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SHORT COMMUNICATION

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Topography of growth hormone receptor expression in the bovine embryo

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Abstract Using in situ hybridization, mRNA encoding the growth hormone receptor (GHR) was localized in preimplantation embryos produced by in vitro fertilization (IVF) as well as in 30- to 70-day-old bovine embryos. In IVF embryos the transcript of GHR was demonstrated in the inner cell mass of 8-day-old blastocysts. In 30-day-old embryos, the mesonephros was the first organ to express the mRNA of GHR. In 40-day-old embryos, the transcript was found in the neurones of the spinal ganglions, the splanchnic nerves and the motoneurones of the spinal cord, in the vascular endothelium, and in the developing striated muscle tissue. Colocalization of the protein by immunohistochemistry showed an identical distribution pattern of GHR in 30- to 70-day-old embryos.

Introduction

Pituitary growth hormone (GH) is known to stimulate postnatal development. The role of this hormone in early embryonic development, however, is still controversial. Hypophysectomized mouse, rat, pig, and rabbit fetuses with GH deficiency reveal almost normal growth (Gluckman et al. 1981). On the contrary, growth of human individuals with Laron-type dwarfism lacking GH receptors (GHRs) is significantly reduced (Godowski et al. 1989). In the human fetus, GH can be demonstrated in the 10th week of gestation for the first time and reaches a peak at the 20th week (Chard 1989). In fetal lambs, the plasma GH level is up to tenfold higher than the postnatal level (Bassett et al. 1970). Under in vitro conditions, many fetal tissues are stimulated by GH (Strain et al. 1987; Swenne et al. 1987; Scheven and Hamilton 1991), indicating the presence of functional GHRs during fetal development.

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Materials and methods

Collection of oocytes, maturation, fertilization, and culture

Oocytes were aspirated from 2- to 8-mm ovarian follicles of cows using a 20-g needle immediately after slaughter. Maturation and fertilization of the oocytes were performed as described by Stojkovic et al. (1995). Embryos were cultured in TCM 199 in a 5% CO_2, 5% O_2, and 90% N_2 humidified atmosphere at 39° C. After 2–15 days, embryos were removed from culture, washed in PBS, and transferred to 3-aminopropylene ethoxysilane-coated slides. After drying, fixation in 4% paraformaldehyde, and dehydratation, the embryos were stored at –70° C.

Collection of 30- to 70-day-old embryos

Uteri of pregant cows were removed immediately after slaughter. The age of the embryos was determined by measuring the crown-rump length. Embryos of 30–70 days old, i.e., embryos with crown-rump lengths of 1.5, 2, 2.5, 4, 5, 6.5, 7, and 7.8 cm were examined. For in situ hybridization, embryos were fixed in 4% paraformaldehyde in PBS for 14 h. After washing in 0.5 M sucrose in PBS for 3 h, they were snap-frozen in liquid N_2 and embedded in tissue-freezing medium (Shandon Southern Products, Cheshire, UK). Sections (7 μm) were mounted on 3-aminopropylene ethoxysilane-coated slides, dried at 50° C for 10 min, and stored at –70° C till use. For immunohistochemistry, embryos were fixed in Bouin's fluid overnight, dehydrated in a graded series of ethanol, and embedded in paraffin. Sections (5 μm) were cut on a Leitz microtome.

Oligonucleotide probes

The biotin-labelled oligonucleotide probe was synthesized according to the cDNA of bovine GHR (Hauser et al. 1990). The sequence of the antisense probe was 5′-TGG TCT GTG CTC ACA TAG CC-3′ (bp 2067–2087).

Production and characterization of monoclonal antibodies

The monoclonal antibody (mAb) 263 (IgG K isotype) was produced by hybridoma technology from mice immunized against a

human GH affinity-purified rabbit and rat liver GHR as described by Barnard et al. (1984). MAb 263 recognizes a cross-species determinant with high affinity and does not cross-react with insulin or prolactin receptors (Barnard et al. 1985). This antibody has been validated extensively for immunohistochemical studies in rats, rabbits, and cattle (Barnard et al. 1988; Lobie et al. 1990; Kölle et al. 1997).

