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Direct Effects of Prolactin on Corticosterone Release by Zona Fasciculata-Reticularis Cells From Male Rats

Ling-Ling Chang,[2] Ming-Jae Lo,[1] Shu-Fen Kan,[1] William Ji-Sien Huang,[3] Jiann-Jong Chen,[1] Mei-Mei Kau,[1] Jui-Ling Wang,[1] Ho Lin,[1] Shiow-Chwen Tsai,[1] Yu-Chung Chiao,[1] Jiun-Yih Yeh,[1] Wan-Song Alfred Wun,[4] and Paulus S. Wang[1]*

[1]Department of Physiology, Schools of Life Science and Medicine, National Yang-Ming University, Taipei, Taiwan 11221, Republic of China
[2]Department of Chemical Engineering, Chinese Culture University, Taipei, Taiwan 11221, Republic of China
[3]Department of Urology, Veterans General Hospital-Taipei,Taipei, Taiwan 11221, Republic of China
[4]Obstetrical & Gynecological Associates, P.A., Houston, Texas 77054

Abstract The role of prolactin (PRL) in the male is not fully defined. The aim of this study was to investigate the function and mechanism of PRL on the production of corticosterone by zona fasciculata-reticularis (ZFR) cells in vitro. The ZFR cells were obtained from male rats under normal, hyperprolactinemic, or hypoprolactinemic situation. PRL stimulated the corticosterone release in a dose-dependent pattern in the ZFR cells from normal male rats. The cellular adenosine 3′-5′-cyclic monophosphate (cAMP) concentration positively correlated with PRL concentration in the presence of forskolin or 3-isobutyl-1-methylxanthine (IBMX). PRL enhanced the stimulatory effects of cAMP mimetic reagents, i.e., forskolin, 8-bromo-adenosine 3′,5′-cyclic monophosphate (8-Br-cAMP), and IBMX on the release of corticosterone. The adenylate cyclase inhibitor (SQ22536) inhibited the corticosterone release in spite of presence of PRL. Nifedipine (L-type calcium channel blocker) did not inhibit corticosterone release. The hyperprolactinemic condition was actualized by transplantation of donor rat anterior pituitary glands (APs) under kidney capsule. By comparison with the cerebral cortex (CX)-grafted group, AP-graft resulted in an increased release of corticosterone, 3β-hydroxysteriod dehydrogenase (HSD) activity and cAMP production by ZFR cells. Acute hypoprolactinemic status was induced by bromocriptine for 2 days. The results showed the productions of corticosterone were lower in hypoprolactinemic group than in control group, which were persistent along with different ACTH concentrations. These results suggest that PRL increase the release of corticosterone by ZFR cells via cAMP cascades and 3β-HSD activity. J. Cell. Biochem. 73:563–572, 1999.

Key words: cAMP; 3β-hydroxysteroid dehydrogenase

In the mammal, the physiological function of PRL on milk production in the female is well established [Houdebine et al., 1985]. The role of PRL in the male has not been fully defined. This study explored the function of PRL other than milk production.

It has been shown that hyperprolactinemia affects sexual behavior [Drago et al., 1981], the functions of the hypothalamus [Kooy et al., 1990; Rasmussen, 1991; Weber and Calogero, 1991], the pituitary gland [Weber and Calogero, 1991], and adrenal glands [Kooy et al., 1990]. In contrast to the inhibition of reproductive functions, hyperprolactinemia seems to activate the hypothalamo-hypophyseal-adrenal axis [Alder, 1986]. The increased corticosterone release in hyperprolactinemic male rats has been explained by a PRL-induced release of corticotropin-releasing hormone (CRH) [Kooy et al., 1990; Weber and Calogero, 1991] and adrenocorticotropin (ACTH) [Calogero et al., 1993]. Since adrenal glands contain PRL receptors, PRL may have a direct effect on the adrenal gland [Frantz et al., 1974; Posner et al., 1974]. This study is to examine the direct effect of PRL on the adrenal zona fasciculata-reticularis cells.

Several early reports have alternatively suggested that G proteins, protein kinase C, or

*Correspondence to: Paulus S. Wang, Ph.D., Department of Physiology, School of Life Science, National Yang-Ming University, Shih-Pai, Taipei, Taiwan 11221, Republic of China. E-mail: pswang@dns.ym.edu.tw

