J. Pharm. Pharmacol. 1998, 50: 1139–1145
Received April 6, 1998
Accepted June 4, 1998

Activation of 11β-Hydroxysteroid Dehydrogenase by Dehydroepiandrosterone Sulphate as an Anti-hypertensive Agent in Spontaneously Hypertensive Rats

MASATO HOMMA, TOSHIAKI ONODERA, MASAKI HIRABATAKE, KITARO OKA, MASAO KANAZAWA*, TAKASHI MIWA* AND TOHRU HAYASHI*

*Department of Clinical Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, and *3rd Department of Internal Medicine, Tokyo Medical College, Nishishinjuku, Shinjuku-ku, Tokyo 160-0023, Japan*

Abstract

The anti-hypertensive properties of dehydroepiandrosterone sulphate (DHEAS) have been investigated by studying its effects on blood pressure, on serum concentrations of corticosterone and dehydrocorticosterone, and on 11β-hydroxysteroid dehydrogenase (11β-HSD) activity in spontaneously hypertensive rats (SHR).

SHR were given intraperitoneal injections of DHEAS (10 mg day^{-1} for 70 days) from six to 16 weeks of age. The blood pressure–time curve was significantly ($P < 0.05$) suppressed immediately after administration of DHEAS. There was no difference between the heart rates of control and DHEAS groups. Serum concentrations of corticosterone and dehydrocorticosterone in the DHEAS group were significantly ($P < 0.05$) lower than those of the control group. The dehydrocorticosterone/corticosterone concentration ratio was, however, significantly ($P < 0.05$) higher in the DHEAS group, suggesting that treatment with DHEAS enhanced the overall interconversion of corticosterone to dehydrocorticosterone. The activity of 11β-HSD in specific organs of the DHEAS group was affected, characteristic changes being increases in the kidney (14–58%), decreases in the liver (11–27%) and no change in the testis. Direct addition of DHEAS to 11β-HSD preparations from the kidneys of control SHR had the same effect as that observed in the in-vivo experiments.

The fall in serum corticosterone in the DHEAS group is considered to be related, at least partly, to increased activity of kidney 11β-HSD. The inverse correlation of kidney 11β-HSD activity with serum corticosterone and blood pressure ($-r = 0.628$, $P < 0.01$, and $-r = 0.478$, $P < 0.05$, respectively) suggest that DHEAS delayed the development of hypertension in SHR by selective promotion of kidney 11β-HSD activity which in turn resulted in lower serum concentrations of corticosterone and its minimal aldosterone-like activity.

Dehydroepiandrosterone sulphate (DHEAS), a conjugated sulphate ester of dehydroepiandrosterone (DHEA), is secreted from the adrenal cortex into the systemic circulation. Although the circulating level of DHEAS is higher than that of any other steroid hormone, the physiological role of this hormone is unclear. DHEAS has a unique secretion pattern, blood levels declining in adults by 2% per year, or by 10–20% of the maximum level when the age of 85 is reached (Baulieu 1996). This pattern is in contrast with those of other adrenal steroids such as cortisol, the blood concentrations of which do not change with ageing. Thus in the early stages of life the hormone-balance of cortisol and DHEAS inclines to DHEAS and in the late stages of life to cortisol, despite the secretion rates of both hormones being regulated by the same adrenocorticotrophic hormone (ACTH). This breakdown of the hormone balance with ageing might enhance the physiological effects of cortisol. If DHEAS plays the role of anti-glucocorticoid (Keith et al 1991; Baulieu 1996) at physiological levels, blood

Correspondence: M. Homma, Department of Clinical Pharmacology, Tokyo University of Pharmacy and Life Science, 1432-1 Horinouchi, Hachioji, Tokyo 192-03, Japan.

pressure elevation, blood glucose increases and deterioration of immune responses could be understood in terms of perturbed hormone balance.

Results from epidemiological studies and clinical trials have implied that the fall in DHEA and DHEAS levels is associated with the increasing prevalence of age-related diseases such as atherosclerosis (Nestler et al 1992), cardiovascular diseases (Connor et al 1986), Alzheimer's disease (Leblhuber et al 1993) and hypertension (Beer et al 1994; Legrain et al 1995). These hormones might, therefore, have pharmacological activity which prevents age-related diseases. Anti-hypertensive effects of these steroids have been demonstrated in animal experiments involving glucocorticoid-induced hypertension (Shafagoj et al 1992). This has been supported further by clinical observations that the lower levels of DHEAS observed in hypertensives with hyperinsulinaemia were restored to normal levels after treatment with calcium antagonists, which inversely reduced cortisol levels (Beer et al 1993). These observations lead us to consider that DHEAS might act as an anti-glucocorticoid by modification of glucocorticoid metabolism preventing glucocorticoid-induced hypertension by promoting cortisol metabolic enzyme(s).

