AHCC on Immobilization Stress in the Rat: Beneficial Effects of Active Hexose Correlated Compound

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SUMMARY

Active Hexose Correlated Compound (AHCC™), an extract from several basidiomycetes, has been known as a biological response modifier (BRM) in humans as well as in animals. In the present study, AHCC was tested for its ability to modulate the hormonal responses to immobilization stress in the rat. AHCC at 3% in drinking water was given to male Wistar rats for one week, then rats were exposed to immobilization for 1 h. At 1 h after immobilization, the serum levels of corticosterone, norepinephrine, epinephrine, dopamine and glucose were increased significantly. Except for the corticosterone levels, all of these changes were restored to control levels by the AHCC pretreatment. These results suggest that AHCC can protect various effects induced by immobilization by attenuating the sympathetic nerve activity.

Key words: immobilization, corticosterone; catecholamines, glucose, AHCC

INTRODUCTION

When animals are exposed to stressors, the sympathoadrenal and hypothalamo- pituitary - adrenocortical system (HP A), are stimulated to maintain the internal environment of the body. Activation of the HPA axis is a common response to many stressors, and is found to be closely associated with increased secretion of adrenocorticotropic hormone (ACTH) and corticosterone in the rat. Stress also activates the sympathoadrenal system which leads to an increase in serum levels of catecholamines and glucose[1, 21]. Thus, plasma levels of ACTH, corticosterone, norepinephrine and epinephrine rise markedly during exposure to stressful events [3]. Immobilization of rats for 1 h results in an increase in heart rate, blood pressure and plasma levels of corticosterone, norepinephrine and epinephrine [4]. Immo bilization stress increases catecholamine turnover, protein and mRNA levels of catecholamine biosynthetic enzymes in the locus coeruleus (LC) cell bodies [5], suggesting transcriptional activation of the genes encoding these enzymes in the LC by stress.

A major physiological function of these "stress hormones" is the regulation of glucose metabolism. Stress- induced hyperglycemia has been attributed to the cooperation of corticosterone and catecholamines [6]. Stress enhances catecholamine secretion through activation of the sympathetic nervous system; pinephrine from the adrenal medulla and norepinephrine from sympathetic nerve endings. Epinephrine increases blood glucose levels through stimulation of hepatic glucose output and interference with peripheral tissue glucose disappearance. Norepinephrine and epinephrine can also inhibit insulin secretion, leading to an increase in blood glucose [7, 8]. Electrical stimulation activates the sympathetic nerve to increase plasma glucose levels [9]. Epinephrine and corticosterone infused into the dog, synergistically increases blood glucose levels [10].

Active Hexose Correlated Compound (AHCC™) (Amino UP Chemical Co. Ltd., Sapporo) is a mixture of

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polysaccharides, amino acids and minerals derived from several fungi. It is obtained by hot water extraction after culturing mycelia of several basidiomycetes in a liquid culture tank and then treating them with some enzymes [11]. AHCC has been proved to be a biological response modifier (BRM) in various disorders. Main active components are thought to be α - and β - glucans. AHCC ameliorates the side - effects induced by antitumor agents such as 5- FU[12]. It is also found that oral administration of AHCC to patients with malignant tumors results in an increase in serum levels of interleukin (IL) - 12, tumor necrosis factor (TNF) - α, interferon (IFN) – y and other cytokines [13]. In addition, AHCC has been shown to prevent experimental diabetes induced by streptozotocin [14]. These findings suggest that AHCC could restore the dysfunction of the immune system and reduce side - effects caused by antitumor agents.

The objective of the present study is to examine whether AHCC can modulate the responses to immobilization stress.

MATERIALS AND METHODS

1. *Materials.*
Lyophilized AHCC was obtained from Amino UP Chemical Co. Ltd. (Sapporo, Japan).

2. *Animals.*
Studies with the rats were approved by Animal Care and Use Committee, Dokkyo University School of Medicine. The animals were treated according to the guidelines for the Care and Use of Laboratory Animals of the Committee. Male Wistar rats of 8- weeks old were purchased from Charles River Japan Inc. (Kanagawa, Japan). The animals were kept in a room at 23 ± 2°C with 12 h light and dark cycle and kept free access to food and water.

