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Effects of Prolactin on Aldosterone Secretion in Rat Zona Glomerulosa Cells

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Abstract Acute effects and action mechanisms of prolactin (PRL) on aldosterone secretion in zona glomerulosa (ZG) cells were investigated in ovariectomized rats. Administration of ovine PRL (oPRL) increased aldosterone secretion in a dose-dependent manner. Incubation of [^{3}H]-pregnenolone combined with oPRL increased the production of [^{3}H]-aldosterone and [^{3}H]-deoxycorticosterone but decreased the accumulation of [^{3}H]-corticosterone. Administration of oPRL produced a marked increase of adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation in ZG cells. The stimulatory effect of oPRL on aldosterone secretion was attenuated by the administration of angiotensin II (Ang II) and high potassium. The Ca^{2+} chelator, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA, 10^{-2} M), inhibited the basal release of aldosterone and completely suppressed the stimulatory effects of oPRL on aldosterone secretion. The stimulatory effects of oPRL on aldosterone secretion were attenuated by the administration of nifedipine (L-type Ca^{2+} channel blocker) and tetrandrine (T-type Ca^{2+} channel blocker). These data suggest that the increase of aldosterone secretion by oPRL is in part due to (1) the increase of cAMP production, (2) the activation of both L- and T-type Ca^{2+} channels, and (3) the activation of 21-hydroxylase and aldosterone synthase in rat ZG cells. J. Cell. Biochem. 72:286–293, 1999.

Key words: cAMP; Ca^{2+}; steroidogenesis

Prolactin (PRL) is a lactotropic hormone that stimulates breast development and milk production in women. In addition, PRL plays a role in reproductive function, fluid balance, and immune function [Nicoll, 1980; Russell, 1989]. Several studies have demonstrated that PRL exerts an inhibitory effect on gonadotropin secretion at the hypothalamus-pituitary levels [Vasquez et al., 1980; Cheung, 1983; Sharkar and Yen, 1985; Voogt et al., 1987] and gonadotropin action at the ovarian level [Gitay-Goren et al., 1989a]. In the rat, gonadotropin secretion is suppressed during chronic hyperprolactinemia [Bartke et al., 1987; Kooy et al., 1989]. These reduced gonadotropin levels are probably due to an inhibitory effect of PRL on pituitary gonadotropin-secreting cells [Vasquez et al., 1980; Cheung, 1983] and on hypothalamic luteinizing hormone-releasing hormone (LHRH) secreting neurons [Sharkar and Yen, 1985; Voogt et al., 1987]. In contrast to the inhibition of reproductive function, hyperprolactinemia seems to activate the hypothalamus-pituitary-adrenal (HPA) activity and may be explained by a direct stimulatory effect of PRL on both hypothalamic corticotropin-releasing hormone (CRH) and pituitary adrenocorticotropic hormone (ACTH) secretion [Weber and Calogero, 1991]. Arafah et al. [1990] have indicated that, during sodium loading, hyperprolactinemic patients have higher blood pressure and higher basal, angiotensin II (Ang II)-, and ACTH-stimulated aldosterone levels than are found in normal subjects. There is evidence that PRL has a direct effect on adrenal function, including PRL-potentiated ACTH-stimulated steroid secretion in rat adrenal slices [Eldridge and Lyman-

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grover, 1984] and enhanced dehydroepiandrosterone (DHEA), cortisol, and aldosterone secretion in human adrenal cells [Glasow et al., 1996].

