Stimulatory Effects of Hyperprolactinemia on Aldosterone Secretion in Ovariectomized Rats

Mei-Mei Kau, Ling-Ling Chang, Shu-Fen Kan, Low-Tone Ho, and Paulus S. Wang

ABSTRACT

Background: **To evaluate the effects of hyperprolactinemia on aldosterone secretion and its mechanisms of action in ovariectomized (OVX) rats.**

Methods: **Hyperprolactinemia was induced by the transplantation of rat anterior pituitary (AP) glands under the kidney capsule for 6 weeks in female rats. Control rats underwent cerebral cortex (CX) transplantation. Four weeks after transplantation, the rats were OVX 2 weeks before decapitation. After decapitation, the trunk blood was collected, and the adrenal glands of CX- and AP-grafted rats were prepared as zona glomerulosa (ZG) cells for in vitro study.**

Results: **Plasma prolactin and aldosterone in the rats were increased by AP gland transplantation. In the in vitro study, the basal aldosterone secretion by the adrenal ZG cells was higher in AP-grafted rats than in CX-grafted rats. The AP-grafted group showed increased responsiveness to angiotensin II (10^{-8} M), KCl (8×10^{-3} M), or 8-bromo-adenosine 3',5'-cyclic monophosphate (8-br-cAMP; 10^{-4} M, a membrane-permeable analogue of cAMP) with regard to aldosterone secretion as compared with the CX-grafted group. *N*-(2-[p-Bromocinnamylamine]ethyl)-5-isoquinolinesulfonamide (H89; 10^{-6}, 10^{-5} M, a protein kinase A inhibitor) or tetrandrine (10^{-5} M, a blocker for both L-type and T-type Ca^{2+} channels) induced a greater suppression of aldosterone secretion in the AP-grafted group than in the CX-grafted group. No significant differences between the CX- and AP-grafted groups were observed, however, with regard to the adrenocorticotropic hormone (10^{-9} M)-, forskolin (10^{-5} M, an adenylyl cyclase activator)-, or nifedipine (10^{-5} M, an L-type Ca^{2+} channel blocker)-induced responsiveness of aldosterone secretion. In addition, there was no difference in the expression of desmolase (i.e., cytochrome P450 side-chain cleavage enzyme) in ZG cells between AP- and CX-grafted rats. The conversions of 25-OH-cholesterol into pregnenolone in the presence of trilostane (an inhibitor of 3β-hydroxysteroid dehydrogenase) and corticosterone into aldosterone, as well as the expression of the steroidogenic acute regulatory protein in ZG cells, were greater in AP-grafted rats than in CX-grafted rats.**

Conclusions: **These results suggest that hyperprolactinemia increases basal, angiotensin II- and KCl-stimulated aldosterone secretion by ZG cells in OVX rats through activation of T-type Ca^{2+} channels, the post-cAMP and protein kinase A pathway, cytochrome P450 side-chain cleavage enzyme, and aldosterone synthase, as well as by causing increased expression of steroidogenic acute regulatory protein in ZG cells. (J Investig Med 2002;50:101–109) Key Words: Ca^{2+} channel• aldosterone synthase• P450scc• StAR protein**

INTRODUCTION

Hyperprolactinemia is a common endocrinological disorder of the hypothalamus-pituitary axis that occurs mainly in women. The major clinical presentations of hyperprolactinemia are hypogonadism, amenorrhea, and galactorrhea.[1] Several studies have demonstrated that these disorders are associated with the inhibition of hypothalamus-pituitary-gonadal activity, including suppressed gonadotropin or luteinizing hormone-releasing hormone secretion.[2–5] Prolactin (PRL) exerts an inhibitory effect on gonadotropic secretion at the pituitary level[6–8] and also

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has gonadotropic action at the ovarian level.[9] In contrast, a stimulatory effect of hyperprolactinemia on hypothalamus-pituitary-adrenal activity has been demonstrated. Hyperprolactinemia induced by the implantation of tumor 7315b or the transplantation of pituitary glands increases adrenal weight,[10,11] hypothalamic content, and the release of immunoreactive corticotropin-releasing factor,[11] adrenocorticotropic hormone (ACTH), and corticosterone.[11] Although PRL has a direct stimulatory influence on aldosterone secretion in human adrenal cells[12] and in rat zona glomerulosa (ZG) cells,[13–15] the effects of chronic hyperprolactinemia on aldosterone steroidogenesis and their mechanisms of action are still unclear.

Arafah et al.[16] indicated that hyperprolactinemic patients, as compared with normal individuals, have higher blood pressure and higher basal level of angiotensin II (Ang II)- and ACTH-stimulated aldosterone during sodium loading. Lehtovirta et al.[17] demonstrated that women who take oral contraceptives and develop hyperprolactinemia are more likely to have hypertension. A positive correlation between the mean arterial pressure and serum PRL level was found in these women. In addition, alterations in vascular reactivity and arterial pressure were reported in PRL-treated animals. Horrobin et al.[18] showed that rabbits that received PRL infusions had increased mean arterial pressure. Bryant et al.[19] indicated that the administration of PRL in rats produces an increase in blood volume, a decrease in blood pressure, and a decrease in angiotensin responsiveness. These observations show that the effects of PRL on the renin-angiotensin-aldosterone system and the regulation of blood volume probably play an important role in the modulation of cardiovascular responsiveness.

