0021-972X/96/$03.00/0
Journal of Clinical Endocrinology and Metabolism

Vol. 81, No. 8
Printed in U.S.A.

Functional Aspects of the Effect of Prolactin (PRL) on Adrenal Steroidogenesis and Distribution of the PRL Receptor in the Human Adrenal Gland*

ANNEGRET GLASOW, MATTHIAS BREIDERT, ANDREA HAIDAN, ULF ANDEREGG, PAUL A. KELLY, AND STEFAN R. BORNSTEIN

Departments of Internal Medicine III and Dermatology (U.A.), University of Leipzig, Leipzig, Germany; and INSERM U-344, Endocrinologie Moleculaire, Faculté de Médicine Neckar (P.A.K.), 75015 Paris, France

ABSTRACT

Hyperprolactinemia is one of the most common disorders in endocrinology. A role for PRL on the human adrenal gland has been postulated in various clinical studies. We have demonstrated for the first time the expression of the PRL receptor in the human adrenal gland and in human adrenal primary cell cultures using PCR and immunohistochemical methods. Using immunostaining, we could detect the PRL receptor in all three zones of the adrenal cortex. Only weak staining was observed in the adrenal medulla. The influence of PRL on the secretion of cortisol, aldosterone, and androgens in human primary cell cultures was investigated. After stimulation with PRL (10^{-7} mol/L), we measured increased concentrations of cortisol (155 ± 9.8%; $P < 0.005$%), aldosterone (122 ± 3.7%; $P < 0.005$), and dehydroepiandrosterone (121 ± 8.6%; $P < 0.05$) in the cell supernatant. PRL did not affect the expression of messenger ribonucleic acid of cytochrome P45017α in human adrenal cell cultures.

In conclusion, we found the PRL receptor in the human adrenal gland. We postulate that PRL has a direct effect on adrenal steroidogenesis, thereby regulating adrenal function, which may be of particular relevance in clinical disorders with hyperprolactinemia. (*J Clin Endocrinol Metab* **81:** 3103–3111, 1996)

IN TERMS OF biological processes, PRL is one of the most versatile hormones produced by the pituitary gland (1). Several attributes of PRL are of particular interest with regard to an effect on the adrenal gland. First, glucocorticoids have a synergistic action with PRL, the classical lactotropic hormone, on breast development (2). This may suggest a coordinated regulation of both hormones.

Secondly, PRL is a stress-related peptide. In addition to activating the hypothalamus-pituitary-adrenal axis, stress can induce the elevation of PRL levels (3–6). In this context, an influence of PRL on human adrenal cortisol secretion was expected.

Thirdly, hyperprolactinemia, a common disorder in clinical endocrinology, is frequently associated with hirsutism, hypogonadism, and amenorrhea (7). It has been speculated that in these disorders, PRL acts directly on the adrenal (8). Therefore, PRL measurements are commonly ordered for the differential diagnosis of hirsutism. Finally, PRL has been reported to act on adrenal aldosterone production (9). There are, however, conflicting and variable results concerning the effect of PRL on the adrenal in different species (3, 10–12). Surprisingly, only a few reports are available on the effects of PRL on isolated human adrenal, with contradictory results in monolayer and suspension cell cultures (13–15). The influence of PRL on steroid hormone production in all three zones of the human adrenal cortex and the effect of PRL on the messenger ribonucleic acid (mRNA) expression of the steroid enzymes have not yet been investigated in human adrenals. The occurrence of the PRL receptor (PRLR) in fetal and adult rat adrenals has been reported recently (16, 17). PRL-binding sites were detected in the adrenals of different species (18, 19), but to date there are no data on the expression of the PRLR in human adrenal tissue. The presence of endogenous PRL in the human adrenal gland has not previously been reported. Immunoreactive PRL has only been described in sections of rat adrenal cortical cells (20).

Therefore, we designed a study to investigate the role of PRL *in vitro* on human adrenal cell cultures. After stimulation with PRL, we measured the concentrations of dehydroepiandrosteron (DHEA), cortisol, androstenedione, testosterone, and aldosterone in the supernatant of cell cultures. We analyzed the influence of PRL on the mRNA expression of 17α-hydroxylase, a key enzyme in steroid biosynthesis, using Northern blotting. After immunostaining the tissue sections with an anti-rat PRLR antibody and an antibody against human PRL, light microscopic evaluations were carried out. The expression of PRLR mRNA was detected by the PCR.

Materials and Methods

Reagents

All reagents were purchased from Sigma Chemical Co. (Munich, Germany) unless otherwise noted.

Human adrenals

Normal human adrenal glands were obtained from 10 patients who underwent surgical excision of renal carcinoma. The ages of the male and

Received December 22, 1995. Revision received March 14, 1996. Accepted March 25, 1996.

Address all correspondence and requests for reprints to: Dr. Annegret Glasow, Department of Internal Medicine III, University of Leipzig, Ph.-Rosenthal-Straße 27, 04103 Leipzig, Germany.

* This work was supported by BMFT, IDZL B1, and Sandoz (Nürnberg, Germany).

female donors varied between 50–75 yr. This investigation was approved by the ethical committee of the University of Leipzig.

Immunohistochemical procedures

Immunostaining for PRLR. Deparaffinized sections of human adrenals were immunostained for PRLR with the avidin-biotin-technique using the UniTect mouse immunohistochemistry detection system from Dianova (Hamburg, Germany) (21). The sections were incubated with 0.5% Triton X-100 for 2 min before blocking the endogenous peroxidase. The cells were then preincubated in 5% normal horse serum and exposed for 1 h to antirat PRLR antibody U5 or T6 (20 μg IgG/mL 5% normal horse serum) (22). The sections were washed in Tris-buffered saline (three times, 10 min each time) and incubated with the biotinylated second antibody diluted 1:30 (horse antirat). After being washed in TBS, the sections were incubated for 30 min with the avidin-biotin peroxidase mixture. As a control, the rat PRLR antibody was replaced by mouse IgG. No staining was observed in these controls. Visualization was achieved by immersion of the sections for 15 min in 3-amino-9-ethyl-carbazole (Dianova-Immunotech, Hamburg, Germany) chromogen solution containing 0.05% H_2O_2. Slides were counterstained with hematoxylin for 1 min, rinsed for 10 min in water, and mounted. The immunostained cells were viewed on a Zeiss Axioskop microscope (Zeiss, Gottingen, Germany).

For immunostaining of PRLR, several primary cultures of human adrenal cells were grown on chamber slides (Nunc, Naperville, IL). After 4 days in culture, the cells were fixed for 30 min in 4% paraformaldehyde and immunostained as described above.

Immunostaining for 17α-hydroxylase. The immunostaining of 17α-hydroxylase was performed using the avidin-biotin technique as described above. The human 17α-hydroxylase antibody from mice was kindly provided by Prof. Watermann (Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN).

