Effect of \(\beta\)-Endorphin on cAMP and Progesterone Accumulation in Rat Luteal Cells

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Abstract. The aim of this study is to investigate the effect of \(\beta\)-endorphin on cAMP and progesterone accumulation in rat luteal cells. Luteal cells of 4-day-old corpora lutea were cultured for 3 h in the absence or presence of 0.001 or 0.01 IU/ml hCG, and cAMP, progesterone and \(\beta\)-endorphin levels in the medium were measured by RIA. hCG stimulated the production of cAMP, progesterone and \(\beta\)-endorphin. In the presence of hCG, treatment with islet-activating protein (IAP) led to overall augmentation of cAMP and progesterone accumulation in comparison with untreated controls. In the absence or presence of low doses of hCG (0.001 IU/ml), \(\beta\)-endorphin did not affect progesterone production, but inhibited cAMP accumulation. This inhibitory effect was abolished by pre-treatment with IAP. In the presence of high doses of hCG (0.01 IU/ml), however, \(\beta\)-endorphin stimulated progesterone production without a corresponding increase in cAMP. This stimulatory effect was also abolished by IAP-treatment. These results suggest that luteal cells produce and release \(\beta\)-endorphin that affects cAMP and progesterone production via IAP-sensitive mechanisms.

Keywords: \(\beta\)-Endorphin, Corpus luteum, Islet-activating protein. (Endocrine Journal 40: 323–328, 1993)
Materials and Methods

Chemicals

The following reagents were obtained from commercial sources: bovine serum albumin (fraction V), HEPES, collagenase (type I), deoxyribonuclease I, 3-isobutyl-1-methylxanthine (IBMX), cAMP, progesterone, IAP, L-glutamine and cAMP-dependent protein kinase (Sigma Chemical Co., St. Louis, MO); pregnant mare serum gonadotropin (PMSG) (Teikoku Hormone MFG, Tokyo, Japan); human chorionic gonadotropin (hCG) (Mochida Pharma., Tokyo, Japan); human β-endorphin (Peptide Institute INC., Osaka, Japan); Eagle's Minimum Essential Medium (Nissui Pharm., Tokyo, Japan); Medium 199 (Gibco, Grand Island, NY); Percoll (Pharmacia LKB, Uppsala, Sweden); Rabbit anti-progesterone-11α-BSA serum (BioMaker, Israel); Rabbit anti-human β-endorphin serum, Goat anti-rabbit IgG serum and normal rabbit serum (Peninsula Laboratories INC., California); [3H]-cAMP (30.5 Ci/mmol), [3H]-progesterone (57.0 Ci/mmol) and 125I-f-endorphin (2200 Ci/mmol) (Dupont New England Nuclear Research Products, Boston, MA); penicillin G potassium and streptomycin sulfate (Meiji Seika, Tokyo, Japan).

Animals

Immature female Sprague-Dawley rats were kept under standardized conditions with lights on between 06:00 and 20:00 h. Food and water were provided ad libitum. To obtain heavily luteinized ovaries, the rats were injected s.c. with 50 IU PMSG at 26 days of age, followed by 25 IU of hCG 54 h later. The animals were killed by cervical dislocation 4 days after the hCG injection and the ovaries were removed for dispersion of luteal cells.

Cell isolation

Luteal cells were isolated by the method of Sender Baum and Rosberg [16]. Briefly, ovaries were cut into pieces and were enzymatically dispersed with 1% collagenase and 0.25% deoxyribonuclease. The crude cell suspension was layered on top of a 40% continuous Percoll gradient and centrifuged for 20 min at 400 × g. Cells with densities between 1.05 and 1.07 g/ml were recovered for use in the experiments with Eagle's MEM supplemented with BSA (0.2%), HEPES (0.01 M), L-glutamine (0.29 mg/ml), penicillin G potassium (100 U/ml) and streptomycin sulfate (10 µg/ml). This is subsequently referred to as the culture medium. The number of cells and viability (95%) were checked by means of the Trypan blue exclusion test.

IAP treatment of luteal cells

IAP was stored in a vehicle consisting of 2 M urea and 0.1 M potassium phosphate buffer (pH 7.0) at 4°C until being appropriately diluted with culture medium for immediate use. Luteal cells were exposed to 30 ng/ml IAP (or its vehicle as control) at a density of 10⁷ cells/ml at 37°C in culture medium for 2 h. The IAP-induced interference was almost complete in NG108-15 hybrid cells under this condition [12]. These IAP-treated (or control) cells, after being washed several times, were subjected to incubation under various conditions as described below.

Incubation of cells

The luteal cells (5 × 10⁴ cells/well) were placed onto multiwell culture plates with culture medium (Costar, Cambridge, MA). The cell cultures were maintained at 37°C in a humidified atmosphere of 95% air and 5% CO₂. In all experiments, the cells were cultured for 3 h with or without the indicated concentrations of hCG and/or β-endorphin in the presence of phosphodiesterase inhibitor IBMX (0.1 mM).

