Demonstration of mRNAs for oxytocin and prolactin in porcine granulosa and luteal cells

Effects of these hormones on progesterone secretion in vitro

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The relative levels of mRNAs for relaxin, prolactin, inhibin and oxytocin have been measured in porcine granulosa as well as luteal cells by hybridisation to single-stranded synthetic DNA. The likelihood of a paracrine function of oxytocin and prolactin in the porcine ovary was inferred from the in vitro effects of both hormones on progesterone secretion of ovarian cells. Both hormones were found to inhibit progesterone secretion of luteal cells. In contrast, only prolactin but not oxytocin stimulated progesterone secretion in granulosa cells.

Oxytocin *Prolactin* *mRNA* *Hybridization* *(Granulosa cell, Luteal cell)* *Progesterone secretion*

1. INTRODUCTION

Oxytocin is synthesized and released in the ovaries of a number of species [1,2]. The concentrations of oxytocin and of oxytocin mRNA were found to be high in bovine ovarian tissue [3]. A possible role for oxytocin in luteolysis was discussed. Recently the production of the pituitary hormone prolactin by placenta and the myometrium was demonstrated [4]. Because concentrations in the follicular fluid were found to be higher than in circulating blood speculation as to its production in ovarian cells was near at hand. To elucidate a possible physiological function of oxytocin as well as prolactin on ovarian cells we attempted to study the effects of the two hormones on progesterone secretion of porcine granulosa and luteal cells. We approached the detection as well as the relative quantitation of oxytocin and prolactin mRNAs in porcine granulosa and luteal cells by hybridisation to specific DNA probes. Principally two techniques, blot hybridisation or solution hybridisation, are employed. The latter represents a true equilibrium method, because hybridisation is carried out in solution and the excess of non-hybridised DNA probe is removed by single-strand specific S_1 nuclease. Whereas blot hybridisation is usually performed with cloned cDNA, specific solution hybridisation requires single-stranded DNA, complementary to the mRNA, to be measured. For this purpose we synthesised single-stranded synthetic DNAs complementary to the respective mRNAs of those peptide hormones.

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2. EXPERIMENTAL

Luteal cells: ovaries were obtained from adult, non-pregnant pigs at the local slaughterhouse within 15 min after death. Cells were dispersed and cultivated in TC199 medium, containing 2.5 mU/ml of highly purified ovine LH and vary-

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ing amounts of oxytocin or purified porcine prolactin. 3×10^5 cells were cultivated and media were changed twice at 24 and 48 h. Progesterone was measured in media obtained from the following 48 h incubation period. Granulosa cells: follicles of porcine ovaries were punctured and the granulosa cells aspirated with the follicular fluid. After washing with TC199 medium cells were cultivated at 10^6 per dish in the presence of 2.5 mIU LH and 2.5 mIU FSH. Oligonucleotides used in hybridisation experiments were synthesised by means of a model 381A DNA-synthesizer from Applied Biosystems utilising phosphoramidite chemistry. Blot hybridisations were carried out on Gen-Screen (NEN, Dreieich) following the instructions of the manufacturer. Solution hybridisation experiments followed the published protocol of Durnam and Palmiter [5]. Poly (A^+) RNA was extracted from cells by the guanidine isothiocyanate method described in [6]. Poly(A^+) RNA was separated in formaldehyde-agarose gels after formamide denaturation [6] and transferred to Gen-Screen by electrophoretical transfer. Specific mRNAs were identified after hybridisation with a radioactive oligonucleotide probe followed by autoradiography. The synthetic oligonucleotides were phosphorylated employing T_4-polynucleotide kinase and [γ-^{32}P]ATP (spec. act. 3000 Ci/mmol) from Amersham-Buchler (Braunschweig) as in [6] to a specific activity of 10 μCi/pmol.

3. RESULTS

We synthesised the synthetic DNAs complementary to mRNAs of oxytocin, prolactin, inhibin as

relaxin (porcine) [7]:

5' GACTAATTCTCGGCCGCATGCCTTAATAAA 3'

oxytocin (bovine) [8]:

5' CCCAGGGGGCAGTTCTCTTGAATGTAGCA 3'

prolactin (human) [9]:

5' CATACCACGTACTTCCGTGACCAGATGATA 3'

inhibin (porcine) [10]:

5' CTGCAGCAGGCGCAGCGCGGCGGGGGA
CCAGGGCCAGGGCCAGGGCAGAGGGGCGGTGGA 3'

Fig.1. Oligonucleotide hybridisation probes.

