Effects of Fasting on Aldosterone Secretion in Ovariectomized Rats

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Abstract

The present study was designed to assess the effect of fasting on aldosterone secretion in ovariectomized (Ovx) rats. Ovx rats were divided into fed (allowed access to food *ad libitum*) and fasted (deprived of food for 24 hours) groups. The trunk blood of fed and fasted rats was collected after decapitation. In the *in vitro* study, adrenal zona glomerulosa (ZG) cells from fed or fasted rats were incubated with angiotensin II (Ang II, 10^{-6} M), adrenocorticotropic hormone (ACTH, 10^{-9} M), or forskolin (an activator of adenylyl cyclase, 10^{-6} M) at 37°C for 30 min. The levels of aldosterone in medium and plasma extracts were measured by radioimmunoassay. Results showed that the levels of plasma aldosterone in fasted rats were lower than those in fed rats. There were no significant differences in basal and Ang II-stimulated aldosterone secretion between fed and fasted groups. The increment of aldosterone induced by ACTH in fasted group was significantly less than that in fed group. Administration of forskolin led to a significant increase in aldosterone secretion in both fed and fasted groups. Fasted group had a decreased aldosterone secretion in response to forskolin as compared with fed group. In summary, these results suggest that fasting decreases aldosterone secretion in Ovx rats through a mechanism in part involving a reduction of aldosterone production in response to ACTH, a decreased activity of adenylyl cyclase, and/or an inhibition of post-cAMP pathway in ZG cells.

Key Words: adenylyl cyclase, forskolin, zona glomerulosa cells, fasting, ACTH, aldosterone

Introduction

Fasting is widely practised in health, devotion and therapeutics in human. In addition to the weight loss, fasting induces many metabolic changes, mineral changes, hormonal changes and medical complications. Many earlier studies demonstrated that fasting causes a marked sodium excretion namely natriuresis of fasting (5, 9, 10). Although the mechanisms responsible for the natriuresis of fasting remain obscure, there are several possible explanations for the natriuresis during fasting. Hoffman *et al*. (9) have indicated that the natriuresis of fasting is reversed by intravenous and oral glucose, and that this effect is independent of changes in circulating insulin and free fatty acids. In addition, glucagon is considered by some investigators to be the natriuretic hormone in fasting (1, 4,13). An important role of the marked decline of insulin has been suggested in the natriuresis of fasting (7, 20).

Aldosterone, a major steroid hormone of the adrenal zona glomerulosa (ZG), regulates Na^+ balance and blood volume mainly through its effects on the distal tubules and cortical collecting ducts of the kidneys (19). The most important regulators of aldosterone secretion are renin-angiotensin system, potassium and adrenocortcotropic hormone (ACTH) (2, 15. 18). Miller et al. (14) have shown that sodium deprivation in the dog is associated with an increase in plasma renin activity which is capable of causing

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changes in aldosterone secretion. However, the role of aldosterone in the regulation of urinary sodium excretion during starvation in human is controversial (5, 8, 10, 16). Rapoport *et al*. (16) found that urinary excretion of sodium decreased and the aldosterone secretion rate rose after 7 days of starvation in patients. Katz *et al*. (10) showed that changes in aldosterone excretion during fasting are not constant. With sodium supplementation (greater than 100 mg per 24 h) during fasting, the rise in aldosterone secretion rate was less conspicuous. Other investigators reported that aldosterone excretory rate is unchanged during fasting (8), and spironolactone (an aldosterone blockade) exaggerates the natriuresis of fasting and blocks the anti-natriuresis of refeeding (6). Boulter *et al*. (5) revealed that during the natriuretic phase of starvation the aldosterone secretory rate rises while plasma renin activity falls in man. The reasons for the dissociation of the renin-aldosterone system during fasting are undefined. According to our observations, some of those contradictory results probably due to the different situations among these studies, including the duration of fasting, the quantity of the sodium supplemented during starvation or health condition of patients.

Therefore, the purpose of the present study was to clarify the effects of fasting on plasma aldosterone level, basal (unstimulated), angiotensin II (Ang II)- and ACTH-stimulated aldosterone secretion by ZG cells in rats. Since the production of aldosterone is stimulated by estradiol (11), the ovariectomized (Ovx) rats were employed in the present study.

Materials and Methods

Animals

Sprague-Dawley female rats of 3 months old were housed in a temperature-controlled room (22±1°C) with 14 h of artificial illumination daily (0600-2000) and given water *ad libitum*.