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sodium/hydrogen antiport may participate in the mechanism of the action of PRL with its receptors [Barkey et al., 1988; Larsen and Dufau, 1988; Rillema et al., 1989; Too et al., 1989, 1990; Banerjee and Vonderhaar, 1992; Koduri and Rillema, 1993]. The recent and striking development in the study of the growth hormone (GH) /PRL/cytokine receptor [Ihle, 1994] indicates that many of these receptors induce rapid activation of a family of tyrosine kinases known as Janus kinases (JAK), whose activation is followed by tyrosine phosphorylation of downstream members of a variety of signal pathways which include the cytoplasmic signal transducer and activator of transcription (STAT) factor complex, and the mitogen activated protein kinase (MAPK) pathway. Esther et al. [1996] demonstrates that the action of PRL on MA-10 Leydig cell steroidogenesis may involve a pertussis toxin-sensitive G protein in PRL-R cellular signal transduction. Furthermore, in the liver, none of the JAK/STAT pathways were observed after PRL treatment [Jahn et al., 1997]. Thus, PRL might express its signal through different routes in different cell types. It was interesting to know whether PRL signal transduction in adrenal cortical cells is mediated by affecting cyclic adenosine 3′-5′-monophosphate (cAMP) production and/or by activating calcium channels.

Bromocriptine, a dopamine agonist, is thought to exert its effects through pituitary D2-like dopamine receptors. D2 receptors inhibit adenylate cyclase. In this study, bromocriptine was used to induce hypoprolactinemic situation in the male rats. SQ22536 is a cell-permeable adenylate cyclase inhibitor [Goldsmith and Abrams, 1991]. It was used to inhibit the cAMP formation.

The purpose of this study is to evaluate the direct effects of PRL on the release of corticosterone and to explore the possible functional mechanisms, i.e., cAMP, calcium channels, under different PRL concentrations. The metabolic pathway of corticosterone was also examined.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (3 months old) provided by National Yang-Ming University were housed in a temperature-controlled room (22 ± 1°C) with 14-h light (06:00–20:00) and 10-h dark photoperiod. Food and water were given ad libitum. For hyperprolactinemic studying, rats were kept either intact, or transplanted with two rat anterior pituitary glands (APs) for each rat, or with a fragment of cerebral cortex under the kidney capsule for 2 months before decapitation [Lu et al., 1998]. For hypoprolactinemic studying, 10 rats received subcutaneous injections twice daily of 0.5 ml of saline or 5 mg/kg of bromocriptine for 2 days as control or hypoprolactinemic group, respectively. The animals were sacrificed by decapitation 2–3 h after the last saline or bromocriptine injection. After decapitation, blood samples were collected. The concentrations of PRL and corticosterone in the plasma samples were measured by radioimmunoassay (RIA).

Preparation of ZFR Cells From Rat Adrenal Glands

Adrenocortical zona fasciculata-reticularis (ZFR) cells for culture were prepared following Purdy et al. [1991] procedure with minor modifications [Lo et al., 1998]. Rat adrenal glands were excised, then kept in an ice-cold 0.9 %(w/v) NaCl solution. The adipose tissues were removed. The encapsulated glands were separated by forceps into capsule (mainly zona glomerulosa) and inner zone (mainly zona fasciculata-reticularis) fractions. The fractions of inner zone from 10–20 adrenals 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% BSA (KRBGA medium) and collagenase 2 mg (Sigma, St. Louis, MO). The tube was aerated with 95% O_2 and 5 % CO_2, then incubated for 1 h at 37°C in a shaker bath oscillating 100 cycles per min. Generally, at least six dispersions (n = 6) of ZFR cells were incubated in each group. At the end of incubation, the ZFR cells were mechanically dispersed by repeated pipetting, then filtering through a nylon mesh. After centrifugation at 200*g* for 10 min, cells were washed with deionized water for disrupting red blood cells, then the osmolarity was immediately restored with 10-fold Hank's balanced salt solution (HBSS). The cell number and cell viability (over 70%) were assessed by 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 (50 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 cells were resuspended in fresh incubation medium for 1 h. Post-incubation and centrifugation, the medium was stored at −20°C for corticosterone RIA. The cells were homogenized in 0.5 ml of 65% ice-cold ethanol by polytron (PT-3000, Kinematica AG., Luzern, Switzerland), then centrifuged at 2,000*g* for 10 min. The supernatants were lyophilized in a vacuum concentrator (Speed Vac, Savant, Holbrook, NY), then reconstituted with assay buffer (0.05 M sodium acetate buffer with 0.01% azide, pH 6.2) before measuring the concentration of cAMP by RIA.

RIA of Corticosterone

The concentrations of corticosterone in plasma and media were determined by RIA as previously described [Chen et al., 1997; Lo et al., 1998]. With this antiserum (PSW#4–9), a RIA was established for the measurement of plasma corticosterone levels. The sensitivity of corticosterone RIA was 5 pg/tube. The intra- and interassay coefficients of variation were 3.3% (n = 5) and 9.2% (n = 4), respectively.

