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A Comparative Study of the Mechanism of Action of Luteinizing Hormone and a Gonadotropin Releasing Hormone Analog on the Ovary

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ABSTRACT

The mechanism of action of a gonadotropin releasing hormone (GnRH) agonistic analog ([D-Ala^6] GnRH) on the rat ovary has been studied in comparison to similar effects of luteinizing hormone (LH). Stimulation of meiosis resumption in vitro in follicle-enclosed oocytes by both LH and [D-Ala^6] GnRH, was blocked by elevated levels of cAMP as demonstrated when either dibutyryl cAMP or the phosphodiesterase inhibitor methylisobutylxanthine was present in the culture medium. In vivo, the prostaglandin synthase inhibitor indomethacin, which blocks LH-induced ovulation, also inhibited ovulation induced by the GnRH analog in hypophysectomized rats. On the other hand, the potent GnRH-antagonist [D-pGlu^1, pClPhe^2, D-Trp^{3,6}] GnRH which blocked the stimulatory effect of the agonist on oocyte maturation and ovulation had no effect on LH action. It is concluded that while a GnRH-like peptide does not seem to mediate LH action on the ovarian follicles, both LH and GnRH agonist share some common mechanistic pathways at a post-receptor locus.

INTRODUCTION

Direct effects of gonadotropin-releasing hormone (GnRH) and its agonistic analogs, both stimulatory and inhibitory, on the rat ovary have been recently demonstrated (for review see Hseuh and Jones, 1981). Among the stimulatory effects, resumption of meiosis in vitro in follicle-enclosed oocytes (Hillensjö and LeMaire, 1980), prostaglandin formation (Clark et al., 1981) and stimulation of both oocyte maturation and ovulation in vivo in hypophysectomized rats (Ekholm et al., 1981, Corbin and Bex, 1981), are included. Inhibition by GnRH is demonstrated on follicle-stimulating hormone (FSH)-induced luteinizing hormone (LH) and prolactin receptor formation, and on LH-induced ovarian weight gain (Hseuh and Jones, 1981). Although GnRH receptor sites are present in ovarian tissue (Clayton et al., 1979) and a GnRH-like peptide was extracted from rat ovaries (Ying et al., 1981), it is not yet clear whether these findings have implications for normal gonadal physiology.

Since GnRH and its analogs seem to mimic the effect of LH on the ovary in terms of prostaglandin formation, oocyte maturation and ovulation, the present study was undertaken to investigate whether LH and GnRH agonists share a common mechanism of action on follicle-enclosed oocytes. Alternatively, experiments were conducted to study whether the locally produced substance with GnRH-like activity may mediate the gonadotropin action and thus participate in the coordination of follicular development and function.

MATERIALS AND METHODS

Hypophysectomy was performed on mature Wistar proestrous female rats and followed by a subcutaneous injection of 15 IU of pregnant mare's serum gonadotropin (PMSG, Gestyl, Organon, Holland) in 0.1 ml of 0.9% NaCl. PMSG treatment was previously shown to preserve the majority of the preovulatory follicles in hypophysectomized rats (Braw et al., 1981). An intraperitoneal injection of either the indicated dose of human chorionic gonadotropin (hCG, Pregnyl, Organon, Holland) or a dose of 500 ng per rat of [D-Ala^6] GnRH (GnRHa, Peninsula Lab., San Carlos, CA) was given 24 h later. A second group of the hypophysectomized rats was injected with one of these hormones in combination with either 5 μg per rat of a GnRH-antagonist [D-pGlu^1, pCl-Phe^2, D-Trp^{3,6}] GnRH (kindly provided by Dr. D. Coy, Tulane University, New Orleans, LA) or 2 mg per rat of indomethacin (1-[p-chlorobenzoyl]-5-methoxy-2-methylindol-3-acetic acid, Assia Chemical Labs., Israel). The rats were killed by cervical disolaction 20 h after injection. The oviducts were removed and examined for the presence of ovulated oocytes. The completeness of hypophysectomy was checked by