Twenty to 21 rats were divided into four groups. Two groups received 3% AHCC in drinking water for a week. This concentration of AHCC was chosen, because maximal responses to AHCC were observed when AHCC was given at the concentrations between 2% to 4% [15]. The AHCC effects were evident only after 4 days of its treatment1ol. Control groups received only tap water for a week. Two groups received immobilization stress; one of them was AHCC - pretreated group, and the other was the control group. All animals were sacrificed by

3. *Immobilization stress.*
Two groups of rats were immobilized for 60 min in a supine position by extending and fastening their limbs to a specially designed wood board by string. During immobilization, though the rats attempt to free themselves, no obvious obstruction of limb blood flow or superficial and deep limb injury was found in any animal.

4. *Glucose assay.*
Rats were sacrificed by decapitation at 1 h after immobilization. Blood samples were collected into plastic blood sampling tubes at 1 h after immobilization. Blood samples were centrifuged at 4°C for 15 min at 1000 x g and the sera thus obtained were used for glucose assay. Serum glucose was determined by glucose assay kit \Wako, Tokyo, Japan), employing the glucose - glucose oxidase method [14]

5. *Corticosterone and catecholamine assay.*
Blood samples were collected into EDTA - coated blood sampling tubes. Bloods were centrifuged at 4°C for 15 min at 1000 x g and the plasma samples thus obtained were used for hormone assay. All the catecholamines in samples were determined by high - performance liquid chromatographv" (HPLC) with electrochemical detection. Corticosterone was determined by radioimmunoassay using a commercial kit, Coat - A - Count provided by Diagnostic Products Corp. (Los Angeles, CA, USA).

6. *Statistical analysis.*
All data were expressed as mean ± SEM. Statistical analysis was performed by Dunnett's multiple comparison test to control and non - parametric method by Scheff's multiple comparison test. A p value less than 0.05 was considered to be statistically significant.

RESULTS

1. *Changes in blood catecholamines levels*
There was no significant difference in the blood levels of norepinephrine, epinephrine and dopamine levels between control and AHCC groups. The immobilization of rats for 1 h resulted in an increase in plasma levels of the three catecholamines. AHCC - pretreatment lowered the

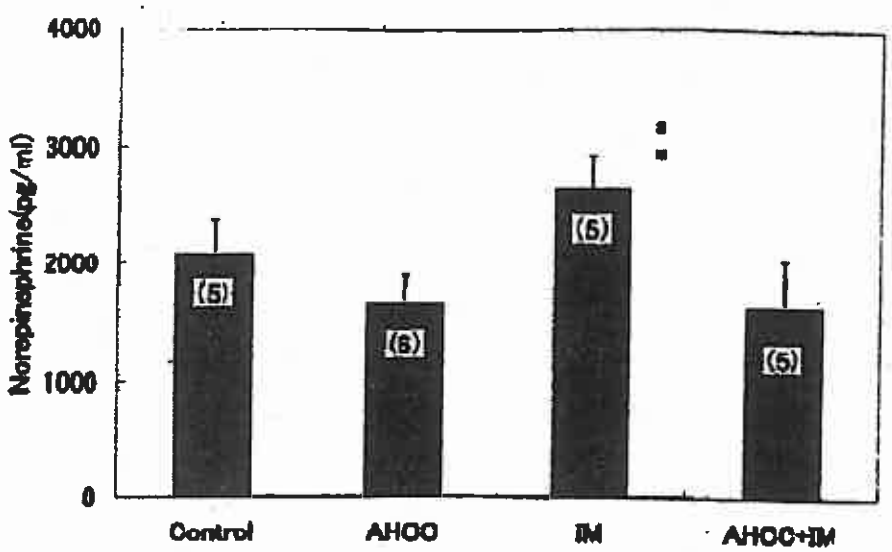

Fig. 1: Effect of immobilization and AHCC on norepinephrine (NE) levels in the plasma. NE was assayed by high- performance liquid chromatography (HPLC) with electrochemical detection. Data are shown as means ± SEM with the number of determinations in parentheses. *, $p < 0.05$ vs. control a, $p < 0.05$ vs. AHCC + IM Abbreviations used are as follows: IM, immobilization; AHCC, Active Hexose Correlated Compound.