Binding sites for PRL and the gene expression of PRL receptors have been demonstrated in rat adrenal cortex [Calvo et al., 1981; Ouhtit et al., 1993]. PRL receptors belong to the cytokine/hematopoietic growth factor receptor family [Kishimoto et al., 1994; Taniguchi, 1995]. It has been shown that Jak2 tyrosine kinase and Stat5 transcription factor play a major role in PRL signal tranduction in mammary gland [Lebrun et al., 1995; Watson and Burdon, 1996; Jahn et al., 1997]. In several cell lines, including the PRL-dependent rat pre-T-cell line (Nb2) [Rui et al., 1994] and differentiated insulin-secreting cell line (INS-1) [Sekine et al., 1996], PRL activated Jak2 tyrosine kinase. However, other signaling mechanisms have been reported for the effect of PRL. For example, PRL produced a rise in cytosolic Ca^{2+} in INS-1 cells [Sekine et al., 1996], increased adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation induced by human chorionic gonadotropin (hCG) in rat luteal cells [Gitay-Goren et al., 1989b], and inhibition of cAMP accumulation induced by hCG in rat granulosa cells [Gitay-Goren et al., 1989a]. These results suggest that the effects of PRL are probably through different signal transduction pathways in different cells.

Several enzymes are involved in the biosynthesis of aldosterone in rat zona glomerulosa (ZG) cells, including cholesterol desmolase (CYP11A), 3β-hydroxysteroid dehydrogenase (3β-HSD), 21-hydroxylase (CYP21), 11β-hydroxylase (CYP11B2), and aldosterone synthase (CYP11B2) [White et al., 1994]. It is well known that the secretagogues of aldosterone production are Ang II, high potassium, and ACTH. The second messengers used by these secretagogues include the rise in cytosolic free calcium and cAMP formation [Quinn and Williams, 1988]. Most of these transduction mechanisms increase phosphorylation and lead to a change in steroid enzyme activity and aldosterone secretion. Thus, the involvement of cAMP or Ca^{2+} in the effect of PRL on aldosterone production is highly suggested. The present study was undertaken to assess the acute effects of PRL on basal, Ang II-, and high potassium-stimulated aldosterone secretion, and the action mechanisms involved in the PRL effects in rat ZG cells. Since the production of aldosterone is increased by ovarian hormones (present authors, unpublished data), the ovariectomized (Ovx) rats were employed in the present study.

MATERIALS AND METHODS

Animals

Female rats of the Sprague-Dawley strain weighing 250–300 g were housed in a temperature-controlled room (22 ± 1°C) with 14 h of artificial illumination daily (0600–2000) and given food and water ad libitum.

Preparation of Adrenal Zona Glomerulosa Cells

Female rats were Ovx for 4 days before decapitation. The adrenal glands were removed and placed in a 0.9% (w/v) NaCl ice bath. The method for the preparation of dispersed ZG cells was modified from the method of Whitehouse and Abayasekara [1994]. Briefly, after removal of excess fat, adrenal glands were separated into capsule (mainly ZG) and inner zone (mainly zona fasciculata/reticularis) fractions. The capsules from 5–8 adrenal glands were assigned as one dispersion, then added to a polyethylene tube containing 1 ml Krebs-Ringer bicarbonate buffer with 3.6 mmol K^+/L, 11.1 mmol glucose/L and 0.2% bovine serum albumin (BSA) (KRBGA medium) and 2 mg collagenase (Sigma Chemical Co., St. Louis, MO). The tube was aerated with 95% O_2 and 5% CO_2 and then incubated for 1 h at 37°C in a shaker bath oscillating at 100 cycles per min. Generally, at least six dispersions (n = 6) of ZG cells were included in each group. At the end of incubation, the capsular tissues were mechanically dispersed into cells by repeated pipetting, then filtering through a nylon mesh. After centrifugation (200*g*, 10 min), cells were washed with deionized water for disrupting red blood cells; the osmolarity was then immediately restored with 10-fold Hank's balanced sodium solution (HBSS). The cell number and cell viability (>72%) were assessed using a hemocytometer and the trypan blue exclusion method. The cells (5×10^4 cells/ml) were preincubated with 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. The supernatant was decanted after centrifugation of the tubes at 200*g* for 10 min. Finally, the cell pellets were resuspended in KRBGA medium.

Effects of Ovine PRL on Basal, Ang II-, or KCl-Stimulated Aldosterone Release in ZG Cells

Aliquots (1 ml) of cell suspensions (5×10^4 cells) were incubated with 0.3 ml KRBGA medium, Ang II (10^{-8}; Sigma), or KCl (8×10^{-3} M) in the absence or presence of ovine prolactin (oPRL) (10^{-9}–10^{-6} M; Sigma) for 30 min. At the end of the incubation, 0.2 ml ice-cold KRBGA medium was added to stop the incubation. The medium was centrifuged at 200*g* for 10 min and stored at −20°C, until it was analyzed for aldosterone by radioimmunoassay (RIA).

