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Changes in Blood Serum Aldosterone Hormone and Some Mineral Levels in Lipopolysaccharide-Induced Hypothermic Rats

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Abstract

In this study, we induced hypothermia in rats by administering LPS (Serotype *E. coli*: O111:B4; 250 µg/kg, ip). Blood samples were taken via intracardiac puncture at the entrance, deepest point, and exit of the induced hypothermia. In the control group, saline (0.09% NaCl, 0.5 ml/kg ip) was administered to rats, and blood samples were taken at the entrance, deepest point, and exit of hypothermia. It was determined that the administered LPS doses did not change the levels of aldosterone and Na, K, Cl, Ca, and P. As a result of the applications, the deepest point of hypothermia (ΔT: -1.48±0.09ºC) was reached in an average of 104.46±4.32 minutes. Analysis of blood serum samples revealed that the differences in aldosterone, Na, K, Cl, Ca, and P levels between the LPS-induced hypothermia groups (entry, trough, and exit) compared to the control group were statistically insignificant. Our results indicate that LPS can cause thermoregulatory hypothermia accompanied by fever in rodents.

Keywords: Lipopolysaccharide, Hypothermia, Aldosterone, Mineral, Rat.

1. Introduction

Gram-negative bacteria are generally hospital and community acquired microorganisms, the source of which is usually the patient himself or herself, living and non-living structures. Lipopolysaccharide (LPS), an endotoxic substance, is a component of Gram-negative bacteria. LPS is a major component of the outer membrane of Gram-negative bacteria and is responsible for the pathophysiological events associated with Gram-negative infections (De Salamanca and García, 2005). Excessive amounts of endotoxins can cause multiple organ failure and death (Natanson et al., 1994).

Experimentally, when LPS is administered systemically at low doses (5-500 micrograms/kg), it elicits a response called the acute phase reaction. This response is controlled by the central nervous system and involves numerous immunological, endocrinological, autonomic, and behavioral changes.

Low-dose studies in rodents indicate that LPS can induce thermoregulatory fever and hypothermia (Dogan et al., 2002). This hypothermia (like fever) is a regulated response. During lipopolysaccharide hypothermia, rodents experience no

passive heat loss, decrease metabolic heat production, and attempt to maintain hypothermia by behaviorally preferring cooler temperatures. These findings suggest that the thermostat, thought to be located in the hypothalamus, is (hypothetically) adjusted to a lower level (Derijk et al., 1994; Dogan et al., 2002; Almeidaet al., 2006). The peripheral signaling molecule that initiates the hypothermic response is suggested to be TNF-α (tumor necrosis factor). TNF-α levels increase initially, and this increase persists throughout the response, in direct proportion to the magnitude of the response (Akarsu and Mamuk, 2007). Blocking the increase or release of TNF-α also inhibits the hypothermic response (Tollner et al., 2000; Akarsu and Mamuk, 2007; Polat et al., 2013). Increased TNF-α stimulates prostaglandin synthesis at the hypothalamic level, allowing the thermostat to be adjusted to a lower level (Akarsu et al., 2008). The hypothermic response can be inhibited by pharmacological agents that block prostaglandin synthesis. It is thought that the cyclooxygenase-1 enzyme, in particular, may play a more important role in this response (Tollner et al., 2000; Akarsu and Mamuk, 2007; Polat et al., 2013).

In recent years, it has been reported that mediators (cytokines) released by immune system cells have an impact on the endocrine system. The hypothalamic-pituitary-adrenal (HPA) axis is altered as a result of interactions between immune-related inflammatory reactions, and cytokines (IL-1, IL-6, and TNF-α) play an important role in this response (Karima et al., 1999; Watanob and Yoneda, 2003). This study aimed to investigate the effects of hypothermia on aldosterone hormone and certain blood mineral levels by inducing hypothermia with LPS in an infection model applied to male Wistar albino rats.