RIA of cAMP

The intracellular or media levels of cAMP were measured by RIA as described elsewhere [Lu et al., 1996; Chen et al., 1997; Lo et al., 1998]. With anti-cAMP provided from Calbiochem-Novabiochem International (San Diego, CA), the sensitivity of cAMP was 14 fmole/tube; the cross-reactivities were 0.0005% with cGMP, 0.02% with cIMP, and less than 0.0001% with cCMP, AMP, ADP, ATP, EDTA, and theophylline.

RIA of PRL

RIA as described previously [Wang et al. 1986] determined the concentrations of PRL in media and plasma samples. Rat PRL RIA kit was provided by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), USA. Rat PRL-I-5 was used for radioiodination. The standard reference was rat PRL-RP-2. The sensitivity of rat PRL RIA was 3 pg/tube. The intra- and interassay coefficients of variability were 3.8% and 3.2%, respectively.

Materials

Ovine PRL (oPRL, 31 IU/mg), SQ22536, 8-Br-cAMP, nifedipine, pregnenolone, ACTH, forskolin, deoxycorticosterone, and progesterone were obtained from Sigma Chemical Company (St. Louis, MO). The non-steroid chemicals were prepared as stock solutions solubilized in double deionized H_2O. The steroids were dissolved in ethanol before diluting with double deionized water.

Statistical Analysis

The treatment means were tested for homogeneity using an analysis of variance, and the difference between specific means was tested for significance using Duncan's multiple range test or Student's *t*-test [Steel and Torrie, 1960]. A difference between two means was considered statistically significant when P was less than 0.05.

RESULTS

1. Hyperprolactinemic Experiments: Effects of oPRL on Corticosterone Release

The results were analyzed by one-way ANOVA then by Duncan's Multiple Comparison Tests. Incubation of oPRL from 10^{-9}–10^{-7} M for 60 min caused a significant difference in the release of corticosterone among the four groups by rat ZFR cells (Fig. 1).The oPRL concentrations of 10^{-11} and 10^{-10} M did not significantly increase the corticosterone release when compared with vehicle group. At the concentration of 10^{-8} M, the release of corticosterone was

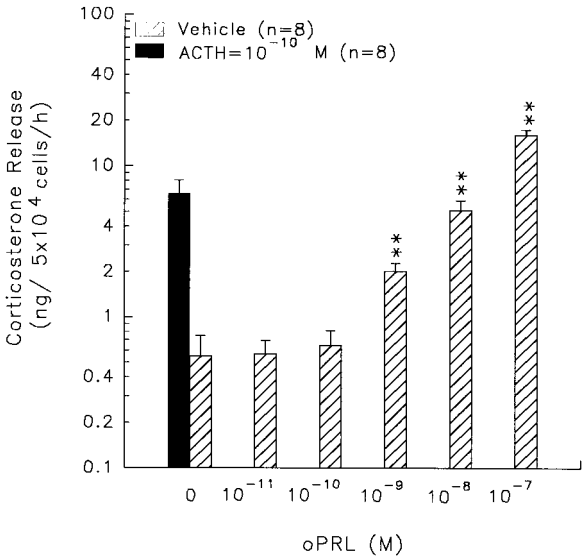

Fig. 1. Ovine prolactin (oPRL, 10^{-11}–10^{-7} M) and ACTH (10^{-10} M)-stimulated release of corticosterone by rat ZFR cells in vitro. $^{**}P < 0.01$, as compared with control. Each value represents mean ± SEM.

significantly higher than all of the previous groups. Its effect was similar to the 10^{-10} M ACTH. At 10^{-7} M, oPRL gave the highest corticosterone release significantly. By the outcome of corticosterone release, ACTH was about 100-fold potent than the PRL did.

Effects of oPRL on the Action of SQ22536, 8-Br-cAMP, Nifedipine, and Pregnenolone

To study the PRL effect on the action of SQ22536, 8-Br-cAMP, nifedipine, and pregnenolone in ZFR cells, cells were incubated with SQ22536 (10^{-4} M), 8-Br-cAMP (10^{-4} M), nifedipine (10^{-7} M), or pregnenolone (10^{-7} M) alone or in combined with PRL (10^{-9}–10^{-7} M) for 1 h. Two way ANOVA showed significant treatment effect (F = 37.4; D.F. = 4,140; P = 0.0001), significant PRL concentration effect (F = 91.2; D.F. = 3,140; P = 0.0001), significant interaction between treatment effect and the PRL concentration (Fig. 2). The releases of corticosterone were significantly increased by increasing the concentration of oPRL in vehicle (P = 0.0001), SQ22536 (P = 0.0033), 8-Br-cAMP (P = 0.0003), nifedipine (P = 0.0003), and pregnenolone (P = 0.0001). Although significant dose-response relationships existed in all five treatment groups, the slopes of the dose-response relationship were varied among the five groups. The slope was largest in the vehicle group, which was followed by nifedipine group, pregnenolone group, 8-Br-cAMP group, and SQ22536 group. The multiple regression analysis suggested that the slopes of dose-response relationship for the 8-Br-cAMP group and for the SQ22536 group were significantly lower than the vehicle group (P = 0.0001 and P = 0.0115, respectively). No significant differences were found among vehicle, nifedipine, and pregnenolone groups.