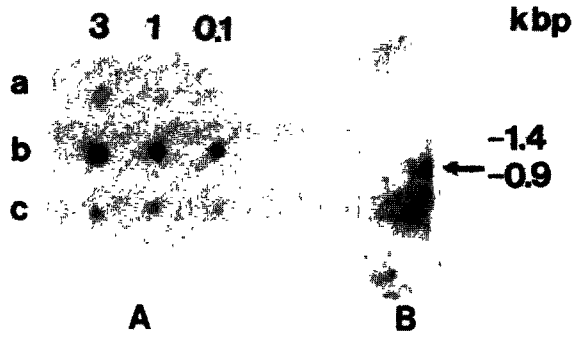

Fig.2. Hybridisation with relaxin-specific oligonucleotide. Numbers in vertical columns give μg poly(A^+) RNA or pBR322 DNA, respectively. (A) Dot blot hybridisation: (a) porcine granulosa cell poly(A^+) RNA, (b) porcine luteal cell poly(A^+) RNA, (c) pBR322 DNA. (B) Northern hybridisation.

well as relaxin making use of the known corresponding cDNA sequences (fig.1). Relaxin was chosen as a model system to evaluate the experimental details because concentrations of this hormone are reportedly high in luteal cells [7]. The results depicted in fig.2A indicate that the relaxin sequence hybridised specifically with relaxin mRNAs in porcine granulosa and luteal cells: Northern blot analysis revealed the presence of an mRNA species of approx. 1000 bp (fig.2B). As expected the levels of relaxin mRNA in granulosa cells were significantly lower than those in luteal cells. Dot blot hybridisation with the oxytocin probe was carried out with poly(A^+) RNA from bovine granulosa cells, porcine granulosa and porcine luteal cells. The former served as an internal reference exhibiting relatively high amounts of oxytocin mRNAs; low but significant levels of oxytocin mRNAs were measured in porcine granulosa and luteal cells (fig.3A). Quantitative evaluation of this dot blot experiment is detailed in table 1. Solution hybridisation with the same system furnished similar results (fig.4) although the quantitative dot blot experiments seemed to us more reproducible. Dot blot experiments demonstrated the presence of low but significant levels of prolactin mRNAs in porcine luteal cells; in granulosa cells such mRNAs were at the borderline of detection (fig.3B). Inhibin mRNAs were practically absent in luteal but present in significant amounts in granulosa cells (fig.3C).

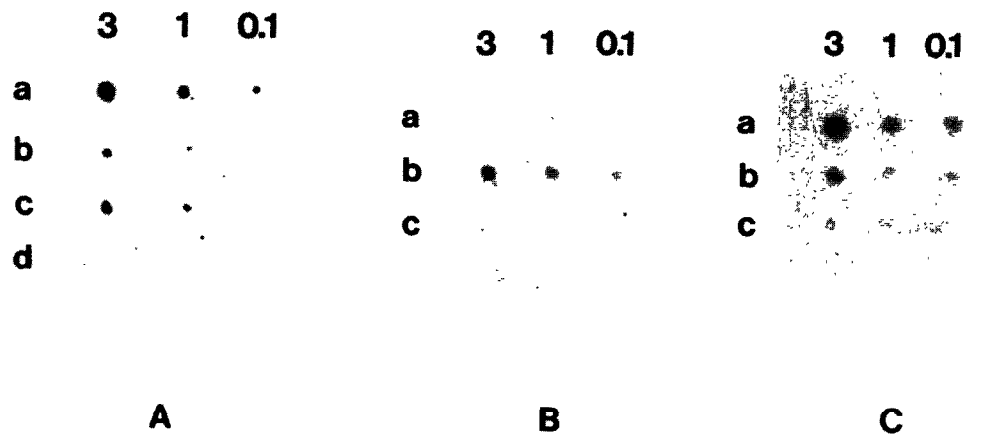

Fig.3. Dot blot hybridisation experiments. (A) Oxytocin-specific oligonucleotide: (a) bovine granulosa cell poly(A^+) RNA, (b) porcine granulosa cell poly(A^+) RNA, (c) porcine luteal cell poly(A^+) RNA, (d) pBR322 DNA. (B) Prolactin-specific oligonucleotide: (a) porcine granulosa cell poly(A^+) RNA, (b) porcine luteal cell poly(A^+) RNA, (c) pBR322 DNA. (C) Inhibin-specific oligonucleotide: (a) porcine granulosa cell poly(A^+) RNA, (b) porcine luteal cell poly(A^+) RNA, (c) pBR322 DNA.

