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RAPID COMMUNICATION

Aldosterone Stimulates Gene Expression of Hepatic Gluconeogenic Enzymes through the Glucocorticoid Receptor in a Manner Independent of the Protein Kinase B Cascade

RYO YAMASHITA*,**, TAISUKE KIKUCHI*, YASUMICHI MORI*, KAZUTAKA AOKI*, YASUSHI KABURAGI**, KAZUKI YASUDA** AND HISAHIKO SEKIHARA*

**Department of Endocrinology and Metabolism, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan*

***Department of Metabolic Disorder, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan*

Abstract. Primary aldosteronism is associated with glucose intolerance and diabetes, which is due in part to impaired insulin release caused by reduction of potassium, although other possibilities remain to be elucidated. To evaluate the *in vivo* effects of aldosterone on glucose metabolism, a single dose of aldosterone was administered to mice, which resulted in elevation of the blood glucose level. In primary cultured mouse hepatocytes, the gene expression of gluconeogenic enzymes such as glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase and phosphoenolpyruvate carboxykinase increased in response to aldosterone in a dose-dependent manner even at a concentration similar to a physiological condition (10^{-9} M). The inhibitory effect of insulin on G6Pase gene expression was partially suppressed by aldosterone. Furthermore, aldosterone enhanced G6Pase promoter activity in human hepatoma cell line HepG2, which was prevented by co-treatment with a glucocorticoid antagonist RU-486, but not a mineralocorticoid antagonist spironolactone. In contrast, aldosterone had no effects on major insulin signaling pathways including insulin receptor substrate-1, protein kinase B, and forkhead transcription factor. These results suggest that aldosterone may affect the inhibitory effect of insulin on hepatic gluconeogenesis through the glucocorticoid receptor, which may be one of the causes of impaired glucose metabolism in primary aldosteronism.

Key words: Aldosterone, Gluconeogenesis, Glucocorticoid, Mineralocorticoid, Primary aldosteronism

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ALDOSTERONE, the major mineralocorticoid hormone, plays a central role in the control of water and electrolyte balance in sodium-transporting epithelia in the distal renal tubules, distal colon, and sweat salivary glands [1]. Primary aldosteronism is characterized by autonomous production of aldosterone causing excessive sodium retention, excessive potassium excretion, suppressed renin activity, hypokalemia and hypertension. Furthermore, it has been known that the patients with primary aldosteronism frequently exhibit glucose intolerance and diabetes mellitus [2]. Many studies have reported the impairment of glucose metabolism and insulin action induced by excess aldosterone, although its mechanism remains unresolved [reviewed in 3]. Potassium reduction in serum and whole body has been considered to be one of the causes of glucose intolerance and decreased insulin release in patients with primary aldosteronism, because clinical studies have indicated that impairment of glucose tolerance could be corrected by potassium repletion [4, 5]. Animal and *in vitro* experiments demonstrated that insulin release from the beta cells of the islets of Langerhans is decreased by low extracellular potassium concentrations [6]. On the other hand, the analysis of cultured rat islets of Langerhans showed that aldosterone has a direct inhibitory effect on glucose-induced insulin release independent of potassium depletion [7]. Several groups also reported that excess aldosterone causes abnormality of

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Correspondence to: Dr. Ryo YAMASHITA, Department of Metabolic Disorder, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan

insulin action [reviewed in 3]. In addition, the analysis using the hyperinsulinemic euglycemic clamp method demonstrated that glucose disposal rates (M), insulin sensitivity (M/I), and metabolic clearance rate of glucose were significantly lower in primary aldosteronism patients as compared with healthy control [8]. However, the effect of aldosterone on insulin action remains controversial because homeostasis model assessment (HOMA) showed that insulin sensitivity is increased in primary aldosteronism [9]. Cushing's syndrome causes insulin resistance and glucose intolerance through stimulation of gluconeogenesis by cortisol. Metabolic defects in primary aldosteronism are similar to Cushing's syndrome, although molecular mechanisms of impaired glucose tolerance in primary aldosteronism, as well as the direct effect of aldosterone on glucose metabolism, have not been clarified in detail. In this study, to determine the role of aldosterone in glucose metabolism, we examined the effects of aldosterone on gene expression of hepatic gluconeogenic enzymes using mouse primary cultured hepatocytes and human hepatoma cell line HepG2 cell.

Materials and methods

Animals and injection

Two-month-old ICR female mice (Charles River, Yokohama, Japan) were placed under a 12-h light/dark cycle. Food and water were given *ad libitum* for at least 1 wk before experiments were performed. Mice were fasted overnight and aldosterone (Sigma, St. Louis, MO, USA) was administered to mice as a single subcutaneous (s.c.) injection of 2 mg in 100 µl of DMSO. After the injection, tail blood was collected at 0 (before s.c. injection), 30 and 60 min. Blood glucose was measured immediately using Glutest Sensor (Sanwa Kagaku, Nagoya, Japan). All animal procedures were approved by the local committee of Yokohama City University School of Medicine.

Cell culture

Primary hepatocytes were isolated from *ad libitum* fed male ICR mice (Charles River) at 3–4 weeks of age. Hepatocytes were obtained using the collagenase method as previously described [10]. The cells were suspended in Dulbecco's modified eagle medium/Nutrient mixture F-12 (DMEM/F12) medium containing 5% fetal bovine serum (FBS) and were plated onto a collagen-coated 10-cm dish (Iwaki, Tokyo, Japan) at a density of 5×10^6 cells per dish. After 4 hours, hepatocytes were washed and cultured in serum-free DMEM/F12 medium containing 5 kIU/ml aprotinin (Sigma), 1 nM dexamethasone (Wako Chemicals, Tokyo, Japan) and 1 nM insulin (Wako) for 16 h. Then, the cells were incubated in serum-free DMEM/F12 medium containing the indicated concentrations of aldosterone for 24 h.

