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DISSOCIATION BETWEEN THE DIRECT STIMULATORY AND INHIBITORY EFFECTS OF A GONADOTROPIN-RELEASING HORMONE ANALOG ON OVARIAN FUNCTIONS

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The paradoxical effects of gonadotropin-releasing hormone (GnRH) on the ovary have hitherto been believed to result from different regimens of administration; an acute treatment was shown to stimulate the ovary while chronic administration of the hormone inhibited LH-induced responses. In the present report we demonstrate that a single injection of a GnRH analog (D-Ala^6)des-Gly^{10}-GnRH-N-ethylamide (GnRHa, 2 μg/rat) is sufficient to obtain a significant inhibition (75%) of hCG-induced ovulation in PMSG-primed, either intact or hypophysectomized, immature rats. Inhibition of ovarian development, in terms of growth and ovulation, by multiple injections with GnRHa (2 μg/rat, twice daily for 3 days) could be obtained only upon administration of the hormone at early stages of follicular development, i.e. concomitantly with the PMSG injection. When administered after PMSG, GnRHa could not inhibit the ovary but rather induced ovulation by itself in the absence of hCG. A 12–24 h delay in initiation of GnRHa treatment triggered 65% of the rats to ovulate while a delay of 48 h resulted in 100% ovulation. Under both regimes of GnRHa administration, either the inhibitory or the stimulatory, the oocytes of the treated rats were induced to resume meiotic maturation. Since under the inhibitory regime ovulation did not occur, maturation was followed by a massive degeneration of the oocytes trapped within their follicles. These findings demonstrate that the follicular stage of development rather than the dose and/or duration of GnRHa administration determines whether GnRHa inhibits ovarian growth and ovulation, while the competence of the oocytes to respond to the GnRHa stimulus and mature is independent of hormonal priming.

Keywords: rat; GnRH analog; oocyte maturation; ovulation.

Gonadotropin-releasing hormone (GnRH) stimulates the biosynthesis and release of pituitary luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Paradoxically, anti-fertility effects of GnRH and its agonistic analogs in vivo have also been observed (Rippel and Johnson, 1976; Corbin et al., 1977; Rivier et al., 1978; Nillius et al., 1978; Hsueh

and Erickson, 1979; Ying and Guillemin, 1979). These reports were followed by the findings that, in addition to its action on the pituitary, GnRH exerts a direct inhibitory effect on the gonads (Hsueh and Erickson, 1979; Ying and Guillemin, 1979; Clayton et al., 1979; Behrman et al., 1980; Hsueh and Jones, 1981). On the other hand it has recently been demonstrated that, besides their negative influence on ovarian functions, GnRH analogs can also stimulate maturation of follicle-enclosed rat oocytes in vitro and induce ovulation in hypophysectomized rats (Hillensjö and LeMaire, 1980; Ekholm et al., 1981; Corbin and Bex, 1981; Dekel et al., 1983).

In an attempt to dissociate the inhibitory from the stimulatory effects of a GnRH analog on the ovary, we have tested its influence at different stages of ovarian development. The hormone was administered to both intact and hypophysectomized rats either concomitantly with PMSG priming or at different time intervals following this treatment. Under these conditions the acute and chronic effects of the hormone were also analysed.

It has been thought that while the acute effects of GnRH analogs upon ovarian functions are stimulatory, the chronic action of the peptide is inhibitory (for review see Hsueh and Jones, 1981). The present report suggests that the follicular stage of development determines whether GnRH stimulates or inhibits ovarian function. In addition it calls for caution in the use of GnRH analogs in fertility control because of excessive recruitment of oocytes followed by a massive degeneration. The report also demonstrates a complete dissociation between GnRH action on oocyte maturation and induction of ovulation.