Immunostaining of PRL. The immunostaining of PRL was performed using paraffin-embedded and frozen sections of human adrenal glands. Monoclonal human PRL antibody from mice was obtained from Dianova-Immunotech (Hamburg, Germany). As a secondary antibody, the biotinylated antibody (horse antimouse) from Dianova (Hamburg, Germany) was used as described above. As a control, the human PRL antibody was replaced by mouse IgG. No staining was observed in these controls. Pituitary paraffin-embedded and frozen sections were stained as positive tissue controls.

Cell culture

Directly after surgery, the adrenals were transferred into ice-cold phosphate-buffered saline. After removal of the adipose tissue, the adrenals were cut into small pieces and washed three times with DMEM-Ham's F-12 (Life Technologies, Eggenstein, Germany).

Dispersed cells were obtained by digestion with collagenase (0.1%, wt/vol) and deoxyribonuclease I (0.01%, wt/vol) and by mechanical disaggregation. The viability of the isolated cells was checked by the trypan blue exclusion test and was found to be greater than 90%. The adrenal cells were placed in 6-well (for RNA isolation; 1×10^6 cells/well) or 24-well (for stimulation experiments; 150,000 cells/well) dishes. They were cultured in DMEM-Ham's F-12 containing penicillin (100 U/mL), streptomycin (0.1% wt/vol), and 10% FCS at 37 C under 5% CO_2.

Incubation procedure

The cells were incubated with human recombinant PRL (49.01 IU/mg; Scripps Laboratories, San Diego, CA), synthetic ACTH (Synacthen, Ciba-Geigy, Wehr, Germany), and PRL in combination with ACTH in serum-free medium. After 3 days in culture, the cells were washed with serum-free medium containing ascorbic acid (10^{-7} mol/L), transferrin (0.001%, wt/vol), and bacitracin (0.01%, wt/vol).

Hormone measurements

The hormone concentrations in the incubation medium were measured by RIA using the following kits: Active Androstenedione (Diagnostic Systems Laboratories, Webster, TX; sensitivity, 0.03 ng/mL; cross-reactivity with androstenedione, 100%; with isoandrosterone, 0.26%; with other steroids, <0.07%; intra- and interassay variations, 5.6% and 9.8%, respectively), Cortisol-RIA (Biermann, Bad Nauheim, Germany; sensitivity, 5.5 nmol/L; cross-reactivity with cortisol, 100%; with prednisolone, 76%; with 11-deoxycortisol, 11.4%; with prednisone, 2.3%; with other steroids, <1%; intra- and interassay variations, 5.1% and 6.4%, respectively); Active DHEA (Diagnostic Systems Laboratories; sensitivity, 0.02 ng/mL; cross-reactivity with DHEA, 100%; with other steroids, <0.88%; intra- and interassay variations, 10.6% and 10.2%, respectively), Aldosterone-RIA (Biermann; sensitivity, 16 pg/mL; cross-reactivity with aldosterone, 100%; with other steroids, < 0.033%; intra- and interassay variations, 5% and 10.4%, respectively), and Testosterone-RIA (Biermann; sensitivity, 0.15 pg/L; cross-reactivity with testosterone, 100%; with other steroids, <0.16%; intra- and interassay variations, 4.3% and 5.5%, respectively).

RNA isolation and Northern blot analysis

The total RNA was isolated from cells maintained in culture for 3 days using the RNAzol-B RNA isolation kit from AGS (Heidelberg, Germany). Five micrograms of total RNA were fractionated by electrophoresis through a 1.2% agarose gel containing 0.61 mol/L formaldehyde and transferred to uncharged nylon membranes (Qiagen, Hilden, Germany). The filters were prehybridized for 1 h and hybridized overnight with 25 ng/mL digoxigen UTP-labeled RNA probe at 68 C. Human cytochrome P45017 (full-length, 1900 bp) complementary DNA (cDNA; kindly provided by Prof. Watermann, Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN) was labeled with digoxigen UTP using the digoxigen RNA labeling kit (T3/T7; Boehringer Mannheim, Mannheim, Germany) by *in vitro* transcription. Sense and antisense probes were constructed. Filters were washed twice for 5 min each time in 2 × SSC (standard saline citrate)-1% SDS at room temperature and twice in 0.1 × SSC-1% SDS for 15 min each time at 68 C. For detection of chemiluminescence, CDP-Star was used according to the manufacturer's protocol provided with the digoxigen luminescence detection kit (Boehringer Mannheim). The resultant blots were exposed to Hyperfilm-ECL (Amersham, Braunschweig, Germany) for 2–30 min. The hybridization signals in the blots were normalized with glyceraldehyde-3-phosphate dehydrogenase and analyzed quantitatively by densitometric scanning. All experiments were repeated at least three times with different samples. The values given are from one representative experiment.

PCR experiments

For the reverse transcription-PCR (RT-PCR), total RNA was isolated from 5×10^6 cultured cells by a single step method using RNAzol-B from AGS (Heidelberg, Germany) according to the manufacturer's protocol. The resulting RNA was washed twice with 80% ethanol, dried, and dissolved in 20 μL DEPC water. The RNA content was determined photometrically. Five micrograms of RNA were taken to synthesize cDNA using the First ScDNA synthesis kit (Boehringer Mannheim)

PCR amplification of the first strand cDNA coding for the cytoplasmatic domain of the PRLR was carried out using the forward primer, 5′-CATCAACAGCTGCCACACTTCTTC-3′, and the complementary reverse primer, 5′-ATCCAAGGGTTTAGCCGAGCCAAA-3′. The PRLR cDNA was amplified for 42 cycles in 25 μL containing 1 × PCR buffer, 200 μmol/L deoxy-NTPs, 12 pmol each of forward and reverse primers, 0.5 U *Taq* polymerase, and 4 mol/L MgCl. The amplification conditions were as follows: denaturation at 94 C for 30 s, annealing of the primers for 30 s at 72 C, and elongation at 72 C for 30 s (42 cycles). Before starting the PCR, the probes were denatured at 94 C for 5 min. The final elongation step was prolonged to 7 min. After amplification, samples were electrophoresed through a 1.0% agarose gel. The amplified bands were identified using ethidium bromide staining.

Statistical analysis

Results are expressed as the mean ± SE. Statistical analysis was performed with unpaired Student's *t* test for comparison of two groups. Differences were considered significant when $P < 0.05$.

Results

Localization of the PRLR: immunostaining

The PRLR could be detected in all three zones of the adrenal cortex (Fig. 1A). In Fig. 1 there are no clear differences in the intensity of staining in the different zones. In some preparations the zona reticularis showed a stronger staining (Fig. 1C) than the zona glomerulosa and fasciculata (Fig. 1B) independent of the sex and age of the patients. Positive staining of the human adrenal gland was achieved with antibodies T6 and U5. In the adrenal medulla, detection of PRLR was low, and staining was weak (Fig. 2). However, islets of strongly stained cells containing PRLR occurred within the medulla (Fig. 3A). These cells turned out to be cortical cells, located within the medulla on serial sections immunostained for 17α-hydroxylase (Fig. 3B). These results demonstrate a high degree of intermingling between the two endocrine tissues. In the human adrenal cell culture, the PRLR was detected on the fourth day of culture in almost all cells (Fig. 4).