Aldosterone increases sodium resorption mainly through its effects on the renal distal tubules and cortical collecting ducts. The biosynthetic enzymes of the two limiting steps in aldosterone steroidogenesis are cytochrome P450 side-chain cleavage (P450scc; conversion of cholesterol to pregnenolone) and aldosterone synthase (conversion of corticosterone to aldosterone).[20,21] It has been indicated that the steroidogenic rate of P450scc in different tissues is determined by steroidogenic acute regulatory (StAR) protein.[22] In the rat adrenal gland, StAR protein has been shown to be the regulator of cholesterol transfer to the mitochondrial P450scc enzyme.[23] Ang II, high potassium, and ACTH are the most important regulators of aldosterone secretion in ZG cells. The signal transduction pathway of these regulators involves increasing intracellular calcium concentration or 3',5'-cyclic adenosine monophosphate (cAMP) production and then the activation of the aldosterone biosynthetic enzymes. Therefore, the purpose of our study was to investigate the effects of hyperprolactinemia induced by pituitary transplantation on 1) plasma aldosterone level; 2) basal, Ang II-, KCl-, and ACTH-stimulated aldosterone secretion in ZG cells; 3) activities of the L-type and T-type Ca^{2+} channels, adenylyl cyclase, protein kinase A (PKA), P450scc, and aldosterone synthase in ZG cells; and 4) protein expression of P450scc and StAR protein in ZG cells from ovariectomized (OVX) rats. Because the production of aldosterone is affected by estradiol,[24] OVX rats were used in the present study.

MATERIALS AND METHODS

Animals

Three-month-old Sprague-Dawley female rats were housed in a temperature-controlled room (22±1°C) with 14 h of artificial illumination daily (6:00 A.M.–8:00 P.M.) and were administered food and water ad libitum.

Effects of Hyperprolactinemia on the Level of Plasma Aldosterone in OVX Rats

Hyperprolactinemia was induced in the rats by pituitary transplantation. Female rats were anesthetized with ether, and then two anterior pituitary (AP) glands were transplanted under the left kidney capsule. Control rats received similar treatment but underwent cerebral cortex (CX) tissue implantation. Four weeks later, these rats were OVX 2 weeks before decapitation. After decapitation, trunk blood samples were collected.

The concentration of PRL in the plasma samples was measured by radioimmunoassay (RIA). The levels of plasma Na^+ and K^+ were determined using a flame photometer (EFOX 5053; Eppendorf, Hamburg, Germany). The plasma was mixed with diethyl ether (10-fold volume), shaken for 30 min, centrifuged at 1,000×*g* for 5 min, and then quickly frozen in a mixture of acetone and dry ice. The organic phase was collected, dried, and reconstituted in 1% bovine serum albumin in borate buffer (pH 7.8) before the concentration of aldosterone was measured by RIA.

Effects of Hyperprolactinemia on the Basal, Ang II-, or KCl-stimulated Aldosterone Release and the Role of Calcium Channels in ZG Cells

After the rats were decapitated, the adrenal glands from the CX- and AP-grafted rats were removed and stored in a 0.9% (w/v) NaCl ice bath. The ZG cells were prepared by a method described previously.[15] After preincubation of ZG cells with Krebs-Ringer bicarbonate buffer containing K^+ 3.6 mM, glucose 11.1 mM, and 0.2% bovine serum albumin (KRBGA medium) for 1 h at 37°C in a shaking

bath (100 cycles/min) aerated with 95% O_2 and 5% CO_2, aliquots (1 mL) of the ZG cells (5×10^4 cells) from the CX- or AP-grafted rats were incubated in KRBGA medium (basal release), Ang II (10^{-8} M; Sigma, St. Louis, MO), and KCl (8×10^{-3} M) for 30 min. To determine the involvement of the different types of Ca^{2+} channels in the hyperprolactinemic effect of aldosterone secretion, ZG cells were incubated with nifedipine (10^{-5} M, an L-type Ca^{2+} channel blocker; Sigma) or tetrandrine (10^{-5} M, a blocker for both L-type and T-type Ca^{2+} channels; Aldrich Chemical Co., Inc., Milwaukee, WI) for 30 min. At the end of the incubation period, 0.2 mL ice-cold KRBGA medium was added to stop the incubation. The medium was centrifuged at $200\times g$ and stored at −20°C until being analyzed for aldosterone by RIA.

Effects of Hyperprolactinemia on the ACTH-, Forskolin-, 8-Br-cAMP-, or H89-induced Aldosterone Release in ZG Cells

To study the effects of hyperprolactinemia on ACTH-stimulated aldosterone secretion as well as the involvement of cAMP, ZG cells from the CX- or AP-grafted OVX rats were incubated with KRBGA medium, ACTH (10^{-9} M; Sigma), forskolin (10^{-5} M, an adenylyl cyclase activator; Sigma), or 8-bromo-adenosine 3',5'-cyclic monophosphate (8-Br-cAMP; 10^{-4} M, a membrane-permeable analogue of cAMP; Sigma) in 0.3 mL of KRBGA medium. To study the effect of hyperprolactinemia on the activity of PKA, ZG cells from CX-grafted or AP-grafted rats were incubated with KRBGA or *N*-(2-[p-Bromocinnamylamine]-ethyl)-5-isoquinolinesulfonamide (H89; 10^{-6} and 10^{-5} M, a PKA inhibitor; Sigma). After 30 min incubation, samples were collected and analyzed for aldosterone by RIA.

