

# The Effect of Moderate Hypothermia on Renin-Angiotensin- Aldosterone System in Male Rats

Kourosh Arami M<sup>a</sup>, Khamenei S<sup>b</sup>, Zarghami N<sup>c</sup>, Vahabian M<sup>a</sup>.

<sup>a</sup>Department of Physiology, Hamedan University of Medical Sciences, Hamedan, <sup>b</sup>Department of Physiology, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, <sup>c</sup>Department of Clinical Biochemistry and RIA, Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

**D**espite the diversity of studies on hypothermia, many of its biologic and physiologic effects are still poorly recognized. In this study the effect of hypothermia on the renin- angiotensin- aldosterone axis was explored.

**Materials and Methods:** Ten male albino wistar rats after anesthetizing by chloral hydrate (0.5 ml/100gr body weights) were cooled to 25°C. Before and just after hypothermia, and once every 24 hours for three days; serum levels of angiotensin I and aldosterone were measured, using the radioimmunoassay method. Plasma renin activity was also determined by the standard formula for angiotensin determinates at two temperatures of 4°C and 37°C.

**Results:** Plasma renin activity increased during hypothermia but then decreased to a level not significantly different from basal values. Angiotensin I increased significantly in the second sample taken 24 hours following hypothermia ( $p < 0.05$ ) and then decreased to the basal range by

72 hours. Serum aldosterone level rose significantly until 48 hours following hypothermia, after which it began to decrease and returned to basal level.

**Conclusion:** Results of this study advocate the stimulating effect of moderate hypothermia on plasma renin activity, angiotensin I and aldosterone.

Key Words: Hypothermia, Renin- angiotensin-aldosterone, Rat

## Introduction

Exposure to stress activates a complex and integrated constellation of behavioral and neurochemical processes to promote successful adaptation to the stressful stimulus. Activation of the hypothalamic-pituitary-adrenal axis represents the major neuroendocrine manifestation of the stress response. In addition, stress activates the noradrenergic system, an important modulator of the many neurobiological components of the response to stress. Indeed, these stress responsive systems are interconnected for instance, activation of the hypothalamic-pituitary-adrenal

*Correspondence:* Kourosh Arami M, Department of Physiology, Hamedan University of Medical Sciences, Hamedan, Iran

*E-mail:* masoomeh\_k@hotmail.com

axis is facilitated by stress-induced release of norepinephrine in certain regions of the brain, including the hypothalamic paraventricular nucleus and stria terminalis.<sup>1</sup>

It has been demonstrated that abrupt exposure to cold results in a rapid increase in mean arterial pressure within 45 min.<sup>2</sup> Hiramatsu et al focussed on noradrenalin as the only hormone responsible for an elevation of blood pressure in response to cold;<sup>3</sup> nevertheless the possible role of Renin-angiotensin-aldosterone system (RAAS) should not be ignored.

It has been shown that RAAS becomes activated in response to hypothermic intervention during cardiopulmonary bypass.<sup>4</sup> Kono et al. demonstrated that aldosterone level increases in pulsatile and non pulsatile cardiopulmonary bypass (CBP).<sup>5</sup> Again, 3 weeks of exposure to cold, increased plasma renin activity which was then followed by a gradual decrease toward control level.<sup>6</sup> However, it is worth noting that CBP studies are confounded by surgical intervention. It is unclear from these studies whether increase in plasma angiotensin concentration is related to hypothermia or the stress induced by a major surgical intervention. The aim of this study is to determine the influence of hypothermia on the RAAS, without the resultant stress associated with cardiopulmonary bypass.

