Role of Gonadotropin-Releasing Hormone (GnRH) During Early Embryonic Development

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Undergraduate Creative Activities & Research Experiences

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Abstract

With human infertility rates on the rise, improved culture of oocytes and embryos can greatly enhance *in vitro* maturation (IVM) and *in vitro* fertilization (IVF) techniques. Commonly, GnRH is used to enhance culture methods utilized by human IVF clinics. Historically, the primary function of GnRH is stimulation of luteinizing hormone (LH) and follicle stimulating hormone (FSH) release from the pituitary gland. In contrast, there is also evidence that GnRH might play an important role in other reproductive organs. In previous *in vitro* studies, GnRH analogs have been associated with improved oocyte maturation, fertilization, and embryo development rates in the cow, mouse, and human (1-4). To examine the role of GnRH on mouse embryo development, we cultured preimplantation embryos in the presents of a specific antagonist of GnRH (SB-75), a specific GnRH agonist (Histrelin), and a combination of treatments. The results from these studies indicated SB-75 (10 μM) dramatically reduced rates of blastocyst formation whereas Histrelin (10 μM) was unable to enhance embryo development. However, treatment of early embryos with Histrelin was able to rescue embryos from SB-75 inhibition. Next, we looked at the effects of Bisindolylmaleimide I Hydrochloride, a protein kinase C (PKC) inhibitor, and SQ 22536, a protein kinase A (PKA) inhibitor, to examine the signaling pathways associated with the interaction between GnRH and it's receptor in early embryos. The Bisindolylmaleimide I Hydrochloride treatment (10 μM) was able to inhibit morula and blastocyst formation. Treatment with SQ 22536 resulted in decreased development to the blastocyst stage with little effect on morula production.

Introduction

Gonadotropin-releasing hormone (GnRH) plays a major role in regulation of reproductive function. The first form of GnRH was isolated and identified in the 1970's. The receptor for this decapeptide was not identified until the 1990's. Since then our understanding of GnRH and its receptor has grown tremendously. Today, analogs of GnRH are being used to treat precocious puberty in children, endometriosis, polycystic ovarian disease, and two prevalent steroid-dependent diseases, prostate and breast cancer. GnRH has also helped enhance reproductive efficiency in food animals by allowing synchronization of estrus. The study of GnRH and its receptor has made major contributions to our understanding of mechanisms and patterns of hormone release, as well as how specific glands are regulated.

GnRH can be regulated by various external signals and is released in a pulsatile fashion from the hypothalamus. GnRH acts on gonadotrope cells of the anterior pituitary gland to stimulate the release of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH initiates ovulation and stimulates the formation and retention of the corpus luteum whereas FSH stimulates follicular development on the ovary. GnRH also triggers several other responses of gonadotropes such as regulation of GnRH receptors and biosynthesis of the gonadotropin subunits (common α-subunit and specific LHβ- and FSHβ-subunits).

In addition to the anterior pituitary gland, receptors for GnRH have been found in other tissues such as the adrenal gland, tumors, the central nervous system as well as placental, uterine, oviductal and embryonic tissues (5). GnRH produced by the oviduct may play an important role in the development of preimplantation embryos. GnRH mRNA and protein are present in the developing mouse embryo from the morula to hatched blastocyst stages (1). Preimplantation embryonic development in mice was significantly enhanced by incubation with increasing concentrations of GnRH agonist and significantly decreased by a GnRH antagonist in previous studies.

Signaling events mediate many processes during embryogenesis to activate the program of early development. Within the cell many of these changes are mediated through the activation or inactivation of kinases and phosphatases. It is well established that PKC is linked to the GnRH receptor in gonadotrope cells of the anterior pituitary gland. Also, PKC has been shown to be involved in at least two developmental transitions during early embryonic development, fertilization and compaction (6). Additionally, seven isotypes of PKC are present during preimplantation embryo development in the mouse including forms derived from both maternal transcripts as well as the embryonic genome (6). However, in studies with cancer cells, GnRH was found to work through another important pathway, PKA, which has also been associated with GnRH signaling.

Objectives

1. Will increasing concentrations of the specific GnRH antagonist, SB-75, inhibit embryo development in culture?
2. Does culture in the presence of increasing concentrations of a GnRH agonist, Histrelin, enhance embryo development?
3. Can we rescue embryos from SB-75 inhibition by treating with Histrelin?
4. How does inhibition of PKA and PKC signaling pathways effect early embryo development?