HepG2 cells were obtained from Cell Resource Center for Biomedical Research, Tohoku University, and were maintained in DMEM medium containing 10% FBS. Before experiments, 80–90% confluent HepG2 cells were starved in serum-free DMEM medium containing 0.1% bovine serum albumin (BSA) overnight. Aldosterone, insulin, spironolactone, or RU-38486 (Sigma) was added to serum-free DMEM medium containing 0.1% BSA at the indicated concentrations. All the cells examined in this study were cultured in a humidified atmosphere of 5% CO_2 at 37°C.

Northern blot analysis

cDNA fragments of glucose-6-phosphatase (G6Pase), fructose-1,6-bisphosphatase (FBPase) and phosphoenolpyruvate carboxykinase (PEPCK) were synthesized from total RNA of hepatocytes and amplified by use of the RNA PCR kit (Takara, Tokyo, Japan). cDNA fragments were subcloned into pGEM®-T Easy Vector (Promega, Madison, WI, USA) and the sequence of these cDNAs were confirmed by ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). cDNAs were labeled with ^{32}P by the random primer method using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech, Piscataway, NJ, USA), and were purified with MicroSpin™G-50 columns (Amersham Pharmacia Biotech) before being used as probes. Total RNA was isolated from the cells with Trizol (Invitrogen, Carlsbad, CA, USA) following the protocol of the manufacturer. About 20 µg of total RNA was electrophoresed on a 1% formaldehyde-denatured-agarose gel using 1 × MOPS running buffer. RNA was transferred to a nylon membrane (Millipore) by capillary action and UV-cross-linked in a Funa-UV-Linker (Funakoshi, Tokyo, Japan). The membrane was then hybridized with ^{32}P-labeled probes at 68°C

for 1 h in ExpressHyb™ Hybridization solution (Clontech, Palo Alto, CA, USA). After hybridization, membranes were washed several times with SSC/SDS buffer. Radioactivity of each hybridization band was quantitated by a Bas 2000 imaging analyzer (Fujifilm, Tokyo, Japan). The signal intensity for each target enzyme mRNA was normalized using that for ribosomal protein S9 cDNA (Clontech) as an internal control.

Immunoprecipitation and immunoblot analysis

HepG2 cells were washed twice with ice-cold phosphate-buffer saline (PBS), and then solubilized for 10 min with ice-cold lysis buffer (1% Triton X-100, 50 mM NaCl, 25 mM HEPES; pH 7.4, 10 mM NaF, 1 mM Na_3VO_4, 0.2 mM Na_2MoO_4, 1 mM PMSF, 10 mg/ml leupeptin). Insoluble materials were removed by centrifugation at 13,000 × g for 10 min, and lysates were incubated with specific antibodies against insulin receptor β chain (IR-β) (Biotechnology, Santa Cruz, CA, USA) or insulin receptor substrate (IRS)-1 (Upstate Biotechnology, Lake Placid, NY, USA) and Protein G beads (Amersham Pharmacia Biotech) for 16 h at 4°C. Immunoprecipitated proteins were washed, and solubilized in SDS sample buffer. Solubilized proteins were separated by electrophoresis on SDS-polyacrylamide gels and electrophoretically transferred onto polyvinylidene difluoride membranes (Millipore, Bedford, MA USA). The membranes were probed with antibodies against phospho-PKB (Ser473) (Cell Signaling Technology, Beverly, MA, USA), phospho-FKHRL1 (Thr32) or phosphotyrosine (4G10) (Upstate Biotechnology). Detection was performed using the ECL system (Amersham Biosciences) according to the manufacture's protocol.

Luciferase assays

G6Pase luciferase reporter plasmid designated pGL-hG6P was generated by subcloning a human G6Pase promoter fragment corresponding to bases –1184 to +112 relative to the transcription start site into the polylinker of plasmid pGL3-Basic (Promega). HepG2 cells on 6-well plates were transfected using Lipofectamine (Invitrogen) with total 1 μg DNA containing 250 ng of pGL3-hG6P and 100 ng pAct-β-gal per well, which was adjusted to 1 μg by adding an empty vector. Luciferase activity was determined using Luciferase Assay Reagent (Promega), and β-gal activity was measured by Luminescent beta-galactosidase Detection Kit II (Clontech). The data were normalized to the co-expressed beta-galactosidase activities.

Statistical analysis

Results are expressed as means ± SEM. The mean values were analyzed by ANOVA followed by Fisher PLSD test for multiple comparisons using StatView 5 (SAS Institute, Cary, NC, USA). A P value of less than 0.05 was considered statistically significant.

