.77±1.63 pmol mL<sup>-1</sup> in adrenal medulla, 138.70±1.79 pmol mL<sup>-1</sup> in hypothalamus (Table 1).

TH enzyme activities were measured as 517±2.17 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in heart, 973±0.99 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in adrenal medulla, 506±2.12 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in hypothalamus. In cold stress application group TH enzyme activities were measured as

Table 1: The effects of cold stress on adrenomedullin levels in some rat tissues (pmol mL<sup>-1</sup>)

Groups	Adrenomedullin levels (pmol mL <sup>-1</sup> )		
	Heart	Adrenal medulla	Hypothalamus
Control	296.68±3.92	694.07±1.9	276.16±1.72
A week cold stress	187.33±2.11	673.77±1.63	138.70±1.79

Table 2: The effects of cold stress on TH enzyme activity in some rat tissues (nmol mg<sup>-1</sup> Protein h<sup>-1</sup>)

Groups	TH activity (nmol mg <sup>-1</sup> Protein h <sup>-1</sup> )		
	Heart	Adrenal medulla	Hypothalamus
Control	517±2.17	973±0.99	506±2.12
A week cold stress	664±3.21	1251±4.11	884±4.21

664±3.21 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in heart, 1251±4.11 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in adrenal medulla, 884±4.21 nmol mg<sup>-1</sup> Protein h<sup>-1</sup> in hypothalamus (Table 2).

The results indicated that AdM levels decreased with cold stress, the differences between control and cold stress application group were found to be statistically significant (p<0.05). TH enzyme activities increased with cold stress (p<0.05).

## DISCUSSION

This study indicated an antagonistic effect of AdM and TH enzyme activities due to cold stress exposure and was designed to compare endogenous AdM and TH activity. These findings may lead to further studies to clarify the mechanisms of physiological action of these two compounds in sequential responses due to stress in living organisms.

An organism's ability to respond to the external and internal environments is explained in terms of the concept of biological stress. The causes of these responses are called stressors. They may be physical, chemical, emotional, or traumatic.

Considering humans, both external and internal risk factors may lead to death as a final step. Among external factors, alcohol, psychic drugs, too light a clothing for the circumstances and wetness and among internal factors leanness, physical exhaustion, traumas in the young, illness and degeneration of the physiological heat conserving and producing responses in the old may be counted (Yüksel *et al.*, 2002).

Catecholamines have an important role in maintaining biological homeostasis to stress. Catecholamine biosynthesis starts from tyrosine and in the first step DOPA is synthesized from tyrosine and catalyzed by the enzyme TH. In rats this enzyme activity varies depending on cold stress, exercise and age (Yürekli and Yarpuzlu, 2006). The chronic cold stress exposure is known to induce TH activity.

Sharma and Khanna have reported increased catecholamine levels following cold Stress (Sharma and Khanna, 1969).

Stachowiak *et al.* (1985) have reported that cold stress increases TH enzyme activity in catecholamine biosynthesis. It has been shown that an increase in TH activity depends on increased TH mRNA levels following administration of reserpine or exposure to cold (Roberts and Tümer, 1987). Baruchin *et al.* (2006) have shown that rats subjected to 5°C cold exposure for 1 h had maximal increases of 300-400%, levels that the controls took 3-6 h to reach and that levels remained constant for the duration of cold exposure.

TH activity was found to be significantly increased in the heart, adrenal medulla and hypothalamus of rats exposed to cold for a week. These findings provide a molecular

approach to the adaptation of the catecholaminergic system during cold stress. Further investigations are required to understand the underlying mechanisms directing the catecholaminergic system of organisms for responding stressors.

Acute stress known to stimulate sympathetic activity as well as the Hypothalamic-Pituitary-Adrenal (HPA) axis, produces a significant increase in AdM levels in the pituitary glands, plasma and adrenal glands, all of which are key components of HPA axis, suggesting a regulatory or protective role for AdM in countering HPA activation following a variety of physiological and psychological stressors (Yüksel *et al.*, 2002).

Increased catecholaminergic and adrenergic activity increases the blood pressure and blood glucose levels. Stress and aging both increase hypothalamo-hypophysial-adrenal axis activity and increase in ACTH, as well as epinephrine and norepinephrine. These compounds initiate a sequence of events including vasoconstriction, glycogen break-down in the liver and stress response (Yüksel *et al.*, 2002; Yürekli and Yarpuzlu, 2006; Tümer *et al.*, 1993).

Kitamura *et al.* (1993) have suggested that AdM as a hormone is important in regulating blood pressure. AdM has been shown to be secreted in stress and in response to glucocorticoids (Minamino *et al.*, 1995; Tanaka *et al.*, 1995). As well as the catecholamines (Kato *et al.*, 1995) AdM is known to be secreted from the adrenal medulla. Plasma AdM levels are known to be increased in response to heart failure, chronic renal failure, cirrhosis, hypertension and hypoxia (Cheung and Leung, 1997). Another indirect evidence to AdM release on cold stress may be derived from studies with plasma AdM in asthmatic patients. It is shown that cold induces asthmatic relapses. AdM has been suggested to take part in the response to acute asthma (Ceyhan *et al.*, 2001).

It has previously been suggested that cold being a form of physiological stress AdM release but in this study we found that AdM levels were decreased with cold stress. AdM and TH activity are suggested here to act as antagonists, such as the antagonizing physiological response due to sympathetic and para-sympathetic nervous system. However, the relation of the biological response to the neural stimuli needs to be clarified.

While doing a literature search, we observed that the studies conducted to reveal the physiological sequelae of AdM response to stress have been conducted on several experimental conditions and variable results which may seemingly be contradictory to interpret have been obtained on different animal models. Especially, the time dependent regulation of AdM response to stress and its mechanisms at cellular level at different end organs is shaded.

Study of stress response is a broad topic and our group intends to further investigate its mechanisms of regulation initially on the suggested rat model.

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