Roles of Ca^{2+}, L-Type Ca^{2+} Channel, T-Type Ca^{2+} Channel, and cAMP in the Effects of oPRL on Aldosterone Secretion in ZG Cells

In order to study the roles of Ca^{2+} and different types of Ca^{2+} channel in the effects of oPRL on aldosterone release, aliquots (1 ml) of cell suspensions (5×10^4 cells) were incubated with 0.3 ml KRBGA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA, 10^{-3} M, 10^{-2} M, a Ca^{2+} chelator; Sigma), nifedipine (10^{-5} M, the L-type calcium channel blocker; Sigma) and tetrandrine (10^{-5} M, the T-type calcium channel blocker; Aldrich Chemical Company, Milwaukee, WI) in the absence or presence of oPRL (10^{-9}–10^{-6} M) for 30 min.

To determine the role of cAMP in regulating the effects of oPRL, 1 ml aliquots of cell suspensions (5×10^4 cells) were incubated with 0.3 ml KRBGA or oPRL (10^{-9}–10^{-6} M). At the end of the 30 min incubation, the medium was collected and stored at −20°C for determination of aldosterone by RIA. The cells were mixed with 0.5 ml of 65% ice-cold ethanol, homogenized by polytron (PT-3000, Kinematica, Luzern, Switzerland), then centrifuged at 2,000*g* for 15 min. The supernatants were lyophilized in a vacuum concentrator (Speed Vac; Savant, Holbrook, NY) and reconstituted with assay buffer (0.05 M acetate buffer with 0.01% sodium azide, pH 6.2) before measuring the concentration of cAMP by RIA.

Effects of oPRL on Aldosterone Steroidogenesis in ZG Cells

Aliquots (1 ml) of cell suspensions were incubated with [^{3}H]-pregnenolone (10,000 cpm, 5 pmol, Amersham International plc., Bucks, UK) or [^{3}H]-pregnenolone combined with oPRL (10^{-9}–10^{-7} M). After 30 min incubation, the medium was collected, then extracted by 1 ml diethyl ether, and then quick-frozen in a mixture of acetone and dry ice. The diethyl ether layer was collected, dried, and reconstituted in 100 µl 100% ethanol containing 5 µg of each of the unlabeled carriers, including progesterone (P_4), deoxycorticosterone (DOC), corticosterone, and aldosterone. Aliquots (50 µl) of samples were applied to a thin-layer chromatography (TLC) plate (0.25 µg-thick silica gel G sheets precoated with fluorescent indicator; Macherey-Nagel, Düren, Germany) and then developed in a mixture of carbon tetrachloride and acetone (4:1; vol/vol). The sheets were dried, and the locations of steroid-containing spots were indicated under ultraviolet (UV) light. The R_f values were 0.97 for P_4, 0.68 for DOC, 0.24 for corticosterone, and 0.11 for aldosterone. The spots were cut off and transferred into vials containing 1 ml of liquid scintillation fluid (Beckman, Fullerton, CA) before the radioactivity was counted in an automatic β-counter (Wallac 1409; Pharmacia, Turku, Finland).

RIA of Aldosterone

Anti-aldosterone antiserum No. 088, provided by the National Institutes of Health (NIH), was diluted with 1% BSA-borate buffer. The cross-reactivities of anti-aldosterone were less than 1% with cortisol, corticosterone, cortisone, testosterone, dehydroepiandrosterone, P_4, estradiol, 18-hydroxycorticosterone and DOC. In this RIA system, a known amount of unlabeled aldosterone, an aliquot of medium samples, adjusted to a total volume of 0.3 ml by a buffer solution (1% BSA–borate buffer, pH 7.8), was incubated with 0.1 ml aldosterone antiserum (1:120,000 dilutions) diluted with 1% BSA-borate buffer and 0.1 ml [^{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) was added and further incubated in an ice bath for 15 min. After incubation, the assay tubes were centrifuged at 1,000*g* for 15 min. The supernatant was mixed with 3 ml liquid scintillation fluid (Beckman) before the radioactivity was counted in an automatic β-counter (Pharmacia). The sensitivity of aldosterone RIA was 4 pg per assay tube. The intra- and interassay coefficients of variation were 7.5% (n = 5) and 8.1% (n = 5). The inhibition curves produced by the incubation medium of