2. Materials and methods

Experimental Animals

Adult male Wistar albino rats weighing 220-300 g were used. Information on the animal groups and numbers used for the experiments is provided in Table 1. For the control group, 0.09% NaCl (physiological serum) was used. Lipopolysaccharide (Serotype *E.coli* O111:B4, Lot no: L2630, 25 mg, 118K4053, Sigma, ISRAEL) was used to create hypothermia.

Table 1. Animal groups created for experiments and the numbers of the groups.

Groups	Number of Animals
Saline	10
LPS (*Hypothermia Enterance*)	10
LPS (Hypothermia Deeppest)	10
LPS (*Hypothermia Exit*)	10

Note: Saline (0,09% NaCl, 0,5 ml/kg ip); LPS (Serotype *E.coli*, O111:B4, 250 µg/kg, ip).

Surgical Procedures (Implant Placement)

Under general anesthesia (ketamine 80 mg/kg+xylazine 10 mg/kg, IM), a temperature transmitter (model VM-FH 3000) implant was placed intraperitoneally in the rats. After this stage, each rat was housed individually in cages. After the surgery, the rats were tested when they reached their preoperative weight.

Experimental Protocol

The temperature of the laboratory where all experiments were conducted was maintained between 24-26°C. Experiments began at 09:00, and body temperature was allowed to stabilize for at least 2 hours. Injections were administered between 11:00 and 12:00, and blood samples were collected based on the response. Body temperature was recorded at 1-minute intervals, and biotelemetrically (Mini Mitter, Bend, OR, USA). TR 3000 series receivers were used to receive temperature signals from the transmitters. Approval and permission were obtained from the Ankara University Animal Experimentation Local Ethics Committee for all experimental protocols implemented in the study (Date: 09.04.2008, No: 2008-24-82).

Blood Sample Collection

Hypothermia was induced by intraperitoneal administration of LPS to rats implanted with biotelemetric transmitters. In the control group, 2-2.5 ml of blood samples were collected intracardially under diethyl ether anesthesia at the beginning, deepest, and end of hypothermia. Different groups of rats were used for each specified time point. The rats in the control group were distributed to different stages of hypothermia (beginning, deepest, and end), and 2-2.5 ml of blood samples were collected at the corresponding time points. The mean body temperatures of each group and the hormone analysis results from the collected blood samples were combined to form a single control group. Following blood collection, rats in all groups were euthanized with diethyl ether. The collected blood samples were kept at 4°C with their mouths closed for one hour and then were centrifuged at 10,000 rpm for 10 minutes. The blood samples were centrifuged and the resulting blood sera were transferred to sterile storage tubes in 75 µl aliquots and stored at -80°C until analysis.

Hormonal and Biochemical Analyses

Commercial ELISA kits (DRG, USA) were used for aldosterone hormone analyses. The operating procedures recommended in the kit package insert were followed, with duplicate measurements performed for each sample, and the lowest and highest measured samples were reanalyzed and averaged. Biochemical analyses were performed using the Architect C8000 (Abbott, USA) instrument and its measurement kits.

Statistical Analyses

The 30-minute pre-injection body temperature was considered the baseline temperature, and the post-injection changes were calculated by calculating the difference from the baseline temperature (λT). Temperature differences were expressed as the mean ≥ standard error and analyzed using parametric statistics. After analyzing hormone levels, the distribution characteristics of the data comprising each group were examined. Differences between the control group and the other groups were analyzed using ANOVA. SPSS 15.0 was used for these analyses.

3. Results

Weight, ΔT, and Blood Collection Times in the Control (Saline) and LPS Experimental Groups

Table 2 shows the average hypothermia levels obtained at different stages of hypothermia in experiments conducted with rats administered saline (0.09% NaCl, 0,5 ml/kg, ip) and LPS (Serotype *E. coli*: O111:B4; 250 µg/kg, ip) and the changes in findings over the corresponding time periods. As can be seen in the same table, the deepest point of hypothermia (ΔT: -1,48±0,09ºC) was reached in an average of 104,46±4,32 minutes. Hypothermia started 71,42±2,16 minutes after LPS injection (ΔT: -0,58±0,03) and ended at 157.65±10.21 minutes (ΔT: -0,08±0,09).