## Materials and Methods

Ten male rats (albino, Wistar) with an average weight of 272 gr and age of 7 months were used. A laboratory made apparatus was used to induce hypothermia. The apparatus had a sensor connected to a thermostat at one end and to the rat rectum at the other. The sensor was also connected to a digital screen, displaying the body temperature of the animal throughout the procedure. After reading the rectal temperature, the animal was generally anesthetized by subperitoneal injection

of chloral hydrate (50 mg/100 gr), and 2mL of blood was drawn from the heart. It was then placed in the specific chamber and the apparatus was set to reduce its body temperature gradually down to 25°C at which temperature the animal was kept for 2 hours. Blood samples were taken immediately after hypothermia, and then every 24 hours for 3 days. To facilitate the procedure, in the 3 later samples, rats were superficially anaesthetized using ether soaked swabs.

All blood samples were centrifuged (2500 rpm, 20 min) and the sera obtained were kept in paraffin-sealed test tubes and stored at -22°C. The hormones were measured by radioimmunoassay kits (Kavoshyar Co, Iran). Angiotensin-I (AT-I) was measured at two different temperatures (4°C and 37°C) using Gamma counter. Plasma renin activity was calculated using the following formula (incubation time was 1.5 hour):

$$\text{PRA} = \text{AT-I at } 37^{\circ}\text{C} - \text{AT-I at } 4^{\circ}\text{C}.$$

Values before induction of hypothermia were considered as basal levels, and paired t-test was used for comparisons (SPSS software) and p values below 0.05 were considered significant.

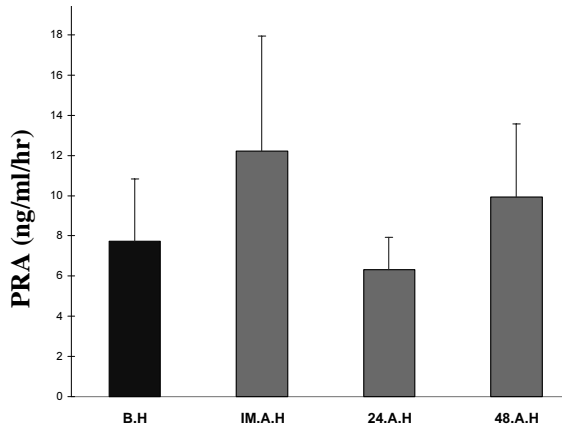
## Results

**Plasma Renin Activity (PRA):** Basal level of PRA was measured to be  $7.73 \pm 3.1$  ng/ml/h. It increased after hypothermia and then decreased to a level not significantly different from basal values (Fig.1).

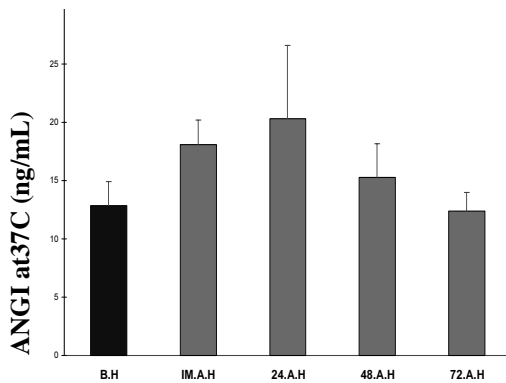
**Angiotensin I:** Basal level of angiotensin I at 37°C was  $12.84 \pm 2.1$  ng/ml. It was subject to a significant increase after induction of hypothermia ( $p < 0.03$ ) reaching its maximum level 24 hours following hypothermia ( $20.31 \pm 6.3$  pg/ml); it then decreased gradually reaching  $12.32 \pm 1.6$  pg/mL after 72 hours, not significantly different from the basal levels (Fig.2). The basal level of angiotensin I at 4°C was  $9.35 \pm 1.15$  ng/mL. Again, it increased significantly after induction of

hypothermia, but its level in the sample taken 24 hours following hypothermia was not statistically different from the control, reaching its nadir within 48 hours (Fig.3).

