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Dietary Salt Modulates the Adrenocortical Expression of P450 11Beta-hydroxylase in Mice

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Abstract: This study was conducted to determine the effect of dietary salt on the synthesis of glucocorticoids in the adrenal cortex of mice. Mice had *ad libitum* access to 3% sodium chloride as the only drinking fluid (high salt diet) for either 4 days or 4 weeks. Adrenocortical expression of cytochrome P450 11beta-hydroxylase, a major regulatory enzyme in the biosynthesis of glucocorticoids, was examined by immunohistochemistry and western blot analysis. Ultrastructure of adrenocortical cell and plasma level of corticosterone were analyzed as well. Size and density of lipid droplets in the cortical cell were increased by high salt diet. Four days of high salt diet decreased P450 11beta-hydroxylase in the adrenal cortex, but 4 weeks increased it. Plasma level of corticosterone changed in parallel with the cortical level of P450 11beta-hydroxylase. These results suggest that high salt diet may modulate the biosynthesis of glucocorticoids, at least partly, via regulating the expression of P450 11beta-hydroxylase in adrenocortical cells.

Key words: Dietary salt, adrenal cortex, glucocorticoid, P450 11beta-hydroxylase, stress

Many studies on the hypertensive effect of dietary salt have been done through modulating the biosynthesis of aldosterone (Lehoux et al., 1974; Aguilera and Catt, 1979; Framer et al., 1979; Muller, 1980; Tremblay and Lehoux, 1992; Lumbers, 1999). Despite the evidence suggesting that adrenal glucocorticoids may induce hypertension through various mechanisms (Griffing et al., 1991; Whitworth, 1994; Saruta, 1996), the molecular mechanism by which dietary salt regulates glucocorticoid biosynthesis is not well known yet. It has been suggested that the plasma Na^+ may play an important role in the control of cell proliferation, mitochondrial density, and steroidogenic activity of the adrenal cortex (Orth et al., 1992; Aguilera, 1993; Greenspan and Baxter, 1994; Biason-Lauber, 1998).

The early steps of steroidogenesis begin with breakdown of cholesteryl esters into free cholesterol by activated protein kinase in lipid droplets. This free cholesterol is then transported into mitochondria by steroidogenic acute regulatory protein, where it undergoes a sequential conversion to pregnolone by cytochrome P450 side-chain cleavage, then to progesterone by 3beta-hydroxysteroid dehydrogenase (Miller, 1988). These early steps are common to all three zones of adrenal cortex, i.e. zona glomerulosa (ZG), zona fasciculata (ZF) and zona reticularis (ZR) *per se*, whereas the late steps of the steroidogenic pathway are zone-specific. In the ZF and ZR, glucocorticoid synthesis is regulated by expressions of cytochrome P450 17alpha-hydroxylase ($P450_{17alpha}$), P450 21alpha-hydroxylase ($P450_{21alpha}$), and cytochrome P450 11beta-hydroxylase ($P450_{11beta}$), which converts pregnolone to cortisol or corticosterone in the mitochondrial inner membrane (Mitani et al., 1982). $P450_{11beta}$, as a major regulatory enzyme, converts 11-deoxycortisol to glucocorticoids in the final step of glucocorticoid synthesis (Simpson and Waterman, 1998). It was reported that the hypertensive effect of dietary salt is accompanied by an elevated 18-hydroxy-corticosterone, which may be caused by an abnormal function of adrenal $P450_{11beta}$ (Hofreiter et al., 1982).

In order to investigate if dietary salt modulates the synthetic activity of glucocorticoids, we examined the adrenocortical expression of $P450_{11beta}$ and plasma level of corticosterone in mice that received high salt diet. Electron microscopic study was carried out to determine if the high salt diet alters ultrastructure of the cortical cells as well.

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MATERIALS AND METHODS

Animals

Male ICR mice at 7 wk of age were purchased (Samtaco Bio, Korea) and acclimated for a wk in the laboratory environment where the temperature (22±1°C) and humidity (55%) were controlled constantly with a 12 h light-dark cycle (light between 07:00 and 19:00). Mice had free access to standard laboratory chow (0.63% sodium content, Purina Rodent Chow, Purina Co., Seoul, Korea) and received either 3% NaCl or tap water as drinking fluid. They were cared for according to The Guide for animal experiments, 2000, edited by The Korean Academy of Medical Sciences, which is consistent with NIH Guideline for the Care and Use of Laboratory Animals, revised 1996.

Mice were sacrificed either 4 d or 28 d (4 wk) after the high salt diet (HSD) (3% NaCl) and the adrenal glands were processed for immunohistochemistry (n=6 in each group), western blot or electron microscopic analysis (n=6 in each group). Trunk bloods were collected for plasma corticosterone assay from the same mice used for western blot or electron microscopy. Age matching mice supplied with tap water instead of 3% NaCl were processed in parallel as the control groups (6 mice in each group for immunohistochemistry and another 6 for western blot, electron-microscopy or corticosterone assay).