In situ hybridization (ISH)

ISH including controls was performed as described in Kölle et al. (1997) with minor modifications. Dehydration and hydration were performed in a graded series of ethanol ranging from 30% to 100% and 100% to 30% respectively. After hydration the slides were fixed in 4% paraformaldehyde in PBS for a second time. The treatment in 0.2 M HCl and the incubation time in proteinase K was shortened to 5 min.

Immunohistochemistry

Immunohistochemistry including controls was performed as described in Kölle et al. (1997). Incubation in 0.5% H_2O_2 in PBS was omitted in the slides with preimplantation embryos. Specimens were counterstained with Mayer's hematoxylin.

Results and discussion

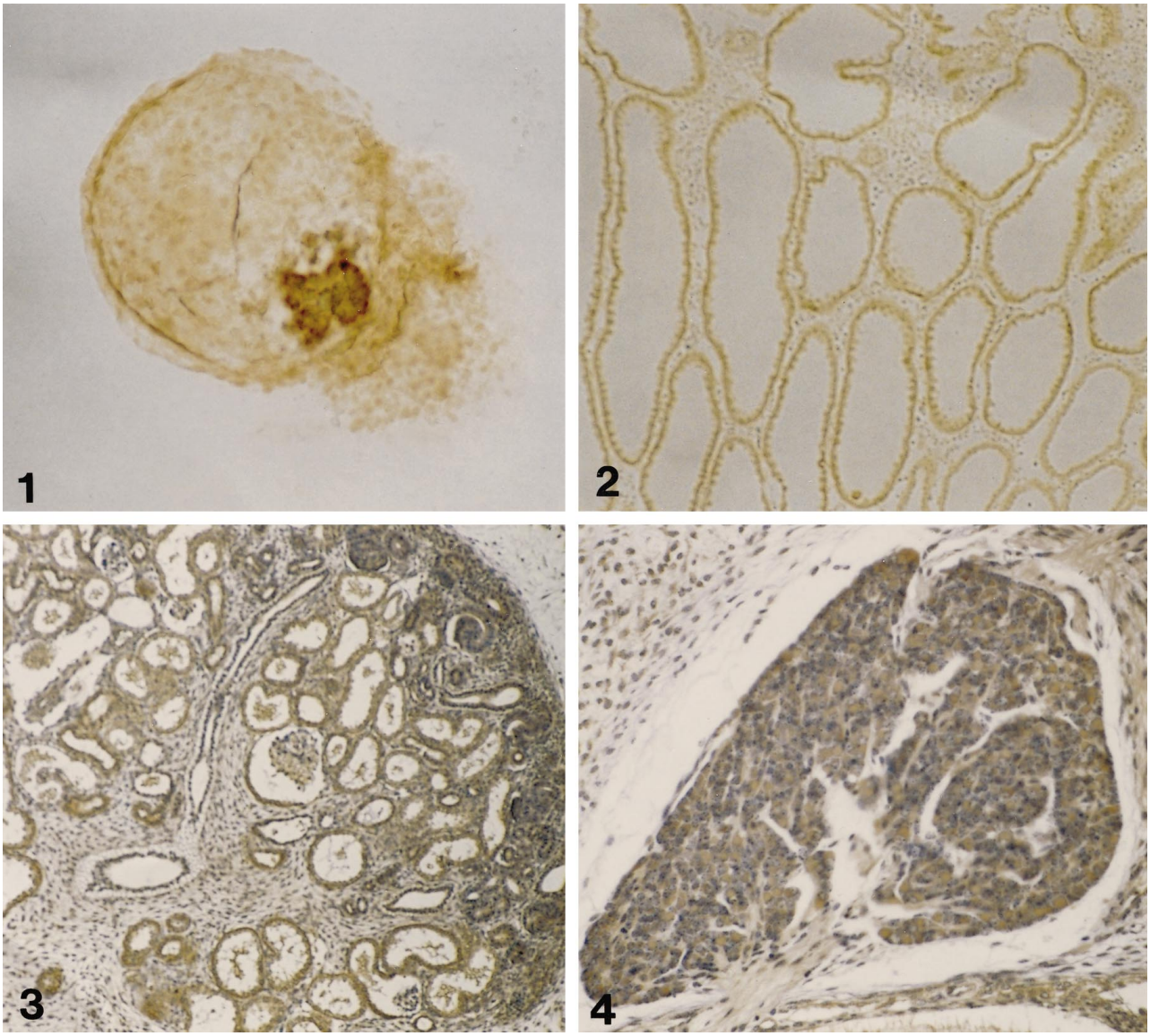
The mRNA encoding GHR was expressed at the early embryonic age of 8 days in the inner cell mass of the blastocyst (Fig. 1). Unlike the transcript, the protein could first be shown in the embryonic disc 13 days after fertilization. Similarly, Ohlsson et al. (1993) were able to demonstrate the transcript of GHR in germline compe-