Results

In vivo effect of aldosterone on the blood glucose level in adult mice

We first examined the *in vivo* effect of aldosterone administration in adult mice. Mice were treated with aldosterone at a single dose of 2 mg after fasting and the blood glucose was measured. As shown in Fig. 1, the blood glucose levels of aldosterone-treated mice

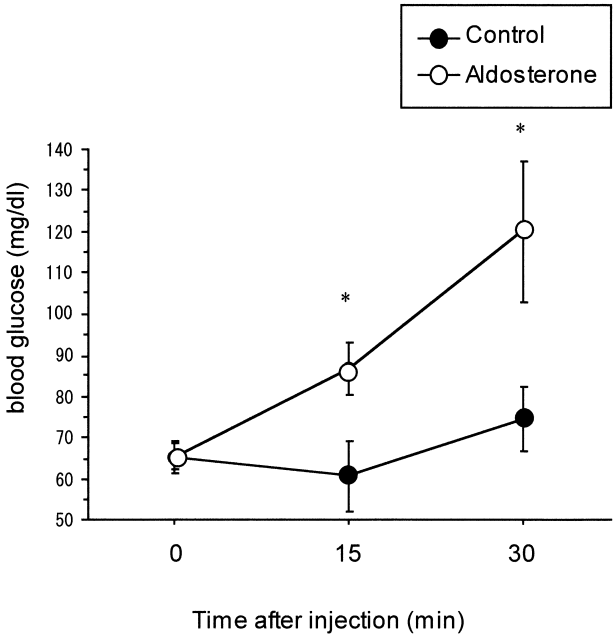

Fig. 1. Blood glucose level after administration of aldosterone. Mice were fasted overnight and aldosterone was administered as a single subcutaneous (s.c.) injection of 2 mg in 100 μl of DMSO. After the injection, tail blood was collected at 0 min (before s.c. injection), 30 and 60 min. ○, vehicle (DMSO) (n = 5); ●, aldosterone (n = 5). Values are means ± SEM. *, P<0.05 *vs*. vehicle.

were significantly (P<0.05) higher than those of vehicle-treated mice at 30 and 60 min after injection.

Regulation of G6Pase, FBPase and PEPCK gene expressions by aldosterone in primary cultured mouse hepatocytes

To test the direct effects of aldosterone on glucose metabolism, we investigated whether aldosterone can directly affect gene expression of enzymes involved in gluconeogenesis. We cultured mouse primary hepatocytes for 24 h with increasing concentrations of aldosterone (10^{-9}–10^{5} M), and isolated RNA for Northern blot analysis to measure G6Pase, FBPase, PEPCK and ribosomal protein S9 (as a control) mRNA levels (Fig. 2). Aldosterone significantly increased gene expression of G6Pase at 10^{-9} M, a concentration similar to a physiological condition, and dose-dependently stimulated G6Pase mRNA accumulation, which were maximized at 10^{-6} μM of aldosterone (320 ± 25%, P<0.001). Aldosterone also significantly elevated the mRNA levels of FBPase and PEPCK. Similar to G6Pase, FBPase mRNA induction was maximally stimulated at 10^{-6} M (162 ± 4.6%, P<0.001), whereas maximal response of PEPCK gene expression was observed at 10^{-5} M (625 ± 10%, P<0.001). Thus, the gene expression of PEPCK was maximally stimulated at a concentration of aldosterone higher than that of G6Pase and FBPase, although the molecular basis of the different response to maximal aldosterone stimulation remains to be elucidated.