Immunohistochemistry

Mice were overdosed with urethane and transcardially perfused with 20 ml of heparinized isotonic saline containing 0.5% $NaNO_2$ (Sigma), then 100 ml of ice-cold 4% paraformaldehyde (Sigma) in 0.1 M phosphate buffer (PB, pH 7.2). Adrenal glands were rapidly dissected, post-fixed in the same fixative for 2 h at 4°C, and then rinsed with 0.1 M PB. The adrenal glands were embedded in wax (Polyethylene glycol 400 diesterate, Polyscience) after dehydration in graded alcohols. The tissue sections were obtained at 6 μm thickness using a microtome (Leica RM 2135) and deposited on gelatin-coated slides and washed twice for 15 min in 0.1 M sodium phosphate buffered saline (PBS), then treated with 0.2% Triton X-100, 1% bovine serum albumin (BSA) in PBS for 30 min. After washing twice in PBS-BSA, sections were incubated overnight with mouse anti-rat cytochrome P450 11beta-hydroxylase monoclonal antibodies (1:200 dilution, Chemicon International Inc., U.S.A.). Sections were washed twice in PBS-BSA and incubated for 1 h with biotinylated anti-mouse IgG (1:200 dilution, Vector Laboratories), then bound secondary antibodies were amplified with the ABC kit (Vectastain Elite Kit, Vector Laboratories). Antibody complexes were visualized with 0.05% of diaminobenzidine (DAB) (Sigma) for 5 min. The sections were washed twice with PB, and then counterstained with hematoxylin, dehydrated, and coverslipped with permount.

Western blot analysis

Adrenal glands were dissected out immediately after decapitation (n=6), and then homogenized in 200 μl of lysis buffer (10 mM Hepes, pH 7.0, 10 mM EDTA, 0.1 mM EGTA, 1 mM DTT, 0.5 mM PMSF, 2 μg/ml leupeptin, and 2 μg/ml aprotinin). Protein concentration of the lysate was measured by the Bradford method. Protein samples were electrophoresed on 12% SDS-polyacrylamide gel and transferred onto nitrocellulose membrane (BioRad Inc.). Loading amounts of the protein samples were consistent over the experimental groups at the concentration base. The membrane was blocked with 2.0% BSA for 1 h at room temperature, incubated with mouse anti-rat P450 11beta-hydroxylase monoclonal antibodies (1:1000 dilution, Chemicon International Inc.) for 2 h. The membrane was washed with 2.0% BSA, incubated with biotinylated anti-mouse IgG (1:1000 dilution, Vector Laboratories Inc.), washed with Tris-buffered saline/Tween-20 (TBST), and then incubated in the ABC kit (Vector Laboratories Inc.) and visualized with DAB reaction. The left adrenal glands were used for western blot analysis, and the right for electron microscopy.

Electron microscopy

Adrenal glands were rapidly dissected out after decapitation, fixed in ice-cold 2% paraformaldehyde, and 2.5% glutaraldehyde in 0.1 M PB for 2 h, and then post-fixed in 2% osmium tetraoxide for 1 h. After dehydration in graded ethanols, the tissues were embedded with Epon (Poly/Bed 812 Resin, Polyscience) mixture and polymerized on the Polimerizer (Reichert-Jung) for 72 h at 60°C. Thin sections of the zona fasciculate were cut with an ultratome (ULTRACUT, Reichert-Jung) and stained with uranyl acetate and lead citrate. The ultrathin specimens were examined under a transmission electron microscope (JEM-1200EX, Japan).

Corticosterone assay

Plasma corticosterone levels of control and HSD mice were determined by a modified method as described previously (Hofreiter et al., 1982). Briefly, trunk bloods were collected in heparinized glass tubes immediately after decapitation, incubated at room temperature for 20 min and then centrifuged at 3,000 rpm for 10 min. Supernatants were aliquoted, rapidly frozen at -70°C and stored until use. Four ml of dichloromethane was added into each glass tube containing 50 μl of the plasma sample. The tubes were capped and shaken gently. After incubation at room temperature for 10 min, aqueous layer was transferred to a new glass tube and vortexed for 10 sec with 2 ml of fluorescence reagent (7 vol. of H_2SO_4 and 3 vol. of ethanol).

After incubation at room temperature for 30 min, tubes were centrifuged at 3,000 rpm for 10 min, and the supernatants removed carefully by aspiration. The bottom layer was transferred to polypropylene tube, the plasma corticosterone level determined using fluorospectrophotometer (EX 475 nm, EM 530 nm). Synthetic corticosterone (Sigma) was used as standard.

Quantitative and Statistical analysis

Percent cellular volume occupied by lipid droplets in the zona fasciculata cell was estimated from photo prints at a magnification of ×10,000 (at least 20 micrographs/mouse). The area of lipid droplets on the micrographs was quantified by Image Analysis System (VIDAS, Kontron, Germany). Quantitative data obtained from each experimental group were averaged per animal, and a calculation was made for the standard error from the mean (SEM). In order to compare the mean values of the different groups, Students t-test was used. The difference between two mean values was considered significant if the probability of error (P) was found to be less than 0.05.