MATERIALS AND METHODS

Immature Wistar-derived female rats (from our departmental colony) were left intact or hypophysectomized (hypox) on day 26. All rats were injected subcutaneously (s.c.) on day 26 with 15 IU PMSG (Gestyl, Organon, The Netherlands). The rats were then divided into two experimental groups: (a) The first group was injected s.c. with (D-Ala^6)des-Gly^{10}-GnRH-N-ethylamide (GnRHa) twice daily (8 a.m. and 4 p.m.) throughout days 26–28 unless otherwise indicated; hCG (Pregnyl, Organon, The Netherlands) (5 or 10 IU, s.c.) was injected into these rats at 2 p.m. on day 28. (b) For the second experimental group, GnRHa treatment was initiated at the time intervals indicated after PMSG injection. These rats were not injected with hCG.

To analyse the stage of development of the ovarian oocytes, rats were

killed at the indicated time points after initiation of GnRHa treatment. Rats sacrificed on the morning of day 29 were examined for incidence of ovulation. In the hypox rats, the sella turcica was examined and only rats without pituitary fragments were examined further. To ensure completeness of hypophysectomy, serum LH levels were determined in selected groups of intact, hypox, and hypox-GnRHa-treated rats, using the rLH RIA kit supplied by the NIAMDD Hormone Distribution Program. Serum LH levels were about 100 ng/ml in control rats and about 14 ng/ml in hypox or in hypox-GnRHa-treated rats.

In both groups the oviducts were checked for the presence of ovulated oocytes. The ovaries were removed, weighed and placed in saline for microscopic examination. In the absence of corpora lutea, the ovarian antral follicles were incised to release the oocytes. Oocytes were examined microscopically by Nomarski Interference Contrast optics. Morphologically normal oocytes, containing a nuclear structure (germinal vesicle, GV), were defined as meiotically arrested or immature. Resumption of meiosis or maturation was indicated by the absence of a GV in the oocyte. Degenerated oocytes usually appeared as cytoplasmic fragments enclosed by the zona pellucida.

RESULTS

Administration of PMSG (15 IU) to immature (26-day-old) intact or hypophysectomized female rats, followed by injection of hCG (5 IU to intact and 10 IU to hypox rats) on day 28, resulted in ovulation of 47 ± 9.5 ($n = 7$) and 65 ± 9.6 ($n = 6$) oocytes/rat in intact and hypox rats, respectively. Since in preliminary experiments induction of ovulation by 5 IU hCG was limited to a small fraction of the hypox rats, the dose of hCG employed throughout the present study for this group of rats was increased to 10 IU. A single injection of GnRHa (2 μg/rat), concomitant with the administration of PMSG, reduced the number of hCG-induced ovulations to 9.7 ± 2.5 oocytes/rat (intact rats) and 16.5 ± 3.2 (hypox rats). Administration of GnRHa twice daily for 3 days effectively blocked hCG-induced ovulation (Fig. 1). The minimal effective dose of GnRHa capable of complete inhibition of ovulation was 0.7 and 2 μg/injection in the intact and hypox rat, respectively. In those rats in which ovulation was only partially inhibited, a progressive decrease in the number of ovulated oocytes/rat was noticed with increasing GnRHa concentrations (Fig. 2).

In the absence of GnRHa treatment the ovaries of either intact or hypox rats contained a large number of corpora lutea in combination