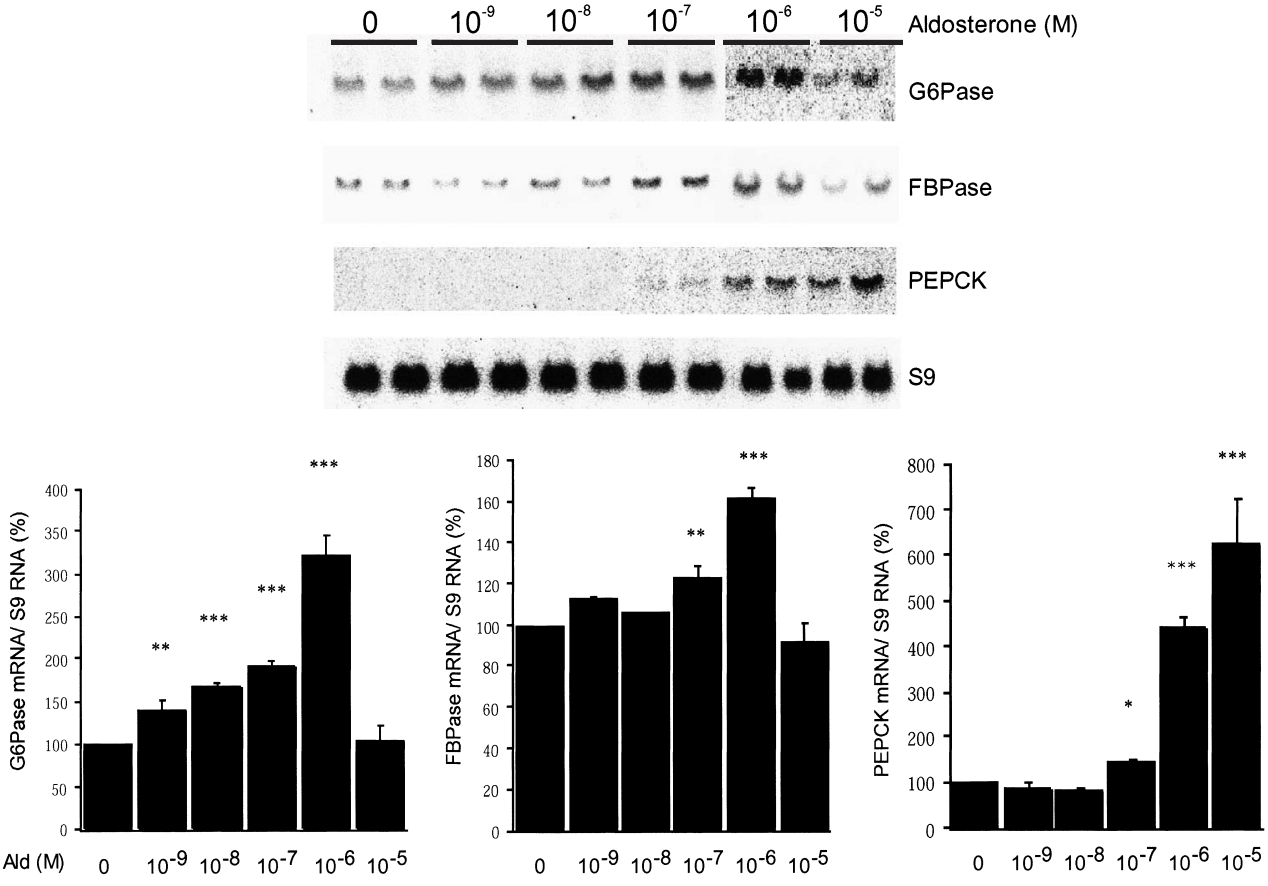

Fig. 2. Effects of aldosterone on G6Pase, FBPase and PEPCK gene expression in primary cultured mouse hepatocytes. Hepatocytes were cultured for 24 h in serum-free DMEM/F-12 containing the indicated concentrations of aldosterone or vehicle (ethanol, 0 M). RNA was extracted and analyzed for expression of G6Pase, FBPase, PEPCK and ribosomal protein S9 mRNA by Northern blot. Results were normalized to S9 signal and expressed relative to vehicle. Values are expressed as means ± SEM (n = 3 separate experiments for each condition). One representative Northern blot is shown. *, P<0.05; **, P<0.01; **, P<0.001 (*vs*. vehicle).

Regulation of G6Pase gene expressions by aldosterone in HepG2 cells

Next, we examined the effect of aldosterone on G6Pase mRNA gene expression using human hepatoma cell line HepG2 cell (Fig. 3). Aldosterone treatment promoted a dose-dependent increase in G6Pase gene expression in HepG2 cells, although a significant effect was detected at 10^{-7} M, and a maximal effect ($250 \pm 15\%$ ($P<0.001$)) was observed at 10^{-5} M of aldosterone. Thus, HepG2 cells exhibited an increase in aldosterone-induced G6Pase gene expression at concentrations higher than those in primary mouse hepatocytes, although the causes of these differences remain unresolved.

Effect of aldosterone on insulin-induced suppression of G6Pase gene expression in mouse primary hepatocytes

It has already been reported that gene expression of gluconeogenic enzymes is decreased by insulin in hepatocytes [11]. To investigate whether aldosterone affects this insulin action in hepatocytes, mouse primary hepatocytes were treated with insulin in the presence or absence of aldosterone for 24 h, and RNA was isolated for Northern blot analysis to measure G6Pase mRNA levels. As shown in Fig. 4, G6Pase gene expression was suppressed to approximately 50% of the control by insulin, whereas it was significantly restored by aldosterone at a concentration similar to a physiological condition (Ins *vs.* Ins + Ald, $P<0.05$). However, insulin significantly suppressed the G6Pase mRNA level even in the presence of aldosterone (Fig. 4).

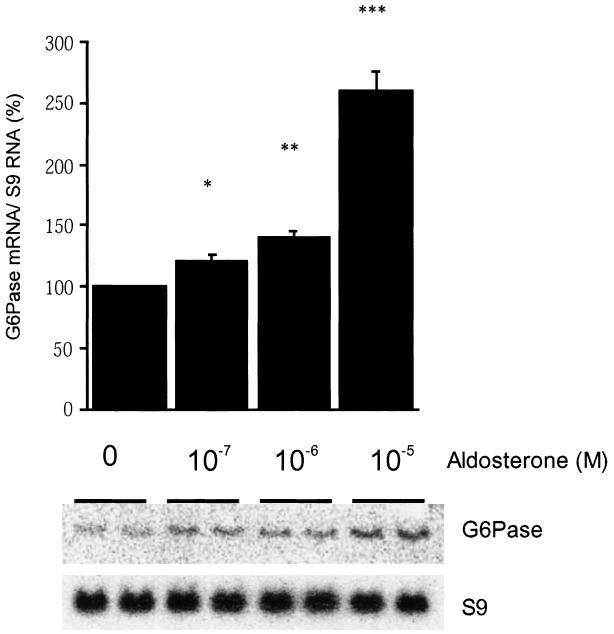

Fig. 3. Effects of aldosterone on G6Pase mRNA in HepG2 cells. HepG2 cells were initially serum-starved for 12 h in serum-free DMEM containing 0.1% BSA and treated with the indicated concentrations of aldosterone for 24 h, or vehicle (ethanol, 0 M). RNA was extracted and analyzed for expression of G6Pase and ribosomal protein S9 mRNA by Northern blot. Results are expressed as described in Fig. 2. Values are expressed as means ± SEM (n = 3 separate experiments for each condition). One representative Northern blot is shown. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (*vs*. vehicle).