Table 2. Findings regarding weight, ΔT and blood collection time in rats.

Gruplar	Ağırlık (g)	ΔT (ºC)	Kan Alma Zamanı (dak.)
Control (n:10)	275,60±4,88	0,03±0,08	106,65±9,34
Hypothermia Enterance (n:10)	279,30±2,94	-0,58±0,03	71,42±2,16
Hypothermia Deepest (n:10)	268,35±5,64	-1,48±0,09	104,46±4,32
Hypothermia Exit (n:10)	289,45±6,82	-0,08±0,09	157,65±10,21

ΔT (ºC): Blood collection temperature – Basal temperature; Saline (0.09% NaCl, 0.5 ml/kg ip); LPS (Serotype *E.coli*, O111:B4, 250 µg/kg, ip).

Findings Regarding Aldosterone Hormone Levels

Table 3 shows the results of blood serum aldosterone hormone analyses conducted in rats using LPS (Serotype *E. coli*: O111:B4; 250 µg/kg, ip) and saline (0,09% NaCl, 0,5 ml/kg ip). Analyses revealed that the differences in aldosterone hormone levels between the LPS-induced hypothermia groups (Enterance, Deepest, and Exit) compared to the control group were statistically insignificant.

Findings from Biochemical Analyses

Based on experiments conducted on rats using saline (0.09% NaCl, 0,5 ml/kg, i.p.) and LPS (Serotype *E. coli*: O111:B4; 250 µg/kg, i.p.), serum Na, K, Cl, Ca, and P levels were determined (Table 3). Analyses revealed that the differences between the control group and the LPS-induced hypothermia groups (Enterance, Deepest, and Exit) in terms of the biochemical parameters Na, K, Cl, Ca, and P were statistically insignificant.

Table 3. Findings regarding blood serum Na, K, Cl, Ca and P levels in control (saline) and LPS-induced hypothermia group rats.

Biochemical Parameters	Control	LPS Groups		
		Hypothermia Enterance	Hypothermia Deepest	Hypothermia Exit
***Aldosterone** (pg/ml)*	38,7±1,63	40,5±1,02	39,8±0,88	40,1±0,95
***Na** (mEq/l)*	149,1±1,29	146,3±1,08	143,3±0,96	143,6±0,87
***K** (mEq/l)*	5,37±0,18	5,30±0,26	5,28±0,20	5,29±0,32
***Cl** (mEq/l)*	108±1,25	109±1,42	109±0,85	110±0,98
***Ca** (mg/dl)*	9,02±0,54	9,16±0,33	9,08±0,47	9,11±0,41
***P** (mg/dl)*	6,38±0,44	6,63±0,39	6,47±0,51	6,52±0,36

4. Discussion

LPS-Induced Body Temperature Change

In this study, hypothermia (ΔT: -1,48±0,09 ºC) was induced in rats by experimentally administering LPS (Serotype *E. coli*: O111:B4, 250 µg/kg, ip) (Table 2). Hypothermia may develop in rodents with low-dose LPS (Derijk et al., 1994; Dogan et al., 2002; Almeidaet al., 2006), and this is thought to be due to the thermostat, thought to be localized in the hypothalamus, being set to a lower level (Derijk et al., 1994; Dogan et al., 2002; Almeidaet al., 2006). During LPS hypothermia, rodents experience no passive heat loss, but metabolic heat production decreases, and they attempt to maintain hypothermia by behaviorally preferring cooler temperatures.