The results were also examined at each individual PRL group. It was analyzed by one-way ANOVA with Duncan's multiple comparison test. In vehicle group, the corticosterone release was significantly increased by 8-Br-cAMP by 10-fold, and pregnenolone by two-fold (Fig. 2). Incubation of ZFR cells with SQ22536 significantly decreased the corticosterone release about 30-fold. No significant differences were observed between the group incubated with nifedipine vs. vehicle.

At 10^{-9} M oPRL, the corticosterone release was significantly increased by 8-Br-cAMP about seven-fold, and significantly decreased by SQ22536 about six-fold (Fig. 2). No significant differences were found among the groups of vehicle, nifedipine, and pregnenolone.

At 10^{-8} M oPRL, similar results were concluded as in 10^{-9} M oPRL. When at 10^{-7} M, the corticosterone release was only significantly decreased by SQ22536 (about eight-fold). No significant differences were found among the groups of vehicle, 8-Br-cAMP, nifedipine, and pregnenolone (Fig. 2).

Plasma PRL and Corticosterone in AP-Grafted and CX-Grafted Rats

The concentrations of plasma PRL in CX-grafted and AP-grafted rats were 68.00 ± 9.43 ng/ml and 565.66 ± 92.28 ng/ml, respectively. The concentrations of plasma corticosterone in CX-grafted and AP-grafted rats were 38.70 ± 7.61 ng/ml and 85.90 ± 15.72 ng/ml, respectively. The levels of both PRL and corticosterone in the plasma were significantly higher ($P < 0.01$) in AP-grafted than in CX-grafted rats.

Effects of Hyperprolactinemia on the Action of Forskolin, SQ22536, Pregnenolone, Progesterone, and Deoxycorticosterone

Administration of forskolin (10^{-6} M), pregnenolone (10^{-7} M), progesterone (10^{-7} M), or deoxycorticosterone (10^{-7} M) increased the release of corticosterone by ZFR cells in both AP- and CX-grafted rats. However, administration of SQ22536 (10^{-5} M) decreased the release of corticosterone by ZFR cells in both AP- and CX-grafted rats. The results were summarized in Figure 3.

Two-way ANOVA found significant differences in treatment (F = 37.46;D.F. = 5,84; P = 0.0001), graft effect (D = 28.09;D.F. = 1,84; P = 0.0001), and interaction between treatment and graft (F = 5.77; D.F. = 5, 84; P = 0.0001). Within each treatment group, two-sample Student's t-test was used to explore the relationship of corticosterone release between the AP- and CX-grafted groups. As shown in Figure 3, the corticosterone releases were significantly higher for the AP-grafted groups than that for CX-grafted groups in the vehicle treatment (P = 0.0002), forskolin treatment (P = 0.0001), SQ22536 treatment (P = 0.0001), and pregnenolone treatment (P = 0.0005). However, no significant differences were found for the corticosterone release between CX- and AP-grafted groups