Table 1

Levels of oxytocin mRNAs in granulosa cells and luteal cells

	Hybridised oligonucleotide/ poly(A^+) RNA (cpm/μg)	Relative levels of mRNA (%)
Bovine granulosa cells	1900	100
Porcine granulosa cells	370	19
Porcine luteal cells	560	29

Appropriate squares of blot hybridisation filters employed in dot blot experiments were cut out and the radioactivity measured

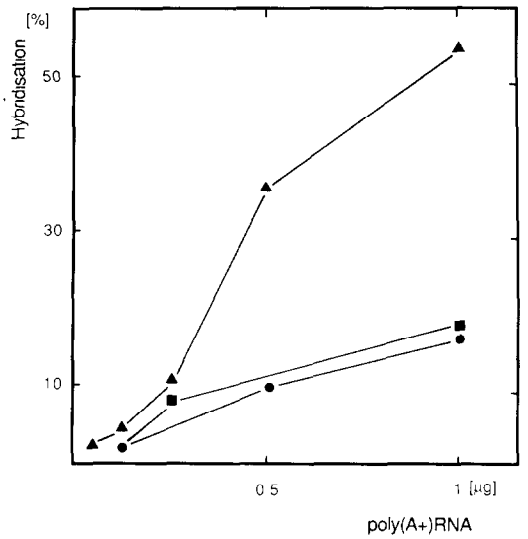

Fig.4. Solution hybridisation with oxytocin-specific oligonucleotide. The values depicted on the ordinate give the degree of hybridisation relative to the total input of oligonucleotide in %. Bovine granulosa cells (▲), porcine granulosa cells (●), porcine luteal cells (■).

Fig.5 details the influence of oxytocin and prolactin on progesterone release of luteal cells in vitro. Both basal and LH-stimulated progesterone secretion was dose-dependently inhibited by oxytocin and prolactin. Fig.6 reveals that oxytocin had no effect on LH/FSH-stimulated progesterone secretion in granulosa cells. Porcine prolactin stimulated progesterone secretion from granulosa cells.

4. DISCUSSION

We report here for the first time detectable levels of mRNAs of relaxin, oxytocin, prolactin as well

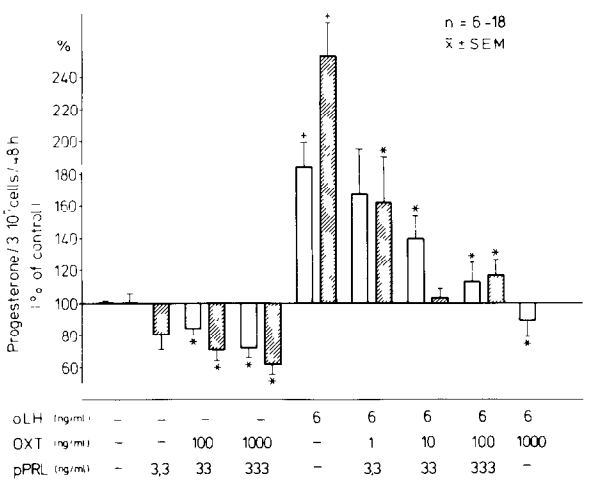

Fig.5. Effects of oxytocin (open bars) and prolactin (hatched bars) on basal and oLH-stimulated progesterone release by porcine luteal cells in vitro.

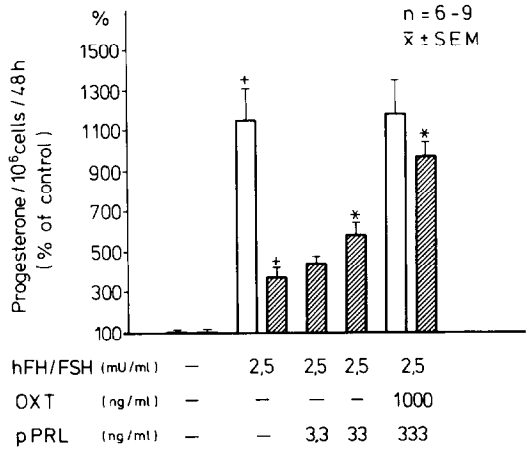

Fig.6. Effects of oxytocin (open bars) and prolactin (hatched bars) on gonadotropin (hLH + hFSH)-stimulated progesterone release by porcine granulosa cells in vitro.

as inhibin in the ovarian cell types, granulosa and luteal cells, indicating the existing potential of those cells to produce the respective hormones. The levels of mRNAs for oxytocin and prolactin are lower in porcine granulosa cells than in luteal cells suggesting lesser production in the former cell type. It is likely that they act in a paracrine fashion possibly by affecting steroidogenesis. Indeed both hormones inhibited progesterone secretion by luteal cells in vitro. While oxytocin was ineffective in altering progesterone secretion from granulosa cells, prolactin had a strong stimulatory effect. The differential effects of these two hormones on progesterone secretion in the two cell types seem to indicate a high degree of specificity.

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