The hypothermia we induced began at an average of 71,42±2,16 minutes after LPS injection, reached its deepest point at 104,46±4,32 minutes, and ended at 157,65±10,21 minutes (Table 2). Akarsu and Mamuk (2007) reported that hypothermia began approximately 40 minutes after LPS injection, reached a minimum point within 30-40 minutes, and ended within the following 60-90 minutes. Polat et al. (2013) also reported a similar study.

Changes in Aldosterone, Na, K, Cl, Ca, and P Levels in LPS-Induced Hypothermia

The adrenal gland functions as a stress response organ by undergoing structural and functional changes under various pathophysiological conditions. This gland plays a crucial role in the stress response in various infections and conditions such as sepsis. Adrenal gland dysfunction has been observed, particularly in cases of endotoxic interactions. LPS, an outer component of the cell wall of all Gram-negative bacteria, is known to be the primary initiating factor in the development of endotoxic or septic shock. LPS is known to activate the HPA axis due to stress in the body. This increase is reported to stimulate HPA axis activity via increased IL-1β, IL-6, and TNF (Karima et al., 1999; Watanob and Yoneda, 2003). Many studies have reported increases in HPA axis hormone levels (CRH and ACTH) following LPS administration (Givalois et al., 1994; Kageyama et al., 1999; Islam and Pestka, 2006). This increase in HPA axis activity is reported to be activated to maintain physiological homeostasis in response to infection (Fukata et al., 1994 Turnbull and Rivier, 1999).

Increased CRH secretion from the hypothalamus stimulates the pituitary gland, leading to increased plasma ACTH levels. Increased ACTH stimulates the adrenal cortex, leading to strong glucocorticoid release, and aldosterone is also reported to be released from the adrenal cortex in response to various stress stimuli (Moncek et al., 2003). It is also reported that aldosterone release by acute and chronic stress factors is mediated by angiotensin II and ACTH hormones (Waanders et al., 2011; Hattangady et al., 2012). However, in different literature reports, it has been reported that aldosterone secretion increases in response to hypotension in the adrenal cortex zona glomerulosa in septic shock, but plasma aldosterone levels are found to be low despite hyperreninemia (De Salamanca and García, 2003; De Salamanca and García, 2005). It has also been found that atrial natriuretic peptide (ANP) concentrations increase during endotoxic shock in mice and ANP inhibits aldosterone release (Ganguly, 1992; Aiura et al., 1995). De Salamanca and García (2005) reported in their study that there was an impairment in the ACTH response of adrenocortical cells isolated from the adrenal glands of endotoxemic rats. Other studies have also reported that patients with serious bacterial infections do not increase cortisol levels in response to ACTH (Tayek and Atienza, 1995). Adrenocortical insufficiency has been reported to develop in some sepsis patients (Tayek and Atienza, 1995; Aygen et al., 1996). Polat et al. (2013) reported that in LPS-induced hypothermic rats, blood serum CRH levels did not change, ACTH levels did not change at the entrance and exit of hypothermia, and decreased at the end point of hypothermia. Steiner et al. (2004) reported that LPS-induced hypothermia resulted in higher ACTH hormone levels in the hypothermia group compared to the control group in rats carrying active leptin receptors, and that hypothermia-induced leptin receptor-deficient rats did not respond to HPA axis activation. As a result of the experiments conducted in this study, it was determined that aldosterone hormone levels did not change in the LPS-induced hypothermia groups (Enterance, Deepest, and Exit) compared to the control group (Table 3 and Figure 1). In literature reports, it has been reported that plasma aldosterone levels do not increase in endotoxic interactions (De Salamanca and García, 2003; De Salamanca and García, 2005), that an increase in ANP inhibits aldosterone release (Ganguly, 1992; Aiura et al., 1995), that there is an impairment in the ACTH response of adrenocortical cells (Tayek and Atienza, 1995), and that adrenocortical insufficiency develops in some sepsis

patients (Tayek and Atienza, 1995; Aygen et al., 1996). LPS-induced aldosterone production has been reported to increase significantly at 24 and 48 hours (Huang et al., 2010). It has been reported that CRH and ACTH levels do not increase in LPS-induced hypothermia studies (Polat et al., 2013).