Effects of Hyperprolactinemia on the Activities of P450scc and Aldosterone Synthase in ZG Cells

To assess the effects of hyperprolactinemia on the activities of P450scc, which converts cholesterol to pregnenolone, and aldosterone synthase, which converts corticosterone to aldosterone, ZG cells from the CX- or AP-grafted OVX rats were incubated with KRBGA medium and 25-OH-cholesterol (10^{-5} M, a membrane-permeable cholesterol, substrate of P450scc) in the presence or absence of trilostane (10^{-6} M, an inhibitor of 3β-hydroxysteroid dehydrogenase) or corticosterone (10^{-5} M, substrate of aldosterone synthase). Sanofi-Synthelabo, Inc. (Malvern, PA) provided trilostane. After 30 min incubation, the medium was collected and measured for pregnenolone or aldosterone by RIA.

Effects of Hyperprolactinemia on the Expression of P450scc and StAR Protein in ZG Cells

To further assess the effects of hyperprolactinemia on the expression of P450scc (a 54-kDa protein) and StAR protein (a 30-kDa protein) in ZG cells, gel electrophoresis and Western blot analysis were used to measure the expression of P450scc protein and StAR protein in ZG cells of the CX- and AP-grafted OVX rats.

RIAs of PRL, Aldosterone, and Pregnenolone

The concentration of plasma PRL was determined by RIA as described previously.[25] The rat PRL RIA kit was provided by the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health (Bethesda, MD). The rat prolactin PRL-I-9 was used for iodination and the rat prolactin PRL-RP-3 served as standard preparation. The sensitivity was 3 pg/assay tube. The intra-assay and interassay coefficients of variation were 3.9% (n=4) and 3.4% (n=4), respectively, for PRL RIA.

The concentrations of aldosterone in plasma and medium samples and of pregnenolone in medium samples were measured by RIA as described previously.[24,26] Anti-aldosterone antiserum no. 088 was provided by the National Institutes of Health. The sensitivity of aldosterone RIA was 4 pg/assay tube. The intra-assay and interassay coefficients of variation were 7.6% (n=5) and 7.9% (n=5), respectively. The antipregnenolone antiserum was purchased from Biogenesis, Inc. (Sandown, NH). The sensitivity of the pregnenolone RIA was 16 pg/assay tube. The intra-assay and interassay coefficients of variation were 2.3% (n=6) and 3.7% (n =4), respectively.

Gel Electrophoresis and Western Blot Analysis

The ZG cells from CX- and AP-grafted OVX rats were washed twice with 0.9% NaCl, then extracted by homogenization buffer as described previously.[27] The proteins of the samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membranes (NEN Life Science Products, Inc., Boston, MA). The membranes were blocked by 120-min incubation in 0.8% NaCl, 0.02 M Tris-base, and 0.3% Tween-20, pH 7.6 (TBS-T) buffer containing 5% nonfat dry milk. The membranes were then incubated overnight with anti-β-actin antibodies (1:8000, for loading control) combined with anti-P450scc antibodies (1:1000, rabbit) or anti-StAR antibodies (1:1000, rabbit) in a 5% solution of nonfat dried milk in TBS-T buffer at 4°C. After one wash for 15 min and three washes for 5 min with TBS-T buffer, the membranes were incubated for 1 h with horseradish peroxidase-conjugated goat antirabbit immunoglobulin G (IgG; 1:6000 dilution) and goat anti-

mouse IgG (1:6000 dilution) in a 5% solution of nonfat dried milk in TBS-T buffer. The membranes were then washed four times with TBS-T buffer. The specific protein bands were detected by electrogenerated chemiluminescence Western blot analysis detection reagents (Amersham International, Buckinghamshire, England).

Statistical Analysis

All data were expressed as mean±SEM. The treatment means were tested for homogeneity using analysis of variance, and the difference between specific means was tested for significance using Duncan's multiple-range test.[28] A difference between two means was considered to be statistically significant at $P<0.05$.

RESULTS

Effects of Hyperprolactinemia on the Level of Plasma Aldosterone in OVX Rats

Six weeks after transplantation, AP-grafted rats had higher plasma PRL levels than did CX-grafted rats (17.6±4.0 ng/mL, n=12 vs 132.2±15.2 ng/mL, n=12; $P<0.01$; Figure 1). The concentration of plasma aldosterone was significantly higher in the AP-grafted OVX rats (253.1±12.1 pg/mL) than in the CX-grafted OVX rats (168.9±14.6 pg/mL) ($P<0.01$; Figure 1). Figure 2 shows the correlation between the levels of plasma PRL and plasma aldosterone in AP-grafted rats. A positive correlation was found between plasma PRL and plasma aldosterone in AP-grafted rats (Pearson's coefficient of correlation; $P<0.05$). The plasma concentrations of Na^+ (139±3 mEq/L vs 142±2 mEq/L) and K^+ (3.9±0.2 mEq/L vs 4.0±0.1 mEq/L) of CX- and AP-grafted rats were not significantly different.

