Oxytocin and vasopressin have no effect on progesterone production and cyclic AMP accumulation by rat luteal cells *in vitro*

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Summary. Enzymically dispersed luteal cells obtained from PMSG–hCG-treated immature pseudopregnant rats were incubated with oxytocin and vasopressin. In response to increasing doses of hCG the rat luteal cells produced progesterone and accumulated intracellular cAMP in a dose-dependent manner. A neuropeptide GnRH agonist (4×10^{-6} M) produced a significant inhibition of hCG-stimulated progesterone production and of accumulation of intracellular cAMP. However, neither the basal nor the hCG-stimulated rate of progesterone production and level of intracellular cAMP was affected by the neurohypophyseal peptides tested. Therefore, it is concluded that oxytocin and vasopressin do not have a direct action on steroidogenesis by rat luteal cells.

Introduction

In several species of animals testicular and ovarian steroidogenesis can be modulated by neurohypophyseal peptide hormones. Several such peptides, including oxytocin and vasopressin, have been shown by Adashi & Hsueh (1981) to be inhibitory to hCG-stimulated steroidogenesis in cultured rat Leydig cells. Tan, Tweedale & Biggs (1982a, b) have reported that oxytocin at a high concentration inhibited basal and hCG-stimulated progesterone production by bovine and human luteal cells, whereas at low concentration it enhanced only the basal rate of steroidogenesis in both types of luteal cells. Oxytocin has been shown to be present in reasonable quantity in ovine (Wathes & Swann, 1982), bovine (Wathes, Swann, Birkett, Porter & Pickering, 1983) and human (Wathes *et al.*, 1982; Khan-Dawood & Dawood, 1983) ovaries. Based on evidence of this type, it has been suggested that oxytocin could play a role in luteal regression. However, there is no report of a direct action of neurohypophyseal peptide on rat luteal cells. We have therefore investigated whether oxytocin or vasopressin can influence basal and hCG-stimulated progesterone production or the accumulation of intracellular cAMP by isolated rat luteal cells *in vitro*.

Materials and Methods

Materials. The sources of chemicals used in this study were as follows: bovine serum albumin, collagenase (Type IA, 165 U/mg), deoxyribonuclease I (2000 U/mg), dibutyryl cAMP, GnRH agonist (des Gly¹⁰-D-Ala⁶-N-ethylamide GnRH), oxytocin (504 i.u./mg) and lysine vasopressin (231 i.u./mg) from Sigma Chemical Company, Munich, Germany; Medium 199, penicillin,

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streptomycin, fungizone and lactalbumin hydrolysate from Grand Island Biological Company, Karlsruhe, Germany; heparin (Liquimin-25000) from Roche, Grenzach-Wyhlen, Germany; hCG from Serono, Freiburg, Germany; PMSG from Ferring, Kiel, Germany; 3-isobutyl-methylxanthine from Aldrich, Beerse, Belgium; and reagents for protein estimation from Bio-Rad, Munich, Germany.

Animals. Wistar rats aged 26 days received a s.c. injection of 50 i.u. PMSG followed 65 h later by an injection of 25 i.u. hCG. The animals (pseudopregnant) were killed by cervical dislocation on the 9th day after the PMSG injection. Depending on the subsequent requirements for incubation, ovaries from 20–25 PMSG–hCG-treated rats were used in one experiment. The ovaries, consisting mainly of luteal tissue, were removed, freed of fat and adhering tissues, pooled and minced with scissors and incubated in Medium 199 with Earle's salts, glutamine and 25 mM-Hepes, pH 7.4, containing 2 mg collagenase/ml, 10 μ g DNase I/ml, 4 mg bovine serum albumin/ml, 2 mg lactalbumin hydrolysate/ml, 20 U penicillin/ml, 20 μ g streptomycin/ml, 0.05 μ g fungizone/ml and 25 U heparin/ml, at a concentration of 50 mg tissue/ml digestion medium. Digestion was carried out under an atmosphere of 95% O₂–5% CO₂ at 37°C with constant shaking (60 cycles/min) for 90 min. The dispersed cells were collected by centrifugation at 100 g at 4°C for 15 min. The cells were washed twice with Medium 199 supplemented as above but without the enzymes (henceforth referred to as Medium 199) to remove any enzyme contamination. The final pellet was resuspended in Medium 199 and cell protein estimated using the Bio-Rad protein estimation kit. The cell suspension was then diluted with Medium 199 to adjust the cellular protein concentration to 800 μ g protein/ml.