Detection of PRL

Using immunohistochemical methods, no PRL was detectable in frozen or paraffin-embedded sections of female and male adult adrenal gland (Fig. 5A). Human pituitary slices were used as a positive tissue control, and the staining was strong for PRL (Fig. 5B).

Detection of the PRLR: PCR

The expression of PRLR mRNA in human adrenal primary cell culture was determined qualitatively by amplification of RT-PCR products. After visualization with ethidium bromide, staining of one band with the expected size of 1044 bp, encoding the cytoplasmatic domain of the human PRLR, was observed (Fig. 6, lane HA). Digestion of the PCR product with *Bst*XI led to two fragments, with specific sizes of 505 and 539 bp (data not shown), identifying the amplified DNA fragment. This experiment was reproduced with different RNA isolations from the same and other adrenal primary cell cultures. In all PCR experiments, negative controls were included in which the amplification reaction was carried out with no cDNA present in the sample (Fig. 6, lane C).

Effect of PRL on the steroidogenesis of cultured adrenal cells

The effect of PRL on the secretion of steroid hormones was determined in monolayer cultures. PRL (10^{-7} mol/L) caused a significant increase in cortisol, aldosterone, and DHEA release. Cortisol secretion was enhanced to 155 ± 9.8% ($P < 0.005$; n = 3), aldosterone release to 122 ± 3.7% ($P < 0.005$; n = 3), and DHEA release to 122 ± 8.6% ($P < 0.05$; n = 3) of the basal concentrations (designated 100%). The concentrations of androstenedione and testosterone did not change significantly, although in both cases a slight increase was observed (Fig. 7).

Dose-dependent increases ($P < 0.05$; n = 2) in cortisol concentrations were observed after incubation with different doses (10^{-9}-10^{-6} mol/L) of PRL. PRL had a stimulatory effect on cortisol synthesis at physiological concentrations (10^{-9} mol/L PRL). After treatment with PRL (10^{-6} mol/L), maximal stimulation was achieved to 160 ± 7.3% of basal cortisol release, corresponding to 82% of the ACTH (10^{-10} mol/L) stimulation result (Fig. 8).

Expression of mRNAs for cytochrome P45017α

PRL (10^{-7} mol/L) did not affect cytochrome P45017α mRNA expression in primary cultures of human adrenal cells, as demonstrated by Northern blotting (Fig. 9). The results were densitometrically evaluated (data not shown). RNA from ACTH (10^{-9} mol/L)-treated cells was used as a positive control. The basal level of cytochrome P45017α mRNA was increased 2-fold. Sense probes were used to confirm the specificity of the antisense cytochrome P45017α probe (data not shown).

Discussion

PRL is the classical lactotropic hormone, but also has many other functions. Its role in steroidogenic tissue in the human adrenal gland is of particular interest. In the study presented, we demonstrated for the first time the occurrence of the PRLR in human adrenal gland and human adrenal cell cultures using two different methods.

We showed the appearance of the PRLR within the human adrenal gland using monoclonal antibodies T6 and U5 against rat liver PRLR. Both antibodies against rat PRLR could also be used for human adrenals because the U5 antigenic region is strongly conserved among different tissues and species. The T6 antibody showed a greater degree of binding to rat adrenal tissues than U5 and also had a high cross-reactivity to tissues from other species, as described previously (22). The staining of human tissue was positive with both antibodies. The specificity of antibodies was confirmed by the fact that no staining occurred in cells from the adrenal capsula and that differential staining within the adrenal itself could be demonstrated. PRL-binding sites were detected in all three zones within the adrenal cortex. Only weak staining in the adrenal medulla was observed, as described previously in rats (22, 23). However, islets of cells in the medulla demonstrated a strong presence. Through staining of serial sections with an antibody against 17α-hydroxylase, these cells could be clearly identified as steroid-producing cortical cells. The occurrence of cortical islets in the adrenal medulla, which stained positive for PRLR, is due to strong intermingling and close contacts between cortical and medullary cells, as described in previous studies (24, 25). In contrast to results in rats (17), no sex difference appeared in the staining of the adrenal cortex, although in some preparations the staining was stronger in the zona reticularis than in the other zones of the adrenal cortex.

We were able to detect PRLR in human adrenal cell culture on the fourth day of culture in almost all cells. We assume that the weakly stained cells could represent chromaffin cells, agreeing with the results in slices from human adrenals.

We also detected PRLR in human adrenal cultured cells after amplification of RT-PCR products, supporting the results of immunostaining. In line with studies on the PRLR in the gastrointestinal tract, we used primers amplifying the region encoding the cytoplasmatic part of human PRLR (26).