Effects of Hyperprolactinemia on Basal, Ang II-, or KCl-stimulated Aldosterone Release and the Role of the Calcium Channels in ZG Cells

The basal (i.e., unstimulated) release of aldosterone by ZG cells was significantly higher in the AP-grafted group than in the CX-grafted group ($P<0.01$; Figure 3). Incubation with Ang II (10^{-8} M) or KCl (8×10^{-3} M) resulted in a marked increase ($P<0.05$, $P<0.01$; Figure 3) in aldosterone secretion as compared with the basal release in both CX- and AP-grafted groups. Ang II increased aldosterone secretion by 44.5±8.1% and 94.8±19.0% for CX- and AP-grafted rats, respectively. KCl increased aldosterone release by 44.0±16.5% and 101.8±15.9% for CX- and AP-grafted groups, respectively. It should be noted that there was a greater response to Ang II or high K^+ for the AP-grafted group than for the CX-grafted group ($P<0.05$).

The administration of nifedipine (10^{-5} M, an L-type Ca^{2+} channel blocker) or tetrandrine (10^{-5} M, a blocker for both L-type and T-type Ca^{2+} channels) markedly decreased aldosterone secretion for both the CX-grafted

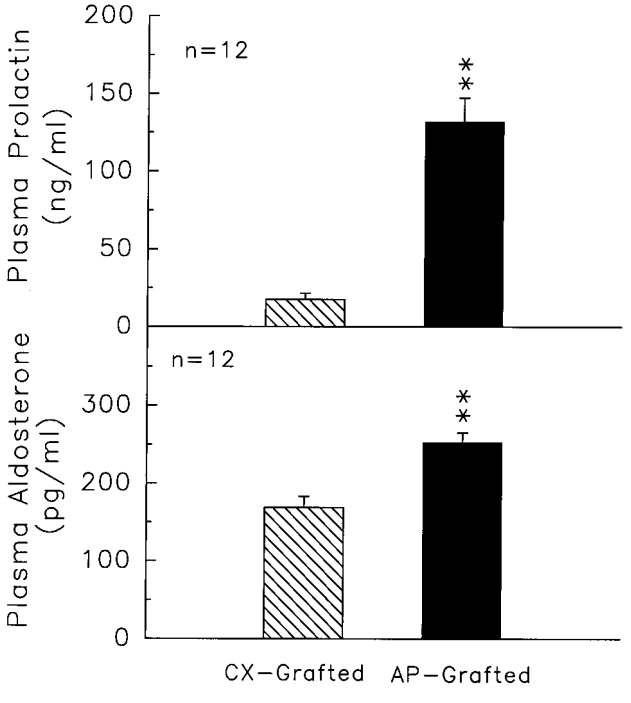

*Figure 1. The concentrations of plasma PRL and aldosterone in CX-grafted and AP-grafted OVX rats. **, $P<0.01$ as compared with CX-grafted rats. Each value represents mean±SEM.*

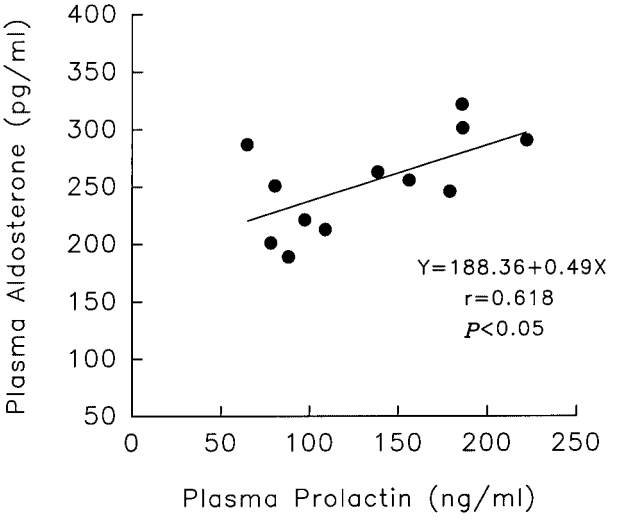

Figure 2. Analysis of correlation between the plasma aldosterone concentration (pg/mL) and plasma PRL level (ng/mL) in AP-grafted OVX rats. Symbols are for individual rats. The plasma aldosterone concentration correlated with plasma PRL level in AP-grafted rats ($P<0.05$).