11β-Hydroxysteroid dehydrogenase (11β-HSD), which facilitates dehydrogenation of cortisol to cortisone in man and corticosterone to 11-dehydrocorticosterone in rats, has been known to modulate the mineralocorticoid-like action of glucocorticoids (Edwards et al 1988; Funder et al 1989). Two isozymes reported are 11β-HSD1, which disperses mainly in the liver, kidney, testes and lungs, and 11β-HSD2, in the kidney, placenta and colon (Walker et al 1992). Activity of 11β-HSD2 in aldosterone-target tissues is believed to be important in blood-pressure regulation by transforming cortisol (or corticosterone) into metabolites which cannot bind to the mineralocorticoid receptor, thus preventing their access to this receptor (Funder et al 1989). Impaired activity of this enzyme leads to over-activation of the mineralocorticoid receptor by cortisol (or corticosterone), resulting in enhanced sodium retention (Edwards et al 1988; Funder et al 1989).

It has recently been reported that the activity of kidney 11β-HSD is impaired in spontaneously hypertensive rats (SHR) (Hermans et al 1995) and that hypercorticoidism is involved in blood-pressure elevation (Ruch et al 1984; Kenyon et al 1993). Because hypertension in SHR seems to be linked to hypercorticoidism and thus to impaired activity of kidney 11β-HSD, SHR was considered to be a suitable animal model for investigating glucocorticoid-induced hypertension. In this study we have investigated the anti-hypertensive pharmacological properties of DHEAS connected with its intervention in glucocorticoid metabolism by determining serum concentrations of corticosterone and dehydrocorticosterone, and tissue 11β-HSD activity.

Materials and Methods

Animals

Five-week-old male SHR (Charles River, Tokyo, Japan) were acclimatized in vivariums for one week before use. During the experiment the animals had free access to food and water and were kept at room temperature (24°C), 55% humidity, with a 12-h light–dark cycle.

The study was approved by the Committee for Animal Welfare and Control at Tokyo University of Pharmacy and Life Science.

DHEAS administration and blood-pressure measurement

Starting at six weeks of age male SHR were given daily intraperitoneal injections of 10 mg DHEAS dissolved in 0·4 mL saline for 10 ($n = 10$) or three ($n = 7$) consecutive weeks. Groups of control animals were given the same volume of saline for 10 ($n = 9$) or three ($n = 6$) consecutive weeks. Systolic blood pressure (SBP) and heart rate were recorded at 4–7-day intervals before and after treatment with DHEAS. SBP and heart rate were measured by the tail-cuff method, using a photoelectric sensor and pulse amplifier (Softron, Tokyo, Japan) connected to a microcomputer (PC-9800, NEC, Tokyo, Japan) for data processing. Before the measurements the rats were kept in a quiet room for 2 h and then placed in a 38·5°C resting cage for 10 min. SBP readings were taken until differences between three sequential readings were within 15 mmHg; the average value was used as the observed SBP. The coefficient of variation for repeated measurements of blood pressure was $<10·4\%$.

Determination of serum concentrations of corticosterone and dehydrocorticosterone

The animals were killed by decapitation 24 h after the last injection and blood samples were obtained. The total time from initial handling to decapitation was <3 min. Serum corticosterone and dehydrocorticosterone were determined by a combination of diatomaceous earth-column extraction and high-performance liquid chromatography (HPLC). The procedures are described in detail elsewhere (Oka et al 1984). In brief, serum (300 μL) containing prednisone (50 ng) as internal standard were

treated in a diatomaceous earth-column system with dichloromethane (7 mL) as extraction solvent. The effluent from the column was evaporated to dryness, reconstituted in methanol–dichloromethane (4:96, v/v; 20 μL) and subsequently injected into an HPLC system (Jasco 880 series, Tokyo, Japan) equipped with a UV-detector and a conventional 4 mm i.d.×250 mm×5 μm particle LiChrosorb Si-60 silica gel column (Merck, Darmstadt, Germany). The mobile phase was water–methanol–dichloromethane–*n*-hexane, 0·1:4:30:65·9 (v/v) at a flow rate of 1·0 mL min^{-1}. The detection wavelength was 245 nm and the sensitivity 0·05 aufs; detection limits for corticosterone and dehydrocorticosterone were 5 and 2·5 ng mL^{-1}, respectively.