Effect of Fasting on Plasma Aldosterone

Female rats were Ovx for four days, and then divided into two groups. The fasted rats were deprived of food for 24 h before decapitation. The control animals were allowed *ad libitum* access to food. After decapitation, the adrenal glands from fed and fasted groups were rapidly removed and stored in 0.9% (w/v) NaCl in an ice bath. The trunk blood was obtained and the plasma was separated by centrifugation at 10,000 × g for 1 min, mixed with diethyl ether (10-fold volume), shaken for 30 min, centrifuged at 1,000 × g for 5 min, and then quick-frozen in a mixture of acetone and dry ice. The organic phase was collected, dried and reconstituted by a 1 % bovine serum albumin (BSA, Sigma, USA) in borate buffer (pH 7.8) before measurement of aldosterone by radioimmunoassay (RIA).

Effect of Fasting on Aldosterone Production by Rat ZG Cells in Vitro

The ZG cells of rat adrenal glands were prepared by a method as described elsewhere (11, 12). After preincubation of ZG cells with Krebs-Ringer bicarbonate buffer containing K^+ 3.6 mmol/l, glucose 11.1 mmol/l and 0.2% BSA (KRBGA medium) for 1 h at 37°C in a shaker bath (100 cycles per min) aerated with 95% O_2 and 5% CO_2, aliquots (1 ml) of ZG cells (5×10^4 cells) from fed or fasted rats was incubated with KRBGA medium (basal release) and angiotensin II (Ang II, 10^{-6} M, Sigma, USA) for 30 min. To determine the effects of fasting on adrenocorticotropic hormone (ACTH)-stimulated aldosterone secretion and the activity of adenylyl cyclase, ZG cells were incubated with ACTH (10^{-9} M, Sigma, USA) or forskolin (10^{-6} M, an activator of adenylyl cyclase, Sigma, USA) for 30 min. At the end of the incubation, 0.2 ml ice-cold KRBGA medium was added to stop the incubation under ice-bath. The medium was centrifuged at 200 × g and stored at -20°C until analysis for aldosterone by RIA.

RIA of Aldosterone

An antiserum to aldosterone was generated by immunizing the rabbit with 4-pregnen-11β, 21-diol-3, 18, 20-trione 3CMO: BSA conjugate (Steraloids Inc., USA). With this antiserum No. JJC-088, a RIA was established for the measurement of aldosterone level in the plasma and medium samples. In this RIA system, a known amount of unlabeled aldosterone, an aliquot of plasma extract, or medium samples was adjusted to a total volume of 0.3 ml by a buffer solution (1% BSA-borate buffer, pH 7.8). Each sample was incubated with 0.1 ml of aldosterone antiserum (1:8000 dilution) diluted with 1% BSA-borate buffer and 0.1 ml of ^{3}H-aldosterone (approximately 8,000 cpm; Amersham, UK) at 4°C for 24 h. Duplicate standard curves with 5 points ranging from 3 to 800 pg of aldosterone were included in each assay. An adequate amount (0.2 ml) of 0.5% dextran-coated charcoal (Sigma, USA) was then added prior to a further incubation in an ice bath for 15 min. At the end of the incubation, the assay tubes were centrifuged at 1,000 × g for 15 min. The supernatant was mixed with 3 ml liquid scintillation fluid (Ready Safe, Beckman, USA) before counting the radioactivity in an automatic beta counter (Wallac 1409, Pharmacia, Finland). The maximum binding of ^{3}H-aldosterone

Table 1. Cross-reactivities of Variant Steroids with Anti-aldosterone Antiserum No. JJC-088

Steroids	Cross-reactivity (%)
Aldosterone	100.00
Deoxycorticosterone	0.07
17α-Hydroxyprogesterone	0.05
Androstenedione	0.04
Corticosterone	0.03
Cortisone	0.01
11-Deoxycortisol	0.01
Estrone	0.01
17α-Estradiol	0.01
Estriol	0.01
Progesterone	0.01
Pregnenolone	0.01
Testosterone	0.01
Androstenediol	<0.01
Cholesterol	<0.01
Cortisol	<0.01
5α-Dihydrotestosterone	<0.01
17β-Estradiol	<0.01