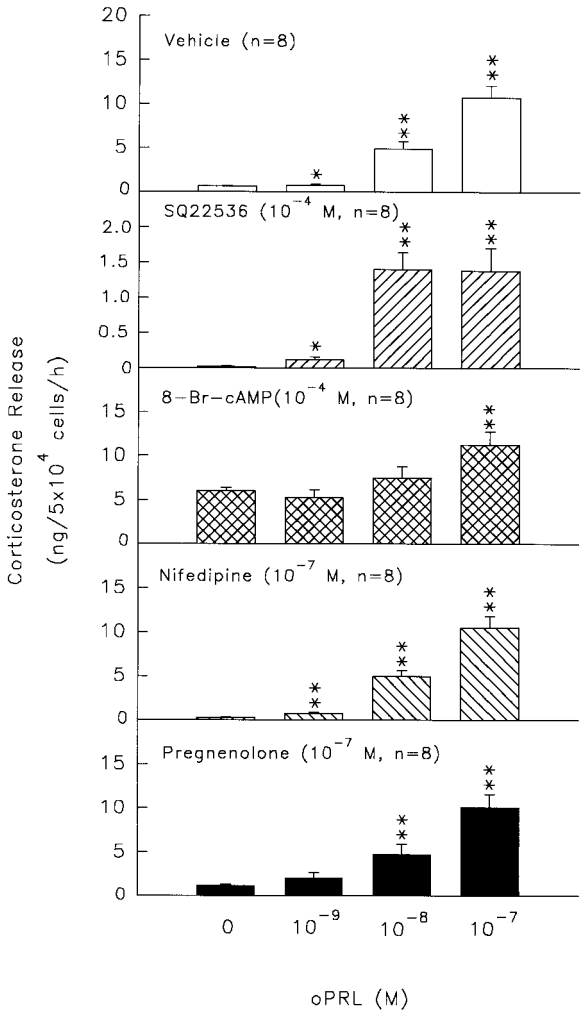

Fig. 2. Effects of oPRL (10^{-9}–10^{-7} M) on the release of corticosterone by rat ZFR cells in vitro in response to the action of SQ22536 (10^{-4} M), 8-Br-cAMP (10^{-4} M), nifedipine (10^{-7} M), and pregnenolone (10^{-7} M). *$P < 0.05$ and **$P < 0.01$, as compared with the group without oPRL. Each value represents mean ± SEM.

in the progesterone and deoxycorticosterone treatments.

Effects of Forskolin and IBMX on the cAMP Production in ZFR Cells of CX- and AP-Grafted Rats

In the presence of forskolin (10^{-6} M), the cellular cAMP was 43.67 ± 3.00 fmol/5 × 10^4 cells/h for CX-grafted group while 100.12 ± 17.27 fmol/5 × 10^4 cells/h for AP-grafted group ($P < 0.01$, Fig. 4).

In the presence of IBMX (5×10^{-4} M), the cellular cAMP was 57.54 ± 9.56 fmol/5 × 10^4 cells/h for CX-grafted group while 201.60 ± 35.14 fmol/5 × 10^4 cells/h for AP-grafted group ($P < 0.01$, Fig. 4).

Effects of oPRL on the Functions of SQ22536, Forskolin, and IBMX on cAMP Production in Normal ZFR cells

In the presence of forskolin (10^{-6} M), IBMX (5×10^{-4} M) or SQ22536 (10^{-5} M), the administration of oPRL (10^{-9}–10^{-7} M) increased the accumulation of cAMP in ZFR cells by a dose-dependent manner (Fig. 5). The results were analyzed by two-way ANOVA. It showed significance on the treatment effect (F = 78.8; D.F. = 2,84; $P = 0.0001$), PRL concentration effect (F = 130.2; D.F. = 3,84; $P = 0.0001$), and the interaction between treatment and PRL concentration effects (F = 20.4; D.F. = 6,84; $P = 0.0001$). The dose-response relationships between PRL concentration and cellular cAMP were analyzed by multiple regression analysis. There was significant dose-response relationship between the concentration of PRL and cellular cAMP ($P = 0.0001$ for all three treatment groups). The slopes of these dose-response relationships varied among the three treatment groups. The slope was the largest for IBMX group, which was followed by forskolin group, and SQ22536 group. No significant difference on the slope between the IBMX and forskolin groups. However, both slopes from IBMX and forskolin groups were significantly higher than the slope from SQ22536 group.

The comparison of cellular cAMP level was also analyzed at each individual PRL concentration. One-way ANOVA with Duncan's multiple comparison tests were used for analysis. The results showed no significant difference of cellular cAMP on each PRL concentration between the IBMX and forskolin groups. The group with SQ22536 showed significant less cellular cAMP than the group with IBMX or forskolin.

2. Hypoprolactinemic Experiments: Plasma PRL and Corticosterone in Control and Hypoprolactinemic Rats

The levels of plasma PRL in control (vehicle treated) and hypoprolactinemic (bromocriptine treated) rats were 82.07 ± 39.14 and 1.99 ± 0.28 ng/ml, respectively. The levels of plasma corticosterone in control and hypoprolactinemic rats were 21.23 ± 3.97 and 7.94 ± 1.17 ng/ml, respectively. Hypoprolactinemic group resulted in a significantly decrease in the con-