rat ZG cells were parallel to those given by unlabeled aldosterone.

RIA of cAMP

The concentration of cAMP in ZG cells extracted by ethanol was measured by an RIA developed in our laboratory as described elsewhere [Lo et al., 1998]. The sensitivity of cAMP RIA was 2 fmol/assay tube. The anti-cAMP CV-27 pool was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDKD, Bethesda, MD). The synthetic tyr-cAMP (Sigma) used for iodination and the unlabelled cAMP (Sigma), which served as standard preparation. The sensitivity of cAMP RIA was 2 fmol per assay tube. The intra- and interassay coefficients of variability were 3.8% (n = 4), and 6.6% (n = 5), respectively.

Statistical Analysis

All the 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 [Steel and Torrie, 1960]. A difference between two means was considered statistically significant at $P < 0.05$.

RESULTS

Effects of oPRL on Basal, Ang II-, or KCl-Stimulated Aldosterone Release in ZG Cells

The effects of oPRL (10^{-9}–10^{-6} M) on basal (unstimulated), Ang II-, or KCl-induced aldosterone secretion in ZG cells are shown in Figure 1. Incubation of oPRL (10^{-8}–10^{-6} M) for 30 min resulted in a significant ($P < 0.05$ or $P < 0.01$, Fig. 1) increase in aldosterone secretion in a dose-dependent manner. Administration of Ang II (10^{-8} M) or potassium chloride (KCl) (8×10^{-3} M) significantly increased aldosterone secretion, as compared with the vehicle (unstimulated) group. Combination of Ang II (10^{-8} M) with oPRL (10^{-7} M or 10^{-6} M) produced a greater secretion of aldosterone than those in Ang II alone-treated group. In comparison with oPRL (10^{-7} and 10^{-6} M) alone-treated groups, Ang II combined with oPRL (10^{-7} and 10^{-6} M) induced less secretion of aldosterone. In the presence of KCl (8×10^{-3} M), oPRL at 10^{-7} and 10^{-6} M led to a significant ($P < 0.01$) increase in aldosterone secretion as compared with those in the KCl alone-treated group. However, the increment of aldosterone induced by KCl plus oPRL (10^{-6} M) was significantly less than that in oPRL (10^{-6} M) alone-treated group.