Results

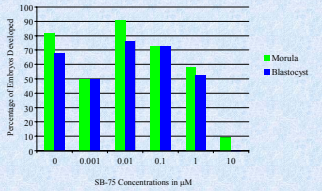

Figure 1. The effects of increasing concentrations of SB-75 on early embryonic development.

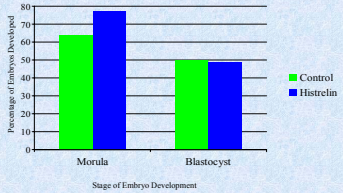

Figure 2. The effects of 10 μM of Histrelin on early embryonic development.

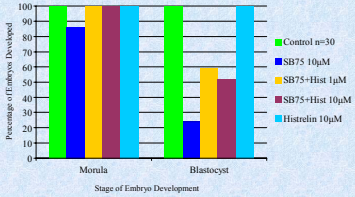

Figure 3. The effects of Histrelin on SB-75 inhibited preimpantation embryos.

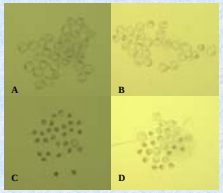

Figure 4. Photomicrographs taken from experiment containing the following treatments; (A) Control, (B) Histrelin, (C) SB-75, (D) SB-75 + 10 μM Histrelin.

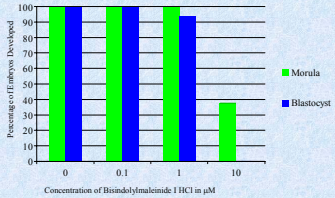

Figure 5. The effects of Bisindolylmaleinide I HCl, a PKC inhibitor, on early embryonic development.

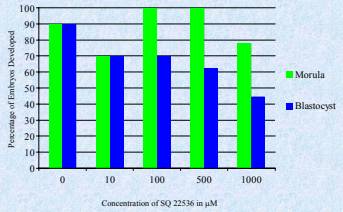

Figure 6. The effects of SQ 22536, a PKA inhibitor, on early embryonic development.

Materials and Methods

Superovulation

- At least three mature female mice were used per experiment.
- Mature female mice were superovulated with I.P. injections of 5 IU PMSG (Day 1) and 5 IU hCG approximately 48 hours later (Day 3).
- Following the hCG injection, each female mouse was mated with a fertile male.
- The following morning (Day 4) the females were checked for cervical plugs, which indicates breeding has taken place.

Media

- M2, an air-buffered medium, was used for short term handling of embryos.
- Hyaluronidase was added to M2 medium and used to remove cumulus cells from the one-cell embryos.
- KSOM, a CO2-buffered medium, was used for long term culture.

Recovery of One-Cell Embryos

- Females with cervical plugs on the morning following hCG injection (Day 4) were sacrificed.
- The oviducts were collected and placed in a petri dish containing a 200 μl drop of 0.5 mg/ml hyaluronidase in M2 medium.
- Embryos were released by tearing the cumulus sac of the oviduct.
- The embryos were washed three times in M2 medium.
- The embryos were then washed through three drops of KSOM medium to ensure the removal of all hyaluronidase and M2 medium.
- The embryos were evaluated, scored for developmental stage and randomly allocated to treatment groups (approximately 25-30 embryos per treatment).

Embryo Culture

- Embryos were cultured in 50 μl microdrops under mineral oil at 37°C in a humidified 5% CO_2 in air environment.
- Media was changed every 12 hours.
- The embryos were monitored daily for development.
- In the first experiment, embryos were cultured with 0, 0.001, 0.01, 0.1, 1.0, or 10.0 μM of the specific GnRH antagonist, SB-75.
- Treatments in the second experiment consisted of 0 or 10.0 μM of the specific GnRH agonist, Histrelin.
- The third experiment consisted of treatments including control, 10 μM SB-75, 10 μM SB-75 + 1 μM Histrelin, 10 μM SB-75 + 10 μM Histrelin, or 10 μM Histrelin alone.
- Embryos in the fourth experiment were cultured in the presence of 0, 0.1, 1.0, or 10.0 μM Bisindolylmaleimide I Hydrochloride, a PKC inhibitor.
- The last treatment used was SQ 22536, a PKA inhibitor, at the concentrations of 0, 10, 100, 500, or 1,000 μM.
- Chi-square analysis was performed.

Conclusions

The results from *in vitro* culture of preimplantation mouse embryos with an antagonist and agonist suggest that GnRH plays a critical role in blastocyst formation during early embryonic development and our data suggests that GnRH is acting in an autocrine manner. In addition, it appears that development to the morula and blastocyst stages are more dependent on the PKC pathway than the PKA second messenger system.

References

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