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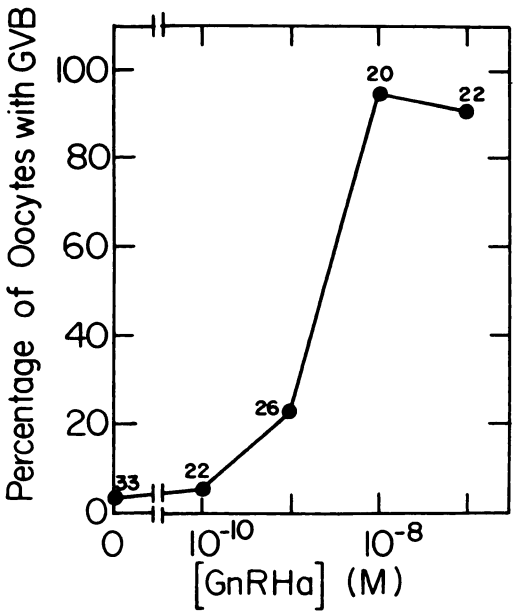

FIG. 1. Dose dependency of GnRHa effect on GV breakdown in follicle-enclosed oocytes. Isolated follicles were incubated in the indicated concentration of GnRHa for 17 h. Cumulus-oocyte complexes were recovered and the oocytes were examined for the presence of GV's as described in *Materials and Methods*. The numbers associated with the graph represent the amount of oocytes examined at each experimental point.

examination of the sella turcica and the peripheral serum was assayed for the presence of LH.

Follicles were dissected from 26-day-old female rats injected subcutaneoulsy with 15 IU of PMSG, and killed by cervical dislocation 48 h later. Groups of 50 individual follicles were incubated in 2.5 ml of Leibovitz's L-15 tissue culture medium (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (Sera-Lab, England), penicillin (100 units/ml) and streptomycin (100 μg/ml) (Gibco), in the presence or absence of the indicated concentrations of the following agents: GnRHa, GnRH antagonist, ovine LH (oLH, NIH LH S-20), dibutyryl 3':5'-cyclic monophosphate (dbcAMP, Sigma Chemical Co., St. Louis, MO) or methylisobutylxanthine (MIX) (Sigma Chemical Co.). Incubations were carried out in a N_2:O_2 (1:1) atmosphere in an oscillating bath at 37°C. At the indicated time the follicles were incised and the cumulus-oocyte complexes were recovered. The oocytes were examined using an inverted microscope equipped with Nomarski interference contrast optics. Resumption of meiosis was indicated by the absence of the germinal vesicle (GV) in the oocyte. For each study, the data of several individual experiments were combined and the results are reported as the fraction of oocytes with GV breakdown (GVB).

RESULTS

In the first part of this study the in vitro effect of LH and GnRHa on resumption of meiosis in follicle-enclosed oocytes was compared and the effect of a potent GnRH-antagonist on the stimulatory action of both these hormones was examined. As shown in Fig. 1, GnRHa induced the resumption of meiosis in follicle-enclosed oocytes in a dose-dependent manner with an ED_{50} at a concentration of $2x10^{-9}$ M. The stimulation of GV breakdown by a maximal effective dose of GnRHa (10^{-7} M) was completed in 93% of the oocytes by 8 h of incubation; 50% of the oocytes responded after 4 h (Fig. 2). Induction of GV breakdown by LH is relatively faster; while 50% of the oocytes responded after less than 3 h, a 5-h incubation period was enough for 95% of the oocytes to resume their meiotic division (Fig. 2). Concomitant addition of a potent GnRH antagonist, [D-pGlu[1], DpClPhe[2], D-Trp[3,6]] GnRH, at a concentration of 10^{-5} M, totally blocked the stimulatory effect of GnRHa (Table 1). However, this antagonist failed to