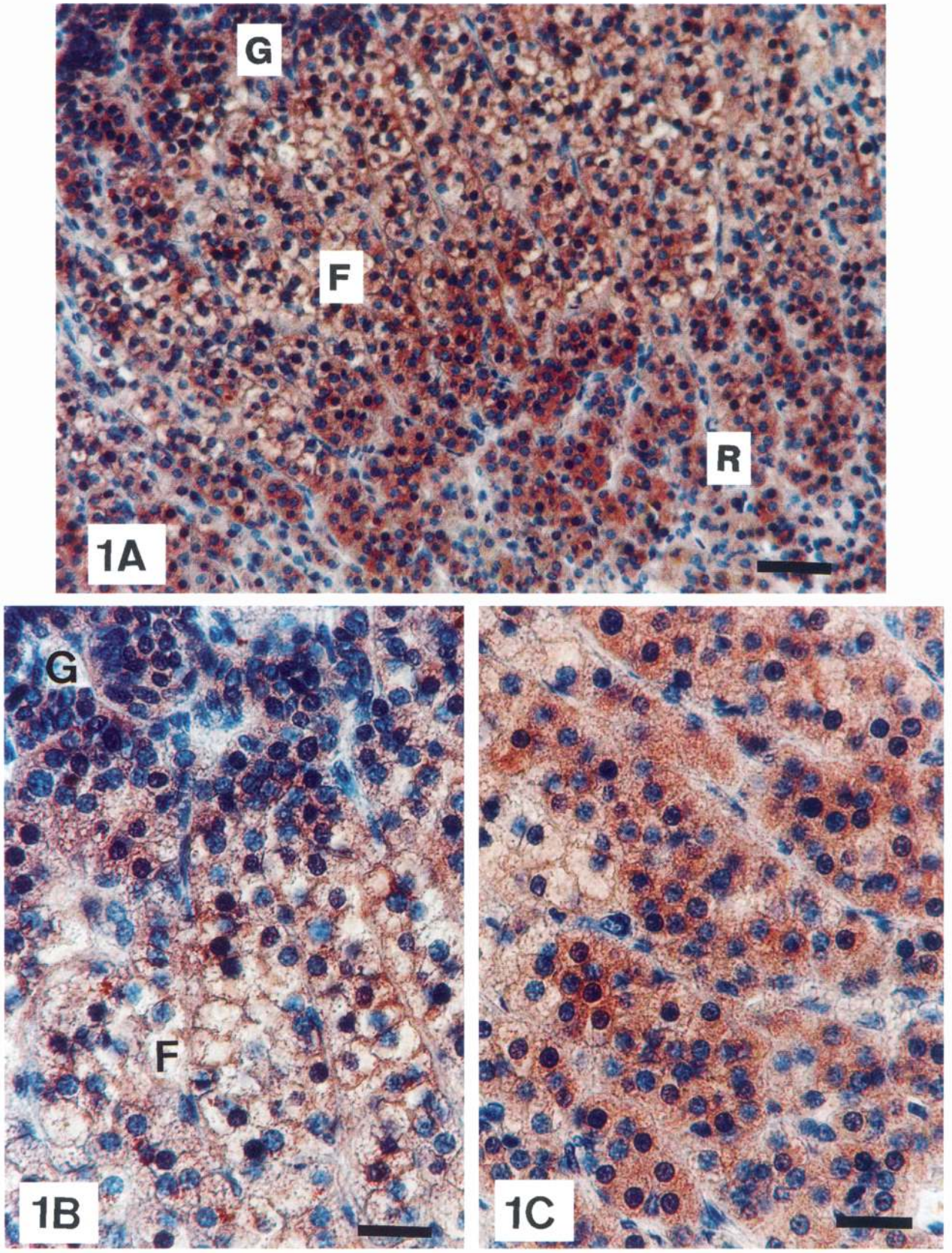
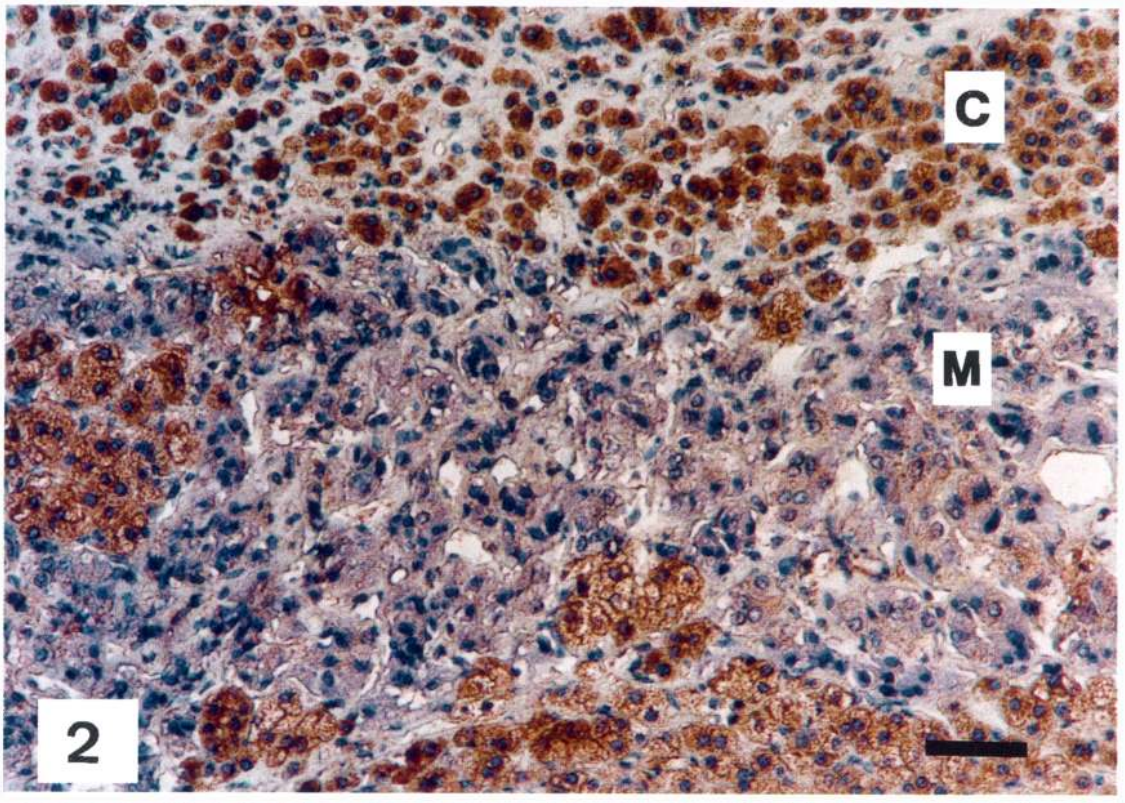


FIG. 1. Paraffin section of a human adrenal immunostained for PRLR. Overview of all three zones of the adrenal cortex using antibody U5. The PRLR occurs in the cytoplasm of cells (A; *bar* = 55 μm). The PRLR in zona glomerulosa (G) and zona fasciculata (F) seems to be equally distributed (B; *bar* = 27.4 μm). Slightly stronger staining of PRLR in the zona reticularis (R) is observed (C; *bar* = 27.4 μm).