RESULTS

Body weight, food and fluid intake

Body weights of mice were in a range of 33–36 g when the experiment was initiated. As shown in Table 1, a significant weight loss was produced by 4 days of HSD ($P<0.001$ vs. control diet), and although the weight gain appeared to be recovered by the 4th week of HSD, it still remained lower than the control ($P<0.01$). Food intake significantly decreased by 4 days of HSD ($P<0.001$), but tended to recover by 4 weeks ($P=0.054$), compared to each control. Fluid intake (3% NaCl in the HSD, water in the control groups) markedly decreased in the 4 day HSD mice ($P<0.01$), and no difference was found by the 4th week. Total amount of sodium consumed by food and fluid was ~8 and ~18 fold higher in the HSD group by the 4th day and the 4th week, respectively, compared to each control diet group.

Effects of high salt diet on $P450_{11beta}$ level

Mice were sacrificed either 4 days or 4 weeks after HSD, and the adrenal tissues processed for $P450_{11beta}$ immunohistochemistry or western blot analysis. In the control group, $P450_{11beta}$ immunoreactivity (-ir) was stronger in the cortex, especially in ZR and ZF, than in the medulla (Fig. 1A; a, c and B; a, c). $P450_{11beta}$-ir appeared to be reduced in the ZR and ZF of the 4 day-HSD mice (Fig. 1A; b, d), compared to the 4 day-control group (Fig. 1A; a, c). On the contrary, the 4 week-HSD group showed a marked increase of $P450_{11beta}$-ir both in ZR and ZF (Fig. 1B; b, d), compared to its control group (Fig. 1B; a, c).

Western blot analysis of $P450_{11beta}$ performed on the whole adrenal lysates showed that $P450_{11beta}$ level significantly decreased (by ~50%) in the 4 day-HSD group ($P<0.05$), but increased (by ~30%) in the 4 week-HSD group ($P<0.05$), compared to each control group (Fig. 2).

Plasma corticosterone level

Plasma corticosterone levels were measured to determine whether the HSD-induced alterations in the adrenocortical level of $P450_{11beta}$ enzyme correlates with the plasma level of its reaction product. To avoid the effect of diurnal variation in the plasma corticosterone level, all bloods were collected 1 to 2 h after the lights were turned on. The plasma corticosterone level was significantly decreased (by ~40%) by 4 days of HSD ($P<0.05$), however, markedly increased (by ~90%) by 4 weeks of HSD ($P<0.05$), compared to each control group (Fig. 3).

Ultrastructural changes induced by high salt diet

Ultrastructure of cells in the ZF was examined to determine whether the modulatory effect of HSD on $P450_{11beta}$ level is accompanied by structural changes of the cortical cells. The mitochondria contained a tubular or sheet structure of cristae, which is a typical feature normally detected in steroidogenic cells (Fig. 4). Lipid droplets, the storage of free cholesterol in steroidogenic cells, were densely distributed over the cytoplasm of ZF cells (Fig. 4). The size of lipid droplets appeared to be enlarged both in the 4 days and 4 weeks HSD groups, compared to the control group (Fig. 4). Quantitative analysis of lipid droplets done with an Image Analysis System revealed that the percent area occupied by lipid droplets in the cytoplasm of ZF cells was significantly increased both by 4 days (~56%, $P<0.05$) and 4 weeks

Table 1. Body weight gain, food and fluid intake (n=6)

Period	Body weight gain (g)		Food intake (g)		Fluid intake (g)	
	CTRL	HSD	CTRL	HSD	CTRL	HSD
4 days	2.53±0.84	-4.92±0.65[a]	25.45±1.44	14.60±1.86[c]	55.17± 3.01	39.83± 3.049[d]
4 weeks	5.30±1.85	-1.85±1.26[b]	164.92±8.10	139.94±8.13	575.75±27.56	602.28±42.94

Body weights of mice were in the range of 33-36 g when the experiment was initiated. A significant weight loss was produced by 4 days of high salt diet (HSD) ([a]$P<0.001$ vs. 4 days control), and the weight gain remained lower in the 4 weeks HSD group than in the control diet group ([b]$P<0.01$). Food intake significantly decreased by 4 days of HSD ([c]$P<0.001$), but tended to recover by 4 weeks (P=0.054), compared to each control group. Fluid intake markedly decreased in the 4 day HSD mice compared with the 4 days control group ([d]$P<0.01$), and no difference was found in the 4 weeks groups. CTRL; control diet, HSD; high salt diet (3% NaCl as the only drinking fluid).