Determination of 11β-HSD activity

The activity of 11β-HSD in tissue-homogenate incubation mixtures was measured by detection of the chemical transformation of cortisol to cortisone in the presence of NADP or NAD as cofactor, as described elsewhere (Homma et al 1994). The incubation mixtures consisted of 820 μL Tris–HCl buffer (pH 8·5) containing Triton-X (0·014%), NADP or NAD (5 mM; 50 μL) and tissue homogenate (100 μL). After 10 min pre-incubation at 37°C, cortisol (0·3 mM; 200 μL) was added and the resulting mixtures were further incubated for 30 or 60 min. The enzyme reaction was terminated by addition of sulphuric acid (5%; 100 μL). Cortisol and cortisone in the reaction mixture were determined by HPLC using Extrashot (Kusano, Tokyo, Japan) which is a disposable syringe-type device for extraction and injection of lipophilic components in biofluids, as described elsewhere (Homma et al 1994).

Statistics

Unpaired Student's or Welch's *t*-tests were used to compare mean values of the SBP of control and DHEAS-treated SHR. Analysis of variance for multiple comparison of serum glucocorticoid levels and 11β-HSD activity of treatment groups of different ages was performed by the Student–Newman–Keuls test. Relationships between blood pressure and other quantitative parameters were examined by calculating Pearson's correlation coefficients (r). Values of $P < 0.05$ were considered to be indicative of significance.

Results

There was no significant difference between basal body weights of rats in the DHEAS and control groups (127·5 ± 6·4 and 131·4 ± 5·3 g, respectively). After onset of the treatment the body weight of the DHEAS group decreased slightly (at day 21, 231·1 ± 10·8 compared with 249·1 ± 11·5, $P < 0.01$; at day 70, 323·8 ± 18·6 compared with 344·8 ± 19·3, $P < 0.05$). However, food intake did not decrease on DHEAS treatment despite a decrease in body-weight gain (data not shown). Organ weights (liver, kidney, testis and lung) in both groups did not differ (data not shown).

The effects of DHEAS (10 mg day^{-1}) injection on blood pressure and heart rates of SHR are shown in Figure 1. Before the onset of treatment there was no difference between the basal SBP of DHEAS and control groups (129 ± 4 and 126 ± 11 mmHg, respectively). The average SBP of the control SHR increased to a maximum value of 179 ± 18 mmHg by day 49. Blood pressures in the DHEAS group were always lower than those in the control group; the maximum value of 165 ± 14 mmHg was observed on day 70. Statistical significance ($P < 0.05$) was observed on days 7, 21, 35, and 49. During the experiments the heart rates were the same for both groups (Figure 1).

Serum concentrations of corticosterone and dehydrocorticosterone and the dehydrocorticosterone/corticosterone concentration ratio were determined at days 21 and 70 (Table 1). Comparison with results from the control group on days 21 and 70

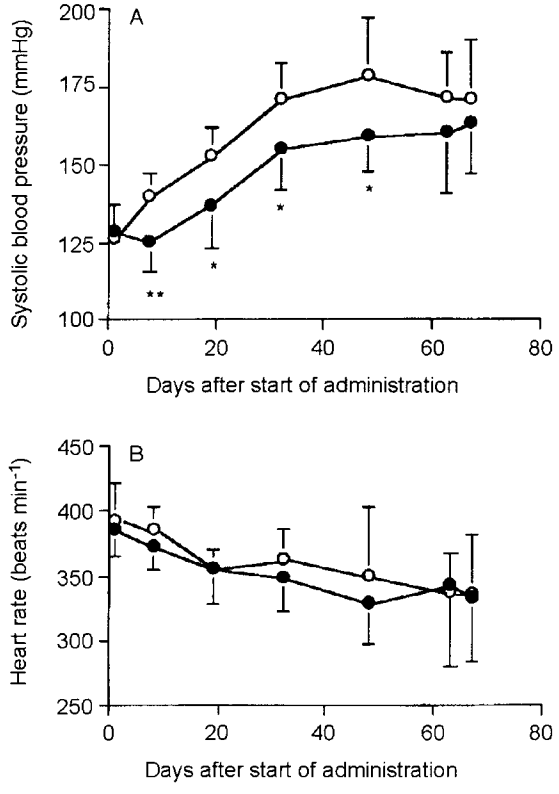

Figure 1. Effects of dehydroepiandrosterone sulphate on systolic blood pressure (A) and heart rate (B) in spontaneously hypertensive rats: ○, control rats (n = 9); ●, dehydroepiandrosterone sulphate-treated rats (n = 10). $*P < 0.05$, $**P < 0.01$, significantly different from control result.