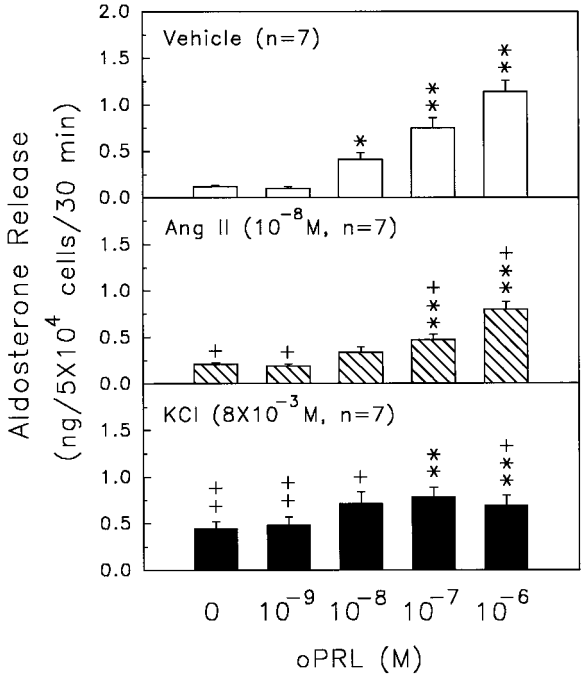

Fig. 1. Effects of oPRL (10^{-9}–10^{-6} M) on the vehicle (basal), Ang II (10^{-8} M)-, and KCl (8×10^{-3} M)-stimulated aldosterone release by ZG cells in Ovx rats. *, **, $P < 0.05$, $P < 0.01$, as compared with oPRL = 0 M, respectively. $^{+}$, $^{++}$, $P < 0.05$, $P < 0.01$, as compared with the vehicle-treated group, respectively. Each value represents mean ±SEM. oPRL, ovine prolactin; Ang II, angiotensin II; KCl, potassium chloride; ZG, zona glomerulosa; Ovx, ovariectomized.

Roles of Ca^{2+}, L-Type Ca^{2+} Channel, T-Type Ca^{2+} Channel, and cAMP in the Effects of oPRL on Aldosterone Release in ZG Cells

Administration of EGTA (10^{-2} M) significantly ($P < 0.01$, Fig. 2) suppressed aldosterone release as compared with the level of control (EGTA = 0 M) group. EGTA (10^{-3} M) alone or EGTA (10^{-3} M) combined with oPRL (10^{-9}–10^{-6} M) did not change the stimulatory effects of oPRL. In the presence of EGTA (10^{-2} M), the stimulatory effects of oPRL on aldosterone secretion were all abolished. Nifedipine (10^{-5} M) or tetrandrine (10^{-5} M) significantly ($P < 0.01$, Fig. 3) inhibited aldosterone secretion. In the presence of nifedipine or tetrandrine, the stimulatory effects of oPRL (10^{-8}–10^{-6} M) were significantly suppressed by nifedipine or tetrandrine. However, oPRL levels of 10^{-8} to 10^{-6} M still has a stimulatory effect on aldosterone secretion. The results of cAMP accumulation in ZG cells

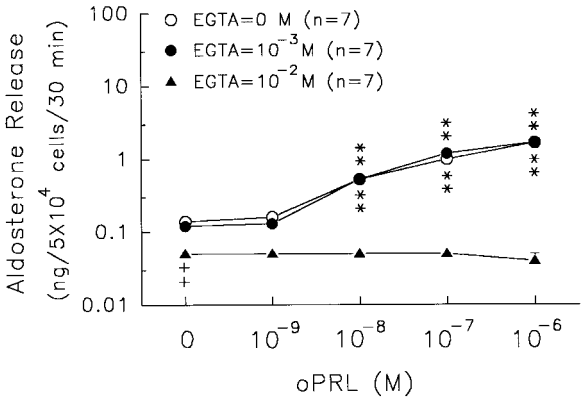

Fig. 2. Effects of oPRL (10^{-9}–10^{-6} M) on the aldosterone release from ZG cells in the presence of EGTA (0, 10^{-3} M, 10^{-2} M) in Ovx rats. **, $P < 0.01$, as compared with oPRL = 0 M. ++, $P < 0.01$, as compared with EGTA = 0 M. Each value represents mean ±SEM. Note log scale on the y-axis. See Fig. 1 for abbreviations.

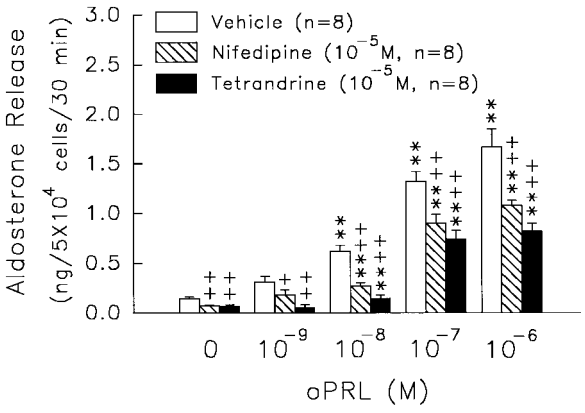

Fig. 3. Effects of oPRL (10^{-9}–10^{-6} M) on the aldosterone release from ZG cells in the presence of nifedipine (10^{-5} M) or tetrandrine (10^{-5} M) in Ovx rats. **, $P < 0.01$, as compared with oPRL = 0 M. +, ++, $P < 0.05$, $P < 0.01$, as compared with vehicle group, respectively. Each value represents mean ±SEM. See Fig. 1 for abbreviations.

are shown in Figure 4. oPRL from 10^{-8} to 10^{-6} M induced a marked increase of cAMP accumulation in ZG cells.