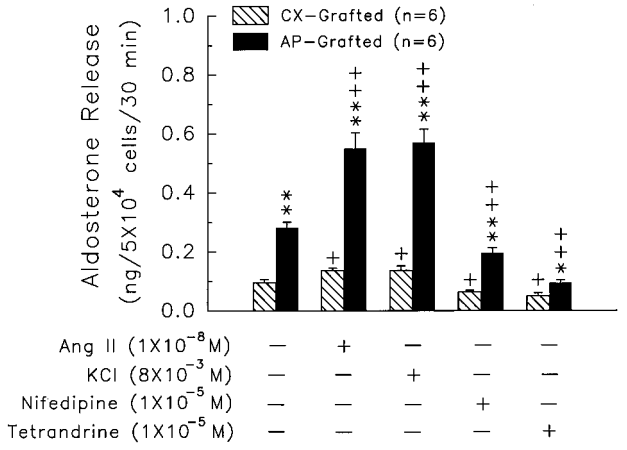

Figure 3. *Effects of hyperprolactinemia on basal (unstimulated), Ang II (10^{-8} M)-, KCl (8×10^{-3} M)-, nifedipine (10^{-5} M)-, or tetrandrine (10^{-5} M)-induced aldosterone release by ZG cells from OVX rats. *, **, $P<0.05$, $P<0.01$, as compared with CX-grafted OVX rats, respectively. +, ++, $P<0.05$ and $P<0.01$ as compared with the basal release, respectively. Each value represents mean±SEM.*

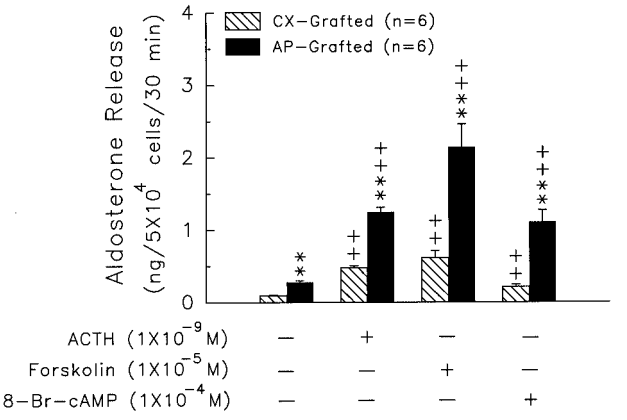

Figure 4. *Effects of hyperprolactinemia on basal, ACTH (10^{-9} M)-, forskolin (10^{-5} M)-, or 8-Br-cAMP (10^{-4} M)-stimulated aldosterone release by ZG cells from OVX rats. **, $P<0.01$ as compared with CX-grafted OVX rats. ++, $P<0.01$ as compared with the basal release. Each value represents mean±SEM.*

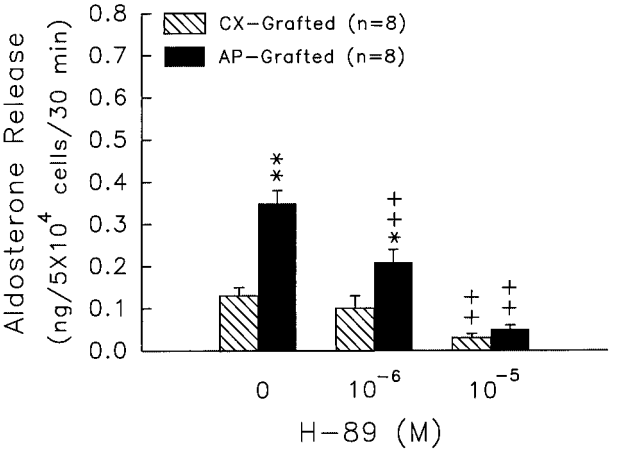

Figure 5. *Effects of hyperprolactinemia on the activity of PKA in ZG cells of OVX rats. *, $P<0.05$, **, $P<0.01$ as compared with CX-grafted OVX rats, respectively. ++, $P<0.01$ as compared with the basal release. Each value represents mean±SEM.*

($P<0.05$; Figure 3) and the AP-grafted ($P<0.01$; Figure 3) groups. Nifedipine inhibited aldosterone secretion by 33.0±5.8% for the CX-grafted group and by 30.7±6.2% for the AP-grafted group. No significant difference between the CX- and AP-grafted groups was observed with regard to nifedipine-decreased aldosterone secretion. Tetrandrine produced a greater inhibitory response in the AP-grafted group (67.7±3.9%) than in the CX-grafted group (44.4±7.6%; Figure 3).

Effects of Hyperprolactinemia on ACTH-, Forskolin-, 8-Br-cAMP-, or H89-induced Aldosterone Release in ZG Cells

ACTH (10^{-9} M), forskolin (10^{-5} M), or 8-Br-cAMP (10^{-4} M) significantly ($P<0.01$, Figure 4) increased the aldosterone secretion by ZG cells from both the CX- and the AP-grafted rats. ACTH increased aldosterone secretion by 405.9±28.3% from CX-grafted group cells and 354.8±18.3% from AP-grafted group cells. Forskolin increased aldosterone secretion by 566.5±83.4% from CX-grafted group cells and by 670.9±114.3% from AP-grafted group cells. No difference in ACTH- or forskolin-stimulated aldosterone secretion was observed between CX- and AP-grafted groups; however, AP-grafted group cells showed a greater response to 8-Br-cAMP (291.5±58.5%) than did the CX-grafted group cells (126.0±34.6%, $P<0.05$).