showed that on day 70 serum corticosterone levels had increased significantly ($P < 0.05$) and the concentration ratio had tended to decrease. Similar changes owing to ageing were also found in the DHEAS group, but these were not statistically significant. Serum dehydrocorticosterone did not change with ageing in either the control or DHEAS groups. Concentrations of corticosterone and dehydrocorticosterone in the DHEAS group on days 21 and 70 were significantly lower ($P < 0.05$) than those in the corresponding control groups. The concentration ratio reflecting interconversion from corticosterone to dehydrocorticosterone increased significantly in the DHEAS group ($P < 0.05$).

The activity of 11β-HSD in the kidney, liver and testis was determined with tissue homogenates and NADP and NAD as specific cofactors for isozymes types 1 and 2, respectively. DHEAS and control-group data obtained on days 21 and 70 were compared (Table 2) and 11β-HSD activity in the kidney and liver was found to be significantly ($P < 0.01$) higher on day 70 than on day 21. This change was common to both cofactors and was observed for both groups during ageing. The effects of DHEAS treatment on 11β-HSD activity were significantly different for the kidney and liver. Compared with the control group 11β-HSD activity in the DHEAS group, determined by use of NADP, was higher in the kidney (18·8% on day 70) and lower in the liver (27·5 and 15·8% on days 21 and 70, respectively). When NAD was used instead of NADP, an increase in 11β-HSD activity (58·4%) in the kidney was also observed on day 21. In the testis, however, enzyme activity did not change during ageing and DHEAS treatment.

Tissue specificity of the effects of DHEAS on 11β-HSD activity were confirmed by an additional in-vitro experiment. By use of tissue homogenates rates of transformation of cortisol to cortisone were measured in the presence or absence of 100 μM DHEAS and the results for liver, kidney and testis were compared. The rate of production of cortisone in kidney homogenate increased 2·4-fold on addition of DHEAS whereas no significant change was observed for liver and testis homogenates. Dose-dependent effects of 10–100 μM DHEAS were compared for kidney and liver homogenates (Figure 2). Only the kidney enzyme was activated dose-dependently by DHEAS.

Relationships between blood pressure and serum concentrations of corticosterone and between blood pressure and kidney 11β-HSD activity were

Table 1. Effects of dehydroepiandrosterone sulphate on serum concentrations of corticosterone and dehydrocorticosterone and on the dehydrocorticosterone/corticosterone concentration ratio in spontaneously hypertensive rats.

Day	Group	n	Serum concentration (ng mL^{-1})		Dehydrocorticosterone/ corticosterone ratio
			Corticosterone	Dehydrocorticosterone	
21	Control	6	328·5 ± 137·0	29·5 ± 5·8	0·104 ± 0·043
	Dehydroepiandrosterone sulphate	7	77·6 ± 55·9†	19·4 ± 9·2*	0·285 ± 0·089*
70	Control	9	465·3 ± 88·4‡	26·3 ± 8·9	0·057 ± 0·019
	Dehydroepiandrosterone sulphate	10	158·8 ± 126·2†	16·3 ± 4·0*	0·201 ± 0·190*

*$P < 0.05$, †$P < 0.01$, significantly different from control result. ‡$P < 0.05$, significantly different from result after administration for 21 days.

Table 2. Effects of dehydroepiandrosterone sulphate on 11β-hydroxysteroid dehydrogenase activity in the kidney, liver and testis of spontaneously hypertensive rats.

Day	Group	n	11β-Hydroxysteroid dehydrogenase activity (pmol min^{-1} (mg protein)$^{-1}$)					
			Kidney		Liver		Testis	
			NADP	NAD	NADP	NAD	NADP	NAD
21	Control	6	149·7 ± 14·0	53·6 ± 7·2	211·0 ± 17·4	115·4 ± 19·7	158·1 ± 18·2	129·0 ± 6·4
	Dehydroepiandrosterone sulphate	7	142·6 ± 31·5	85·0 ± 21·8*	153·2 ± 27·0†	89·7 ± 18·5†	167·4 ± 29·1	107·1 ± 19·0
70	Control	9	258·8 ± 31·3‡	209·7 ± 29·8‡	255·6 ± 25·5‡	168·8 ± 15·8‡	138·0 ± 30·8	134·8 ± 25·3
	Dehydroepiandrosterone sulphate	10	307·4 ± 29·5†‡	238·4 ± 30·5*‡	215·2 ± 27·9†‡	149·6 ± 16·0*‡	133·1 ± 11·0	120·6 ± 10·9

*$P < 0.05$, †$P < 0.01$, significantly different from control result. ‡$P < 0.01$, significantly different from result after administration for 21 days.