Effects of oPRL on Post-Pregnenolone Steroidogenesis in ZG Cells

Figure 5 illustrates the effects of oPRL (0, 10^{-9}–10^{-7} M) on the activities of 3β-HSD (conversion of [^{3}H]-pregnenolone to [^{3}H]-P_4), 21-hydroxylase (conversion of [^{3}H]-pregnenolone to [^{3}H]-DOC), 11β-hydroxylase (conversion of [^{3}H]-pregnenolone to [^{3}H]-corticosterone), and aldosterone synthase (conversion of [^{3}H]-pregnenolone to [^{3}H]-aldosterone) in ZG cells from

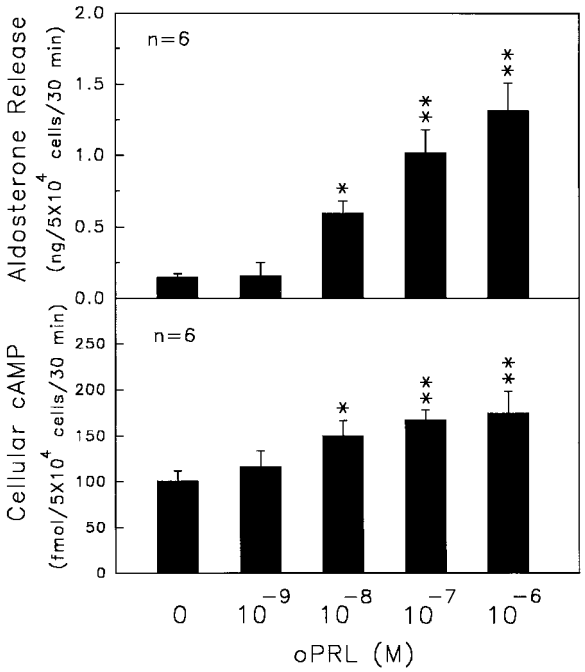

Fig. 4. Effects of oPRL (10^{-9}–10^{-6} M) on aldosterone production and cAMP accumulation in ZG cells of Ovx rats. *, **, $P < 0.05$, $P < 0.01$, as compared with oPRL = 0 M, respectively. Each value represents mean ±SEM. See Fig. 1. for abbreviations.

Ovx rats. In the presence of [^{3}H]-pregnenolone, oPRL (10^{-8}–10^{-7} M) produced a significant ($P < 0.05$ and $P < 0.01$) increase in [^{3}H]-aldosterone production. oPRL (10^{-9}–10^{-7} M) did not alter the accumulation of [^{3}H]-P_4 but increased the accumulation of [^{3}H]-DOC at 10^{-8} and 10^{-7} M oPRL. The accumulation of [^{3}H]-corticosterone was decreased by oPRL (10^{-7} M). These results suggest that the activities of 21-hydroxylase and aldosterone synthase are increased by oPRL treatment.

DISCUSSION

The present study indicated that PRL stimulates aldosterone release from ZG cells in Ovx rats. In the presence of [^{3}H]-pregnenolone, PRL resulted in an increased accumulation of [^{3}H]-DOC and [^{3}H]-aldosterone and decreased [^{3}H]-corticosterone production. These results suggested that PRL stimulates aldosterone production through the activation of 21-hydroxylase (the conversion of P_4 into DOC) and aldosterone synthase (the conversion of corticosterone into aldosterone).