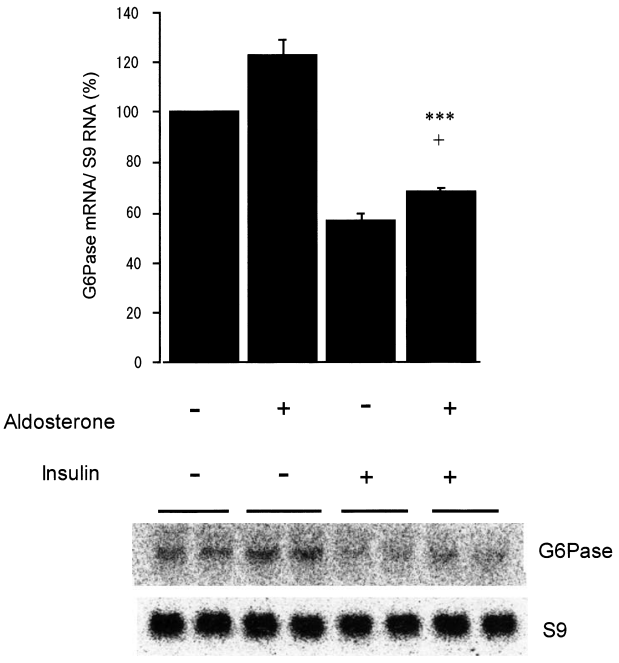

Fig. 4. Aldosterone prevents the suppression of G6Pase gene expression induced by insulin in primary cultured mouse hepatocytes. Hepatocytes were incubated for 24 h with 10^{-8} M aldosterone and 10 nM insulin, individually or in combination. RNA was extracted and analyzed for expression of G6Pase and ribosomal protein S9 mRNA. Results are expressed as described in Fig. 2. Values are expressed as means ± SEM (n = 3 separate experiments for each condition). One representative Northern blot is shown. +, $P<0.05$ *vs.* Ald; ***, $P<0.001$ *vs*. vehicle.

Effect of aldosterone on major insulin-signaling pathways

The phosphatidylinositol 3-kinase (PI3K)-Akt/protein kinase B (PKB) cascade, which is activated through tyrosine-phosphorylated IRS proteins in response to insulin, is reportedly required for inhibition of G6Pase and PEPCK gene expression [12, 13]. To test whether this major insulin-signaling pathway plays a role in aldosterone-induced gene expression of gluconeogenetic enzymes, we examined the effects of aldosterone on the tyrosine phosphorylation of the β-subunit of the insulin receptor (IR-β) and insulin receptor substrate-1 (IRS-1) in HepG2 cells. We also evaluated insulin-induced phosphorylation of PKB and the transcription factor FKHRL1, one of the PKB substrates. As shown in Fig. 5, the insulin-induced tyrosine phosphorylation of neither IR-β nor IRS-1 was impaired by 10^{-6} M aldosterone, a concentration at which G6Pase mRNA was maximally accumulated (Fig. 3). Moreover, 10^{-6} M aldosterone did not affect the phosphorylation of either PKB or FKHRL1.

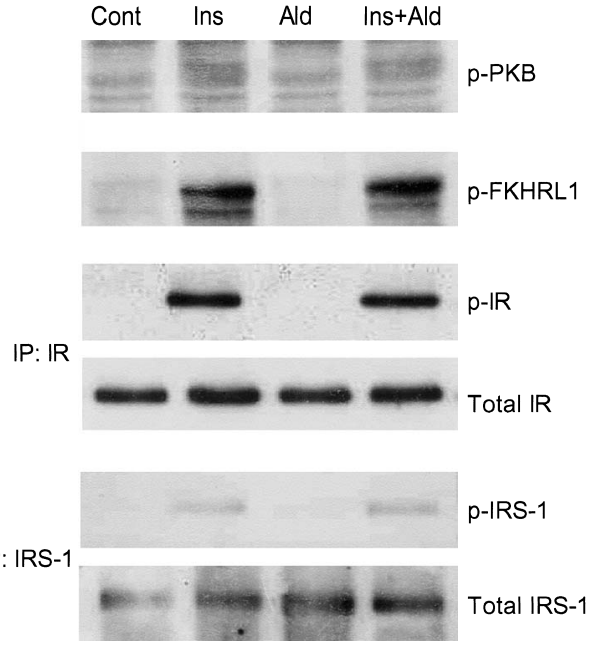

Fig. 5. Aldosterone does not affect major insulin-signaling pathway. After treatment with or without 10^{-6} M aldosterone for 24 h followed by an additional 1 h incubation with 10 nM insulin, cell lysates from HepG2 cells were separated by SDS-PAGE and analyzed by immunoblot analysis with anti-phospho-PKB or anti-phospho-FKHRL1 antibodies. Cell lysates were also immunoprecipitated with anti-IR or anti-IRS-1 and the immunoprecipitated protein were analyzed by immunoblotting with an anti-phosphotyrosine antibody. The figure shows the result of a typical experiment, repeated between two and four times.

Effects of mineralocorticoid and glucocorticoid receptor antagonists on aldosterone-induced G6Pase promoter activity

To study the molecular mechanism whereby aldosterone induces G6Pase gene expression, we constructed a reporter gene plasmid (pGL3-Basic) containing the portion of the G6Pase 5' region (–1184 to +112) and transiently transfected it into HepG2 cells. The promoter activity of G6Pase was significantly enhanced by aldosterone (10^{-7} M, $P<0.01$; 10^{-6} M, $P<0.001$), which confirmed the stimulation of G6Pase gene transcription to be attributable to a genomic effect of aldosterone. We next studied the effects of mineralocorticoid and glucocorticoid antagonists on aldosterone-induced G6Pase promoter activation. The effect of aldosterone was not altered by the addition of 1 μM spironolactone, a mineralocorticoid antagonist, but was suppressed by 10 μM RU-38486, an anti-glucocorticoid compound (100 nM A *vs.* A + R, $P<0.01$; 1 μM A *vs.* A + R, $P<0.001$). These results suggest that the effect of aldosterone on G6Pase gene expression is mediated through its interaction with the glucocorticoid receptor (GR), but not with the mineralocorticoid receptor (MR).