The viability of the cells thus prepared was over 90% as assessed by trypan blue exclusion (Tennant, 1964). The cell suspension consisted of single luteal cells, cells in clumps and red blood cells.

Incubation of luteal cells. Incubation of luteal cells was carried out in quadruplicate in a total volume of 0.55 ml containing 200 μ g protein equivalent of cells, at 37°C under an atmosphere of 95% O₂ and 5% CO₂ for a period of 3 h. Test substances were diluted in Medium 199 before addition. The incubations were terminated by placing the tubes in an ice-water bath followed by addition of ice-cold 0.1 M-phosphate-buffered saline, pH 7.0. The tubes were centrifuged at 1500 g for 20 min and the supernatant stored frozen at –20°C until the progesterone content was measured by radioimmunoassay. The cell pellet was extracted with 1.0 M-perchloric acid for intracellular cAMP determination. In experiments in which intracellular cAMP was measured the incubation mixture contained 0.5 mM-3-isobutyl-methylxanthine, a phosphodiesterase inhibitor.

Radioimmunoassay of progesterone. Progesterone concentration in the supernatant was measured by using a commercial radioimmunoassay kit supplied by Radioassay Systems Laboratories, Carson, CA, U.S.A. According to the information provided with the kit the following materials were checked for cross-reactivity (the percentages indicate cross-reactivity at 50% displacement compared to progesterone): progesterone, 100%; 20 α -dihydroprogesterone, 6.25%; desoxycorticosterone, 3.2%; corticosterone, 0.42%; 17 α -hydroxyprogesterone, 0.15%; pregnenolone, 0.06%; androstendione, 0.04%; testosterone, 0.03% and 11-deoxycortisol, pregnenolone sulphate, cholesterol dihydroepiandrosterone, oestradiol-17 β , oestrone, oestriol, androsterone, aldosterone and cortisol, all <0.01%. The sensitivity of the assay, defined as the minimum detectable amount, was 0.2 ng progesterone/ml. Inter- and intra-assay coefficients of variation were <8% ($n = 16$) and 5% ($n = 20$) respectively. Progesterone concentration is expressed as ng progesterone per 200 μ g protein equivalent of luteal cells per 3 h.

Radioimmunoassay of cAMP. Intracellular cAMP was extracted from the cell pellet by adding 500 μ l 1.0 M-perchloric acid followed by thorough mixing, incubation at 4°C for 30 min and centrifugation at 1500 g for 10 min at 4°C. The supernatant obtained was neutralized by adding 500 μ l 0.71 M-K₃PO₄ and centrifuged. The clear supernatant obtained was radioimmunoassayed for

cAMP by the method of Steiner, Parker & Kipnis (1972) as described by Schumacher, Schaefer, Lichtenberg & Hilz (1979). The sensitivity of the assay was 5 fmol cAMP/tube. Interassay variation was <10% ($n = 10$) and intra-assay variation was <5% ($n = 10$).

Statistical analysis. The statistical significance of the observed differences was determined by Student's *t* tests.

Results

As shown in Text-fig. 1, the production of progesterone and accumulation of intracellular cAMP were stimulated by hCG in a dose-dependent manner. However, neither oxytocin nor vasopressin, added in doses up to 4×10^{-6} M (equivalent to 2 i.u. oxytocin/ml and 1 i.u. vasopressin/ml) had any significant effect on basal rate of steroidogenesis or the level of intracellular cAMP.

The effects of oxytocin, vasopressin and a GnRH agonist on hCG-stimulated steroidogenesis and intracellular cAMP formation were studied. The dose of hCG used was submaximal, as it is known that GnRH can inhibit gonadotrophin-stimulated steroidogenesis only when a submaximal dose of hCG is used, and that at a supramaximal dose of hCG, GnRH is ineffective (Clayton, Harwood & Catt, 1979). In the experiment in which progesterone was measured, hCG was used at a dose of 25 mi.u. and in the other part of the experiment, in which cAMP was measured, a dose of 2.5 mi.u. hCG was used. These two doses of hCG were selected on the basis of the data in Text-fig. 1.

Text-fig. 1. Response of rat luteal cells to the addition of increasing doses of hCG, oxytocin and vasopressin. The luteal cells were incubated in Medium 199 under an atmosphere of 95% air and 5% CO₂ in presence of various doses of hCG (Δ , $\blacktriangle$), oxytocin ($\circ$, $\bullet$) and vasopressin ($\square$, $\blacksquare$) for 3 h and the progesterone (Δ , $\circ$, $\square$) produced and intracellular cAMP ($\blacktriangle$, $\bullet$, $\blacksquare$) accumulated were measured in quadruplicate incubations. The results shown represent mean $\pm$ s.d. (vertical bars); vertical bars are omitted when the s.d. was smaller than the symbol. Each value presented is the concentration measured, corrected by subtracting the basal amount estimated in the absence of any addition. The basal amounts of progesterone produced and intracellular cAMP accumulated were 11.3 ± 0.6 ng and 17.3 ± 3.8 pmol per 200 μ g cell protein respectively. The concentration of 4×10^{-6} M was equivalent to 1 i.u. vasopressin/ml and 2 i.u. oxytocin/ml.