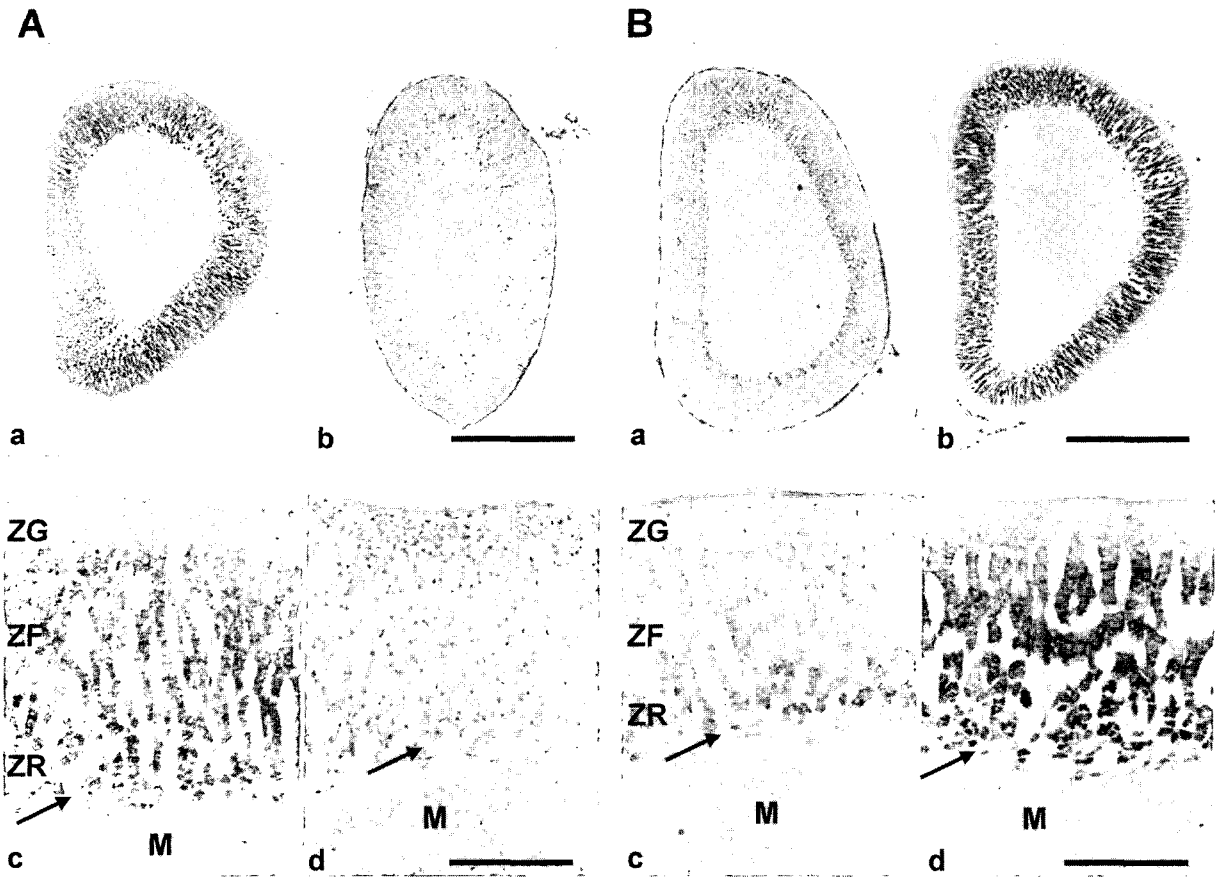

Fig. 1. Immunohistochemistry of P450 11beta-hydroxylase ($P450_{11beta}$) in the adrenal gland. Mice were sacrificed either 4 days or 4 weeks after free access to high salt diet (HSD; 3% NaCl as the only source of drinking fluid instead of water). A, A decrease in $P450_{11beta}$ immunoreactivities (-ir) was observed in the adrenal cortex of 4 days HSD mouse (b, d) compared to the control mouse (a, c). B, On the contrary, $P450_{11beta}$-ir in the cortex of 4 weeks HSD mouse (b, d) appeared to be stronger than its control (a, c). Changes in $P450_{11beta}$-ir were detected in the fasciculata and the reticularis layers, but not in the glomerulosa. M; medulla, ZG; zona glomerulosa, ZF; zona fasciculata, ZR; zona reticularis. Arrows indicate border lines between the cortex and the medulla. Scale bars= 50 μm (a, b) and 10 μm (c, d).

(~53%, $P<0.05$) of HSD, compared to each control group (Fig. 5).

DISCUSSION

Many lines of evidence suggest that adrenal glucocorticoids as well as dietary salt may be implicated in the pathogenesis of hypertension through various mechanisms (Okamoto, 1969; Griffing et al., 1991; Whitworth, 1994; Saruta, 1996), and the plasma salt may modulate activity of adrenocortical cells (Orth et al., 1992; Aguilera, 1993; Greenspan and Baxter, 1994; Biason-Lauber, 1998). However, the mechanism by which dietary salt regulates glucocorticoid biosynthesis is not well known. In this study, we demonstrated that short-term (4 days) HSD decreases cytochrome P450 11beta-hydroxylase ($P450_{11beta}$) level, but long-term (4 weeks) increases it, in the ZR and ZF of adrenal cortex. $P450_{11beta}$ is a major regulatory enzyme in the final step of glucocorticoids biosynthesis, which converts 11-deoxycortisol to glucocorticoids (Simpson and Waterman, 1998; Sewer and Waterman, 2003). Thus, altered expression of $P450_{11beta}$ may result in an alteration of the plasma glucocorticoids level. Indeed, in the present study we found a parallel change in the plasma corticosterone level with $P450_{11beta}$ expression in the HSD mice, i.e. decreased by 4 days and increased by 4 weeks of HSD. Therefore, it is suggested that HSD may modulate the plasma glucocorticoids level, at least partly, via regulating the gene expression of $P450_{11beta}$ in the cortical cells.