Discussion

The renin-angiotensin-aldosterone (RAA) system mainly plays a role in the regulation of blood pressure and extracellular fluid volume. However, the RAA system has been found to correlate not only with hypertension, but also with insulin resistance. One explanation for the relationship between RAA and insulin resistance is that angiotensin II inhibits insulin-stimulated IRS-1- and IRS-2-associated PI3K activity and negatively modulates insulin signaling [14]. Moreover, several studies have reported that elevated plasma aldosterone might be associated with insulin resistance [15], suppression in plasma leptin level [16] and obesity [17]. In addition, single-nucleotide polymorphisms in the aldosterone synthase were shown to

be correlated with plasma glucose levels and diabetic states [18]. But the molecular mechanisms of insulin resistance caused by activated RAA systems have yet to be clarified in detail.

The present study showed that the administration of aldosterone to mice elevated blood glucose level (Fig. 1) at a superphysiological concentration of aldosterone, suggesting that aldosterone exerted gluconeogenesis-promoting effects rather than impairing insulin secretion, because mice were fasted in this experimental condition. However, there remains the possibility that aldosterone may also play a role in impairing insulin actions through other mechanisms in various tissues. Next, we analyzed whether aldosterone directly affects hepatic gluconeogenesis *in vitro*. It is well known that hepatic gluconeogenic enzymes are very important for glucose metabolism. Hepatic gluconeogenic key enzymes, such as G6Pase, FBPase and PEPCK have been implicated in the control of blood glucose levels. In fact, both the activities and mRNA levels of these hepatic gluconeogenic enzymes are elevated in the livers of diabetic mice as compared with non-diabetic mice [19–21]. The gene expression of these enzymes is regulated by hormones such as insulin, glucocorticoid and glucagons, because the promoter regions of these genes have the elements involved in the responsiveness to these hormones [22]. In the present study, we observed that aldosterone up-regulated gene expression of these enzymes in hepatocytes (Fig. 2, 3). G6Pase gene was increased by physiologic doses of aldosterone (Fig. 2). The genes of gluconeogenic enzymes are also reportedly controlled by the PI3K/PKB pathway in a fashion independent of the activation of mitogen-activated protein kinase and p70 S6 kinase [23, 24]. In rat hepatoma cell line H4IIE cell, it was also shown that insulin increased tyrosine phosphorylation of IR-β and IRS-1, while G6Pase and PEPCK gene expression was reduced in a PI3K-dependent manner [12]. In addition, several recent reports have shown that G6Pase gene expression was inhibited by insulin through PKB-mediated inactivation of forkhead transcription factor FKHR [25–27]. In contrast, angiotensin II treatment reportedly decreased IRS-1 tyrosine phosphorylation and IRS-1-associated PI3K activity [28]. In the present study, we observed that aldosterone partially restored insulin-induced inhibition of G6Pase gene expression (Fig. 4), although aldosterone did not affect the insulin-regulated IRS/PI3K/PKB/forkhead cascade (Fig. 5). These findings suggest that a pathway independent of this major insulin-signaling pathway regulated the effect of aldosterone on gluconeogenesis. In addition, we also observed that G6Pase mRNA level and promoter activity were not elevated by angiotensin II treatment (data not shown).

Aldosterone signals are mediated not only through the MR, but also through the GR [29, 30]. The GR is ubiquitously expressed, and has considerable structural and functional homology with the MR [31]. On the other hand, recent reports have shown the MR to be expressed in several tissues, including non-epithelial cells of the brain, heart, and skin, as well as leukocytes and brown adipocytes [32, 33]. In fact, we also detected MR expression in hepatocytes by RT-PCR (data not shown), raising the possibility that MR may play a role in the regulation of gluconeogenic gene expression. A

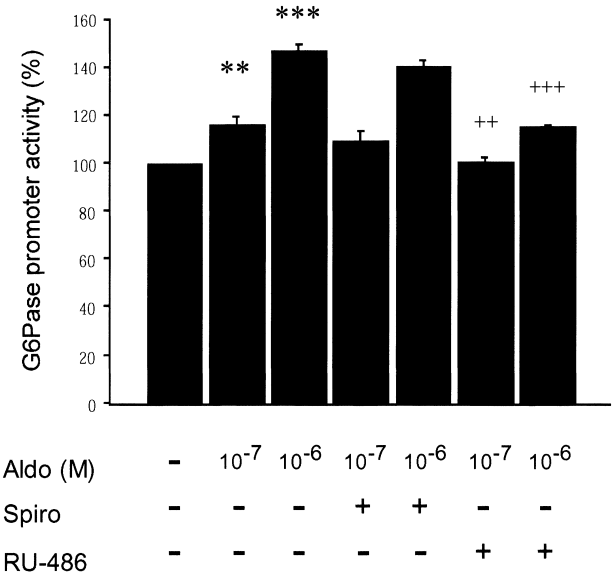

Fig. 6. Effects of mineralocorticoid and glucocorticoid receptor antagonists on aldosterone-induced G6Pase promoter activity in HepG2 cells. Cells were transiently transfected with a G6Pase reporter construct (G6Pase –1184 / +112-pGL3) or a control plasmid (pAct-β-gal). Twenty-four h later, the cells were serum-starved for 8 h and incubated for 16 h in serum-free DMEM medium containing 0.1% BSA in the presence of 10^{-7} M or 10^{-6} M aldosterone (Aldo), and together with 10 μM RU-38486 (RU-486), or 10 μM spironolactone (Spiro) for 20 h, and the relative luciferase activity was determined. The basal G6Pase promoter activity from cells treated with vehicle (ethanol) was set as 100%. Values are expressed as means ± SEM (n = 5 separate experiments for each condition). **, $P<0.01$; ***, $P<0.001$ *vs*. vehicle; ++, $P<0.01$ *vs*. 10^{-7} M Aldo; +++, $P<0.001$ *vs*. 10^{-6} M Aldo.