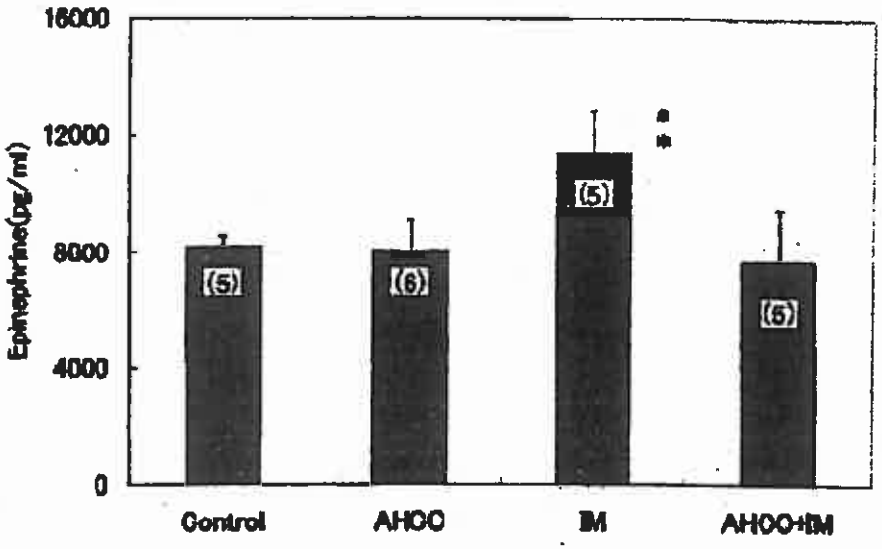

Fig. 2: Effect of immobilization and AHCC on epinephrine (EP) levels in the plasma. EP was assayed by high- performance liquid chromatography (HPLC). Data are shown as means ± SEM with the number of determinations in parentheses. *, $p < 0.05$ vs. control a, $p < 0.05$ vs. AHCC + IM.

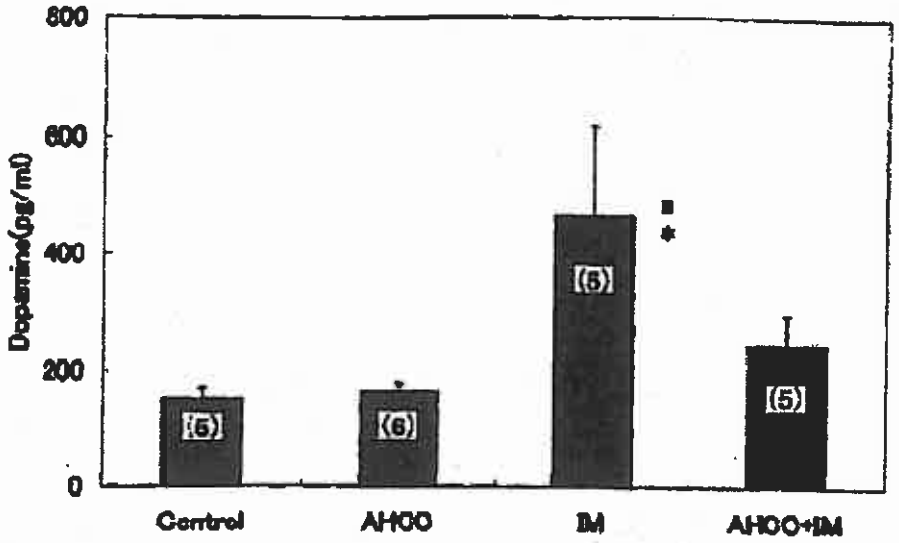

Fig. 3: Effect of immobilization and AHCC on dopamine (DA) levels in the plasma. DA was assayed by high- performance liquid chromatography (HPLC). Data are shown as means ± SEM with the number of determinations in parentheses. *, $p < 0.05$ vs. control a, $p < 0.05$ vs. AHCC + IM