**Aldosterone:** Prior to hypothermia, serum concentration of aldosterone was  $321 \pm 53$  pg/ml. After induction of hypothermia, it elevated to a level significantly different from the basal value ( $p < 0.03$ ) and reached its



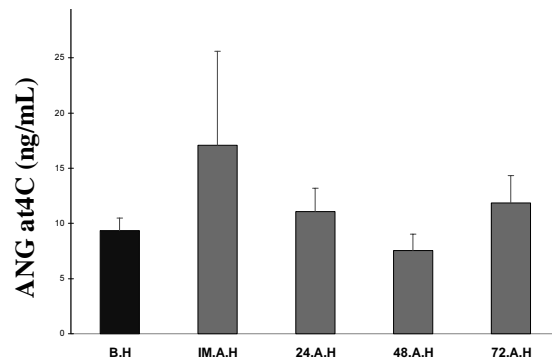
**Fig.1.** Serum plasma renin activity levels before and just after hypothermia (2 hours) 24, 48 and 72 hours after hypothermia. Data represent mean  $\pm$  SEM (n=10),  $p < 0.05$  (BH: before hypothermia, IMAH: immediately after hypothermia, 24, 48, 72AH = 24, 48, 72 hours after hypothermia)



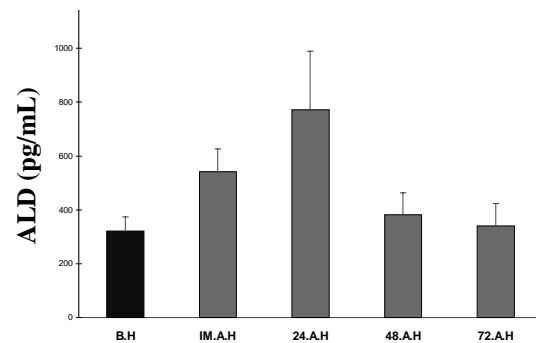
**Fig.2.** Changes of Angiotensin I during and after hypothermia at 37C. There is a significant difference between values before hypothermia and immediately after hypothermia. Rest of the legend is as described for Fig.1.

maximum level 24 hours following hypothermia ( $771 \pm 218$  pg/mL), decreasing gradually to the range of the basal levels after 72 hours (Fig.4).

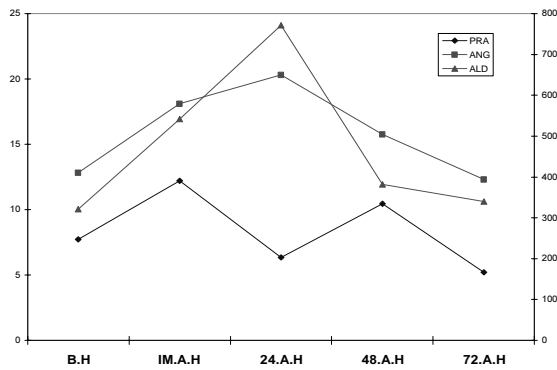
**Correlation studies:** There was a high degree of correlation between Angiotensin I and PRA ( $r = 0.7$ , Fig.5).



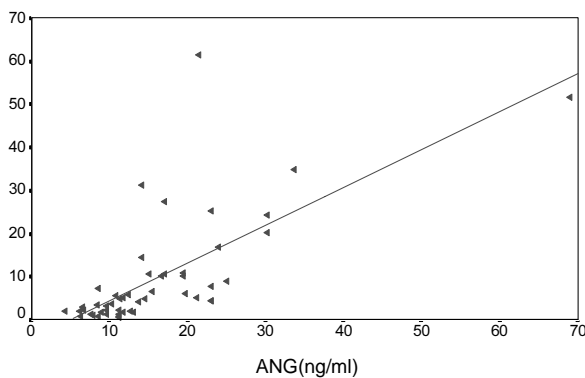
**Fig. 3.** Serum concentrations of Angiotensin I during and after hypothermia at 4C. There is a significant difference between values before and immediately after hypothermia. Rest of the legend is as described for Fig.1.



**Fig.4.** Serum aldosterone levels before and just after hypothermia (2 hours) 24, 48 and 72 hours after hypothermia. Rest of the legend is as described for Fig.1.