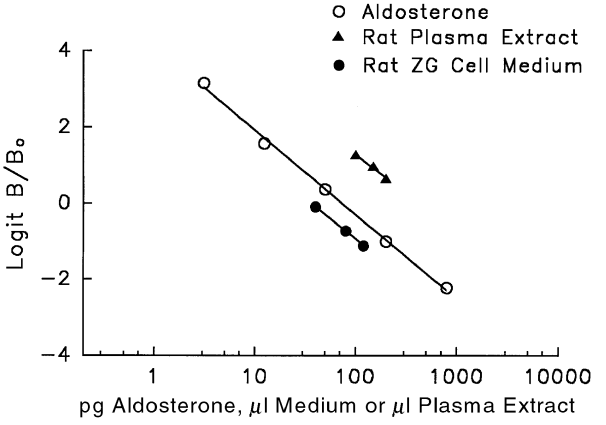

Fig. 1. The inhibition curves produced by anti-aldosterone antiserum No. JJC-088 with unlabeled aldosterone, ether-extracted rat plasma and the incubation medium of rat ZG cells.

with anti-aldosterone antiserum No. JJC-088 was 29%. The sensitivity of aldosterone RIA was 4 pg per assay tube. The inhibition curves produced by ether-extracted rat plasma and the incubation medium of rat ZG cells were parallel to that given by unlabeled aldosterone (Fig. 1). The specificity of the antiserum (JJC-088) was evaluated by determining the relative cross-reaction of 17 other steroids. These data are summarized in Table 1. The intra- and interassay coefficients of variation were 3.9% (n=5) and 8.2% (n=4), respectively. For confirming the fasting effects, all samples were measured by RIA using anti-aldosterone antisera of both JJC-088 and NIH-088

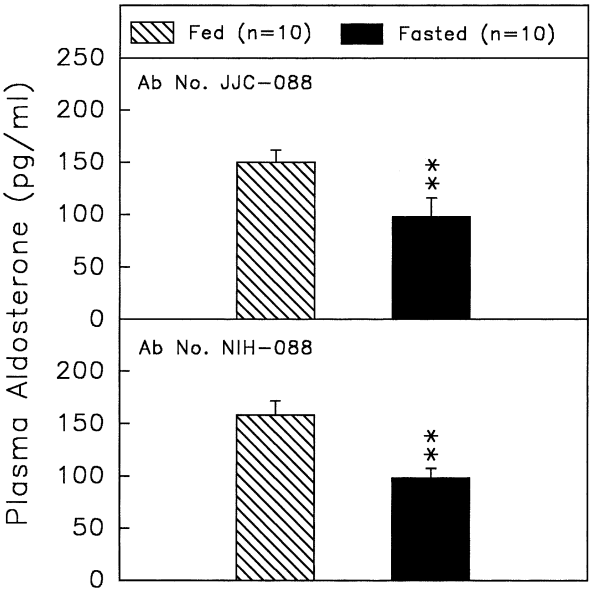

Fig. 2. The concentrations of plasma aldosterone measured by RIAs using anti-aldosterone antiserum No. JJC-088 (upper panel) and antiserum No. NIH-088 (lower panel) in fed and fasted ovariectomized (Ovx) rats. **, $P<0.01$ as compared with fed rats. Each value represents the mean±SEM.

(provided by the National Institutes of Health, USA). The RIA system using anti-aldosterone antiserum NIH-088 has been described elsewhere (11, 12).

Statistical Analysis

All data were expressed as mean±SEM. The treatment means were tested for homogeneity using analysis of variance (ANOVA), and the difference between specific means was tested for significance using Duncan's multiple-range test (17). A difference between two means was considered to be statistically significant when P was less than 0.05.

Results

Effect of Fasting on Plasma Aldosterone in Ovx Rats

The levels of plasma aldosterone in fed and fasted rats were shown in Figure 2. A 24-h fasting resulted in a significant decrease ($P<0.01$) on the level of plasma aldosterone in Ovx rats. The results of plasma aldosterone determined by anti-aldosterone antiserum JJC-088 were similar to those obtained by anti-aldosterone antiserum NIH-088.