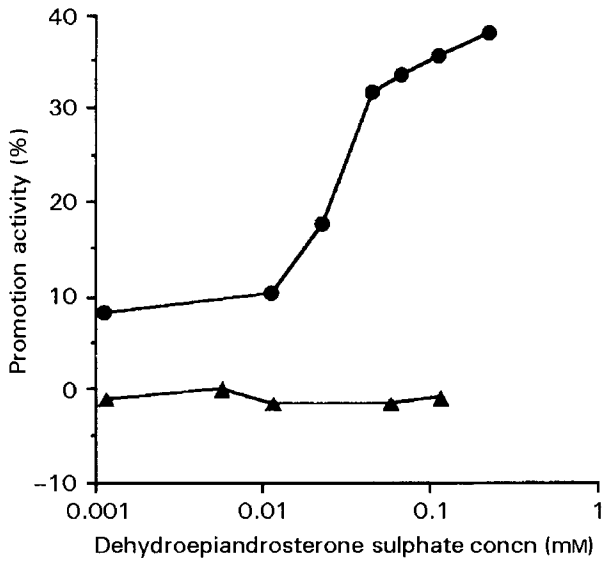

Figure 2. Dose-dependent dehydroepiandrosterone sulphate-induced promotion of 11β-hydroxysteroid dehydrogenase activity in kidney (●) and liver (▲) homogenate. NAD and NADPH were used as cofactors for determination of activity in the kidney and liver, respectively.

examined at day 70 in the DHEAS and control groups. As shown in Figure 3, blood pressure correlated positively with serum corticosterone concentration ($r = 0.511$, $P < 0.05$) and inversely with kidney 11β-HSD activity determined with NAD ($-r = 0.478$, $P < 0.05$). An inverse correlation between kidney 11β-HSD activity and serum corticosterone concentrations ($-r = 0.628$, $P < 0.01$) was also apparent (Figure 3). We found that the enzyme activity which correlated with blood pressure and serum corticosterone was that of the kidney enzyme manipulated with NAD and not NADP. This enzyme activity correlated significantly with the dehydrocorticosterone/corticosterone serum concentration ratio ($r = 0.817$, $P < 0.01$) (data not shown).

Discussion

The SHR has been widely used and intensively studied as an animal model for essential hypertension. Because the adrenalectomized SHR does not have elevated blood pressure (Ruch et al 1984; Hashimoto et al 1989), adrenal function is essential for development of hypertension. Comparison of results from inherited strains, SHR and normotensive Wistar–Kyoto rats has implied that corticosterone, rather than aldosterone, contributes to the occurrence of hypertension. SHR have significantly higher basal corticosterone levels than Wistar–Kyoto rats, whereas no difference in aldosterone production was apparent (Kenyon et al 1993). In addition to elevated levels of circulating glucocorticoid, recent investigations have demonstrated that local activity of corticosterone in SHR is enhanced by reduced 11β-HSD activity. This is especially important in mineralocorticoid target tissues such as mesenteric arteries (Takeda et al 1993) and the kidney (Hermans et al 1995), offering us a possible mechanism for hypertension in this animal. It seems likely that the SHR hypertension might be reversed if 11β-HSD activity could be recovered.

In this study we found that pharmacological doses of DHEAS prevented blood-pressure elevation in SHR, improving both hypercorticoidism and kidney 11β-HSD activity. The suppressive effects