H89 at 10^{-6} or 10^{-5} M produced a significant decrease in aldosterone secretion in the AP-grafted group ($P<0.01$; Figure 5). However, H89 at 10^{-6} M did not inhibit aldosterone secretion in the CX-grafted group until the 10^{-5} M level. Considering the responsiveness of aldosterone to 10^{-5} M H89, the AP-grafted group had greater inhibition to H89 (85.7±2.6%) than did the CX-grafted group (71.8±5.4%, $P<0.05$).

Effects of Hyperprolactinemia on the Activities of P450scc and Aldosterone Synthase in ZG Cells

Figure 6 illustrates the results of the conversion of 25-OH-cholesterol (10^{-5} M) to pregnenolone in the presence or the absence of trilostane (10^{-6} M) in the ZG cells of CX- and AP-grafted rats. The administration of 25-OH-

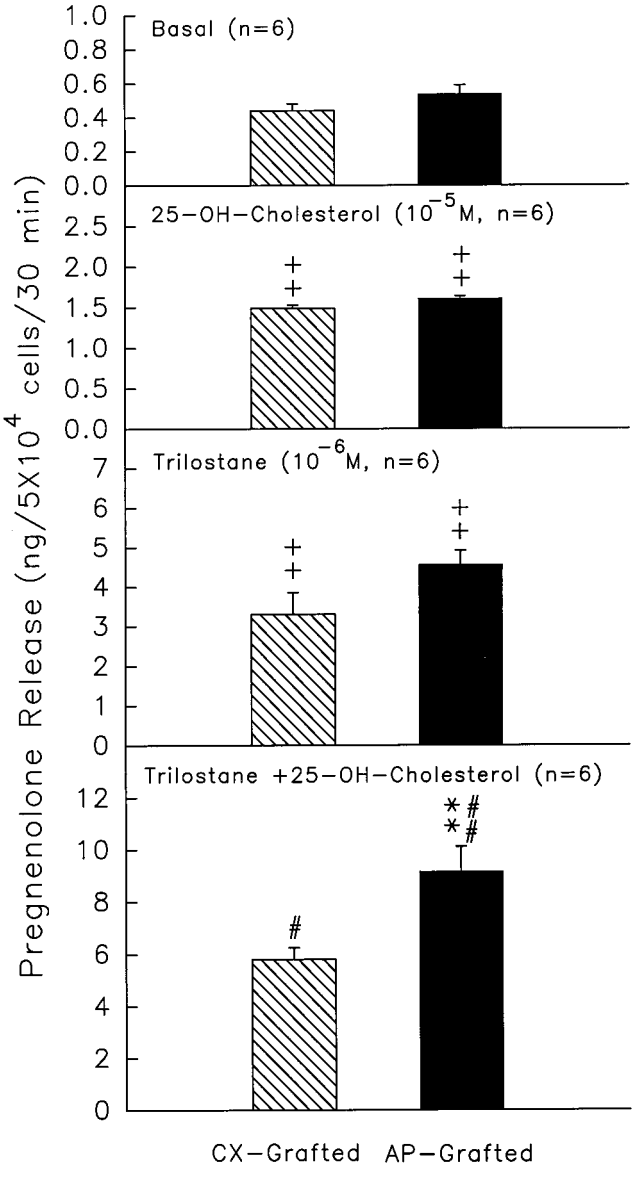

Figure 6. *Effects of hyperprolactinemia on the activity of P450scc in ZG cells of OVX rats. **, P<0.01 as compared with CX-grafted OVX rats. ++, P<0.01 as compared with the basal release. #, ##, P<0.05, P<0.01 as compared with trilostane 10^{-6} M, respectively. Each value represents mean±SEM.*

cholesterol or trilostane resulted in a significant increase of pregnenolone release in both groups (P<0.01). The combination of 25-OH-cholesterol and trilostane caused greater pregnenolone release as compared with the group of trilostane alone in CX- and AP-grafted rats (P<0.05 and P<0.01, respectively). There were no significant differences in basal, 25-OH-cholesterol-, or trilostane-induced pregnenolone release between the CX- and AP-grafted groups. In the presence of trilostane, however, the AP-grafted group had greater (P<0.01) conversion of 25-OH-cholesterol to pregnenolone.

Figure 7 shows the results of the conversion of corticosterone (10^{-5} M) to aldosterone in the CX- and AP-grafted groups. Corticosterone produced a significant increase in aldosterone secretion in both groups (P<0.01). As compared with the CX-grafted group, the AP-grafted group showed greater conversion of corticosterone to aldosterone (P<0.01). These results demonstrate that the activities of P450scc and aldosterone synthase are higher in AP-grafted rats than in CX-grafted rats.

Effects of Hyperprolactinemia on the Expression of P450scc and StAR Protein in ZG Cells

Western blot analysis showed that the expression of P450scc protein (54 kDa) in ZG cells is no different in CX- than in AP-grafted rats (Figure 8). StAR protein (30 kDa) expression by ZG cells was higher in AP-grafted rats than in the CX-grafted rats (Figure 9).