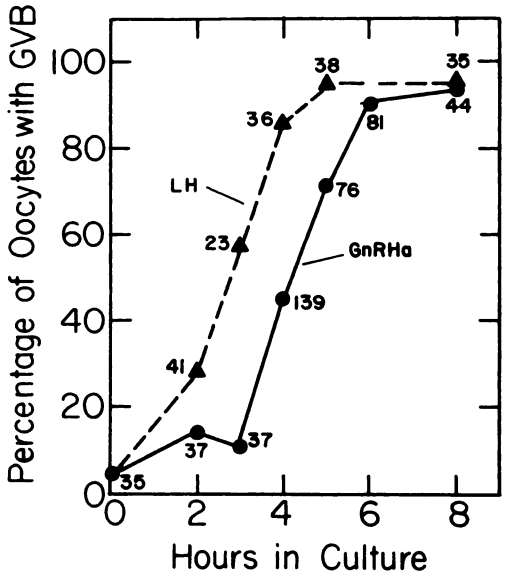

FIG. 2. Time course of GV breakdown in follicle-enclosed oocytes. Isolated follicles were incubated in the presence of either GnRHa (10^{-7} M) or oLH (5 μg/ml) for the indicated times. Cumulus-oocyte complexes were recovered and the oocytes were examined for the presence of GV's. The numbers associated with the graph represent the amount of oocytes examined at each experimental point.

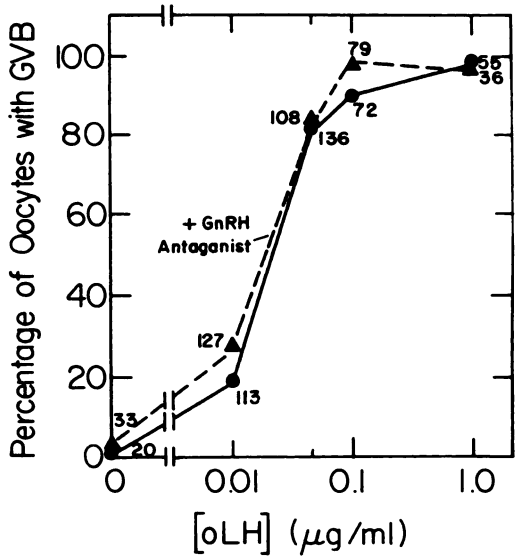

FIG. 3. Effect of GnRH antagonist on LH-induced maturation in follicle-enclosed oocytes. Isolated follicles were incubated in the presence of the indicated concentrations of oLH with or without GnRH antagonist (10^{-5} M) for 17 h. Cumulus oocyte complexes. were recovered and the oocytes were examined for the presence of GV's. The numbers associated with the graph represent the amount of oocytes examined at each experimental point.

inhibit GV breakdown in follicle-enclosed oocyte stimulated by LH (Table 1 and Fig. 3). cAMP effectively blocks LH-induced maturation in follicle-enclosed oocytes (Dekel et al., 1981). To examine whether this nucleotide can also interfere with the stimulatory action of GnRHa in this system, the incidence of GV breakdown was monitored when either the membrane soluble dibutyryl derivative of cAMP or the phosphodiesterase inhibitor MIX was present in the culture medium. As seen in Table 1, both modulators of cAMP levels totally blocked LH as well as GnRHa stimulatory action on oocyte maturation. A dose-dependent analysis of the effect of MIX (Fig. 4) revealed that a concentration as low as 50 μM totally prevented the action of the maximal effective doses of both hormones.