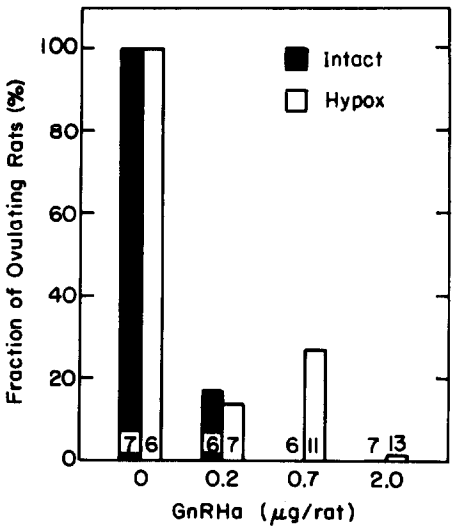

Fig. 1. Effect of GnRH agonist analog on hCG induction of ovulation. Immature (26-day-old) intact or hypophysectomized (hypox) female rats were injected with 15 IU PMSG. (D-Ala^6)des-Gly^{10}-GnRH-N-ethylamide (GnRHa) was injected twice daily (s.c.) for 3 days (8 a.m. and 4 p.m.) and hCG was injected at 2 p.m. on day 28 (5 and 10 IU in intact and hypox rats, respectively). Rats were sacrificed on the morning of day 29 and their oviducts were checked for the presence of ovulated oocytes. The number of rats is indicated in the bars.

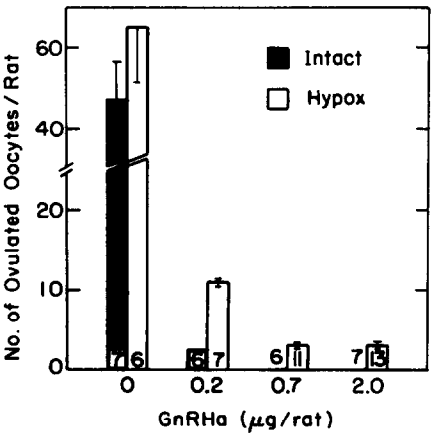

Fig. 2. Effect of GnRH analog (GnRHa) on the number of ovulated oocytes in PMSG-primed, hCG-treated immature rats. For protocol see legend to Fig. 1. The higher ovulation rate in the hypox rats is due to a higher dose of hCG (10 IU vs. 5 IU in the intact rat).

with a population of antral follicles of variable size (in the range of 0.2–0.8 mm diameter). Following multiple injections of GnRHa the ovarian follicles were uniformly small (< 0.4 mm diameter) and the overall weight was greatly decreased (Fig. 3). When submaximally effective doses of GnRHa were used, few corpora lutea could be observed, corresponding in number to the oocytes present in the oviduct. The follicles in these ovaries were small in size (< 0.4 mm diameter), similar to those in the fully inhibited rats. A significant decrease in the ovarian weight (intact 28.3 ± 0.5 vs. hypophysectomized 23.2 ± 1.36) could also be observed following a single GnRHa (2 μg/rat) injection.

Although the above regime was inhibitory in terms of follicular development and ovulation, GnRHa administration triggered to oocytes to resume meiotic maturation. Mature oocytes could be isolated from the ovaries as early as 12 h after GnRHa administration in either intact or hypox rats. The decrease in the fraction of mature ovarian oocytes observed at 48 h after GnRHa administration in the hypox rats corresponds to a significant increase in the fraction of degenerated oocytes (Fig. 4). The process of degeneration of the mature oocytes trapped in their follicles was completed within 72 h after starting GnRHa treatment. At this time 86% of the oocytes isolated from the follicles of the hypox rats appeared as fragments of cytoplasm enclosed by the zona pellucida.

When administration of GnRHa was delayed for various time inter-

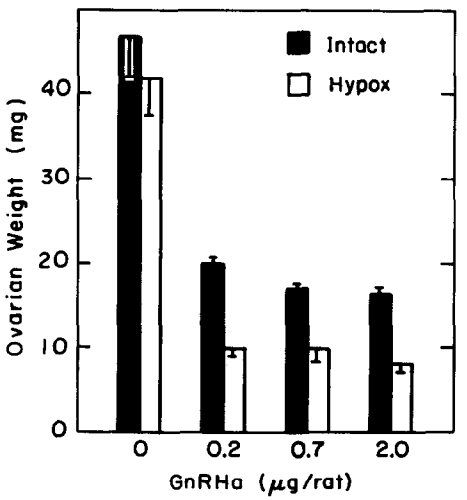

Fig. 3. Effect of GnRH analog (GnRHa) on ovarian weight gain in PMSG-primed, hCG-treated immature rats. For protocol see legend to Fig. 1. Bars indicate mean ± SEM of 6 determinations.