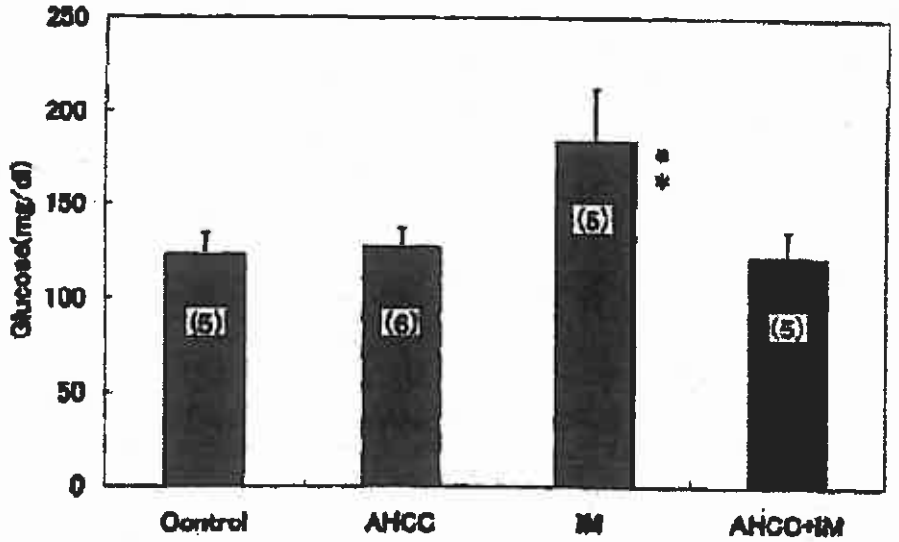

Fig. 4: Effect of immobilization and AHCC on glucose levels in the serum. Glucose in the serum was assayed by glucose assay kit. Data are shown as means ± SEM with the number of determinations in parentheses. *, $p < 0.05$ vs. control a, $p < 0.05$ vs. AHCC + IM

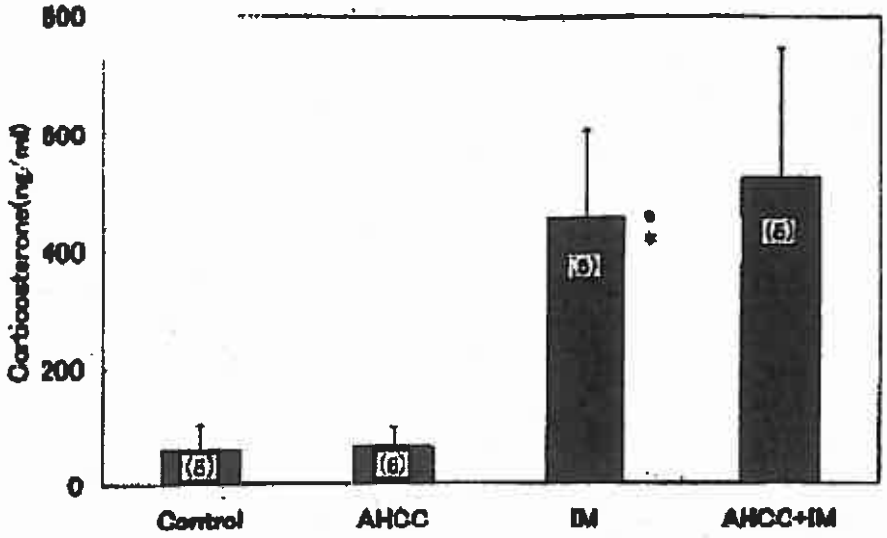

Fig. 5: Effect of immobilization and AHCC on corticosterone levels in the plasma. Corticosterone was assayed by high- performance commercial radioimmunoassay. Data are shown as means ± SEM with the number of determinations in parentheses. *, $p < 0.05$ vs. control a, $p < 0.05$ vs. AHCC + IM

elevated levels of norepinephrine, epinephrine and dopamine to near control levels. (Figs. 1 ~ 3)