Assays of cAMP, progesterone and β-endorphin

cAMP, progesterone and β-endorphin were determined in aliquots taken from the culture medium immediately after the incubation. The medium was kept frozen at −80°C until analysis. β-endorphin was determined in unextracted aliquots by radioimmunoassay (RIA). Incubation was conducted at 37°C in the presence of anti-human β-endorphin serum at 4°C overnight. 125I-β-endorphin was added, and the incubations continued at 4°C for an additional 24 h. After the
second 24 h, goat anti-rabbit IgG serum and normal rabbit serum were added to separate free and bound β-endorphin. The precipitates were collected by centrifugation at 1700 × g for 20 min. The pellets were counted for 125I in a scintillation well gamma counter. The sensitivity of the β-endorphin assay was approximately 14 pg/tube (IC50). The cAMP assay was performed by the method of Gilman [17], modified as previously described [18]. Our laboratory data have shown that the extracellular cAMP content roughly parallels that of intracellular cAMP [19]. Progesterone was determined in nonextracted medium by RIA, as previously described [20]. The coefficients of the intra- and inter-assay variations in each RIA were smaller than 10%.

Statistics

Experimental values are given as the mean ± SEM of triplicate samples. Statistical analysis was by unpaired Student’s t-test. A P value less than 0.05 was considered significant.

Results

Effect of hCG on cAMP, progesterone and β-endorphin production by IAP-treated or untreated luteal cells

Luteal cells of 4-day-old corpora lutea were cultured for 3 h in the absence or presence of 0.001 or 0.01 IU/ml hCG, and extracellular CAMP, progesterone and β-endorphin levels were measured. As shown in Fig. 1a, b and 2, hCG stimulated the production of cAMP, progesterone and β-endorphin by untreated luteal cells. In the presence of hCG, treatment with IAP led to an overall augmentation of luteal cell cAMP and progesterone accumulation in comparison with untreated controls.

Effect of β-endorphin on cAMP and progesterone production by untreated luteal cells

To investigate the possibility that β-endorphin may regulate basal and hCG-induced CAMP and progesterone accumulation, luteal cells were cultured with various concentrations of β-endorphin (10–1000 ng/ml) in the presence or absence of hCG. In the absence of hCG, β-endorphin at a concentration of 1000 ng/ml significantly decreased CAMP accumulation (Fig. 3, P<0.05), but did not affect progesterone production (data not shown). In the presence of 0.001 IU/ml of hCG, β-endorphin (1000 ng/ml) significantly decreased hCG-stimulated CAMP accumulation (Fig. 3, P<0.05), but did not suppress progesterone production (data not shown). In the presence of high doses of hCG (0.01 IU/ml), CAMP was not affected by β-endorphin (Fig. 3); while progesterone production was significantly increased when the cells were treated with 1000 ng/ml of β-endorphin (Fig. 4).
To investigate the possibility that β-endorphin suppresses cAMP production via Gi, the effects of β-endorphin on IAP-treated and untreated cells were compared. Luteal cells were cultured with increasing concentrations of hCG (0.001 IU/ml to 0.01 IU/ml) for 2 h. At the end of the incubation, the media were collected and β-endorphin content was measured by RIA. The results represent the mean ± SEM of three determinations.

**Effect of β-endorphin on IAP-treated luteal cell**

Luteal cells were cultured in the presence or absence of IAP (30 ng/ml) for 2 h. Then the IAP-treated cells or untreated cells were cultured with β-endorphin (1000 ng/ml) in the absence or presence of hCG (0.001 IU/ml). As mentioned above, β-endorphin suppressed cAMP accumulation by untreated cells in the absence or presence of hCG (0.001 IU/ml). In contrast, β-endorphin did not suppress cAMP accumulation by IAP-treated cells in the absence or presence of hCG. Moreover the stimulatory effect of β-endorphin on progesterone production in the presence of hCG (0.01 IU/ml) by IAP-treated or untreated luteal cells. Luteal cells were treated with IAP (30 ng/ml) as shown in Fig. 1. IAP-treated or untreated luteal cells were reincubated for an additional 3 h with β-endorphin (1000 ng/ml) and hCG (0.01 IU/ml). At the end of the incubation, the media were collected and progesterone content was assayed. The results represent the mean ± SEM of three determinations. *, P<0.05.