Effect of Fasting on Aldosterone Release by ZG Cells in Vitro

Figure 3 illustrated the results of basal and Ang II (10^{-6} M)-stimulated aldosterone release from ZG

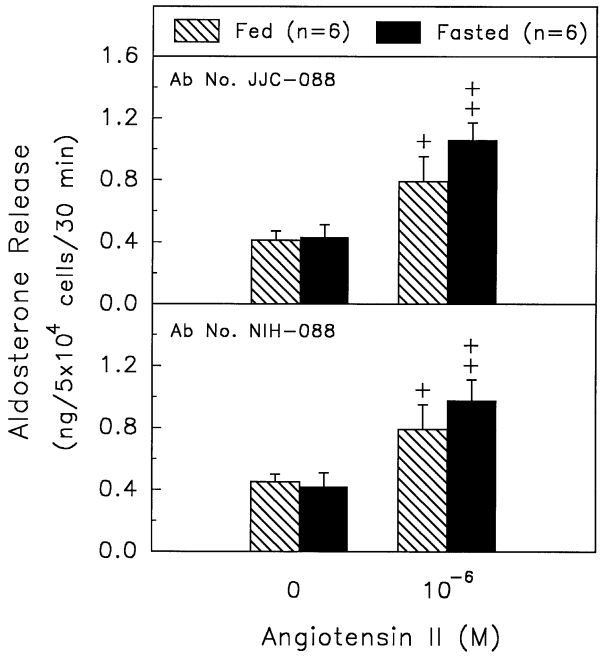

Fig. 3. Effects of fasting on basal (unstimulated) and angiotensin II (10^{-6} M)-induced aldosterone release by zona glomerulosa (ZG) cells from Ovx rats. The concentrations of medium aldosterone were measured by RIAs using anti-aldosterone antiserum No. JJC-088 and antiserum No. NIH-088. +, ++, $P<0.05$ and $P<0.01$ as compared with the basal release, respectively. Each value represents the mean±SEM.

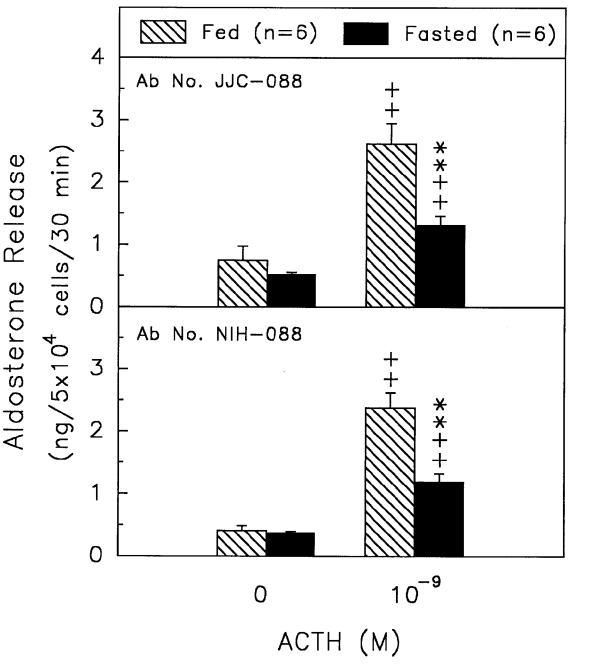

Fig. 4. Effects of fasting on basal and ACTH (10^{-9} M)-stimulated aldosterone release by zona glomerulosa (ZG) cells from Ovx rats. The concentrations of medium aldosterone were measured by RIAs using anti-aldosterone antiserum No. JJC-088 and antiserum No. NIH-088. **, $P<0.01$ as compared with fed rats. ++, $P<0.01$ as compared with the basal release. Each value represents the mean±SEM.

cells in fed and fasted rats. Ang II induced a marked ($P<0.05$ and $P<0.01$) elevation in aldosterone secretion in both fed and fasted animals. There were no significant differences in basal (unstimulated) and Ang II-stimulated aldosterone secretion between fed and fasted groups.

As shown in Figure 4 (top, determined by antiserum JJC-088), administration of ACTH (10^{-9} M) significantly ($P<0.01$) increased aldosterone secretion (2.07±0.23 ng/5×10^4 cells) in fed rats 2.7-fold relative to fasted rats (0.76±0.11 ng/5×10^4 cells). The fasted group had a less response to ACTH as compared with the fed group. Similar results were shown in the bottom of Figure 4 (determined by antiserum NIH-088).

Effect of Fasting on Forskolin-Stimulated Aldosterone Release by ZG Cells

Incubation of forskolin (10^{-6} M) significantly ($P<0.01$) increased the release of aldosterone by ZG cells in both fed and fasted groups (Fig. 5, top, determined by JJC-088). Forskolin increased aldosterone release (2.33±0.10 ng/5×10^4 cells) in fed rats 1.8-fold relative to fasted rats (1.27±0.11 ng/5×10^4 cells). In other words, fasted group had lower response to foskolin as compared with the fed group. The results of fasting on forskolin-stimulated aldosterone secretion determined by antiserum JJC-088 (Fig. 5, top) were similar to those obtained by antiserum NIH-088 (Fig. 5, bottom).