recent study reported that the inhibitory effect of 0.1–10 μM aldosterone on steroidogenic acute regulatory protein (StAR) mRNA induction was completely prevented by both the anti-mineralocorticoid compound spironolactone and the anti-glucocorticoid compound RU-486, suggesting the presence of cross-reaction between MR and GR [34]. In contrast, we showed that the aldosterone-induced G6Pase promoter activity was elevated at concentrations similar to that study [34], while it was prevented by RU-486 but not by spironolactone even in the presence of MR, indicating that this action was GR-specific (Fig. 5). These results suggest that MR and GR may mediate aldosterone signals in distinct fashions in various tissues. 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) is highly expressed in liver and effectively amplifies glucocorticoid action, which may affect the aldosterone-induced action through GR [35]. In fact, we observed that the aldosterone-induced G6Pase promoter activity was not suppressed to less than control level (Fig. 6) by RU-486 inhibitor, indicating that endogenously-produced glucocorticoid plays a minor role in this model.

In patients with primary aldosteronism, impaired insulin action improved after excision of an aldosterone-producing adenoma, whereas spironolactone treatment did not significantly influence insulin action [8]. Interestingly, our results are consistent with this clinical study because the effect of aldosterone was suppressed by RU-486 but not by spironolactone (Fig. 6). Thus, we speculate that impaired insulin action in patients with primary aldosteronism may have been caused at least in part by the effect of aldosterone through GR but not MR in liver.

In conclusion, we have shown that aldosterone itself can influence the hepatic gluconeogenesis through GR at least in hepatocytes. Although it is known that elevating aldosterone level is correlated with many diseases such as hypertension, nephrosis and heart failure, little is known about its role in glucose metabolism. The findings presented in this study raise the possibility that aldosterone excess may also play a role in impaired glucose metabolism in these diseases, although the contributions of MR- and GR-mediated signals remain to be investigated.

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References

1. Fuller PJ, Lim-Tio SS, Brennan FE (2000) Specificity in mineralocorticoid versus glucocorticoid action. *Kidney Int* 57: 1256–1264.
2. Conn JW (1965) Hypertension, the potassium ion and impaired carbohydrate tolerance. *N Engl J Med* 273: 1135–1143.
3. Corry DB, Tuck ML (2003) The effect of aldosterone on glucose metabolism. *Curr Hypertens Rep* 5: 106–109.
4. Gorden P (1973) Glucose intolerance with hypokalemia. Failure of short-term potassium depletion in normal subjects to reproduce the glucose and insulin abnormalities of clinical hypokalemia. *Diabetes* 22: 544–551.
5. Podolsky S, Melby JC (1976) Improvement of growth hormone response to stimulation in primary aldosteronism with correction of potassium deficiency. *Metabolism* 25: 1027–1032.
6. Henquin JC, Lambert AE (1974) Cationic environment and dynamics of insulin secretion. II. Effect of a high concentration of potassium. *Diabetes* 23: 933–942.
7. Pierluissi J, Navas FO, Ashcroft SJ (1986) Effect of adrenal steroids on insulin release from cultured rat islets of Langerhans. *Diabetologia* 29: 119–121.
8. Sindelka G, Widimsky J, Haas T, Prazny M, Hilgertova J, Skrha J (2000) Insulin action in primary hyperaldosteronism before and after surgical or pharmacological treatment. *Exp Clin Endocrinol Diabetes* 108: 21–25.
9. Ishimori M, Takeda N, Okumura S, Murai T, Inouye H, Yasuda K (1994) Increased insulin sensitivity in patients with aldosterone producing adenoma. *Clin Endocrinol* (Oxf) 41: 433–438.
10. Berry MN, Friend DS (1969) High-yield preparation of isolated rat liver parenchymal cells: a biochemical and fine structural study. *J Cell Biol* 43: 506–520.
11. Lange AJ, Argaud D, el-Maghrabi MR, Pan W, Maitra SR, Pilkis SJ (1994) Isolation of a cDNA for the catalytic subunit of rat liver glucose-6-phosphatase: regulation of gene expression in FAO hepatoma cells by insulin, dexamethasone and cAMP. *Biochem Biophys Res Commun* 201: 302–309.
12. Waltner-Law ME, Wang XL, Law BK, Hall RK,

Nawano M, Granner DK (2002) Epigallocatechin gallate, a constituent of green tea, represses hepatic glucose production. *J Biol Chem* 277: 34933–34940.