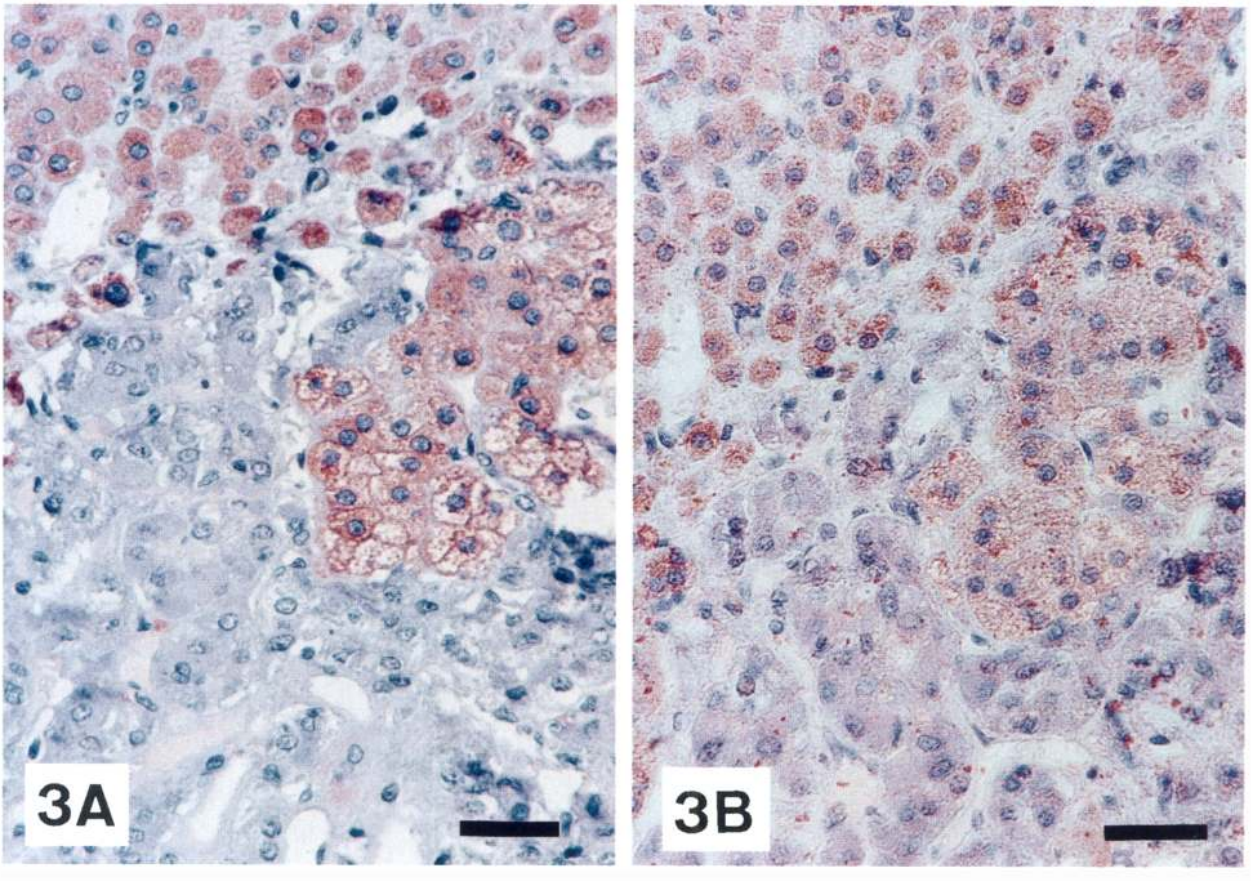
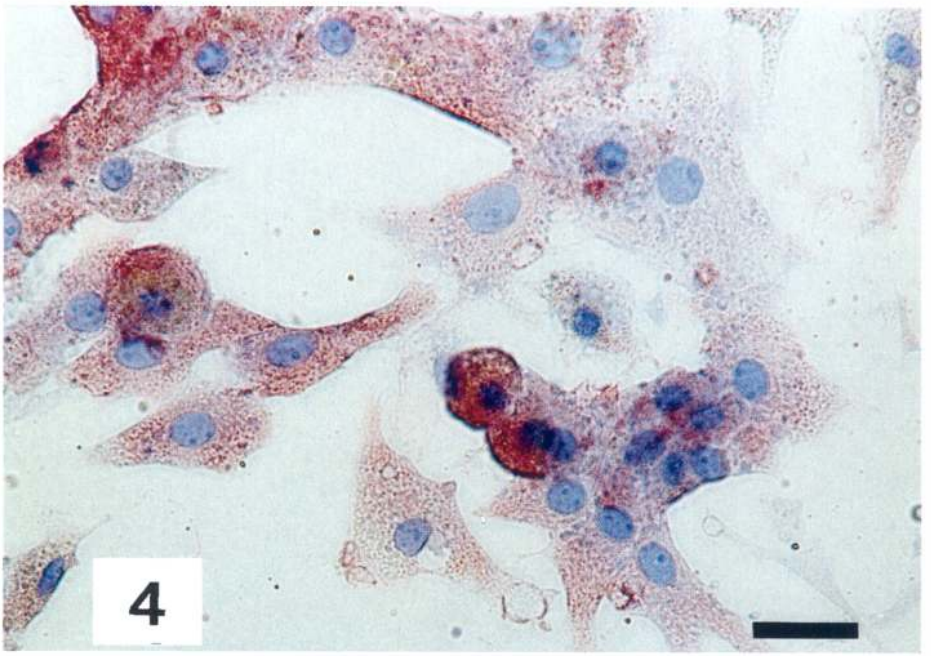
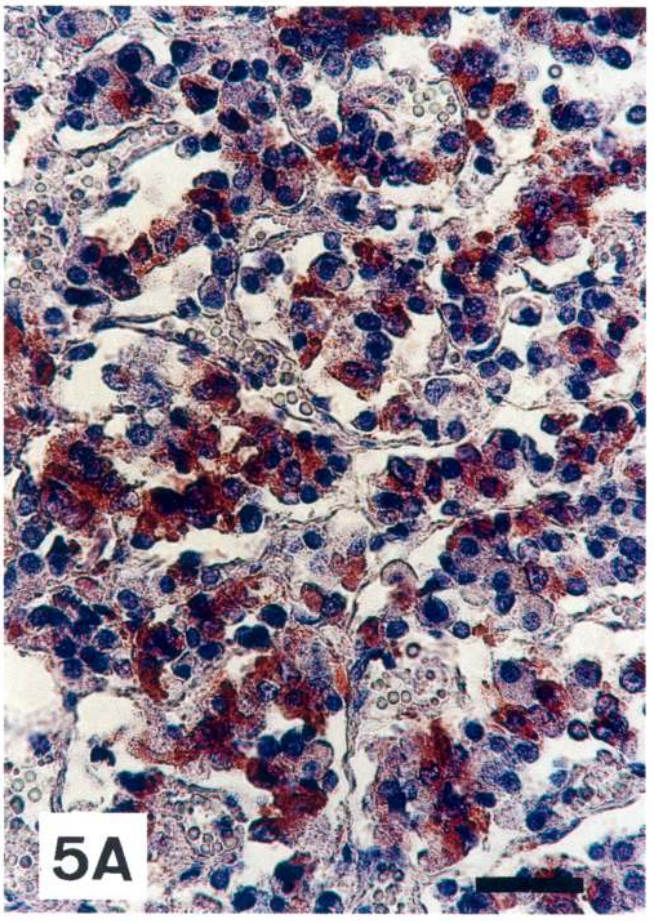


FIG. 2. Occurrence of PRLR in islets of cortical cells (C) within the adrenal medulla (M). Only weak staining was detected in medullary cells (antibody T6; *bar* = 55 μm).

FIG. 3. Intermingling of cortical and medullary cells in the human adrenal. Identification of cortical cells within the adrenal medulla by detection of 17α-hydroxylase in these areas (A; anti17α-hydroxylase; *bar* = 37.8 μm). Serial sections of adrenal gland with detection of the PRLR in cortical cells within the adrenal medulla. (B; antibody T6; *bar* = 37.8 μm).