**Fig.5. Relation between plasma renin activity and changes in angiotensin I with complete correlation ( $p=0$ ,  $r=0.7$ ).**



**Fig.6. Changes of plasma renin activity, angiotensin I and aldosterone levels during and after hypothermia. Rest of the legend is as described for Fig.1.**

## Discussion

This study demonstrated the influence of hypothermia on the renin-angiotensin-aldosterone system in rat. Our data indicated remarkable alterations in plasma renin activity, angiotensin I and aldosterone levels, in that, angiotensin I and aldosterone level increased compared to basal values during hypothermia and kept increasing for up to 24 hours afterwards.

Plasma renin activity was seen to increase during cooling though its alteration was not significant. PRA increment seen in the Fukuhara and Picotti studies was considered to be

an effect of sympathetic activation; in their studies they observed that plasma concentration of norepinephrine which increases as early as 30 minutes to one hour after cold exposure, remains elevated as long as the exposure lasts.<sup>7-8</sup> Sympathetic input to kidney increases the release of renin, which in turn contributes to the production of angiotensinII. A compromise in cardiac performance might be responsible for the reduction of arterial pressure. Talwar et al. observed a significant drop in heart rate, cardiac output, arterial pressure and left ventricular contractility during hypothermia.<sup>9</sup> As could be expected, reductions in arterial pressure stimulate renin secretion by juxtaglomerular cells, and thereby activate angiotensin production.

Although a non-significant increase was observed in angiotensin I level in comparison to the basal level, nevertheless some correlation was seen between PRA and angiotensin I. As a matter of fact, angiotensin I concentration is determined by factors acting at two extremes, ie; plasma renin activity and angiotensin converting enzyme. Therefore, the elevation in serum concentration of angiotensin I during hypothermia might be attributed to enhancement of angiotensin converting enzyme activity during such conditions. Serum aldosterone level showed remarkable alterations; according to our data, it increased dramatically compared to basal values, during and 24 hours after hypothermia. Various factors such as increased sodium, potassium, adrenocorticotrophic hormone (ACTH) levels and reduction of hormone metabolism due to cardiopulmonary bypass and hypothermia are thought to be involved in this process.

Pardon et al demonstrated that chronic continuous or intermittent exposure to cold has been shown to be effective in sensitizing both neuroendocrine and noradrenergic stress reactivity in rats. Prior exposure to chronic cold stress increases the subsequent activation of the hypothalamic-pituitary-adrenal axis, measured by plasma ACTH and corticosterone levels, upon exposure to a novel acute stressor. If this holds true for a subacute pe-

riod of hypothermia incorporated in the present study, then it could be assumed that ACTH elevation resulting from hypothermia stimulates zona glomerulosa, which would then induce aldosterone secretion.<sup>1</sup>

In a study of athletes during a cold weather race it was shown that serum sodium concentration decreased significantly from  $140.8 \pm 1.2$  mmol/L to  $138.4 \pm 2.2$  mmol/L<sup>10</sup>; decrement of sodium is another stimulatory factor for aldosterone secretion.

Cold exposure reduces or inhibits Na-K ATPase pump activity that causes K<sup>+</sup> elevation and then stimulation of both early and late processes of steroid genesis in the corticorenal region leading to a rise of aldosterone

level synthesis and secretion there.<sup>11,12</sup> Again, information about Na and K changes come from normothermic cold exposure and their application to our data requires further investigation.

It has been shown that liver blood flow is significantly reduced to 66.9% during hypothermic cardiopulmonary bypass, Therefore it might be argued that hypothermia, through reduction of hepatic blood flow dampens metabolic clearance of aldosterone.<sup>13</sup>

Collectively, these results demonstrate that a short-term moderate degree hypothermia, devoid of surgical intervention, leads to systemic activation of the renin-angiotensin-aldosterone system in rats.

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