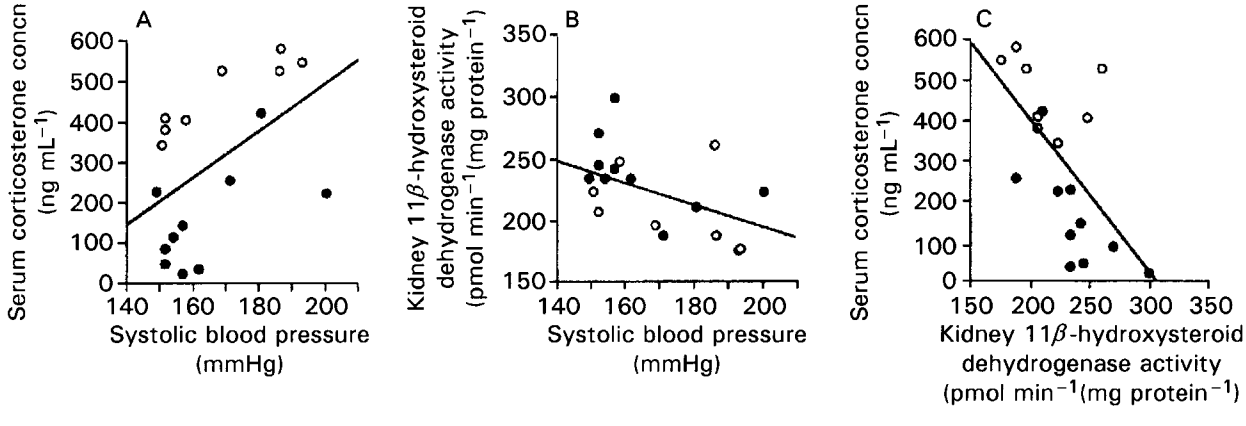

Figure 3. Correlation between systolic blood pressure and serum corticosterone (A; $r = 0.511$, $P < 0.05$) or kidney 11β-hydroxysteroid dehydrogenase determined with NAD (B; $r = 0.478$, $P < 0.05$), and between kidney 11β-hydroxysteroid dehydrogenase and serum corticosterone (C; $r = 0.628$, $P < 0.01$): ○, control rats ($n = 9$); ●, dehydroepiandrosterone sulphate-treated rats ($n = 10$).

of DHEAS on blood pressure were not caused by changes in the sympathetic nervous system because heart-rate changes during treatment were observed equally in DHEAS and control groups. It is still controversial whether changes in serum corticosterone and kidney 11β-HSD activity could account for the significant effects on blood pressure we observed. Significant correlations were observed both between blood pressure and serum corticosterone and between blood pressure and kidney 11β-HSD activity. Ruch et al (1984) found that SHR plasma corticosterone correlated with blood pressure, and that the suppressed blood pressure in adrenalectomized SHR was recovered by betamethazone replacement. These observations suggested that the serum glucocorticoid level was the most important factor in the pathogenesis of hypertension in SHR and that the anti-hypertensive effects of DHEAS were mediated by a fall in serum corticosterone.

The dose used in this study, 10 mg day^{-1}, equivalent to 30–40 mg kg^{-1} day^{-1}, is 100–200-fold higher than physiological secretion of DHEAS (0·2–0·3 mg kg^{-1} day^{-1}) in healthy male subjects. At a similar dose DHEA induces several physiological changes. Milewich et al (1995) reported that administration of DHEA to mice or rats resulted in a decrease in body weight gain and peroxisome proliferation. In the current experiment we also observed reductions in weight gain in the DHEAS group. Because DHEAS is the major source of circulating DHEA, it seems likely that the reduction in weight gain is because of the anti-obesity property of DHEA, which has been observed in C57BL/6 obese mice (Milewich et al 1995), rather than a delay in normal development. This was also implied by the absence of differences between food intake and organ weights for the DHEAS and control groups.

The mechanism(s) of action of the DHEAS-induced decrease in serum corticosterone concentration was of considerable interest. Because a higher dehydrocorticosterone/corticosterone serum concentration ratio was observed in the DHEAS group, it seems reasonable to suggest that DHEAS enhanced the overall rate of conversion of corticosterone to dehydrocorticosterone by promoting kidney 11β-HSD activity. This hypothesis is supported by the significant correlation between the dehydrocorticosterone/corticosterone concentration ratio and kidney 11β-HSD level. However, another possibility should also be considered—the fall in serum corticosterone in the DHEAS group might involve alteration of glucocorticoid biosynthesis. Sharma et al (1963) reported that DHEAS suppressed corticosterone production in adrenocortical cells by inhibiting 11β-hydroxylase, a key enzyme for glucocorticoid biosynthesis. It is therefore possible that the lower serum corticosterone concentration in the DHEAS group measured in our current experiments was a result of inhibition of corticosterone biosynthesis.