Genetic mutations in CYP11B1 gene, which encodes $P450_{11beta}$, have been reported to result in hypertension with cortisol deficiency in human (Ferrari et al., 2001). Thus, decrease in the adrenal expression of $P450_{11beta}$ and the plasma corticosterone in the 4 days HSD group may represent perhaps a short-term effect of extra sodium loading on hypertension. It has been reported that dietary salt loading with 2% NaCl ad libitum for 7 days decreases plasma corticosterone level and the hypothalamic expression of corticotropin-releasing hormone (CRH) in rats (Amaya

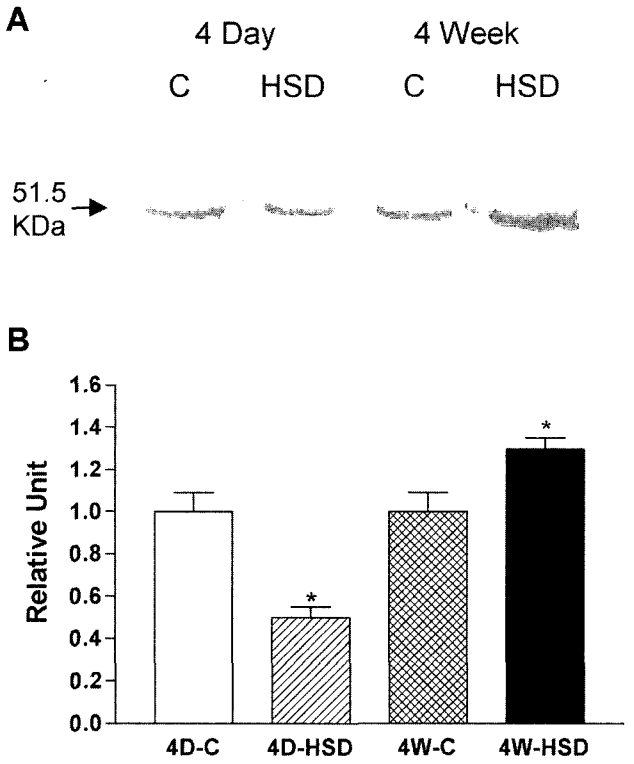

Fig. 2. Western blot analysis of the adrenal gland tissue lysates with specific antibodies against P450 11beta-hydroxylase ($P450_{11beta}$). A, Four days HSD appeared to decrease, but 4 weeks HSD increase, $P450_{11beta}$ in the adrenal tissues, compared to each control. B, Relative optical densities of the immuno-bands showed a significant decrease in 4 days HSD (~50% decrease), increase in 4 weeks HSD (~30% increase), compared to each control group. n=6 in each group. *P<0.05 vs. each control.

et al., 2001). This report supports our short-term (4 days HSD) result, and together it is suggested that short-term salt loading may reduce plasma corticosterone level via suppressing the hypothalamic expression of CRH. Adrenocorticotrophic hormone (ACTH), which is released by CRH stimulation, increases transcription of CYP11B1 gene in the

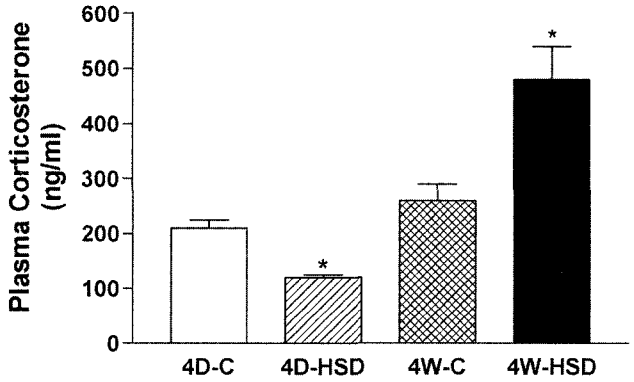

Fig. 3. Plasma corticosterone level. Four days of HSD significantly decreased, but 4 weeks increased, the plasma level of corticosterone. *P<0.05 vs. each control.

