0013-7227/88/1232-1205$02.00/0
Endocrinology
Vol. 123, No. 2
Printed in U.S.A.

RECEPTORS FOR GONADOTROPIN RELEASING HORMONE ARE PRESENT IN RAT OOCYTES.

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ABSTRACT: Specific receptors for gonadotropin releasing hormone (GnRH) in the rat oocyte have been identified by using two independent methods. Light microscopic autoradiography, utilizing an iodinated biologically active photoaffinity derivative of GnRH, revealed specific binding of the neurohormone to rat oocytes. Furthermore, the presence of GnRH-receptor is also evident from indirect fluorescent immunocytochemistry that shows binding of GnRH-receptor antibodies to rat oocytes which is neither detected with non immune serum nor with antiserum depleted of GnRH-receptor antibodies. These antibodies to the GnRH-receptor, also bind to both cumulus and granulosa cells but not to rat basophilic leukemia cells. The presence of specific GnRH receptors on rat oocytes provides an experimental basis for understanding the molecular events involved in GnRH-induced oocyte maturation.

Gonadotropin-releasing hormone (GnRH) mediates the hypothalamic control of pituitary gonadotropin secretion and biosynthesis. However, recent studies have shown that, at least in the rat, GnRH and its agonist analogs could also elicit ovarian responses, both in vitro and in vivo (1). One of the direct responses to GnRH in the rat ovary is stimulation of oocyte maturation (2-4).

It is well established that the follicular oocyte in mammals is arrested at the prophase of the first meiotic division and that the physiological stimulus for resumption of meiosis is provided by the preovulatory surge of luteinizing hormone (LH) (5). Since the presence of LH receptors on the oocyte has not been shown, it was suggested that LH induces oocyte maturation indirectly by interaction with the somatic follicular cells (6,7). Similar to LH, GnRH could also induce oocyte maturation indirectly, via activation of specific GnRH receptors on granulosa cells (8-11). Alternatively, GnRH could stimulate meiosis reinitiation by direct interaction with the oocyte.

The presence of receptors in general and GnRH receptors in particular on mammalian oocytes has not been tested as yet, probably due to the great technical difficulty in obtaining sufficient cellular material to allow the use of the common binding assays. However, utilization of a photoaffinity labeling technique combined with autoradiography (12,13) and the recent production of antibodies to the GnRH receptor (14) enabled us to analyze oocytes for the presence of GnRH receptors at the single cell level. We now report that both a GnRH analog and the antibody to the GnRH receptor bind to rat oocytes in a specific manner suggesting the presence of receptors for this neurohormone on the gamete of the female rat. These findings provide an experimental basis for the understanding of the molecular events involved in GnRH-induced oocyte maturation, which has so far been virtually totally ambiguous.

MATERIALS AND METHODS

[Azidobenzoyl-D-Lys^6]-GnRH was prepared and iodinated as previously described (11). Cumulus-oocyte complexes were isolated from the ovarian follicles of twenty-seven-day-old Wistar-derived female rats of our departmental colony, injected with pregnant mare's serum gonadotropin (PMSG, Gestyl Organon, 15 IU/rat) and killed 48 h later. Some oocytes were mechanically treated to remove the attached cumulus cells and the zona pelucida was digested by proteolysis (5 min exposure to 0.01% α-chemotrypsin). For autoradiography either cumulus-oocyte complexes or cumulus-free oocytes were incubated (90 min at 4°C, in the dark) with 10^{-11}M of the ^{125}I-labeled photoreactive GnRH analog, [azidobenzoyl-D-Lys^6]GnRH, in the absence or presence of 10^{-7}M unlabeled [D-Lys^6]GnRH. Cells were transferred through two washes of phosphate buffered saline, photolyzed (5 min., 4°C) and fixed in glutaraldehyde. Following washes, cells were transferred to poly-L-lysine-coated slides. The slides were coated with Ilford L-4 liquid emulsion, stored in the dark (21 days at 4°C) and subsequently developed using standard procedures. Light microscopy was performed using a Zeiss Photomicroscope III.

Indirect immunofluorescence was performed on either cumulus-oocyte complexes or cumulus-free oocytes fixed in methanol and incubated (overnight at 4°C) with antibodies to the GnRH-receptor (diluted 1:100 in PBS). The cells were washed and subsequently incubated (1 h at room temperature) with fluorescein-conjugated anti-rabbit IgG (Bio-Makor, Israel). The slides were washed, mounted and the samples examined using a Zeiss Fluorescent Photomicroscope III.