Hypophysectomized rats were used in the second part of this study to examine and compare the in vivo effects of both LH and GnRHa on ovulation. As seen in Table 2, injection of either hCG (either 2 or 4 IU) or GnRHa (500 ng) induced ovulation in PMSG-treated hypophysectomized rats. However, while the effect of GnRHa was completely blocked by the GnRH antagonist (5 μg), no inhibition by GnRH antagonist could be demonstrated even when the minimal effective dose of hCG (2 IU) was used to induce ovulation. On the other hand, indomethacin, which is an effective inhibitor of prostaglandin synthesis and is known to block LH-induced ovulation (Tsafriri et al., 1973) could also block ovulation in the GnRHa-stimulated rats (Table 3).

TABLE 1. Effect of GnRH antagonist, dbcAMP and MIX on either LH or GnRHa-induced GV breakdown in follicle-enclosed oocytes.

Additions to Medium[a]					GVB	
oLH (0.1 μg/ml)	GNRHa (10^{-7} M)	GNRH antagonist (10^{-5} M)	dbcAMP (5 mM)	MIX (0.2 mM)	Percentage of oocytes	Total no. of oocytes
–	–	–	–	–	2	65
+	–	–	–	–	93	96
–	+	–	–	–	97	149
–	–	+	–	–	3	33
–	–	–	–	+	9	47
–	+	+	–	–	11	56
+	–	+	–	–	99	79
–	+	–	–	+	13	70
+		–	–	+	1	70
–	+	–	+	–	5	42
+	–	–	+	–	4	45

[a]Isolated follicles were incubated in the presence of the indicated concentrations of either oLH or GnRHa with or without either GnRH antagonist, dbcAMP or MIX. After 20 h the oocytes were recovered and examined for the presence or absence of GVs.

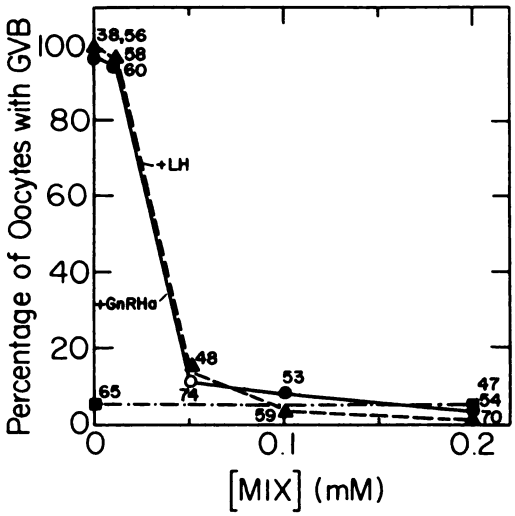

FIG. 4. Effect of MIX on LH- or GnRH-induced maturation in follicle-enclosed oocytes. Isolated follicles were incubated in the presence of the indicated concentrations of MIX with or without either oLH (5 μg/ml) or GnRHa (10^{-7} M) for 17 h. Cumulus-oocyte complexes were recovered and the oocytes were examined for the presence of GV's. The numbers associated with the graph represent the amount of oocytes examined at each experimental point.

DISCUSSION

Similar to earlier reports (Hillensjö and LeMaire, 1980; Ekholm et al., 1981; Corbin and Bex, 1981) we have also demonstrated that a GnRH agonistic analog is a potent inducer of the resumption of meiosis in follicle-enclosed oocytes in vitro, as well as a trigger of both oocyte maturation and ovulation in vivo, in hypophysectomized rats. In addition, we have shown that a GnRH antagonist which effectively blocked these stimulatory actions of GnRHa on the ovary failed to interfere with LH action. However, both hormones were inhibited in vitro by elevated levels of cAMP and in vivo when prostaglandin synthesis was blocked.

It seems likely that the ovulatory response of the follicles to GnRHa is a result of the stimulatory effect of this hormone on prostaglandin synthesis, which has been demonstrated in whole ovaries in vivo (Ekholm et al., 1982), as well as in cultured granulosa cells (Clark et al., 1981). The fact that indomethacin, which completely abolished the rise in PGE synthesis in vivo also blocked ovulation in the GnRH-treated rats, as shown originally by Ekholm et al. (1982) and confirmed in our study, clearly supports this idea. However, the mechanism by which reinitiation of meiosis occurs under the influence of GnRH is not clear as yet.