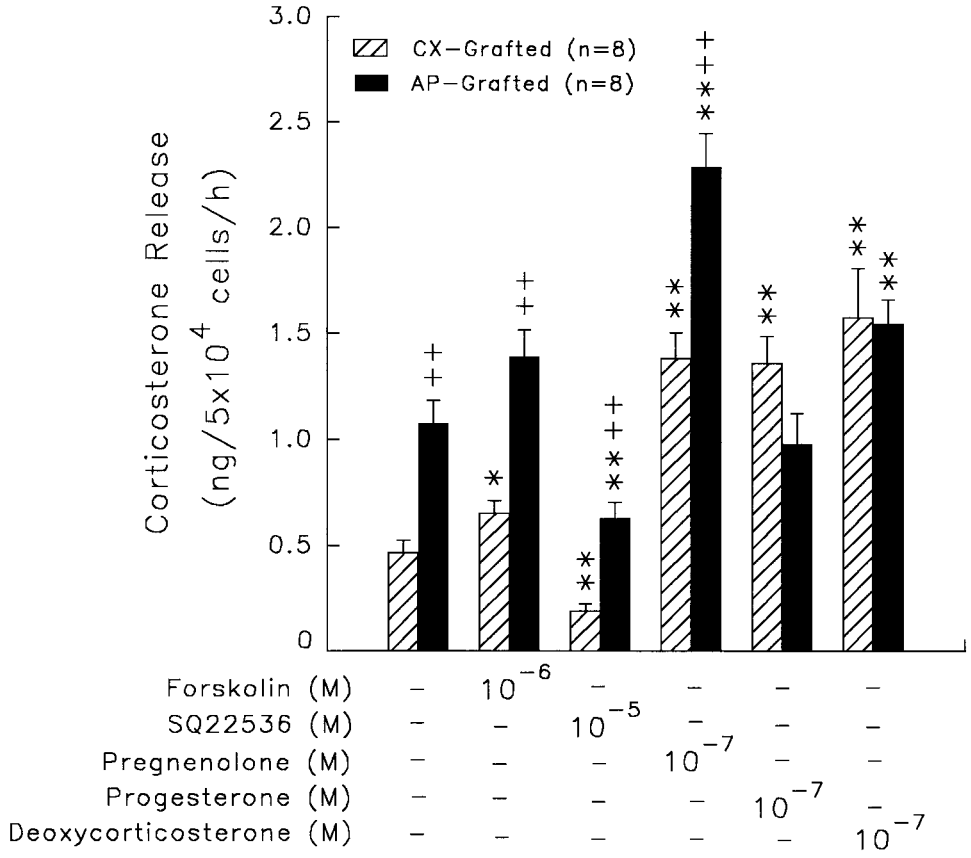

Fig. 3. Effects of forskolin (10^{-6} M), SQ22536 (10^{-5} M), pregnenolone (10^{-7} M), progesterone (10^{-7} M), or deoxycorticosterone (10^{-7} M) on the release of corticosterone in ZFR cells prepared from CX-grafted and AP-grafted rats in vitro. $^{*}P < 0.05$ and $^{**}P < 0.01$, as compared with the vehicle group; $++P < 0.01$ as compared with CX-grafted group, respectively. Each value represents mean ± SEM.

centrations of PRL ($P < 0.05$) and corticosterone ($P < 0.01$).

Effects of Hypoprolactinemia on the Action of ACTH

ACTH (10^{-10}–10^{-9} M) significantly increased ($P < 0.01$) corticosterone release by ZFR cells in both control (vehicle treated) and experimental (bromocriptine treated) groups (Fig. 6). The corticosterone release from control group was always significantly higher than that from experimental group ($P < 0.05$).

DISCUSSION

The present results demonstrated that: 1) PRL had direct effect on corticosterone release; 2) hyperprolactinemia, induced by AP-graft, increased the release of corticosterone by ZFR cells; and 3) hypoprolactinemia, induced by injections of bromocriptine, decreased the release of corticosterone by ZFR cells.

ACTH is the major hormone which regulates not only the acute glucocorticoid secretion, but also the expression and maintenance of adrenal cell specific functions, i.e., ACTH receptor number [Penhoat et al., 1994], which is in agreement with those obtained in mouse and human adrenocortical cell lines [Mountjoy et al., 1994]. Thus, the increased steroidogenic responsiveness following ACTH treatment is due not only to the regulatory effects of ACTH on the expression of the genes encoding the steroidogenic enzymes [Simpson and Waterman, 1988], but also, to the effects of ACTH on the expression of its own receptors. Most, if not all, of the actions of ACTH on the adrenal cortex are believed to be mediated through the cAMP second messenger system with the activation of cAMP-dependent protein kinases (PKAs) [Clegg et al., 1992; Schimmer, 1980; Wong et al., 1992]. In this study, physiological concentration of ACTH increased corticosterone secretion by rat ZFR cells showing the in vitro system worked properly.

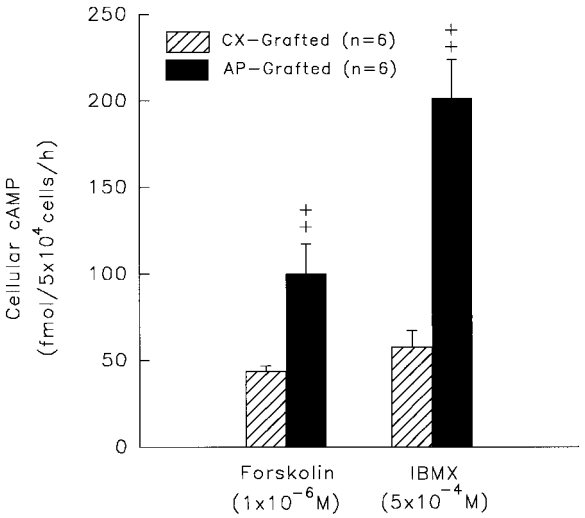

Fig. 4. Effects of forskolin (10^{-6} M) and IBMX (5×10^{-4} M) on the accumulation of cAMP in ZFR cells prepared from CX-grafted and AP-grafted rats in vitro. $++P < 0.01$ as compared with the CX-grafted group. Each value represents mean ± SEM.