RESULTS

Photoactivation of rat oocytes and cumulus cells with the [^{125}I]-labeled GnRH analog and subsequent autoradiographic analysis revealed that the silver grains were associated with the oocytes (Fig. 1a). Essentially all the binding was displaced by an excess of unlabeled hormone (Fig. 1b), indicating that GnRH binds specifically to oocytes. Specific binding of the GnRH analog was also demonstrated in cumulus (Fig. 1c,d), and granulosa cells (data not shown) isolated from the same ovarian follicles.

The binding of the GnRH-receptor antibody to the oocyte was demonstrated by indirect fluorescent immunocytochemistry (Fig. 2a,b). The possibility that the second antibody binds to the oocytes in a non-specific manner can be excluded, since no fluorescence could be detected when the first antibody was replaced by non immune serum (Fig. 2c,d). Supportive evidence for the specificity of the GnRH-receptor-antibody binding is provided by the fact that the fluorescence was not observed when the antiserum was previously incubated with granulosa

Received in Iowa City, May 23, 1988.

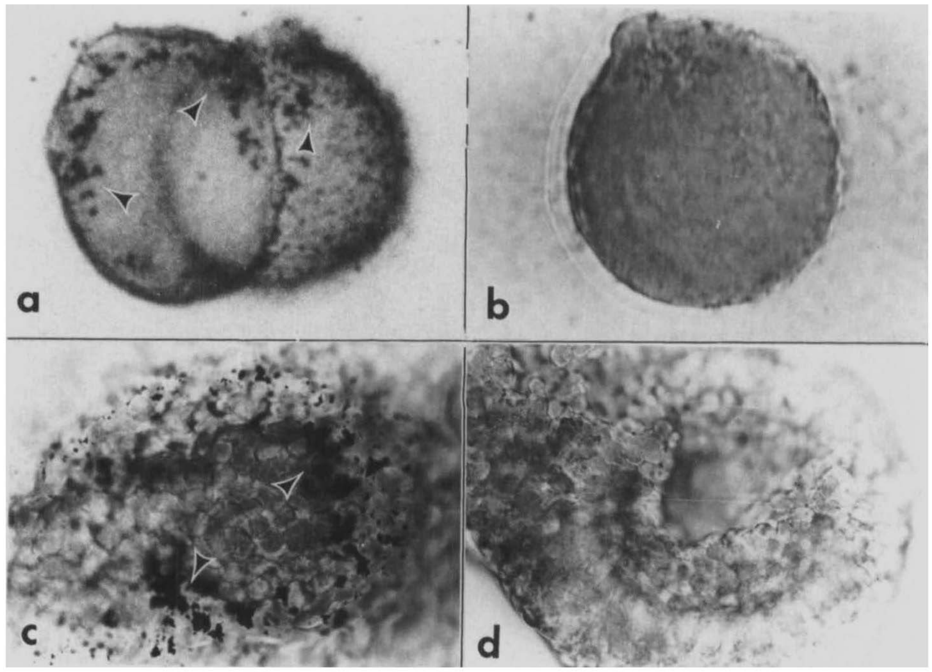

Fig. 1: Autoradiographs of rat oocytes and cumulus cells incubated with [^{125}I] labeled-photoreactive GnRH analog. Isolated oocytes (a,b) and cumulus-oocyte complexes (c,d) were incubated (4ºC, in the dark) with the [^{125}I]GnRH analog in the presence (b,d) or absence (a,c) of 10^{-7} M unlabeled GnRH analog. After 90 min, the cells were photolysed, washed and processed for autoradiography as described in the Methods section. Arrows (a,c) indicate the presence of clusters of silver grains on oocytes and cumulus cells. Experiments were repeated at least 8 times.

cells to deplete the GnRH-receptor antibodies, or when the oocytes were preincubated with antibodies to an irrelevant hormone, e.g., αTSH (data not shown). Using indirect fluorescent immunocytochemistry we could also demonstrate binding of the anti GnRH-receptor antibodies to both cumulus and granulosa cells (Fig. 2e,f), but failed to detect any interaction of the antibody with rat basophilic leukemia cells that do not possess receptors for GnRH (data not shown).

