Prolactin Interacts with Gonadotropin through a Suppression of c-AMP Production Probably as a LH-Sensitive Adenylate Cyclase Inhibitor.

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Abstract

The interaction between gonadotropin and prolactin (PRL) on ovarian steroidogenesis as well as c-AMP production was studied in rat ovaries. Ovaries obtained from adult female Wistar rats in a morning of proestrus were chopped into 30-40 pieces and subjected to short term incubation studies using various buffers. HCG-stimulated c-AMP, estradiol (E2), progesterone (P) secretions were suppressed in a dose-dependent manner by ovine (0) PRL in a plain Gey-Gey (G-G) buffer. Addition of 3-isobutyl-1-methyl-xanthine (IBMX) increased c-AMP accumulation as well as E2 and P secretions. Deletion of Ca++ from the IBMX buffer stimulated c-AMP production, but suppressed steroid secretion. The inhibitory effect of PRL on E2 and P was not demonstrated in IBMX buffer at any Ca++ concentration examined despite suppression of c-AMP production. In conclusion, it was demonstrated that 1) PRL inhibited gonadotropin-stimulated production of E2 and P by inhibiting c-AMP production. 2) IBMX stimulated accumulation of c-AMP, E2 and P and counteracted with the antigonadal effect of PRL. 3) Ca++ inhibited c-AMP accumulation but stimulated E2 and P secretions. 4) The data suggested that PRL exerts its antigonadal effect through an inhibition of adenylate cyclase action in a manner similar to that of Ca++.
Materials and Methods

Ovaries were obtained from mature female Wistar rats weighing about 250 gr in the morning of proestrus. A pair of ovaries were chopped into 30-40 pieces and aliquoted into 6 vials. Each vial was incubated in 2 ml of 4 different kinds of buffers. 1) Plain Gey-Gey (G-G) buffer, 2) G-G with 0.5 mM IBMX, 3) G-G deleting Ca++ with 0.5 mM IBMX and 4) G-G deleting Ca++ and 1 mM EGTA with 0.5 mM IBMX. In those situations without Ca++, Mg++ was substituted in an equimolar amount. Incubations were performed in a 37°C water bath under continuous aeration with 95% O₂ and 5% CO₂ gas for 90 min. There was a 60 min. preincubation period under the same conditions. After the addition of HCG and/or PRL, the medium was changed at 30 min. intervals and c-AMP, E₂ and P were measured.

IBMX was obtained from Sigma Chemical Co. (St. Lois, Mo.). HCG was purchased from Mochida Pharmaceutical Co. Tokyo, Japan. Ovine (o) PRL was purchased from Ferring A.B., Sweden.

E₂ and P were measured by radioimmunoassay (RIA) after extraction of medium using antibodies supplied by Eiken, ICL, Tokyo, Japan. C-AMP was measured with a Yamasa c-AMP RIA kit commercially available in Japan. Coefficients of variation of intra- and inter-assay for these methods were all less than 10%.

Data represented in the figures the mean of 6 replicates performed on at least 2 occasions. Statistical analyses were done by Student's t-test.

Results

During the course of incubation, basal secretion of both E₂ and P decreased and became constant after 90 min. Experiments were therefore started after 90 min. of preincubation.

E₂ and P secretions in response to HCG and/or PRL in a plain G-G buffer are shown in Figs. 1 and 2 as the % change from the basal values. 1 IU/ml of HCG significantly stimulated both E₂ and P secretions with a peak response at 90 min. Although PRL, 1 IU/ml, did not significantly affect the responses obtained by 1 IU/ml of HCG, 10 IU/ml of PRL suppressed both the E₂ and P responses. Suppression of E₂ was statistically significant (p<0.05).

Maximum c-AMP accumulation during an incubation period in G-G buffer with 0.5 mM IBMX in response to HCG with or without PRL is shown in Fig. 3. HCG stimulated c-AMP accumulation in a dose-dependent manner. PRL, 1 IU/ml, did not
Fig. 2. % change in HCG-stimulated P response and effects of PRL. PRL, either 1 IU/ml or 10 IU/ml, did not significantly affect the response. However, 10 IU/ml PRL significantly suppressed c-AMP accumulation either individually or in the presence of 1 IU/ml of HCG. No suppressible effect was observed in combination with the same amount of HCG. Thus, PRL was less potent than HCG on a unit basis in inhibiting production of c-AMP.

Fig. 3. HCG-stimulated c-AMP accumulation in a dose-dependent manner. PRL suppressed the response in a dose-dependent manner with ten times as much as HCG.

Fig. 4. Comparison of basal E₂ secretion in 4 different buffers. E₂ secretion was significantly high in IBMX added buffers and significantly low in Ca²⁺ depleted EGTA buffer.
Fig. 5. E2 response to HCG and/or PRL in 4 different buffers. The response did not differ greatly among the 4 groups. PRL suppressed HCG-stimulated E2 response only in plain G-G buffer.

Fig. 6. Basal P secretion in 4 different buffers. P secretion was very high in IBMX added buffers and very low in Ca++ depleted EGTA buffer.