The demonstrations of the direct action of GnRH on gonadal cells were followed by the characterization of stereospecific, tissue-specific high-affinity gonadal sites for the hormone (Clayton et al., 1981). Our and other reports (Behrman et al., 1980) suggest that GnRH does not share its receptors with LH, since a potent GnRH antagonist blocked GnRH but not LH actions on the ovary. Nevertheless, our present study implies that these two hormones may operate in the follicle via a related or common mechanism at a post-receptor locus. LH-induced maturation in follicle-enclosed oocytes is blocked by cAMP (Dekel et al., 1981), as well

TABLE 2. Effect of GnRH antagonist on either GnRHa or hCG-induced ovulation in hypophysectomized rats.[a]

Hormone administered (dose/rat)	Fraction of ovulating rats	Oocytes/ovulating rat
GnRHa (500 ng)	6/6	8.3
GnRHa (500 ng) + GnRH antagonist (5 μg)	1/7	9.0
hCG (4 IU)	4/5	11.0
hCG (4 IU) + GnRH antagonist (5 μg)	5/6	12.0
hCG (2 IU)	5/8	8.8
hCG (2 IU) + GnRH antagonist (5 μg)	10/14	8.4

[a]GnRH antagonist (5 μg) was injected concomitantly with the indicated doses of either GnRHa or hCG to hypophysectomized mature rats. The oviducts were isolated and examined for the presence of ovulated oocytes 20 h after the injection.

TABLE 3. Effect of indomethacin on GnRHa-induced ovulation in hypophysectomized rats.[a]

Agent administered (dose/rat)	Fraction of ovulating rats	Oocytes/ovulating rat
GnRHa (500 ng)	3/3	7.0
GnRha (500 ng) + indomethacin (2 mg)	0/8	—

[a]Indomethacin (2 mg) was injected concomitantly with an ovulatory dose (500 ng) of GnRHa to hypophysectomized mature rats. The oviducts were isolated and examined for the presence of ovulated oocytes 20 h after the injection.

as by phosphodiesterase inhibitors (Tsafriri et al., 1972; Hillensjö, 1976). The present study demonstrates that elevated levels of cAMP caused by the presence of either dbcAMP or the phosphodiesterase inhibitor MIX, not only inhibit LH, but also GnRHa action. It seems likely that the effect of MIX in our study is attributed solely to its inhibitory influence on phosphodiesterase activity, since relatively low concentrations (IC_{50}=30 μM) of this agent exhibit a complete inhibitory effect. Our findings, that a phosphodiesterase inhibitor interferes with GnRH action, agree with a recent study which demonstrates that GnRH increases phosphodiesterase activity in cultured granulosa cells (Knecht and Catt, 1981). Since oocyte maturation is blocked by cAMP, we suggest that GnRH may induce maturation by inhibition of cAMP accumulation leading to decreasing cAMP levels in the oocyte. A drop in cAMP levels in the oocyte can be a result of a direct interaction of the hormone with the gamete, as well as an outcome of its effect on the somatic cells of the follicle. Determination of the cellular target for GnRH action is open for further investigation.

Whether GnRH has a physiological role in the control of ovarian function is still not clear. Although the gonadal binding sites to GnRH fulfill all the requirements of classical hormone receptor interaction, the ovary is normally not exposed to effective concentrations of endogenous GnRH of hypothalamic origin. However, since recent studies suggest the possible production of a GnRH-like peptide(s) by the ovarian cells (Ying et al., 1981), a possible role for the GnRH-like factor in regulation of oocyte maturation and release may still be considered. Our present findings, that the time course of GnRH action on meiosis is much more retarded than that of LH, suggest that this hormone cannot act as a local mediator for LH action. In addition, the fact that the GnRH antagonist failed to inhibit the LH effects on both oocyte maturation and ovulation, can also serve as a support for this speculation. However, it is still possible that the gonadal peptide is similar but not identical to the pituitary GnRH. Thus, the antagonist to the pituitary hormone may not interfere with the action of the ovarian ligand. Until a specific antagonist to the ovarian GnRH-like substance is used, this question remains open.