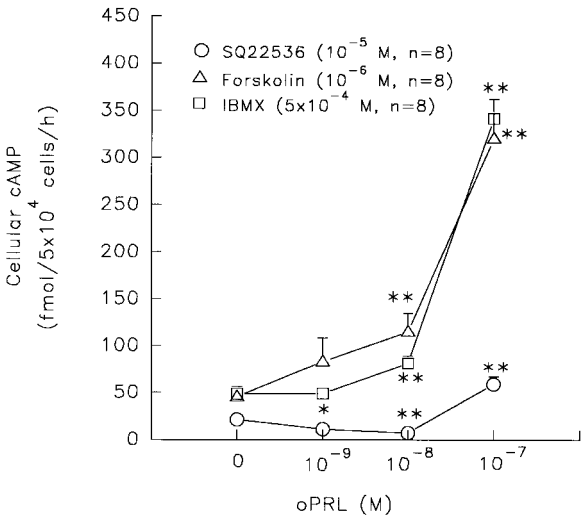

Fig. 5. Effects of oPRL on the accumulation of cAMP in normal rat ZFR cells in vitro in response to SQ22536 (10^{-5} M), forskolin (10^{-6} M), or IBMX (5×10^{-4} M). $*P < 0.05$ and $**P < 0.01$, as compared with the group treated without oPRL. Each value represents mean ± SEM.

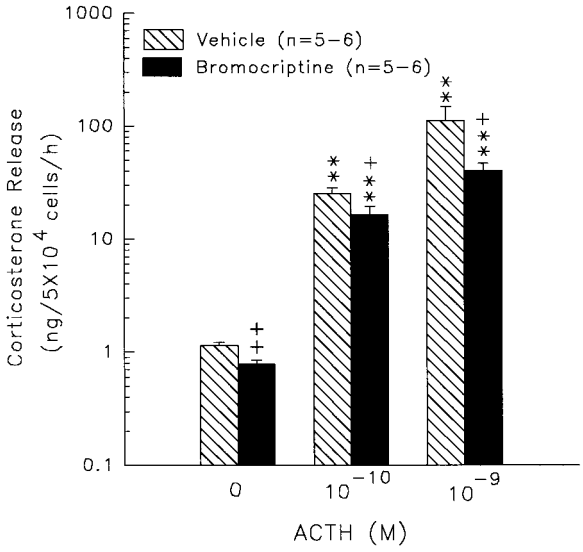

Fig. 6. Effects of ACTH (10^{-10}–10^{-9} M) on the release of corticosterone in ZFR cells prepared from vehicle and hypoprolactinemia rats in vitro. $**P < 0.01$ as compared with the group treated without ACTH; $+P < 0.05$ and $++P < 0.01$, as compared with the vehicle group, respectively. Each value represents mean ± SEM.

At the physiological concentration (10^{-9} M), PRL significantly increases the corticosterone release by ZFR cells (Figs. 1 and 2). The plasma PRL concentration in hyperprolactinemic rats, induced by AP-graft, was in the range of 10^{-8}–10^{-7} M. That was the similar range of PRL increased the corticosterone release (Figs. 1 and 2) and the cellular cAMP accumulation in vitro (Fig. 5). We therefore suggest that PRL has direct effect on corticosterone release at either physiological or pharmacological concentrations.

By supplement with 10^{-6} M forskolin, the adenylate cyclase activity was higher in AP-grafted than in CX-grafted group. Since forskolin acts directly on adenylate cyclase, we suggest the activity of adenylate cyclase increased by chronic increasing PRL. The data with 5×10^{-4} M IBMX showed the basal cAMP production was higher in the AP-grafted than in the CX-grafted group. This evidence suggest that chronic hyperprolactinemia could sensitize the receptor-G-protein-adenylate cyclase coupling and cAMP production. Based on these chronic data, it seems hyperprolactinemia enhances the production of cAMP.