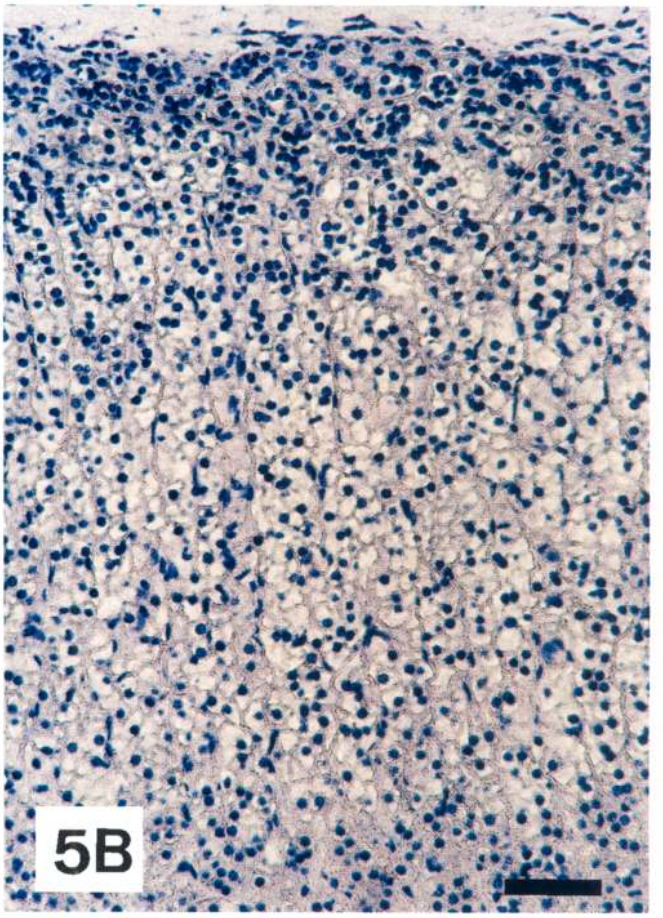


FIG. 4. Occurrence of PRLR in human adrenal cell culture. After 3 days in culture, human adrenal cells were immunostained for PRLR with the antibody T6 (*bar* = 27.4 μm).

FIG. 5. PRL was clearly detectable in the human pituitary gland (A; *bar* = 37.8 μm). In the human adrenal, no PRL was detected after immunostaining (B; *bar* = 75.6 μm).

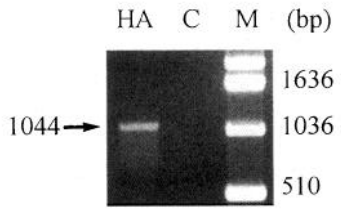


FIG. 6. Detection of the cytoplasmatic domain of the PRLR mRNA by RT-PCR in human adrenal primary cell cultures (HA). PRLR was not detected in controls in the absence of cDNA (C). PCR product size was identified using DNA standard X (M).

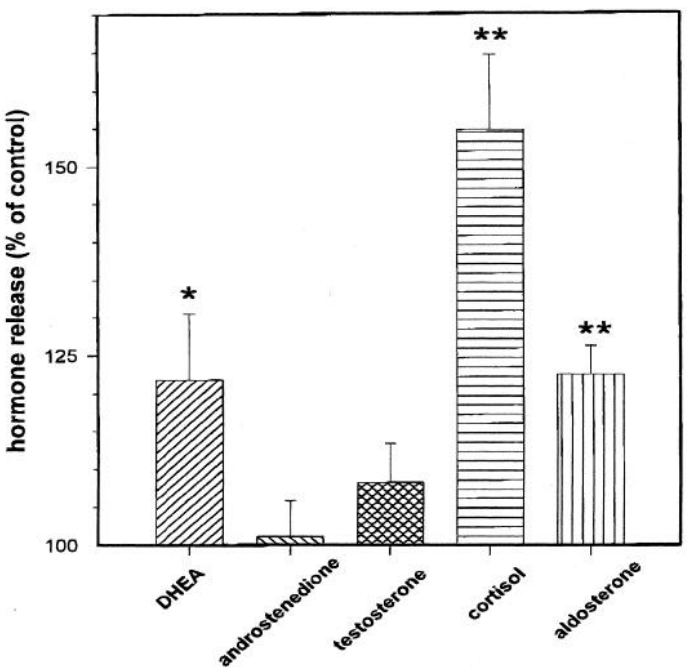


FIG. 7. Effect of PRL (10^{-7} mol/L) on the secretion of DHEA, androstenedione, testosterone, cortisol, and aldosterone after 24 h of incubation. Data are presented as a percentage of basal secretion (quadruplicate samples; n = 3; mean ± SE). *Asterisks* indicate significant differences from unstimulated controls (*, $P < 0.05$; **, $P < 0.005$).

PRL-like immunoreactivity has been detected in rat adrenal (1). This suggests a paracrine role for PRL in rat adrenal. We could not detect any PRL in paraffin-embedded and frozen sections of the human adrenal with immunohistochemical methods. Therefore, PRL seems not to play a role in paracrine processes in the human adrenal gland. PRL was clearly identified in slices of human pituitary that were used as a positive control.

The occurrence of PRLR in the adrenal gland indicates a direct effect of PRL on adrenal cells. Therefore, we investigated the influence of PRL on adrenal steroidogenesis in all three cortical zones.

PRL concentrations similar to those in hyperprolactinemic patients significantly stimulated steroidogenesis in the zona fasciculata and zona glomerulosa. Our results confirm the effect of PRL on the zona glomerulosa by the increased levels of aldosterone in human aldosterone-producing adenoma cell suspension (9). In patients with acromegaly, hypertension, and increased tetrahydroaldosterone-3-glucuronide levels, a relationship between simultaneously elevated PRL

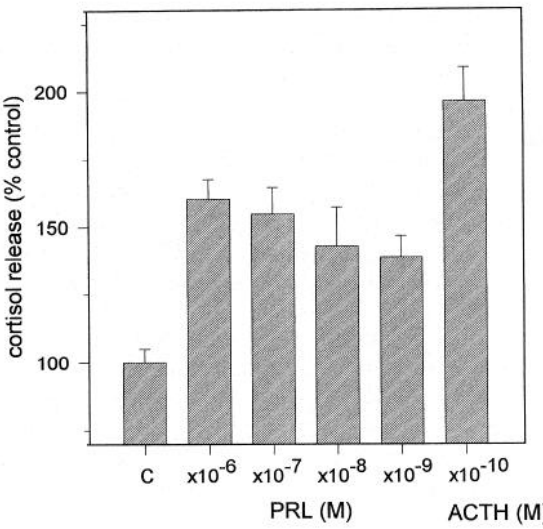


FIG. 8. Cortisol release from adrenal cells incubated with different doses of PRL (10^{-6}-10^{-9} mol/L) for 24 h. For comparison, treatment with ACTH (10^{-10} mol/L) is shown. Data are presented as a percentage of basal secretion (five wells per experiment; n = 2; mean ± SE).

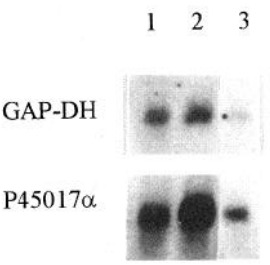


FIG. 9. Effects of PRL and ACTH on cytochrome P45017α mRNA levels. On the third day of culture, the cells were treated with the indicated hormones for 24 h: lane 1, basal; lane 2, (10^{-9} mol/L) ACTH; lane 3, (10^{-7} mol/L) PRL. The amount of cytochrome P45017α mRNA was determined by Northern analysis and normalized to the amount of glyceraldehyde-3-phosphate dehydrogenase mRNA. The experiment was repeated with another cell culture with the same result, *i.e.* cytochrome P45017α mRNA was not increased after PRL stimulation.

levels is suspected (27). In addition, it is known that PRL administration partially counteracts the fall in blood aldosterone levels in rats after dexamethasone treatment (28). In rat adrenocortical slices superfused *in vitro* with PRL, aldosterone release was significantly revealed (29).