DISCUSSION

The results obtained by using these two independent methods indicate, for the first time, that specific receptors for GnRH are not only present on the somatic components of rat ovaries, such as granulosa and luteal cells (8-11), but also present on the oocyte. Virtually all the oocytes examined by both experimental methods were labeled.

Several studies have demonstrated that GnRH, like LH, can stimulate rat oocytes to mature (2-4). Furthermore, we have recently reported that oocytes undergoing maturation in response to either GnRH or LH are equally able to be fertilized and develop further into a 2-cell embryo (15). Nevertheless, our results strongly suggest that GnRH actions are not mediated through the LH receptor since a GnRH antagonist, that totally blocked GnRH-induced oocyte maturation, failed to affect LH action on the oocyte (16).

The present findings that rat oocytes can respond to GnRH via direct interaction of the hormone with specific receptors on the female gamete raises a question concerning the origin of the hormone. Because of the specialized portal blood system, which transports GnRH from the hypothalamus to the pituitary, and because the GnRH concentration in the systemic circulation is undetectable, it seems that GnRH secreted by the hypothalamus is too low to exert any physiological effect on the oocyte. However, Aten et al. (17,18) have recently identified in rat, bovine and ovine ovaries, a substance which has binding properties similar to those of GnRH, but is immunologically distinct from GnRH. Thus, it is possible that a GnRH-like peptide is synthesized locally in the ovary and could interact with GnRH receptors on the oocyte to induce oocytes maturation.

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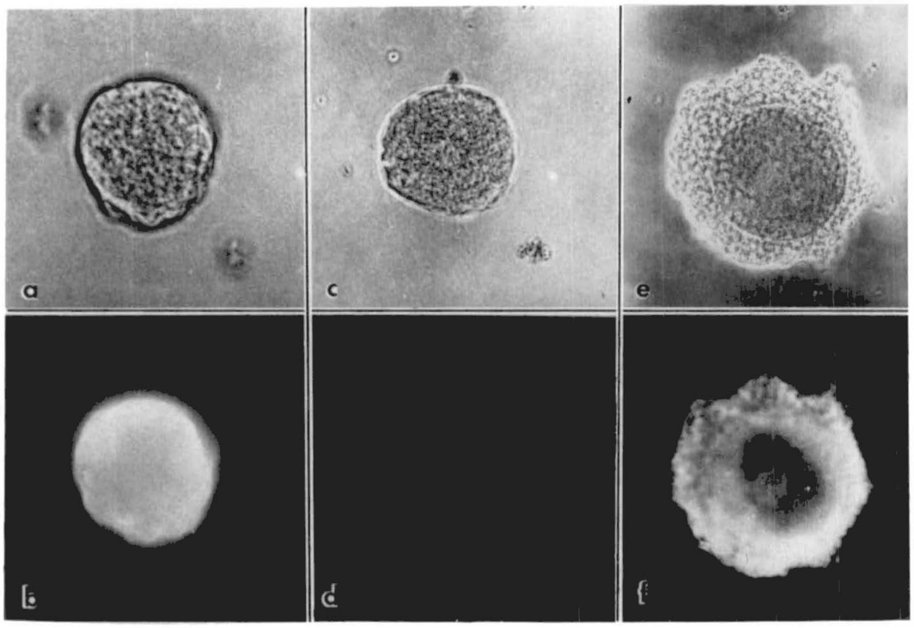

Fig. 2: Immunofluorescent micrographs of oocytes and cumulus-oocyte complexes treated with anti GnRH-receptor antibodies. Oocytes and cumulus-oocyte complexes were isolated, fixed and incubated with antibodies to GnRH-receptor. Following washes, the cells were incubated with fluorescein-conjugated anti-rabbit IgG, and examined using a Zeiss Fluorescent Photomicroscope III. Top micrographs: phase contrast microscopy; bottom micrographs: fluorescence microscopy of the same fields; a,b. An isolated oocyte incubated with anti GnRH-receptors antibodies followed by fluorescein-conjugated second antibody; c,d. An isolated oocyte was treated as in a,b but with non immune serum replacing the first antibody; e,f. A cumulus-oocyte complex incubated with the anti-GnRH-receptor antibodies followed by fluorescein-conjugated second antibody. Experiments were repeated at least 8 times.

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