In our acute study, the cellular cAMP increased along with increasing the PRL concentration (Fig. 5). It seemed that PRL had acute effect on cAMP production. The detail action mechanism of PRL was still unclear. There was no evidence of the action of PRL action mediated through activation of adenylate cyclase. It has been shown that the action of PRL might mediate through G-binding protein [Too et al., 1989, 1990]. In the present study, several evidence demonstrated that PRL worked through cAMP system in rat ZFR cells. One was the dose-response relationship of PRL and cellular cAMP concentration (Fig. 5). The second evi-

dence was that the inhibitory effect of SQ22536 on the release of corticosterone was not altered by incubation of high concentration of PRL. It has been well-known that SQ22536 inhibits the adenylate cyclase activity, which blocks hormone or messenger worked through it. This indirect evidence suggested that the activation of adenylate cyclase mediated the stimulatory effects of PRL on corticosterone release. The third reason was from the AP-grafted study. In the hyperprolactinemic rats induced by AP-graft, the level of plasma corticosterone was enhanced two-fold compared with CX-grafted rats. The significant accumulation of cellular cAMP by IBMX in ZFR cells from AP-grafted rats also linked the PRL function with cAMP. These indirect data suggested that cAMP was the common mediator for the action of ACTH and PRL on the production of corticosterone.

It is known that forskolin increases cAMP production by activating adenylate cyclase [Uneyama et al., 1993]. The mechanism of forskolin's positive inotropic effect is related to cAMP-dependent increase in Na^+ permeability which results in an indirect augmentation of calcium release [Yanagibashi et al., 1989]. Our in vitro data indicates that forskolin increased but SQ22536 decreased the corticosterone release by ZFR cells in CX-grafted rats (Fig. 3). Since forskolin and SQ22536 did not alter the differences between CX- and AP-grafted treatments in corticosterone release, a higher production of cAMP in ZFR cells induced by hyperprolactinemia is suggested. This aspect was demonstrated by a greater level of cAMP in ZFR cells of AP-grafted rats than in that of CX-grafted rats after exposure to IBMX (Fig. 4). The forskolin could enhance the basal adenylate cyclase activity but not much in the already active stage. It reflected the results that forskolin significantly increased the corticosterone release in CX-grafted group while not significant in the AP-grafted group (Fig. 3).

It has been shown that nifedipine is a selective blocker of L-type voltage-operated calcium channels [Inukai et al., 1993]. Since there was no significant effect of nifedipine on PRL effect (Fig. 2), the action of PRL was not mediated through the L-type calcium channel.

The main synthesis pathway of corticosterone is the cholesterol → pregnenolone → progesterone → 11-deoxycorticosterone → corticosterone. From pregnenolone to progesterone depends on 3β-hydroxysteroid dehydrogenase (3β-HSD). The step from progesterone to deoxycorticosterone depends on 21-hydroxylase. The step from deoxycorticosterone to corticosterone depends on 11β-hydroxylase. In normal rats, pregnenolone increased the corticosterone release by ZFR cells in the absence of PRL (Fig. 2). In the presence of PRL, the effect of pregnenolone seemed masked by the overwhelming stimulatory effect of PRL (Fig. 2). From AP- and CX-grafted studies (Fig. 3), pregnenolone was the only steroid that increased the corticosterone release in AP-grafted group as compared to CX-grafted group. The increase of the supplementation of progesterone or deoxycorticosterone on the release of corticosterone in both groups should be due to the increase of substrate for corticosterone production. In CX-grafted group, the three experiments with steroid supplementation had similar corticosterone release. It supported the catalytic activities of three enzymes were similar (Fig. 3). The general corticosterone release pattern was AP-group released more than the CX-group did, that disappeared by the progesterone or deoxycorticosterone supplementation. It might be the endproduct feedback inhibition on the 3β-HSD. These data revealed that 3β-HSD activity was enhanced by chronic hyperprolactinemia. Neither 21-hydroxylase nor 11β-hydroxylase activity was altered by hyperprolactinemia.

Bromocriptine is a dopamine agonist and exerts its effects through pituitary dopamine receptors. It is used to treat in Parkinson's disease, and hyperprolactinemic patients. This study showed bromocriptine injections decreased the levels of plasma PRL and corticosterone in male rats. Another possible mechanism of bromocriptine action might be due to its effects through dopamine effects by inhibition of adenylate cyclase [Kebabian et al., 1986; Vanllar and Meldolesi, 1989]. The ZFR cells released less corticosterone at basal and ACTH-stimulated conditions in the bromocriptine group than in the control group (Fig. 6). These data could not differentiate the low corticosterone production in ZFR cell was due to the hypoprolactinemic situation or direct effect of bromocriptine on the adrenal cells.

In summary, this study demonstrated pharmacological concentration of PRL increased the release of corticosterone by rat ZFR cells via the mechanisms including of cAMP cascades and the activity of 3β-HSD. Hypoprolactinemia de-

creased the release of corticosterone by rat ZFR cells.

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