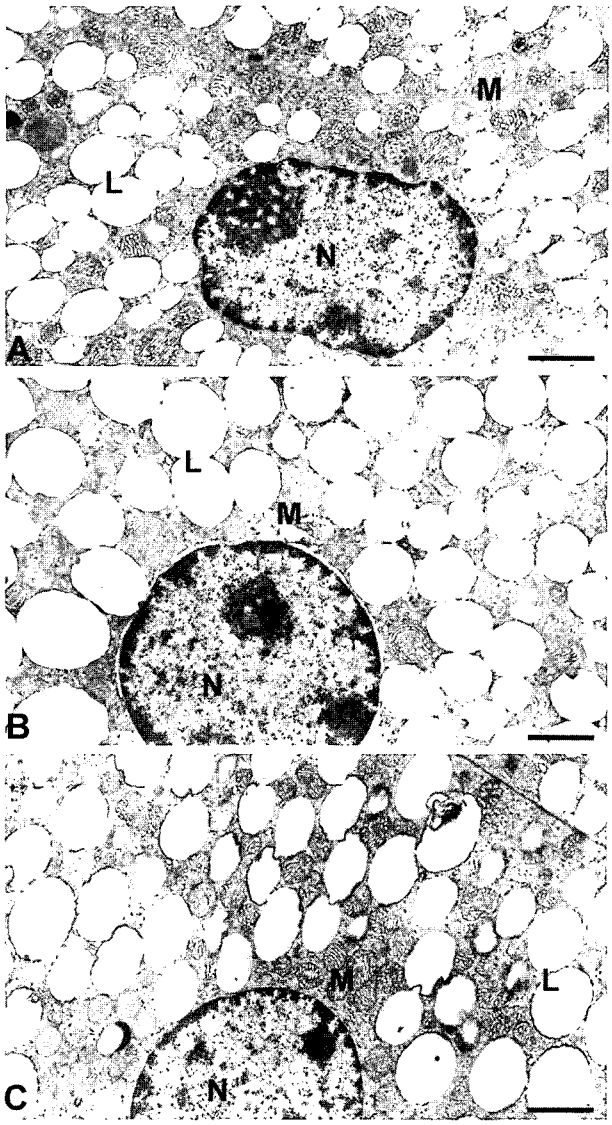

Fig. 4. Electron micrographs of cells in the zona fasciculata of adrenal cortex. Mitochondrial cristae were developed in a tubular or sheet structure, which is a typical feature normally detected in steroidogenic cells. Lipid droplets, the storage of free cholesterol in steroidogeic cells, appeared to be enlarged both in 4 days (B) and 4 weeks (C) HSD groups, compared to the control diet group (A). M; mitochondria, L; lipid droplet, N; nucleus. Scale bar=0.1 μm.

fasciculata of adrenal glands by cAMP-mediated pathway (Sewer and Waterman, 2003). Taken all together, it is concluded that decreased expression of the adrenal $P450_{11beta}$ in the 4 day-HSD group may be due to a decrease in CRH expression, likely by short-term salt loading. However, the mechanism by which the plasma Na^+ suppresses CRH expression should be further studied.

Whereas short-term (4 days) salt loading decreased the plasma corticosterone level, long-term (4 weeks) salt loading markedly increased it. This result concurs with previous report that short- and long-term salt loading

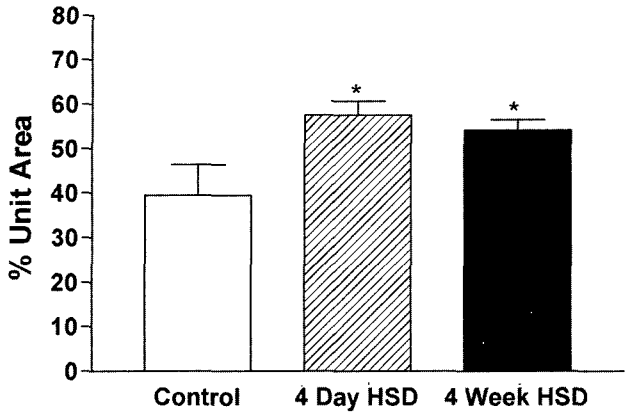

Fig. 5. Percent area of lipid droplets in the cytoplasm of zona fasciculata cell. Area of lipid droplets on the micrographs at ×10,000 magnification was quantified by Image Analysis System (VIDAS, Kontron, Germany). The percent area occupied by lipid droplets significantly increased both in 4 days (~56%) and 4 weeks HSD (~53%), compared to the control. *P<0.05 vs. control.

produces effects opposite to each other on the pituitary-adrenal axis activity (Elias et al., 2002). It has been reported that 2% NaCl ad libitum for 7 days decreases (Amaya et al., 2001), but water deprivation for 48 h or longer (Aguilera et al., 1993; Kiss et al., 1994) or feeding with high salt chow (7.5% NaCl) for 14 days (Sechi et al., 1994) increases plasma corticosterone level with increased osmolarity in rats. Six days of water restriction with 30 min daily water supply also increases plasma osmolarity, but, does not change the plasma corticosterone level (Wotus et al., 2003). These reports further support our result, and together it is suggested that extended and/or higher doses of salt loading may increase plasma corticosterone level, in contrast to the effect of mild salt loading.