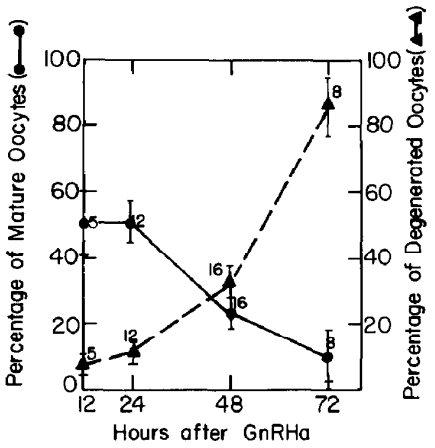

Fig. 4. Effect of GnRH analog (GnRHa) on the oocytes in PMSG (15 IU) primed immature hypophysectomized rats (26 days). GnRHa (2 μg/rat twice daily) was administered and the animals were sacrificed at the times indicated. The ovaries were removed and all the isolated oocytes examined microscopically. Degenerated oocytes appeared as cytoplasmic fragments enclosed by the zona pellucida. The numbers on the graph indicate the group size of rats used for each experimental point. About 200 oocytes were examined at each time point.

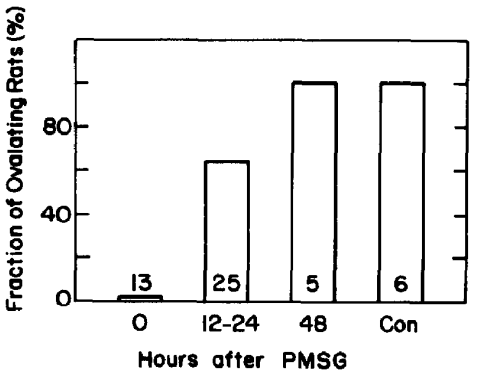

Fig. 5. Effect of delay in GnRHa administration on the fraction of ovulating rats in PMSG-primed hypophysectomized rats. Immature rats were hypophysectomized on day 26 and injected with 15 IU PMSG. GnRHa injections (2 μg/rat twice daily) were initiated at the time indicated after PMSG administration. Rats were sacrificed on the morning of day 29 and their oviducts were checked for the presence of ovulated oocytes. Control rats (Con) were given 10 IU of hCG on day 28. The number of rats is given in the bars.

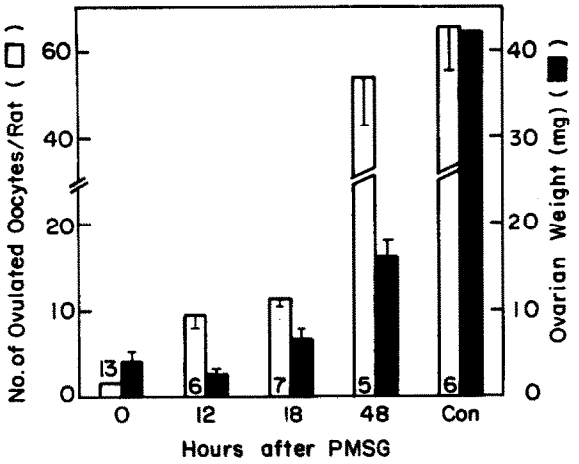

Fig. 6. Effect of delay in GnRHa administration on the number of ovulated oocytes/rat and ovarian weight in PMSG-primed hypophysectomized immature rats. For protocol see legend to Fig. 5.

vals after the PMSG injection, induction rather than inhibition of ovulation was observed (Fig. 5). Thus, when GnRHa injections were initiated between 12 and 24 h after PMSG administration, 65% of the treated rats ovulated; a delay of 48 h in GnRHa treatment resulted in induction of ovulation in all the rats examined. As seen in Fig. 6, when GnRHa treatment was initiated at 48 h after PMSG, not only the ovulation rate but also the number of ovulated oocytes/rat was restored to control levels; the ovaries in these rats, although larger in size than those of the fully inhibited rats, weighed 50% less than the ovaries of control hCG-treated rats.