**Table.** Effect of β-endorphin (βend, 1000 ng/ml) on extracellular cAMP accumulation in the presence and absence of IAP (30 ng/ml) pretreatment

<table>
<thead>
<tr>
<th>cAMP Accumulation (pmol/3 h)</th>
<th>Untreated</th>
<th>IAP-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>hCG(-)</td>
<td>2.40±0.30</td>
<td>2.60±0.28</td>
</tr>
<tr>
<td>hCG(-)+βend</td>
<td>0.28±0.18</td>
<td>1.80±0.16</td>
</tr>
<tr>
<td>hCG 0.001 IU</td>
<td>4.00±0.45</td>
<td>5.94±0.26</td>
</tr>
<tr>
<td>hCG 0.001 IU+βend</td>
<td>1.90±0.13</td>
<td>8.04±0.86</td>
</tr>
<tr>
<td>hCG 0.01 IU</td>
<td>5.80±0.40</td>
<td>7.61±0.47</td>
</tr>
<tr>
<td>hCG 0.01 IU+βend</td>
<td>6.80±0.30</td>
<td>8.99±0.33</td>
</tr>
</tbody>
</table>

Luteal cells were cultured in the absence or presence of IAP (30 ng/ml) for 2 h. At the conclusion of this period, media were discarded and the cells were washed, and reincubated for an additional 3 h with or without β-endorphin (1000 ng/ml) and the indicated concentrations of hCG. After the incubation period, aliquots of the medium were frozen for analysis of cAMP content. The results are shown as means±SEM of three determinations. *, P<0.05; **, P<0.01.
EFFECT OF β-ENDORPHIN ON LUTEAL CELLS

hCG (0.01 IU/ml) was also abolished by IAP treatment (Fig. 4).

Discussion

We showed that hCG stimulated β-endorphin release from luteal cells in a dose-dependent manner in a primary culture of rat luteal cells. This finding is in accordance with the fact that gonadotropin regulated the expression of POMC mRNA in ovaries [5], and that β-endorphin was produced and secreted in ovaries and the production was related to the estrous cycle [8, 10].

We also demonstrated that β-endorphin suppressed luteal cell cAMP accumulation in both the absence and presence of lower doses of hCG. A statistical significance was observed only between 0 and 1000 ng/ml of β-endorphin in this work since the analysis was performed only at triplicate. However, it seems likely that β-endorphin at the concentration of more than 100 ng/ml exhibits a remarkable reduction of cAMP accumulation. These data are supported by a report stating that the [D-Ala²-Met⁵] analog of enkephalin was found to inhibit forskolin-activated adenylate cyclases in rabbit corpora lutea membranes [21]. With higher concentrations of hCG, however, β-endorphin did not suppress cAMP accumulation. These results indicate that hCG stimulation of adenylate cyclase activity competes with inhibition by β-endorphin. Moreover, our data showed that the inhibitory effect of β-endorphin on cAMP accumulation was abolished when cells were pre-treated with IAP, suggesting that β-endorphin suppressed adenylate cyclase activity, presumably via Gi.

Our data showed that increases in cAMP and progesterone levels in the presence of hCG were larger in IAP-treated luteal cells than in nontreated cells. The basal content of cAMP in luteal cells in the absence of hCG was not altered by pre-treatment with IAP. Similar potentiation of receptor-mediated cAMP accumulation by IAP treatment has been reported for opiate receptors in NG 108–15 cells [12]. This paper showed IAP-induced enhancement of receptor mediated activation of adenylate cyclase [12]. One way to explain our results is that IAP-induced abolition of the Gi function is responsible for enhancement of adenylate cyclase activity, resulting in the enhancement of cAMP and progesterone production. Further studies will be required to identify Gi in rat luteal cells and investigate the coupling of LH receptor to Gi.

The observed stimulation of progesterone synthesis by hCG (0.01 IU/ml) + β-endorphin (1000 ng/ml) was not accompanied by cAMP accumulation. Moreover, in the presence of hCG (0.001 IU/ml) β-endorphin (1000 ng/ml) inhibited cAMP accumulation, but did not that of progesterone. This disparity between steroid synthesis and cAMP levels suggests the existence of cAMP-independent steroidogenesis. In fact, it was reported that LH increased inositol 1, 4, 5-triphosphate (IP₃) and cytosolic free Ca²⁺ as well as cAMP in bovine luteal cells [22]. It was also shown that the opioid [D-Ala²-Met⁵]-enkephalin regulated calcium channels through opioid receptors in NG 108–15 cells [23]. These reports raise the possibility that cross talk of second messenger systems might be involved in mediating the actions of hCG and β-endorphin in the corpus luteum. In some cell types G protein-mediated activation of phospholipase C was IAP-sensitive [24]. Taking into account the data showing that the stimulation by β-endorphin of progesterone production in response to hCG was abolished by treatment with IAP, these mechanisms which might mediate such cross talk seem to be IAP-sensitive.

Considering the fact that β-endorphin alone had no effect on steroidogenesis in corpora lutea, it appears to act as modulator of the steroidogenic effects of other hormones such as hCG. Though no modulatory effects on steroidogenesis were apparent at low doses of hCG, there was a positive modulatory effect on progesterone production stimulated by high doses of hCG. The concentration of 1000 ng/ml of β-endorphin is, however, not physiological. The possibility exists that a high dose of β-endorphin might interfere with luteal cell steroidogenesis. Since the chief aim of this study was the suppression by β-endorphin of cAMP accumulation, high doses of β-endorphin were used in our experiment. Further studies including low dosage β-endorphin experiment are therefore needed before the physiological effect of β-endorphin on luteal cells can be established.
References