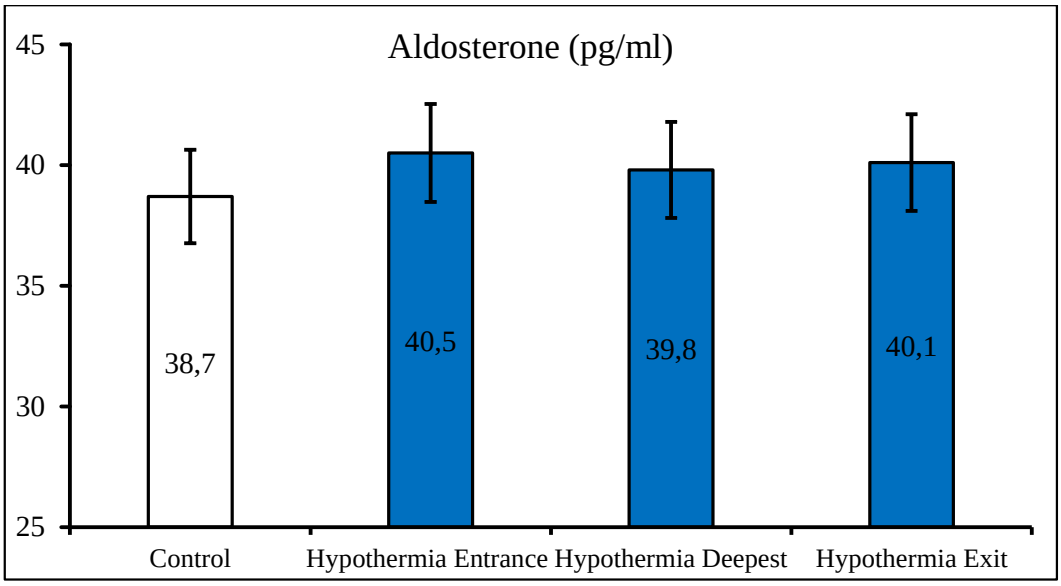

Figure 1. Illustration of changes in aldosterone hormone in kontrol (saline) and LPS group rats.

Aldosterone binds to mineralocorticoid receptors (MR) located on the cell membranes of the kidney's collecting ducts and activates Na+/K+ pumps by increasing sodium and potassium permeability across these cell membranes (Gilbert and Brown, 2010; Waanders et al., 2011). Therefore, it affects energy production (ATP), resulting in the absorption of water and Na into the blood and the excretion of K into the urine (Gilbert and Brown, 2010). In this study, it was determined that Na, K, Cl, Ca and P levels remained unchanged in the LPS-induced hypothermia groups (Enterance, Deepest, and Exit) (Table 3, Figures 2 and 3). Aldosterone is known to regulate water-electrolyte balance and blood sodium and potassium balance. These results suggest that aldosterone hormone levels remained unchanged during LPS-induced hypothermia.

Research results indicated that aldosterone, Na, K, Cl, Ca, and P levels remained unchanged. Aldosterone is known to physiologically regulate water-electrolyte balance, regulate sodium and potassium balance in the blood, and bind to mineralocorticoid receptors (MR) located on the cell membranes of the renal collecting duct, increasing sodium and potassium permeability across these cell membranes. As aldosterone hormone levels remained unchanged during LPS-induced hypothermia, mineral (Na, K, Cl, Ca, and P) levels were unchanged.