13. Dickens M, Svitek CA, Culbert AA, O'Brien RM, Tavare JM (1998) Central role for phosphatidylinositide 3-kinase in the repression of glucose-6-phosphatase gene transcription by insulin. *J Biol Chem* 273: 20144–20149.
14. Folli F, Saad MJ, Velloso L, Hansen H, Carandente O, Feener EP, Kahn CR (1999) Crosstalk between insulin and angiotensin II signalling systems. *Exp Clin Endocrinol Diabetes* 107: 133–139.
15. Widimsky J Jr, Strauch B, Sindelka G, Skrha J (2001) Can primary hyperaldosteronism be considered as a specific form of diabetes mellitus? *Physiol Res* 50: 603–607.
16. Torpy DJ, Bornstein SR, Taylor W, Tauchnitz R, Gordon RD (1999) Leptin levels are suppressed in primary aldosteronism. *Horm Metab Res* 31: 533–536.
17. Goodfriend TL, Kelley DE, Goodpaster BH, Winters SJ (1999) Visceral obesity and insulin resistance are associated with plasma aldosterone levels in women. *Obes Res* 7: 355–362.
18. Ranade K, Wu KD, Risch N, Olivier M, Pei D, Hsiao CF, Chuang LM, Ho LT, Jorgenson E, Pesich R, Chen YD, Dzau V, Lin A, Olshen RA, Curb D, Cox DR, Botstein D (2001) Genetic variation in aldosterone synthase predicts plasma glucose levels. *Proc Natl Acad Sci USA*. 98: 13219–13224.
19. Aoki K, Saito T, Satoh S, Mukasa K, Kaneshiro M, Kawasaki S, Okamura A, Sekihara H (1999) Dehydroepiandrosterone suppresses the elevated hepatic glucose-6-phosphatase and fructose-1,6-bisphosphatase activities in C57BL/Ksj-db/db mice: comparison with troglitazone. *Diabetes* 48: 1579–1585.
20. Li Y, Mechin MC, van de Werve G (1999) Diabetes affects similarly the catalytic subunit and putative glucose-6-phosphate translocase of glucose-6-phosphatase. *J Biol Chem* 274: 33866–33868.
21. Aoki K, Kikuchi T, Mukasa K, Ito S, Nakajima A, Satoh S, Okamura A, Sekihara H (2000) Dehydroepiandrosterone suppresses elevated hepatic glucose-6-phosphatase mRNA level in C57BL/KsJ-db/db mice: comparison with troglitazone. *Endocr J* 47: 799–804.
22. Schmoll D, Allan BB, Burchell A (1996) Cloning and sequencing of the 5' region of the human glucose-6-phosphatase gene: transcriptional regulation by cAMP, insulin and glucocorticoids in H4IIE hepatoma cells. *FEBS Lett* 383: 63–66.
23. Sutherland C, O'Brien RM, Granner DK (1995) Phosphatidylinositol 3-kinase, but not p70/p85 ribosomal S6 protein kinase, is required for the regulation of phosphoenolpyruvate carboxykinase (PEPCK) gene expression by insulin. Dissociation of signaling pathways for insulin and phorbol ester regulation of PEPCK gene expression. *J Biol Chem* 270: 15501–15506.
24. Dickens M, Svitek CA, Culbert AA, O'Brien RM, Tavare JM (1998) Central role for phosphatidylinositide 3-kinase in the repression of glucose-6-phosphatase gene transcription by insulin. *J Biol Chem* 273: 20144–20149.
25. Ayala JE, Streeper RS, Desgrosellier JS, Durham SK, Suwanichkul A, Svitek CA, Goldman JK, Barr FG, Powell DR, O'Brien RM (1999) Conservation of an insulin response unit between mouse and human glucose-6-phosphatase catalytic subunit gene promoters: transcription factor FKHR binds the insulin response sequence. *Diabetes* 48: 1885–1889.
26. Schmoll D, Walker KS, Alessi DR, Grempler R, Burchell A, Guo S, Walther R, Unterman TG (2000) Regulation of glucose-6-phosphatase gene expression by protein kinase Balpha and the forkhead transcription factor FKHR. Evidence for insulin response unit-dependent and -independent effects of insulin on promoter activity. *J Biol Chem* 275: 36324–36333.
27. O'brien RM, Streeper RS, Ayala JE, Stadelmaier BT, Hornbuckle LA (2001) Insulin-regulated gene expression. *Biochem Soc Trans* 29: 552–558.
28. Folli F, Kahn CR, Hansen H, Bouchie JL, Feener EP (1997) Angiotensin II inhibits insulin signaling in aortic smooth muscle cells at multiple levels. A potential role for serine phosphorylation in insulin/angiotensin II crosstalk. *J Clin Invest* 100: 2158–2169.
29. Fuller PJ, Lim-Tio SS, Brennan FE (2000) Specificity in mineralocorticoid versus glucocorticoid action. *Kidney Int* 57: 1256–1264.
30. Viengchareun S, Penfornis P, Zennaro MC, Lombes M (2001) Mineralocorticoid and glucocorticoid receptors inhibit UCP expression and function in brown adipocytes. *Am J Physiol Endocrinol Metab* 280: E640–E649.
31. Arriza JL, Weinberger C, Cerelli G, Glaser TM, Handelin BL, Housman DE, Evans RM (1987) Cloning of human mineralocorticoid receptor complementary DNA: structural and functional kinship with the glucocorticoid receptor. *Science* 237: 268–275.
32. Wickert L, Watzka M, Bolkenius U, Bidlingmaier F, Ludwig M (1998) Mineralocorticoid receptor splice variants in different human tissues. *Eur J Endocrinol* 138: 702–704.
33. Zennaro MC, Souque A, Viengchareun S, Poisson E, Lombes M (2001) A new human MR splice variant is a ligand-independent transactivator modulating corticosteroid action. *Mol Endocrinol* 15: 1586–1598.
34. Casal AJ, Silvestre JS, Delcayre C, Capponi AM (2003) Expression and modulation of steroidogenic acute regulatory protein messenger ribonucleic acid in rat cardiocytes and after myocardial infarction. *Endocrinology* 144: 1861–1868.
35. Seckl JR, Walker B (2001) Minireview: 11β-hydroxysteroid dehydrogenase type 1-a tissue specific amplifier of glucocorticoid action. *Endocrinology* 142: 1371–1376.