Discussion

The present study demonstrated that 24-h fasting induces a decreased level of plasma aldosterone in Ovx rats. There were no significant differences in basal and Ang II-stimulated aldosterone secretion from ZG cells between the fed and fasted groups. In addition to renin-angiotensin system, ACTH also stimulates aldosterone secretion, but its effect is short-lived (less than 24 h) (19). It is well known that ACTH increases cytosolic 3':5'-cyclic adenosine monophosphate (cAMP) production through activation of the adenylyl cyclase in ZG cells (3, 15). In the *in vivo* condition, several circulating regulators are involved in the secretion of aldosterone in adrenal ZG cells, eg, Ang II, ACTH, and high potassium. In our present study, the fasted group had diminished aldosterone secretion to ACTH and forskolin as compared with the fed group. We therefore suggest that fasting-induced decrease in plasma aldosterone is associated with the attenuated ACTH response, decreased adenylyl cyclase activity, and/or blunted

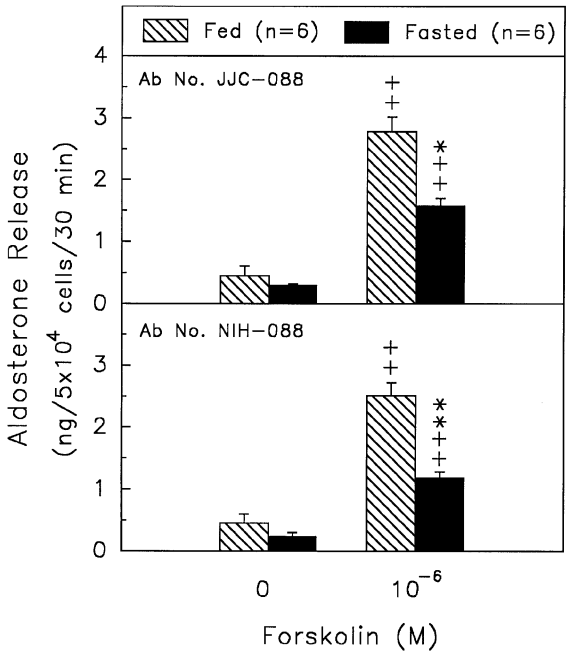

Fig. 5. Effects of fasting on forskolin (10^{-6} M)-stimulated aldosterone release by zona glomerulosa (ZG) cells from Ovx rats. The concentrations of medium aldosterone were measured by RIAs using anti-aldosterone antiserum No. JJC-088 and antiserum No. NIH-088. *, **, $P<0.05$, $P<0.01$ as compared with fed rats, respectively. ++, $P<0.01$ as compared with the basal release. Each value represents the mean±SEM.

post-cAMP pathway in ZG cells. Since we did not measure the levels of plasma Ang II or ACTH, the role of these regulators in the alteration of plasma aldosterone during fasting remains unclear at the present time.

Some earlier studies indicated that fasting is accompanied by a natriuresis (5, 9, 10). The natriuresis may be related to the hormonal changes in fasting, including glucagon (1, 4, 13) and insulin (7, 20). Although the renin-aldosterone system is a major modulator in sodium homeostasis, the effects of fasting on renin-aldosterone system were conflicting (5, 8, 10, 16). The reasons for the controversial results are unknown. However, notable differences exist among these studies, including sodium intake, fasting duration, sex, body weight or health condition of patients. Under the controlled experimental condition, fasting induced a decreased plasma aldosterone in Ovx rats, which would provide an explanation in the mechanisms of natriuresis during fasting. The natriuresis activated by fasting could be an homeostatic adaptive mechanism and hence defend the acid-base balance and minimize the risk of uric acid stone formation.

In summary, the present data suggests that the fasting-induced decrease in plasma aldosterone is in part due to the decreased ACTH response, attenuated adenylyl cyclase activity, and/or inhibitory post-cAMP pathway in ZG cells. Since both *in vivo* and *in vitro* data assessed by antiserum No. JJC-088 were in agreement with those assessed by antiserum No. NIH-088, the anti-aldosterone antiserum No. JJC-088 is suitable for measuring the change of aldosterone levels in either plasma or cell medium.

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