Fig. 1 In situ hybridization of an 8-day-old bovine embryo produced by in vitro fertilization (×200). The mRNA encoding growth hormone receptor (GHR) is localized in the inner cell mass

Fig. 2 In situ hybridization of a 5-week-old embryo (×170). The mesonephric tubules reveal the transcript of GHR

Fig. 3 Immunohistochemical localization of GHR in an 8-week-old embryo (×80). The tubules of the kidney show distinct staining

Fig. 4 Immunohistochemical localization of GHR in a 40-day-old embryo (×180). The spinal ganglions show distinct amounts of GHR



tent embryonic stem cells and preimplantation mouse embryos. The early expression of the GHR gene only a few days after conception may imply a significant role for GHR in the implantation process.

In 30- to 70-day-old embryos, the mesonephros was the first organ to express the transcript of GHR at the early embryonic age of 5 weeks (Fig. 2). Both the mRNA and the protein showed the same localization of the protein in the epithelium of the tubules. With the formation of the definitive kidney in the 7th week, the amount of GHR in the mesonephric tubules was significantly reduced, but increased within the tubules of the kidney (Fig. 3). In rabbit fetuses, the highest level of GHR mRNA of all fetal organs was also found in the kidney (Ymer and Herington 1992).

The second organ system expressing GHR and its transcript was the nervous system. In 40-day-old embryos, strong signals for GHR and its transcript were found in the motoneurones of the ventral horn of the spinal cord, in the spinal ganglions (Fig. 4), and in the splanchnic nerves. Similarly, GHR expression has been found in fetal rat ganglia (Garcïa-Aragón et al. 1992). The present data support the concept that GH may play a role in the development of the nervous system. In 40-day-old embryos, GHR and its transcript were also found in developing muscle cells, indicating that GHR is involved in growth and differentiation of striated muscle tissue. GHR was also localized in the endothelial cells of vessels at the early embryonic age of 8 weeks. Whereas liver is known to be rich in GHR after birth, embryonic liver did not express GHR until the 6th week of life. In 10-week-old fetuses, single cells showed GHR labelling. Similar results have been found in sheep fetuses; at day 95 of pregnancy, muscle cells expressed significantly higher levels of GHR mRNA than did liver cells (Klempt et al. 1993).

The function as well as the mode of action of GHR in the embryo are unknown. The presence of GHR in the vascular and nervous system and in muscle tissue implies that GH is involved in differentiation processes of the early embryo. Whether the effects of GH at the embryonic GHR are mediated by direct action of the hormone or by insulin-like growth factor is currently under investigation.

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