Whether a GnRH-like substance acts as a local regulator of ovarian function and development or not, it still can provide a very useful means to get an insight into the mechanism of maturation of the mammalian oocyte which is obscure as yet.

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REFERENCES

Behrman, H. R., Preston, S. L. and Hall, A. K. (1980). Cellular mechanism of the antigonadotropic action of luteinizing hormone releasing hormone in the corpus luteum. Endocrinology 107: 656–664.

Braw, R. H., Bar-Ami, S. and Tsafriri, A. (1981). Effect of hypophysectomy on atresia of rat preovulatory follicles. Biol. Reprod. 25: 989–996.

Clark, M. R. (1982). Stimulation of progesterone and prostaglandin E accumulation by luteinizing hormone releasing hormone (LHRH) and LHRH analogs in rat granulosa cells. Endocrinology 110:146–152.

Clayton, R. N., Harwood, J. P. and Catt, K. J. (1979). Gonadotropin-releasing hormone analogue binds

to luteal cells and inhibits progesterone production. Nature 282:90–92.

Corbin, A. and Bex, F. J. (1981). Luteinizing hormone releasing hormone agonists induce ovulation in hypophysectomized proestrous rats: Direct ovarian effect. Life Sci. 29:185–192.

Dekel, N., Lawrence, T. S., Gilula, N. B. and Beers, W. H. (1981). Modulation of cell-to-cell communication in the cumulus-oocyte complex and the regulation of oocyte maturation by LH. Dev. Biol. 86:356–362.

Ekholm, C., Clark, M. R., Magnusson, C., Isaksson, O. and LeMaire, W. J. (1982). Ovulation induced by gonadotropin releasing hormone analog in hypophysectomized rats involves prostaglandins. Endocrinology 110:288–290.

Ekholm, C., Hillensjö, T. and Isaksson, O. (1981). Gonadotropin-releasing hormone agonists stimulate oocyte meiosis and ovulation in hypophysectomized rats. Endocrinology 108:2022–2024.

Hillensjö, T. (1976). Oocyte maturation and glycolysis in isolated preovulatory follicles of PMS-injected immature rats. Acta Endocrinol. 82:809–830.

Hillensjö, T. and LeMaire, W. J. (1980). Gonadotropin releasing hormone agonists stimulate meiotic maturation of follicle-enclosed rat oocytes in vitro. Nature 287:145–146.

Hsueh, A.J.W. and Jones, P.B.C. (1981). Extrapituitary actions of gonadotropin-releasing hormone. Endocr. Rev. 2:437–461.

Knecht, M. and Catt, K. J. (1981). Gonadotropin-releasing hormone: Regulation of adenosine 3′,5-monophosphate in ovarian granulosa cells. Science 214:1346–1348.

Tsafriri, A., Lindner, H. R., Zor, U. and Lamprecht, S. A. (1972). In vitro induction of meiotic division in follicle-enclosed rat oocytes by LH, cyclic AMP and prostaglandin E_2. J. Reprod. Fertil. 31:39–50.

Tsafriri, A., Koch, Y. and Lindner, H. R. (1973). Ovulation rate and serum LH levels in rats treated with indomethacin or prostaglandin E_2. Prostaglandins 3:461–467.

Ying, S. Y., Ling, N., Bohlen, P. and Guillemin, R. (1981). Gonadocrinins: Peptides in ovarian follicular fluid stimulating the secretion of pituitary gonadotropins. Endocrinology 108: 1206–1215.