DISCUSSION

It has been proposed that acute administration of GnRH analogs stimulates ovarian function, whereas continuous treatment with high doses of GnRH analogs is inhibitory (for review see Hsueh and Jones, 1981). Our present results clearly indicate that the ovarian status at the time of exposure to GnRHa determines whether GnRHa is stimulatory or inhibitory to ovarian function. Thus, a continuous administration of high doses of GnRHa can exhibit a stimulatory action, provided that the ovary has been exposed earlier to PMSG, while a single relatively low

dose of GnRHa injection, administered concomitantly with PMSG, will inhibit hCG-induced ovulation. These findings indicate that a non-primed follicle responds to a GnRHa challenge in an inhibitory fashion while a PMSG-primed follicle is stimulated by the same hormonal trigger.

Recent studies support this notion. It was demonstrated that the inhibitory action of a GnRH agonist on PMSG, hCG-induced ovarian weight gain was dependent upon the peptide being present from the start of PMSG treatment (Harwood et al., 1980). Also it was shown that in granulosa cells from preantral follicles, both FSH-induced LH receptors formation and steroidogenesis are inhibited by GnRH analogs (Hsueh et al., 1980). On the other hand, it has recently been reported that a GnRH analog stimulates prostaglandin and progesterone production in cells from preovulatory follicles and that GnRH analogs do not inhibit gonadotropin action in this cell preparation (Clark, 1982). The stimulatory effect of GnRH analogs on prostaglandin production in preovulatory follicles may account for the ability of GnRHa to induce ovulation in hypox rats pretreated with PMSG, since administration of indomethacin completely blocked GnRH-induced ovulation (Ekholm et al., 1982; Dekel et al., 1983). On the other hand, it is possible that the failure of hCG to induce ovulation following exposure to GnRHa at earlier stages of follicular development is the result of a lack of LH receptors in these ovaries. However, the fact that GnRHa, which stimulated ovulation by itself in the PMSG-primed rats, was unable to elicit this response under the inhibitory regime suggests that a factor other than just the absence of receptors to LH is responsible for the ovarian failure to ovulate. In any case, since earlier exposure to PMSG did make the ovary competent to respond to the GnRHa stimulus and ovulate, it seems very likely that the inhibitory action of GnRHa on the immature ovary, observed in our study, results from interference with FSH-induced processes in follicular development.

Our finding that, even under the inhibitory regime, GnRHa stimulated the oocyte to undergo maturation suggests a complete dissociation between GnRHa effect on resumption of meiosis and on the induction of ovulation. Furthermore, our data indicate that while PMSG-priming of the ovary is a prerequisite for GnRHa to induce ovulation, the same hormone can trigger GV breakdown independently of any hormonal priming.

A dissociation between the stimulatory and inhibitory effects of GnRH at the ovarian level could be demonstrated in the intact rat as well as in the hypophysectomized rat. However, the intact rat appeared to be more sensitive to the GnRHa action, since a lower dose of GnRHa per rat was required to obtain both its inhibitory and stimulatory actions. It

is possible that elevation of endogenous LH, triggered by GnRHa in the intact rats, contributes to the GnRHa stimulatory effect on the ovary and also amplifies its inhibitory action, perhaps by causing down-regulation of gonadal LH receptors. In the male, the inhibitory effect of GnRHa on testicular function results primarily from increased endogenous LH levels (Sequin et al., 1982).

This study should raise some concern regarding the potential use of GnRH analogs in fertility control. The finding that, under an inhibitory regime, GnRH does stimulate a large fraction of the oocytes to resume meiosis and that oocyte maturation is followed by a massive degeneration, calls for caution in the use of high doses of analogs.

Our present report demonstrates that the action of GnRH analogs can be either inhibitory or stimulatory, depending on the developmental stage of the ovarian follicle at the time of exposure to the peptide hormone, and that complete dissociation exists between GnRH effects on resumption of meiosis and induction of ovulation.

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