The adrenal expression of $P450_{11beta}$ markedly increased concomitantly with the plasma corticosterone level in the 4 weeks HSD mice, compared to the control diet mice. This result reveals that an increase in the plasma corticosterone by extended salt loading may be correlated with increased expression of CYP11B1 gene in the adrenal cortex. The plasma Na^+ has been suggested to be a physiological stressor breaking the cellular homeostasis (Ogishima et al., 1992) and a stress activation of the hypothalamic-pituitary-adrenal axis elevates plasma corticosterone level via ACTH release. It has been reported that surgical stress and nutritional stress, such as food deprivation, all increase $P450_{11beta}$ expression in the adrenal cortex in relation with increased plasma glucocorticoids (Engeland et al., 1997; Chang et al., 2002). Therefore, it appears that the extended salt loading, likely as physiological stress, may increase the adrenal expression of $P450_{11beta}$ by activating the hypothalamic-pituitary-adrenal axis. As mentioned above, $P450_{11beta}$ expression in the adrenal cortex is stimulated by ACTH, a hormone released in response to stress (Ferrari et al., 2001; Sewer and Waterman, 2003). However, no report has shown an increase in plasma ACTH by chronic salt loading. Further study should be done on the molecular mechanism by which extra salt loading induces $P450_{11beta}$ gene expression.

Many studies have reported that food deprivation or restriction elevates plasma glucocorticoid levels (Garcia-Belenguer et al., 1993; Kiss et al., 1994; van Haasteren et al., 1996). Furthermore, Chang et al. (2002) reported that food deprivation enhances enzyme activity of $P450_{11beta}$ and elevates plasma corticosterone level. In this study, food intake and body weight gain decreased both in the 4 day- and 4 week-HSD groups, compared to each age-matching control. However, $P450_{11beta}$ expression and the plasma corticosterone level decreased in the 4 day-HSD, but increased in the 4 week-HSD, respectively. Furthermore, the reduction in food intake in the 4 week-HSD mice was not statistically significant, compared to the control diet group. Therefore, a tentative effect of reduced intake on the plasma corticosterone level and/or $P450_{11beta}$ expression can be excluded in this study.

In the ultrastracture study of adrenocortical cells, the number of lipid droplets as well as the size of each droplet appeared to be increased by HSD regardless of HSD duration. The percent area occupied by lipid droplets per unit area was markedly increased (53–56%) in the HSD groups. Lipid droplets in steroidogenic cells are known to be the storage of free cholesterol and it has been reported that cholesterol contents and the compartment of lipid droplets in the adrenocortical cells are increased by sustained stimulation of steroidogenic activity (Rocco et al., 1994; Cheng et al., 1998). Thus, the increase in lipid droplets, together with increases in $P450_{11beta}$ expression and plasma corticosterone level, in the 4 week-HSD group supports chronic increase in steroidogenic activity. However, in the 4 day-HSD mice, $P450_{11beta}$ expression and plasma corticosterone level were decreased despite the increase in lipid droplets. One plausible explanation is that the enlarged lipid droplets in the 4 day-HSD group may be due to a feedback regulation to recover the short-term decrease in plasma corticosterone level. Further study is required to prove this.

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REFERENCES

Amaya F, Tanaka M, Hayashi S, Tanaka Y, and Ibata Y (2001) Hypothalamo-pituitary-adrenal axis sensitization after chronic salt loading. *Neuroendocrinology* 73: 185-193.

Aguilera G and Catt KJ (1979) Loci of action of regulators of aldosterone biosynthesis in isolated glomerulosa cells. *Endocrinology* 104: 1046-1052.

Aguilera G (1993) Factors controlling steroid biosynthesis in the zona glomerulosa of the adrenal. *J Steroid Biochem Mol Biol* 45: 147-151.

Aguilera G, Lightman SL, and Kiss A (1993) Regulation of the hypothalamic-pituitary-adrenal axis during water deprivation. *Endocrinology* 132: 241-248.

Biason-Lauber A (1998) Molecular medicine of steroid hormone biosynthesis. *Mol Aspects Med* 19: 155-220.

Chang LL, Kau MM, Wun WS, Ho LT, and Wang PS (2002) Effects of fasting on corticosterone production by zona fasciculate-reticularis cells in ovariectomized rats. *J Investig Med* 50: 86-94.

Cheng B, Chou SC, Abraham S, and Kowal J (1998) Effects of prolonged ACTH-stimulation on adrenocortical cholesterol reserve and apolipoprotein E concentration in young and aged Fischer 344 male rats. *J Steroid Biochem Mol Biol* 66: 335-345.

Elias LL, Dorival Campos A, and Moreira AC (2002) The opposite effects of short- and long-term salt loading on pituitary adrenal axis activity in rats. *Horm Metab Res* 34: 207-211.

Engeland WC, Levay-Young BK, Rogers LM, and Fitzgerald D (1997) Differential gene expression of cytochrome P450 11beta-hydroxylase in rat adrenal cortex after *in vivo* activation. *Endocrinology* 138: 2338-2346.