It has been shown that potassium-stimulated aldosterone secretion depends on extracellular calcium and that it can be suppressed by nifedi-

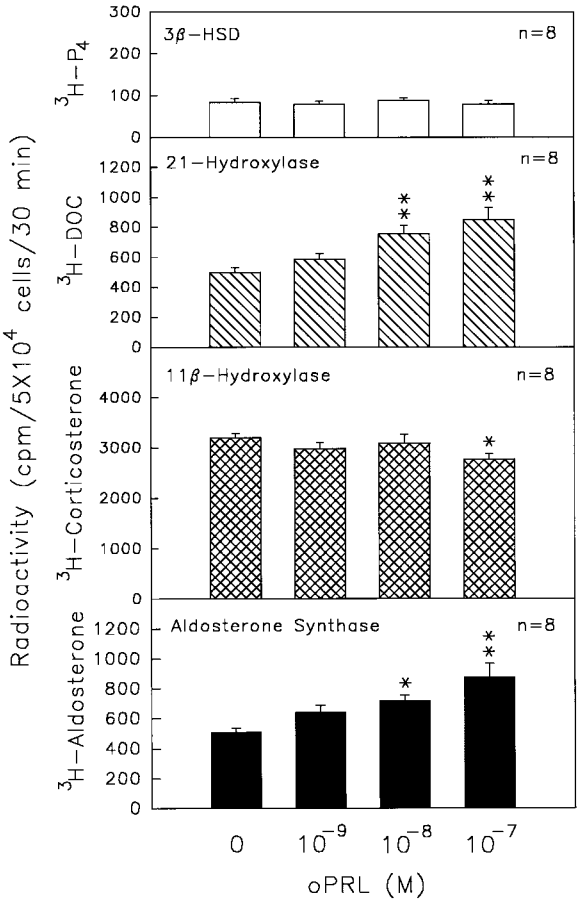

Fig. 5. Effects of oPRL (10^{-9}–10^{-7} M) on the activities of 3β-HSD, 21-hydroxylase, 11β-hydroxylase, and aldosterone synthase in ZG cells from Ovx rats. Rat ZG cells were incubated with ^{3}H-pregnenolone (10,000 cpm) and different doses of oPRL at 37°C for 30 min. Radioactive products in the medium were extracted by ether and then analyzed by thin-layer chromatography. *, **, $P < 0.05$, $P < 0.01$, as compared with oPRL = 0 M, respectively. Each value represents mean ±SEM. See Fig. 1 for abbreviations.

pine (an L-type calcium channel blocker) in cultured ZG cells of rats [Yagci and Muller, 1996]. Rossier et al. [1993] demonstrated that tetrandrine is a potent inhibitor of aldosterone production and that this effect is mediated by a decrease in Ca^{2+} influx in bovine adrenal ZG cells. The patch-clamp technique demonstrated that tetrandrine blocks T-type voltage-activated Ca^{2+} channel [Rossier et al., 1993]. In the present study, EGTA (a chelator of Ca^{2+}) at 10^{-2} M completely abolished the stimulatory effects of PRL on aldosterone secretion. Nifedipine (L-type) or tetrandrine (T-type calcium channel blocker) significantly diminished the effects of PRL, while PRL still has a stimulatory effect on aldosterone secretion. Tetrandrine also affected L-type currents at higher concentration in bovine adrenal ZG cells [Rossier et al., 1993]. Although we cannot exclude the possibility that tetrandrine also acts on L-type Ca^{2+} channel, its inhibitory effect on oPRL-induced aldosterone secretion was more stronger than that in the nifedipine-treated group. We suggest that the stimulatory effects of PRL depend on Ca^{2+} influx. The activation of both L-type and T-type Ca^{2+} channels also plays a partial role in the aldosterone secretion induced by PRL. In addition to Ca^{2+} pathway, cAMP formation is one of the transduction mechanisms used by the ZG cells that stimulates aldosterone steroidogenesis through activation of the cAMP-dependent protein kinase [Quinn and Williams, 1988]. In the present study, PRL increased the cAMP accumulation in ZG cells. We therefore suggested that the PRL-induced increase in aldosterone secretion is associated with an increase in cAMP accumulation.