Organ-specific activity of DHEAS on 11β-HSD was also observed. When DHEAS was added to the enzyme preparations in-vitro, promotive effects on kidney 11β-HSD were apparent whereas no effect was observed on liver 11β-HSD. Although the promotion of the kidney isozyme by DHEAS seems to be a direct effect of DHEAS, the inhibitory effect of DHEAS on the liver isozyme was attributed to DHEAS metabolite(s). It is known that in the liver DHEAS is converted to DHEA and several other androgens such as testosterone and dihydrotestosterone (Baulieu 1996) and these metabolites were considered to be possible inhibitors of liver 11β-HSD. Two isozymes of 11β-HSD, type 1 in the liver and type 2 in the kidney, have been shown to require different cofactors, NADP and NAD, respectively (Walker et al 1992). In this account the activity of DHEAS on kidney isozyme was much higher in the presence of NAD than NADP, suggesting that DHEAS selectively enhanced type-2 isozyme activity.

Similar type-2-selective 11β-HSD activity has been reported for captopril and ramipril (Riddle & McDaniel 1994). These typical anti-hypertensive agents are also known to be angiotensin-converting enzyme inhibitors. The effect of inhibitors of angiotensin-converting enzyme on kidney type-2 11β-HSD might contribute to the natriuretic action of the agents (Dullaart et al 1995). It is worth mentioning that angiotensin II suppresses 11β-HSD activity and that this suppression is recovered by administration of an angiotensin II receptor antagonist, saralasin, and so the above-mentioned activation of kidney 11β-HSD by inhibitors of angiotensin converting enzyme might be mediated through inhibition of angiotensin II production in the kidney. This, however, might not be the only mechanism by which these agents affect 11β-HSD, because endogenous production of angiotensin II in the kidney is not enough to achieve an effective concentration (Riddle & McDaniel 1994). Despite the uncertainties, it has been confirmed that inhibition of angiotensin-converting enzyme accompanied by enhancement of 11β-HSD activity prevented blood-pressure increases associated with microalbuminuria in insulin-dependent diabetes mellitus (Dullaart et al 1995). This, with our current observations, provides evidence which underscores the clinical importance of renal 11β-HSD activity in the regulation of volume and sodium homeostasis.

These studies have confirmed our hypothesis that DHEAS, the serum levels of which are lower in hypertensive than in healthy subjects, has anti-hypertensive properties involving promotion of kidney 11β-HSD activity. It has also been suggested that DHEAS might be a regulator of renal 11β-HSD activity associated with glucocorticoid-induced hypertension, proposed as being latent in essential hypertension (Walker 1994).

Acknowledgements

We are grateful to Mr H. Horigome, Miss A. Wakita and Miss K. Shiraishi for their technical assistance. This work was supported by the Ministry of Education in Japan (Grant in Aid No. 08772175).

References

Baulieu, E. E. (1996) Dehydroepiandrosterone (DHEA): a fountain of youth? J. Clin. Endocrinol. Metab. 81: 3147–3151

Beer, N. A., Jakubowicz, D. J., Beer, R. M., Nestler, J. E. (1993) The calcium-channel blocker amlodipine raises serum dehydroepiandrosterone sulphate and androstenedione but lowers serum cortisol, in insulin-resistant obese and hypertensive men. J. Clin. Endocrinol. Metab. 76: 1464–1469

Beer, N. A., Jakubowicz, D. J., Beer, R. M., Nestler, J. E. (1994) Disparate effects of insulin reduction with diltiazem on serum dehydroepiandrosterone sulphate levels in obese hypertensive men and women. J. Clin. Endocrinol. Metab. 79: 1077–1091

Connor, E. B., Khaw, K. T., Samuel, S. C. (1986) A prospective study of dehydroepiandrosterone sulphate, mortality, and cardiovascular disease. N. Eng. J. Med. 315: 1519–1524

Dullaart, R. P. F., Ubels, F. L., Hoogenberg, K., Smit, A. J., Pratt, J. J., Muntinga, J. H. J., Sluiter, W. J., Wolthers, B. G. (1995) Alterations in cortisol metabolism in insulin-dependent diabetes mellitus: relationship with metabolic control and estimated blood volume and effect of angiotensin-converting enzyme inhibition. J. Clin. Endocrinol. Metab. 80: 3002–3008

Edwards, C. R., Stewart, P. M., Burt, D., Brett, L., McIntyer, M. A., Sutanto, W. S., Kloet, E. R., Monder, C. (1988) Localisation of 11beta-hydroxysteroid dehydrogenase-tissue specific protector of the mineralocorticoid receptor. Lancet 2: 986–989