Table 1. Effect of addition of oxytocin, vasopressin and GnRH agonist on hCG-stimulated progesterone production and accumulation of intracellular cAMP in rat luteal cells

Additions	Progesterone (ng/200 µg protein)	Intracellular cAMP (pmol/200 µg protein)
None	9.05 ± 2.02	17.29 ± 3.81
hCG	45.44 ± 7.05*	62.22 ± 9.63*
hCG + oxytocin	49.41 ± 1.75	65.79 ± 12.47
hCG + vasopressin	49.21 ± 4.04	54.74 ± 5.74
hCG + GnRH	20.15 ± 1.27†	45.10 ± 2.27†

Values are mean ± s.d. for quadruplicate determinations.

* $P < 0.001$ compared to control group.

† $P < 0.05$; ‡ $P < 0.001$ compared to hCG-treated group.

The results (Table 1) indicate that neither oxytocin (4×10^{-6} M) nor vasopressin (4×10^{-6} M) had any effect on hCG-stimulated steroidogenesis and intracellular cAMP formation. On the other hand GnRH agonist (4×10^{-6} M) produced a significant inhibition of hCG-stimulated progesterone production and of intracellular cAMP formation. Lower doses (4×10^{-9} – 4×10^{-7} M) of oxytocin and vasopressin have also been used to investigate the effects on hCG-stimulated steroidogenesis but were found to be ineffective (R. Tams, unpublished observations). Each experiment was repeated at least twice and some experiments were also repeated using oxytocin from Serva (Heidelberg, Germany). The results obtained were similar to those presented here. The negative data were also reproducible between several different preparations of luteal cells.

Discussion

Ott & Scott (1910) were the first to suggest that oxytocin-like activity was associated with the corpus luteum when they reported that administration of luteal extract to goats enhanced the milk-ejection response. Neurohypophysial peptides have now been shown to be present in the ovary of several animal species (Wathes *et al.*, 1982, 1983; Wathes & Swann, 1982; Khan-Dawood & Dawood, 1983). However, physiological functions of such ovarian oxytocin have yet to be elucidated. It has been suggested that the ovarian oxytocin could have an intraovarian function and act directly at the luteal cells, thereby effecting rapid luteal regression (Tan *et al.*, 1982a, b). Flint & Sheldrick (1983), on the other hand, suggested that ovarian oxytocin may be indirectly responsible for luteal regression by enhancing the secretion of uterine prostaglandins.

The results obtained in the present study do not support the view that neurohypophysial peptides directly modulate steroidogenesis by the luteal cells, at least in the rat. The luteal cells obtained by enzymic dispersion of ovaries from immature pseudopregnant rats responded, in a dose-dependent manner, to stimulation by hCG in terms of both progesterone production and accumulation of intracellular cAMP. The GnRH agonist, at a dose of 4×10^{-6} M, significantly inhibited progesterone production and intracellular cAMP formation, as previously reported (Clayton *et al.*, 1979; Behrman, Preston & Hall, 1980). However, in contrast to bovine (Tan *et al.*, 1982a) and human (Tan *et al.*, 1982b) luteal cells, the rat luteal cells did not respond to low or high doses of oxytocin used. Vasopressin was also without any effect. Furthermore, neither oxytocin nor vasopressin, used at doses equimolar to that of GnRH showing an antigonadotrophic effect, could modify the response of rat luteal cells to the stimulation by hCG. It may be concluded, therefore, that the luteal cells of the rat are insensitive to a direct action by the neurohypophysial peptides, oxytocin and vasopressin. Possibly the direct action of oxytocin on the luteal cells is limited to certain species of animals only, or at a particular physiological state, e.g. during early pregnancy only as shown by Tan *et al.* (1982a).

When an effect of GnRH has been reported for the testis, it is also found in the ovary, as in the case of rat (Hsueh & Jones, 1981). However, although an inhibitory effect of neurohypophysial peptides on cultured rat testicular cells has been reported (Adashi & Hsueh, 1981), no effect on rat luteal cells was observed in the present study.

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