Fig. 7. P response to HCG and/or PRL. The response was much lower in Ca++ deleted groups. PRL only suppressed P response in a plain buffer, though the difference was not statistically significant.

4 conditions is shown in Fig. 4. Basal E2 secretion was significantly greater in IBMX added buffer (p<0.05). Among the IBMX buffers, E2 secretion was significantly lower in the absence of Ca++ (p<0.05). Maximal E2 response to 1 IU/ml of HCG in four different buffers is shown in Fig. 5. E2 response in % change from basal secretion was not significantly different in 4 buffers. PRL suppressed E2 only in a plain G-G buffer (p<0.05). IBMX prevented the PRL suppression in the presence or absence of Ca++.

Comparison of basal P secretion in the four buffers is shown in Fig. 6. Basal P secretion was significantly greater in IBMX added buffer (p<0.05). Among the three
buffers with IBMX added, P secretion was significantly lower in Ca\(^{++}\) depleted EGTA buffer. P response to HCG with or without PRL is demonstrated in Fig. 7. P response was significantly lower in Ca\(^{++}\) deleted groups than in G-G or G-G with IBMX. PRL suppressed HCG-stimulated P response only in the plain G-G buffer.

C-AMP accumulation, both basal and in response to HCG with or without PRL, is shown in Figs. 8 and 9. Though a statistical analysis was impossible because of extremely low values in a plain G-G buffer, basal secretions were markedly elevated in IBMX buffer. Among these, basal c-AMP secretion increased with decreasing Ca\(^{++}\) levels. With respect to maximal accumulation in response to HCG, essentially the same results were obtained as in basal secretion. PRL, 10 IU/ml suppressed HCG-stimulated c-AMP production approximately 50% in an IBMX added buffers. Suppression was significant in each pair (p<0.05) compared to those values obtained with 1 IU/ml of HCG alone.

**Discussion**

These results show that the suppressible effect of PRL on E\(_2\) and P in both basal and HCG-stimulated secretions is only observed in plain G-G buffer and not in the presence of IBMX with or without Ca\(^{++}\). In G-G buffer, PRL suppressed both the E\(_2\) and P responses as well as c-AMP accumulation in a dose-dependent manner. It was also shown that PRL inhibited gonadotropin-related c-AMP accumulation, as we previously observed in human ovaries (Ono et al, 1982). In addition, on a unit basis, significantly more PRL was necessary to
counteract the gonadotropin effect. The addition of IBMX, a phosphodiesterase inhibitor, stimulated basal as well as gonadotropin-stimulated secretions of c-AMP to a greater extent than with a plain buffer. It also abolished the suppressible effect of PRL on E_2 and P secretions; nevertheless c-AMP accumulation was inhibited to the same degree as in the G-G buffer alone. This may indicate that PRL suppressed gonadotropin-stimulated c-AMP production rather than affecting its degradation rate and that the total c-AMP accumulation, potentiated by IBMX, overcame the suppressant effect of PRL on c-AMP production in the experimental conditions used.

Depletion of extracellular Ca^{++} ions, by omitting Ca^{++} or by adding EGTA, stimulated c-AMP, but suppressed basal as well as gonadotropin-stimulated E_2 and P secretions. Dorflinger et al. (1984) recently demonstrated that removal of extracellular Ca^{++} approximately doubled gonadotropin-stimulated c-AMP accumulation but blunted LH-stimulated progesterone secretion in luteal cells in rats. In addition, they found that intra-cellular Ca^{++} inhibited activation of adenylate cyclase by LH in the rat luteal cells. The results of the present studies are consistent with their observations and may indicate that there exists a Ca^{++} dependent process in steroid secretion beyond that of c-AMP.

PRL suppressed c-AMP production, but not E_2 and P secretions, in IBMX added buffer irrespective of the Ca^{++} concentration. Higuchi et al. (1976) also suggested that Ca^{++} may be necessary for progesterone secretion. Recently, there has been evidence that PGF_2 (Thomas et al., 1978), and LH-RH (Lahav et al., 1976) blocked the stimulatory response of c-AMP and steroid secretion to LH in rat luteal cells. It was suggested that agonadal effects of these agents may be exerted through an inhibition of LH-sensitive adenylate cyclase by Ca^{++}. The results obtained with PRL were similar to those with these agents, although the mechanism is not yet known.

In conclusion, 1) PRL inhibited gonadotropin-stimulated production of c-AMP, E_2 and P in a plain G-G buffer. 2) IBMX stimulated an accumulation of c-AMP, E_2 and P by prohibiting a degradation of c-AMP and counteracted agonadal effects of PRL. 3) Ca^{++} inhibited c-AMP accumulation but oppositely stimulated E_2 and P secretions after c-AMP. Ca^{++} did not interfere with the c-AMP suppressible effect of PRL. 4) It appeared that PRL exerted its agonadal effect through an inhibition of c-AMP production similar to Ca^{++}.

**Acknowledgements**

This study was partly supported by a research grant for “Specific Diseases” from the Japanese Ministry of Health and Welfare and by a research grant from the Japanese Ministry of Education.

**References**


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