In contrast to these findings, PRL did not affect blood levels of aldosterone and cortisol or corticosterone in guinea pigs and rats (30). There is also a lack of consistent correlation between PRL and aldosterone blood concentrations in patients with primary aldosteronism and PRL-secreting pituitary tumors (31) and in women with various endocrine diseases connected with water-salt metabolism disturbances (32). Possible explanations for this inconsistency may be the predominant influence of other aldosterone stimuli overlapping the PRL effect and the variability of factors inhibiting aldosterone secretion. Small effects also could be masked by the high blood volume.

It is well known that PRL has a stimulating effect on salt and water metabolism in lower vertebrates (33, 34). In mam-

mals, PRL increases the renal tubular reabsorption of Na^+, Cl^-, and water (35, 36). In humans, we were able to show that PRL has a moderate stimulatory effect on aldosterone secretion. Therefore, an influence on sodium, potassium, and water reabsorption may be relevant to high blood PRL levels.

It is known that during stress situations, PRL secretion in humans is elevated (37, 38). We showed that PRL stimulates cortisol secretion in human adrenal cell cultures. In this context we suspect a subtle regulation of PRL on cortisol secretion. It could possibly be of some importance in a stress response also.

Cortisol seems to inhibit PRL secretion, acting as a possible feedback mechanism (39). The postulated glucocorticoid upregulation of PRLR in kidneys and adrenals of rats support the suspected mutual interaction of adrenal and pituitary hormones. In accordance with our finding of a lesser effect of PRL on aldosterone levels, mineralocorticoids seem to have only a small, if any, influence on PRLR regulation (39).

The stimulation of cortisol can be increased by elevated expression of cytochrome P45017α mRNA. However, a stimulatory effect of cytochrome P45017α mRNA expression could not be observed by Northern blotting. Therefore, the treatment of adrenal cells with PRL may only affect posttranslational steroidogenesis. Nevertheless, we cannot exclude a weak activation of cytochrome P45017α mRNA expression, because the Northern blot method is not sensitive enough to discriminate a 1.5-fold stimulation. Further investigations are necessary to clarify whether PRL can regulate the activity of other enzymes in steroid biogenesis.

A stimulatory influence of PRL on androgen synthesis is often suggested. In 20% of patients with hirsutism, hyperprolactinemia also occurs. However, conflicting data exist regarding the influence of PRL on adrenal androgen synthesis in human cell cultures (13–15). Patient studies have reported variable success in finding correlations between hyperprolactinemia and blood androgen levels (40–42).

In our experiments there was a lack of significant changes in androstenedione and testosterone levels and only a small increase in DHEA concentrations after PRL stimulation. Because of the well known decline in androgen levels with age, it cannot be excluded that in younger patients the results are deviant. We obtained human adrenals from patients between 50–75 yr of age. There was no correlation between androgen levels and age in this small range. The modest effects of PRL on androgen synthesis suggest no direct influence of PRL on adrenal steroidogenesis in the normal zona reticularis. However, with the high PRL concentrations associated with hirsutism, the mild stimulatory effects may become relevant.

In summary, we demonstrated the distribution of PRLR in the human adrenal gland with immunohistochemical methods. We were able to detect positive signals in all zones of the adrenal cortex and only weak staining in medullary cells. PRL stimulated the release of aldosterone, cortisol, and DHEA in a dose-dependent manner. An effect of PRL on the expression of cytochrome P45017α was not be observed. PRL does not appear to be produced in the human adrenal gland itself.

The effect of PRL on adrenal steroidogenesis can be of particular relevance in clinical settings associated with hyperprolactinemia. The high expression of PRLR in the normal adrenal gland suggests other functions of PRL, possibly as a growth factor for adrenal cells. Hyperprolactinemia can be associated with adrenal hyperplasia and could affect the proliferation of adrenocortical cells in mice (43). Therefore, continuing studies are important to elucidate the role of PRL in adrenal physiology and pathophysiology.

Acknowledgments

The authors gratefully acknowledge the donation of human adrenals by Prof. Dieterich, Department of Urology, University of Leipzig. The technical assistance of Silke Brauer was greatly appreciated.