DISCUSSION

In our study, AP gland transplantation induced higher plasma PRL levels in the female rats during a 6-week than

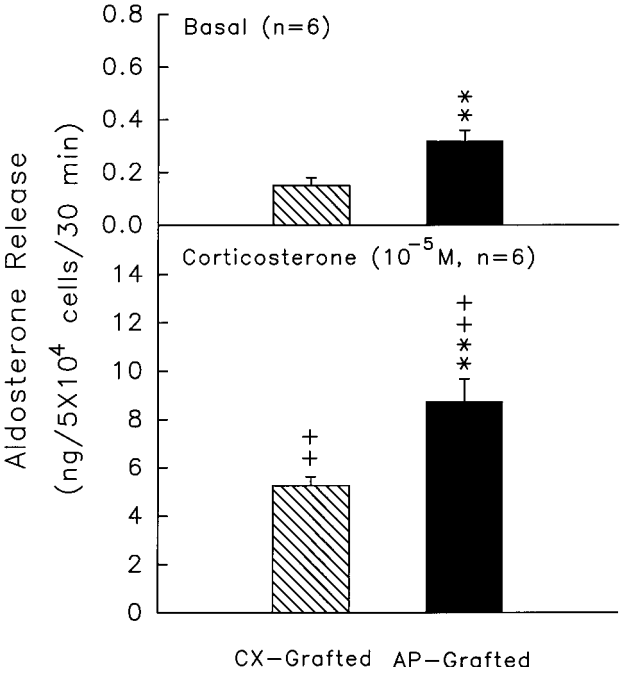

Figure 7. *Effects of hyperprolactinemia on the activity of aldosterone synthase in ZG cells of OVX rats. **, P<0.01 as compared with CX-grafted OVX rats. ++, P<0.01 as compared with the basal release. Each value represents mean±SEM.*

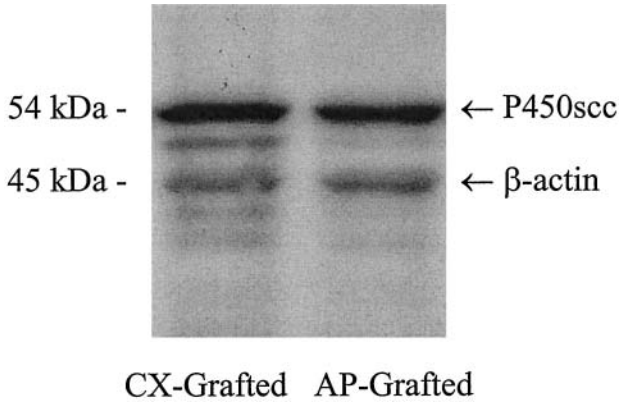

Figure 8. *Western blot analysis of P450scc protein in ZG cells of CX- and AP-grafted OVX rats. Protein extracts (10 μg) were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically blotted into the transfer membrane. The membrane was incubated with the anti-P450scc and anti-β-actin antibodies overnight at 4°C, then incubated with antirabbit IgG and antimouse IgG for 1 h. The bands for P450scc and β-actin were visualized by electrogenerated chemiluminescence reaction. This experiment was performed twice with similar results.*

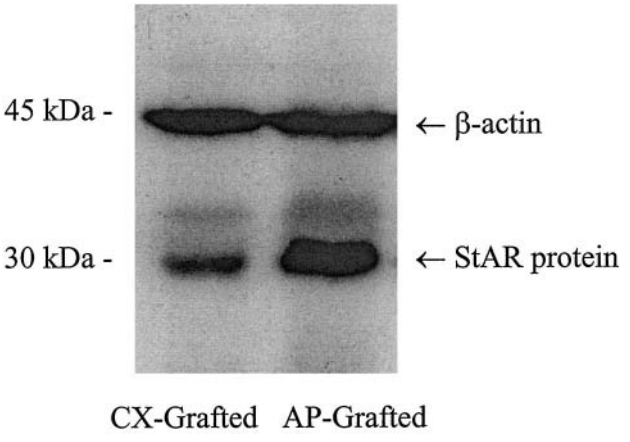

Figure 9. *Western blot analysis of StAR protein in ZG cells of CX-grafted and AP-grafted OVX rats. Protein extracts (30 μg) were subjected to 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrophoretically blotted into the transfer membrane. The membrane was incubated with the anti-StAR and anti-β-actin antibodies overnight at 4°C, and then incubated with antirabbit IgG and antimouse IgG for 1 h. The bands for StAR protein and β-actin were visualized by electrogenerated chemiluminescence reaction. This experiment was performed twice with similar results.*

it did in control rats (i.e., CX-grafted). Also, AP-grafted (i.e., hyperprolactinemic) rats had higher levels of plasma aldosterone and greater basal release of aldosterone from the ZG cells than did the CX-grafted rats. Although hyperprolactinemia did not change the expression of P450scc in ZG cells, the AP-grafted group exhibited more production of pregnenolone or aldosterone from 25-OH-cholesterol or corticosterone and greater expression of StAR protein in ZG cells than did the CX-grafted group. These results indicate that the stimulatory effect of hyperprolactinemia induced by the transplantation of AP glands on aldosterone secretion is associated with the activation of P450scc and aldosterone synthase as well as increased StAR protein expression in the ZG cells. This increased expression of the StAR protein in the ZG cells showed that an alteration in the upstream pathway of P450scc, the transport of cholesterol to mitochondria, may play an important role in the stimulatory effect of hyperprolactinemia on aldosterone secretion.