In addition, PRL markedly potentiated the Ang II- and KCl-stimulated secretion of aldosterone in ZG cells. However, the increments of aldosterone secretion induced by PRL combined with Ang II or KCl were significantly less than those in the PRL alone-treated groups. It appears that Ang II or KCl blunts the stimulatory effect of PRL on aldosterone secretion. The precise mechanisms remain undefined. Besides the rise of intracellular calcium and the activation of protein kinase C, Ang II also induced an inhibition of adenylyl cyclase in ZG cells [Quinn and Williams, 1988]. We therefore suggested that the inhibitory effect of Ang II on PRL is probably associated with an inhibition of adenylyl cyclase. It has been well established that the increase of intracellular calcium in ZG cells is involved in the action mechanisms of Ang II and high potassium. Our results did not show any additive effect on aldosterone secretion between the groups of PRL and Ang II or PRL and high potassium and imply that PRL might share partial mechanisms with Ang II or high potassium. Taken together, we suggest that the inhibitory effect of Ang II or KCl on PRL-stimulated aldosterone secretion might be attributable to an interaction between different signal pathways caused by PRL and Ang II or KCl or to a compensatory effect of PRL on the aldosterone production via cAMP pathway.

Analysis of PRL receptor polypeptides sequence showed that PRL receptor is a member of the cytokine/hematopoietic growth factor re-

ceptor superfamily [Kishimoto et al., 1994; Taniguchi, 1995]. Recent data suggest that the key intracellular components of the PRL signaling pathway are the kinase Jak2 and the transcription factor Stat5 in the mammary gland [Lebrun et al., 1995; Watson and Burdon, 1996; Jahn et al., 1997]. Results from other studies implicated that other signaling factors are involved in the effects of PRL in the nonmammary cells. For example, the protein tyrosine kinase p^{59fyn} may serve as a signaling intermediary necessary for PRL-induced tyrosine phosphorylation and proliferation in T lymphocytes [Clevenger and Medaglia, 1994]. Gitay-Goren et al. [1989a] demonstrated that PRL inhibits hCG-induced cAMP accumulation and estradiol secretion in rat granulosa cell cultures. In rat luteal cell cultures, PRL significantly increased hCG-induced cAMP accumulation and P_4 secretion [Gitay-Goren et al., 1989b]. Therefore, it raises the possibility that PRL may act through different signaling pathways in different cell types. Our results demonstrated that cAMP accumulation and Ca^{2+} influx are involved in the stimulatory effect of PRL on aldosterone secretion in ZG cells.

Besides the mammary gland, PRL also acts at other sites, such as the suppressed hypothalamus-pituitary-gonad activity in hyperprolactinemia [Vasquez et al., 1980; Sharkar and Yen, 1985; Voogt et al., 1987; Bartke et al., 1987; Kooy et al., 1989]. The suppressed hypothalamus-pituitary-gonad activity probably results from PRL-mediated inhibition of LHRH secretion, which leads to impaired gonadotropin secretion and inhibition of gonadal function [Sharkar and Yen, 1985; Voogt et al., 1987]. Additionally, PRL activates the HPA axis, including stimulatory effect on CRH, ACTH [Weber and Calogero, 1991], and adrenal steroids [Eldridge and Lymangrover, 1984]. In the present study, PRL was found to act directly at the level of adrenal ZG cells and to stimulate aldosterone steroidogenesis. PRL is also a stress hormone, and the levels of PRL rise in response to psychological and physical stress. These results suggest that the effects of PRL on HPA activity may play an important role in stress responses. Several studies have reported that estrogen stimulates PRL release at the level of pituitary [Nicoll and Meites, 1964; Ben-David et al., 1964]. Taken together, the stimulation of PRL on aldosterone secretion may be responsible for the physiological requirement in blood volume expansion and body fluid turnover during pregnancy and lactation.

In summary, we demonstrated a direct stimulatory effect of PRL on basal, Ang II-, and KCl-stimulated aldosterone secretion in ZG cells. The increase of aldosterone secretion by oPRL is due in part to increased cAMP production, the activation of both L- and T-type Ca^{2+} channels, and the activation of 21-hydroxylase and aldosterone synthase in rat ZG cells.

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