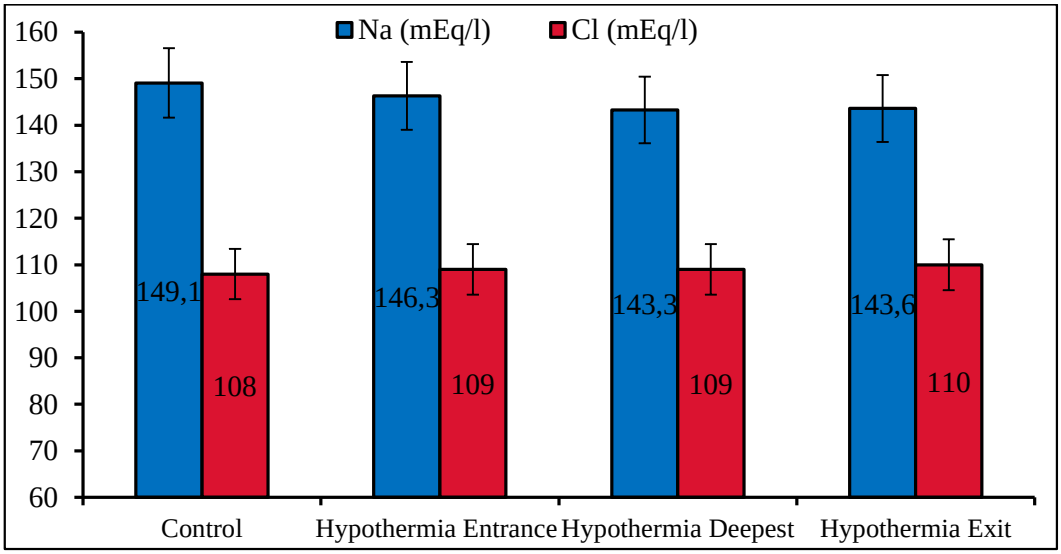

Figure 2. Illustration of Na and Cl changes in control (saline) and LPS group rats.

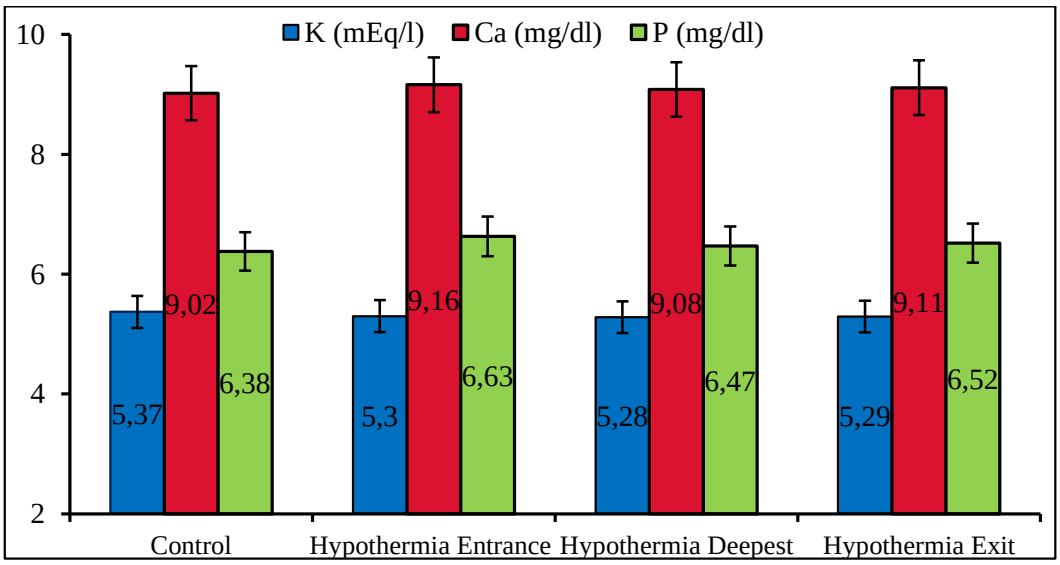

Figure 3. Illustration of K, Ca and P changes in control (saline) and LPS group rats.

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