References

1. **Sinha YN.** 1995 Structural variants of prolactin: occurrence and physiological significance. Endocr Rev. 16:354–369.
2. **Reinwein D, Benker G.** 1992 Brusterkrankungen. In: Reinwein D, Benker G, eds. Klinische Endokrinologie und Diabetologie, 2nd ed. Stuttgart, New York: Verlag Schattauer; 348–352.
3. **Milenkovic L, Bogic L, Martinovic JV.** 1986 Effects of oestradiol and progesterone on stress-induced secretion of prolactin in ovariectomized and/or adrenalectomized female rats. Acta Endocrinol (Copenh). 112:79–82.
4. **Conte-Devolx B, Guillaume V, Grino M, et al.** 1993 Stress, aspects neuroendocriniens. Encephale. 19:143–146.
5. **Klemcke HG, Mayes HF, Hahn GL, Becker BA, Nienaber JA.** Effects of food and water deprivation and transportation of plasma prolactin in pigs. Proc of the 3rd Int Lvst Env Symp, Am Soc Agricultural Engineers. 1988;369–374.
6. **Bánky Z, Nagy GM, Halász B.** 1994 Analysis of pituitary prolactin and adrenocortical response to ether, formalin or restraint in lactating rats: rise in corticosterone, but no increase in plasma prolactin levels after exposure to stress. Neuroendocrinology. 59:63–71.
7. **Thorner MO, Besser GM.** 1990 Prolactin and its disorders. In: Becker KL, Bilezikiab JP, Bremmer WJ, et al, eds. Principles and practice of endocrinology and metabolism. Philadelphia: Lippincott; 134–139.
8. **Carter J, Tyson J, Warne G, McNeilly A, Faiman C, Friesen H.** 1977 Adrenocortical function in hyperprolactinemic women. J Clin Endocrinol Metab. 45:973–980.
9. **Caroll JE, Campanile CP, Goodfriend TL.** 1982 The effect of prolactin on human aldosterone-producing adenomas *in vitro*. J Clin Endocrinol Metab. 54:689–692.
10. **O'Connell Y, McKenna TJ, Cunningham SK.** 1994 The effect of prolactin, human chorionic gonadotropin, insulin and insulin-like growth factor1 on adrenal steroidogenesis in isolated guinea-pig adrenal cells. J Steroid Biochem Mol Biol. 48:235–240.
11. **Walker ML, Pepe GJ, Albrecht ED.** 1988 Regulation of baboon fetal adrenal androgen formation by pituitary peptides at mid- and gestation. Endocrinology. 122:546–551.
12. **Lewinski A, Bartke A, Esquifino A, Sewerynek E, Steger RW.** 1986 Adrenal catecholamine content: effects of congenital GH, PRL and TSH deficiency and of hormone replacement therapy in the male mouse. Exp Clin Endocrinol. 87:176–182.
13. **Higuchi K, Nawata H, Maki T, Higashizima M, Kato K, Ibayashi H.** 1984 Prolactin has a direct effect on adrenal androgen secretion. J Clin Endocrinol Metab. 59:714–718.
14. **Keymolen V, Dor P, Borkowski A.** 1976 Output of oestrogens, testosterone and their precursors by isolated human adrenal cells as compared with that of glucocorticoids. J Endocrinol. 71:219–229.
15. **Fehèr T, Szalay KS, Sziliàgi G.** 1985 Effect of ACTH and prolactin on dehydroepiandrosterone, its sulfate ester and cortisol production by normal and tumourous human adrenocortical cells. J Steroid Biochem. 23:153–158.
16. **Freemark M, Nagano M, Edery M, Kelly PA.** 1995 Prolactin receptor gene expression in the fetal rat. J Endocrinol. 144:285–292.
17. **Outhit A, Morel G, Kelly PA.** 1993 Visualization of gene expression of short and long forms of prolactin receptor in the rat. Endocrinology. 133:135–147.
18. **Rose J, Wert C.** 1993 Prolactin binding sites in the adrenal glands of mink (*Mustela vison*). Comp Biochem Physiol. 104:759–763.
19. **Klemcke HG, Pond WG, Nienaber JA.** 1989 Porcine adrenal prolactin receptors: characterization, changes during neonatal development and effects of hypoprolactinemia. Comp Biochim Physiol Acta. 92:197–206.
20. **Nolin JM.** 1978 Intracellular prolactin in rat corpus luteum and adrenal cortex. Endocrinology. 102:402–406.
21. **Bourne JA.** 1983 Handbuch I der Immunperoxidase Färbemethoden. Carpinteria: Dako Corp.; 14–26.
22. **Okamura H, Zachwieja J, Raguet S, Kelly PA.** 1989 Characterization and applications of monoclonal antibodies to the prolactin receptor. Endocrinology. 124:2499–2508.
23. **Dubé D, Kelly PA, Pelletier G.** 1980 Comparative localization of prolactin-

binding-sites in different rat tissues by immunohistochemistry, radioautography and radioreceptor assay. Mol Cell Endocrinol. 18:109–122.
24. **Bornstein SR, Gonzalez-Hernandez JA, Erhart-Bornstein M, Adler G, Scherbaum WA.** 1994 Intimate contact of chromaffin and cortical cells within the human adrenal gland forms the cellular basis for important intraadrenal interactions. J Clin Endocrinol Metab. 78:225–232.
25. **Bornstein SR, Ehrhart-Bornstein M.** 1992 Ultrastructural evidence for a paracrine regulation of the rat adrenal cortex mediated by the local release of catecholamines from chromaffin cells. Endocrinology. 131:3126–3128.
26. **Nagano M, Chastre E, Choquet A, Bara J, Gespach C, Kelly PA.** 1995 Expression of prolactin and growth hormone receptor genes and their isoforms in the gastrointestinal tract. Am J Physiol. 268:431–442.
27. **Marks P, Vincent R, Wilson B, Delassale A.** 1984 Aldosterone in acromegaly. Am J Med Sci. 287:16–19.
28. **Rebuffat P, Robba C, Mazzocchi G, Nussdorfer GG.** 1986 Further studies on the effect of prolonged prolactin administration on the zona glomerulosa of the rat adrenal cortex. Res Exp Med. 186:307–315.
29. **Eldridge JC, Lymangrover JR.** 1984 Prolactin stimulates and potentiates adrenal steroid secretion *in vitro*. Horm Res. 20:252–260.
30. **Sautin YY, Chelnakova IS, Tronko ND, Mikosha AS.** 1992 Trophic effect and modulation of ACTH-dependent stimulation of steroidogenesis by prolactin in guinea pig adrenal cortex. Endocr Regul. 26:35–39.
31. **Holland OB, Gomez-Sanchez CE, Kem DC, Weinberger MH, Kramer NJ, Higgins JR.** 1977 Evidence against prolactin stimulation of aldosterone in normal human subjects and patients with primary aldosteronism, including a patient with primary aldosteronism and a prolactin-producing adenoma. J Clin Endocrinol Metab. 45:1064.
32. **Atamanova TM, Egart FM, Dobracheva AL.** 1992 Interrelationships between prolactin and the renin-aldosterone system in patients with various endocrine diseases. Prob Endocrinol. 38:43–45.
33. **Nicoll CS, Bern HA.** 1972 On the actions of prolactin among the vertebrates: is there a common denominator? In: Wolstenholme GEW, Knight J, eds. Lactogenic hormones. Edinburgh: Churchill Livingstone; 299.
34. **Loretz ZA, Bern HA.** 1982 Prolactin and osmoregulation in vertebrates. Neuroendocrinology. 35:292–304.
35. **Bliss DJ, Lote CJ.** 1982 Effect of prolactin on urinary excretion and renal haemodynamics in conscious rats. J Physiol (Lond). 322:399–407.
36. **Stier CT, Cowden EA, Friesen HG, Allison EM.** 1984 Prolactin and the rat kidney; a clearance and micropuncture study. Endocrinology. 115:362–367.
37. **Kirschbaum C, Pirke K, Hellhammer DH.** 1993 The 'Trier social stress test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychology. 28:76–81.
38. **Rohrer T, Richthofen Vv, Schulz C, Beyer J, Lehnert H.** 1994 The stress-, but not corticotropin-releasing hormone-induced activation of the pituitary-adrenal axis in man is blocked by alprazolam. Horm Metab Res. 26:200–206.
39. **Marshall S, Huang HH, Kledzik GS, Campbell G, Meites J.** 1978 Glucocorticoid regulation of prolactin receptors in kidneys and adrenals of male rats. Endocrinology. 102:869–875.
40. **Parker LN, Odell WD.** 1980 Control of adrenal androgen secretion. Endocr Rev. 1:392–410.
41. **Vermeulen A, Suy E, Rubens R.** 1977 Effect of prolactin on plasma DHEA(S) levels. J Clin Endocrinol Metab. 44:1222.
42. **Parker L, Change S, Odell W.** 1978 Adrenal androgens in patients with chronic marked elevations of prolactin. Clin Endocrinol (Oxf). 8:1–5.
43. **Lewinski A, Sewerynek E, Webb S, Esquifino A, Bartke A.** 1988 Stimulatory effect of prolactin on the mitotic activity of the adrenal cortex in Snell mice with hereditary dwarfism. Res Exp Med. 188:87–94.