Funder, J. W., Pearce, P. T., Smith, R., Smith, A. I. (1989) Mineralocorticoid action: target tissue specificity is enzyme, not receptor, mediated. Science 242: 583–585

Hashimoto, K., Makino, S., Hirasawa, R., Takao, T., Sugawara, M., Murakami, K., Ono, K., Ota, Z. (1989) Abnormalities in the hypothalamo–pituitary–adrenal axis in spontaneously hypertensive rats during development of hypertension. Endocrinology 125: 1161–1167

Hermans, J. J. R., Steckel, B., Thijissen, H. H. M., Janssen, B. J. A., Netter, K. J., Maser, E. (1995) Comparison of 11β-hydroxysteroid dehydrogenase in spontaneously hypertensive and Wistar–Kyoto rats. Steroids 60: 773–779

Homma, M., Oka, K., Niitsuma, T., Ito, H. (1994) A novel 11β-hydroxysteroid dehydrogenase inhibitor contained in Saiboku-to, a herbal remedy for steroid-dependent bronchial asthma. J. Pharm. Pharmacol. 46: 305–309

Keith, L. B., Merrily, P., William, M. R., Edward, W. B. (1991) Dehydroepiandrosterone antagonizes the suppressive effects of dexamethasone on lymphocyte proliferation. Endocrinology 129: 3174–3179

Kenyon, C. J., Panarelli, M., Holloway, C. D., Dunlop, D., Morton, J. J., Connell, J. M. C., Fraser, R. (1993) The role of glucocorticoid activity in the inheritance of hypertension: studies in the rat. J. Steroid Biochem. 45: 7–11

Leblhuber, F., Neubauer, C., Peichl, M., Reisecker, F., Steinparz, F. X., Windhager, E., Dienstl, E. (1993) Age and sex differences of dehydroepiandrosterone sulphate (DHEAS) and cortisol (CRT) plasma levels in normal controls and Alzheimer's disease (AD). Psychopharmacology 111: 23–26

Legrain, S., Berr, C., Frenoy, N., Gourlet, V., Debuire, B., Baulieu, E. E. (1995) Dehydroepiandrosterone sulphate in a long-term care aged population. Gerontology 41: 343–351

Milewich, L., Catalina, F., Bennett, M. (1995) Pleotropic effects of dietary DHEA. Ann. NY Acad. Sci. 774: 149–170

Nestler, J. E., Clore, J. N., Blackard, W. G. (1992) Dehydroepiandrosterone: missing link between hyperinsulinemia and atherosclerosis? FASEB J. 6: 3073–3075

Oka, K., Ohki, N., Noguchi, M., Matsuoka, Y., Irimajiri, S., Abe, M., Takizawa, T. (1984) Extraction-monitoring and rapid flow fractionation for determination of serum corticosteroids. Anal. Chem. 56: 2614–2617

Riddle, M. C., McDaniel, P. A. (1994) Renal 11β-hydroxysteroid dehydrogenase activity is enhanced by ramipril and captopril. J. Clin. Endocrinol. Metab. 78: 830–834

Ruch, W., Baumann, J. B., Hausler, A., Otten, U. H., Siegl, H., Girard, J. (1984) Importance of the adrenal cortex for development and maintenance of hypertension in spontaneously hypertensive rats. Acta. Endocrinol. 105: 417–424

Shafagoj, Y., Opoku, J., Qureshi, D., Regelson, W., Kalimi, M. (1992) Dehydroepiandrosterone prevents dexamethasone-induced hypertension in rats. Am. J. Physiol. 263: E210–E213

Sharma, D. C., Forchielli, E., Dorfman, R. I. (1963) Inhibition of enzymatic steroid 11β-hydroxylation by androgens. J. Biol. Chem. 238: 572–575

Takeda, Y., Yoneda, T., Miyamori, I., Gathiram, P., Takeda, R. (1993) 11β-Hydroxysteroid dehydrogenase activity in mesentric arteries of spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 20: 627–631

Walker, B. R., Campbell, J. C., Williams, B. C., Edowards, C. R. W. (1992) Tissue-specific distribution of the NAD^+-dependent isoforms of 11β-hydroxysteroid dehydrogenase. Endocrinology 131: 970–972

Walker, B. R. (1994) Organ-specific actions of 11β-hydroxysteroid dehydrogenase in humans: implications for the pathophysiology of hypertension. Steroids 59: 84–89