Ferrari P, Bianchetti M, and Frey FJ (2001) Juvenile hypertension, the role of genetically altered steroid metabolism. *Horm Res* 55: 213-223.

Garcia-Belenguer S, Oliver C, and Mormede P (1993) Facilitation and feedback in the hypothalamo-pituitary-adrenal axis during food restriction in rats. *J Neuroendocrinol* 5: 663-668.

Greenspan FS and Baxter JD (1994) Basic and Clinical Endocrinology. Lange Medical Publishers, New York.

Griffing GT, Melby JC, Holbrook M, Wilson T, Azar S, Delaney M, and Weiss S (1991) Elevated 18-hydroxy-corticosterone in inbred salt-sensitive rats. *Clin Exp Hypertens A* 13: 371-382.

Hofreiter BT, Allen JP, Mizera AC, Powers CD, and Masi AM (1982) High-performance liquid chromatography and radioimmunoassay of rat plasma corticosterone. *Steroids* 39: 547-555.

Kiss A, Jezova D, and Aguilera G (1994) Activity of the hypothalamic pituitary adrenal axis and sympathoadrenal system during food and water deprivation in the rat. *Brain Res* 663: 84-92.

Kramer RE, Gallant S, and Browine AC (1979) The role of cytochrome P-450 in the action of sodium depletion on aldosterone biosynthesis in rats. *J Biol Chem* 254: 3953-3958.

Lehoux JG, Sandor T, Henderson IW, and Jones IC (1974) Some aspects of the dietary sodium intake on the regulation of aldosterone biosynthesis in rat adrenals. *Can J Biochem* 52: 1-6.

Lumbers ER (1999) Angiotensin and aldosterone. *Regul Pept* 80: 91-100.

Miller WL (1988) Molecular biology of steroid hormone synthesis. *Endocr Rev* 9: 295-318.

Mitani F, Shimizu T, Ueno R, Ishimura Y, Izumi S, Komatsu N, and Watanabe K (1982) Cytochrome P450 11 beta and $P450_{scc}$ in adrenal cortex: zonal distribution and intramitochondrial localization by the horseradish peroxidase-labeled antibody method. *J Histochem Cytochem* 30: 1066-1074.

Muller J (1980) 11β-Hydroxylation of 18-hydroxy-11-deoxy-corticosterone by rat adrenal tissue: zone specificity and effect of sodium and potassium testriction. *J Steroid Biochem* 13: 253-257.

Ogishima T, Suzuki H, Hata J, Mitani F, and Ishimura Y (1992) Zone-specific expression of aldosterone synthase cytochrome P-450 and cytochrome P-450 11β in rat adrenal cortex: histochemical basis for the functional zonation. *Endocrinology* 130: 2971-2977.

Okamoto K (1969) Spontaneous hypertension in rats. *Int Rev Exp Pathol* 7: 227-270.

Orth DN, Kavacs WJ, and Rowan DeBold C (1992) William's Textbook of Endocrinology. Saunders, Philadelphia, pp 489-619.

Rocco S, Rebuffat P, Cimolato M, Opocher G, Peters J, Mazzocchi G, Ganten D, Mantero F, and Nussorfer GG (1994) Zona glomerulosa of the adrenal gland in a transgenic strain of rat: a morphologic and functional study. *Cell Tissue Res* 278: 21-28.

Saruta T (1996) Mechanism of glucocorticoid-induced hypertension. *Hypertens Res* 19: 1-8.

Sechi LA, Griffin CA, and Schambelan M (1994) Effect of dietary sodium chloride on insuline receptor number and mRNA levels in rat kidney. *Am J Physiol* 266: F31-F38.

Sewer MB and Waterman MR (2003) ACTH modulation of transcription factors responsible for steroid hydroxylase gene expression in the adrenal cortex. *Microsc Res Tech* 61: 300-307.

Simpson ER and Waterman MR (1998) Regulation of the synthesis of steroidogenic enzymes in adrenal cortical cells by ACTH. *Annu Rev Physiol* 50: 427-440.

Tremblay A and Lehoux JG (1992) Influence of captopril on adrenal cytochrome P-450s and adrenodoxin expression in high potassium or low sodium intake. *J Steroid Biochem Mol Biol* 41: 799-808.

van Haasteren GA, Linkels E, van Toor H, Klootwijk W, Kaptein E, de Jong FH, Reymond MJ, Visser TJ, and de Greef WJ (1996) Effects of long-term food reduction on the hypothalamus-pituitary-thyroid axis in male and female rats. *J Endocrinol* 150: 169-178.

Whitworth JA (1994) Studies on the mechanisms of glucocorticoid hypertension in humans. *Blood Press* 3: 24-32.

Wotus C, Osborn JW, Nieto PA, and Engeland WC (2003) Regulation of corticosterone production by vasopressin during water restriction and after drinking in rats. *Neuroendocrinology* 78: 301-311.

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