Our study results also show that the ZG cells in hyperprolactinemic rats have a greater response in aldosterone secretion to Ang II and KCl than they do in the control group. It is well known that extracellular Ca^{2+} influx and the Ca^{2+} signal transduction pathway are involved in the mechanisms of action of Ang II and KCl.[29,30] Our results demonstrate that the responsiveness of aldosterone to tetrandrine (a blocker for both L-type and T-type Ca^{2+} channels) in the AP-grafted group is greater than that in the CX-grafted group. However, there still is a significant difference between AP- and CX-grafted groups. There was no difference in the response of aldosterone to nifedipine (L-type Ca^{2+} channel blocker) in the AP- and CX-grafted groups. We therefore suggest that all three effects induced by hyperprolactinemia—increases in aldosterone basal release, Ang II-stimulated aldosterone secretion, and KCl-stimulated aldosterone secretion—are due in part to the increased activity of T-type Ca^{2+} channels.

Several investigators[31–33] have reported that AP gland transplantation did not alter the levels of plasma thyroid-stimulating hormone and growth hormone in rats. Kooy et al.[11] found that AP-grafted rats have higher plasma ACTH levels than control, but Drago et al.[34,35] obtained different results. Although the effect of ACTH on aldosterone secretion cannot be ruled out, the major purpose of our study was to evaluate the effects of chronic hyperprolactinemia on aldosterone secretion and its mechanisms of action with regard to the level of ZG cells. In addition, numerous studies have shown that ACTH activates adenylyl cyclase via its G protein-coupled receptor and increases the intracellular cAMP level. Subsequently, cAMP activates the PKA pathway and then increases the activities of the steroidogenic enzymes.[29] Our study indicates that there is no difference between CX- and AP-grafted rats regarding aldosterone responsiveness to ACTH or forskolin. However, the AP-grafted group showed a greater responsiveness in aldosterone secretion to 8-Br-cAMP (a cAMP analogue) and H89 (a PKA inhibitor). The reasons for these discrepant results are unknown at present. These

discrepancies imply that the AP-grafted group probably has a blunted cAMP response to ACTH or forskolin and an augmented post-cAMP and PKA pathway, and then has a level of responsiveness to ACTH or forskolin similar to that seen in the CX-grafted group. However, more studies are required to prove this hypothesis. On the basis of these observations, we suggest that the higher aldosterone release in AP-grafted group is related to the greater activity of the post-cAMP and PKA pathway in ZG cells.

Many biological functions of PRL in various vertebrates have been reported. These include effects on growth and development, endocrine function and metabolism, brain and behavior, reproduction, immunoregulation, and protection.[36] In addition to the usual target organs of PRL, the mRNA encoding the PRL receptor is expressed in the renal tubular epithelia of mammals.[36,37] Several studies have indicated that PRL reduces renal Na^+ and K^+ excretion,[38] stimulates Na^+-K^+ adenosine triphosphatase,[39] decreases Na^+ and Cl^- in sweat,[40] and increases water and salt absorption in all regions of the intestine[41] in mammals. In addition to the direct effect of PRL on the kidneys, the indirect action of PRL should be considered. Our previous studies[15] and the present study demonstrate that the stimulatory effects of PRL on aldosterone secretion may provide evidence for the involvement of PRL in the regulation of water and sodium. The physiological significance of hyperprolactinemia-increased aldosterone secretion may be responsible for the physiological requirement in the body fluid turnover and blood pressure maintenance that occur during lactation, pregnancy, and stress.

CONCLUSIONS

Hyperprolactinemia induced by AP gland transplantation increases the level of plasma aldosterone and basal, Ang II-, and KCl-stimulated aldosterone secretion in ZG cells. This stimulatory effect of hyperprolactinemia on aldosterone secretion is due in part to five factors: the activation of T-type Ca^{2+} channels, the post-cAMP and PKA pathway, P450scc, aldosterone synthase, and an increased expression of StAR protein in ZG cells.

ACKNOWLEDGMENTS

This study was supported by Grant No. 89-B-FA22-2-4 in the Program for Promoting Academic Excellence of Universities from the Ministry of Education, Executive Yuan, Taiwan, Republic of China, Grant No. VTY89-P5-40 from the VGH-NYMU Joint Research Program, Tsou's Foundation, Republic of China, and awards from the Medical Research and Advancement Foundation in memory of Dr. Chi-Shuen Tsou, Republic of China, to PSW. The anti-aldosterone antiserum no. 088 and rat PRL RIA kit were kindly supplied by the National Hormone and Pituitary Program, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Child Health and Human Development, and the U.S. Department of Agriculture. The anti-P450scc antibody was kindly supplied by Dr. Bon-Chu Chung, Institute of Molecular Biology, Academia Sinica, Republic of China. The anti-StAR protein antibody was kindly supplied by Dr. D.M. Stocco, Department of Cell Biology and Biochemistry, Texas Tech University Health Science Center, Lubbock, TX.

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Stimulatory Effects of Hyperprolactinemia on Aldosterone Secretion in Ovariectomized Rats

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J Investig Med 2002 50: 101-109
doi: 10.2310/6650.2002.31271

Notes