2. *Serum glucose levels*

The immobilization of rats for 1 h increased the glucose levels in the serum. AHCC - pretreatment restored the elevated levels of blood glucose to near control levels. (Fig. 4)

3. *Serum corticosterone levels*

A significant rise in serum corticosterone levels was observed in the immobilization group, while the elevated levels of corticosterone were not significantly affected by AHCC pretreatment. (Fig. 5)

DISCUSSION

Stress- induced release of norepinephrine, epinephrine and dopamine levels has been well established in the literature [1-2]. Our results have also demonstrated a significant increase in the plasma levels of norepinephrine, epinephrine and dopamine levels after immobilization. Immobilization stress can stimulate the peripheral norepinephrine - containing nerve endings, leading to an increase in plasma norepinephrine levels.

Dopamine has been considered to represent a norepinephrine precursor pool, and dopamine and norepinephrine coexist within the norepinephrine axon terminals in the hippocampus [16]. Epinephrine synthesis is a five steps pathway in the adrenal medulla that begins with tyrosine. This amino acid is first converted into dihydroxyphenylalanine (DOPA) by tyrosine hydroxylase. When immobilization stress stimulates the sympathetic nervous system, tyrosine hydroxylase increases its activity and rapid synthesis of epinephrine occurs [17]. The release of epinephrine from the adrenal medulla is also carried out by the direct connection of nerve fibers from the hypothalamus to the adrenal medulla [18]. Under stressful conditions nerve cell activity in the hypothalamus is stimulated. As a result, preganglionic sympathetic neurons send impulses to the adrenal medulla with a higher frequency which ultimately leads to the increased release of epinephrine into the blood stream to maintain homeostasis [18].

A number of results suggest that short term immobilization produces a dramatic and sustained increase in blood glucocorticoid levels [19, 20]. Our present results have also shown that the levels of corticosterone in the serum increase significantly after immobilization. Wong et al [21] reported that immobilization stimulated the hypothalamic - pituitary - adrenal axis responses,

resulting in an increase in corticosterone secretion. Auby et al [22] found that corticotrophin - releasing factor (CRF) neurons enhance corticosterone secretion upon exposure to stress.

AHCC pretreatment suppressed the increase in the norepinephrine, epinephrine and dopamine levels in the plasma after 1 h by immobilization, but the increased serum levels of corticosterone were not decreased. Makara et al [23] have found that the elevation in plasma levels of epinephrine and norepinephrine during immobilization does not participate in the changes in plasma corticosterone. Kvetnansky et al [24] also found that catecholamines released from sympathoadrenomedullary system do not affect the acute release of corticosterone during immobilization. These data show that the increase in catecholamine secretion and the change in glucocorticoid secretion in response to immobilization are indepenently regulated. Possibly AHCC can suppress the increased levels of norepinephrine, epinephrine and dopamine without affecting serum corticosterone levels after 1 h by immobilization.

Our study showed that immobilization stress increase glucose levels in the serum. The effects of various stressors on serum glucose levels have been reported in animals [25, 26]. The effects of various stressors on glycemia differ depending on the type of stress. Immobilization stress can cause a rise in glucose levels in the rat, while cold stress does not [25,26]. There are two factors that contribute to the immobilization - induced increase in serum glucose; an increase in hepatic glucose output (gluconeogenesis and/ or glycolysis in the liver) and a decrease in glucose clearance in the peripheral tissues [27] Epinephrine increases serum glucose levels through stimulation of hepatic glucose output and interference with peripheral tissue glucose disappearance[7].

AHCC pretreatment restored the increased serum glucose levels induced by immobilization to normal. The finding suggests that AHCC inhibits serum glucose increase at least partially by suppressing the increase in epinephrine production and/ or secrection from adrenal medulla and that corticosterone plays a minor role in the regulation of blood glucose.

In conclusion, AHCC can suppress the immobilization - induced increase in circulating levels of norepinephrine, epinephrine, dopamine and glucose in the plasma by immobilization.

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