Growth hormone in neural tissues of the chick embryo

S Harvey, C D M Johnson and E J Sanders

Department of Physiology and Perinatal Research Center, University of Alberta, Edmonton, Alberta, Canada T6G 2H7

(Requests for offprints should be addressed to S Harvey; Email: steve.harvey@ualberta.ca)

Abstract

Growth hormone (GH) gene expression predominantly occurs in the pituitary gland, although it also occurs in many extrapituitary sites, including the brain. The cellular location and ontogeny of neural GH production is, however, largely unknown. This has therefore been determined during chick embryogenesis.

In chicks, the brain develops from the neural tube at embryonic day (ED) 3. At this age, the divisions of the brain (the telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon) have intense GH immunoreactivity (GH-IR) (detected by two polyclonal antibodies and a monoclonal antibody for chicken GH). The otic and optic vesicles were also strongly GH immunoreactive, as were the Vth (semi-lunar), VIIth (facial), VIIIth (acoustic) and IXth (glossopharyngeal) nerve ganglia. This GH-IR was specific for GH and was lost when the antibodies were preabsorbed with recombinant chicken GH. The widespread distribution of GH-IR in the neural tissues of ED 3 embryos was mirrored by the distribution of GH receptor (GHR) immunoreactivity, detected by an antibody raised against the chicken GHR.

In ED 6/ED 7 embryos, the neural retina of the eye and the epithelial and lens fiber cells were intensely stained for GH-IR, as was Rathke’s pouch and the wall of the diencephalon. In contrast, only a few scattered cells were immunoreactive in the surrounding mesoderm. At ED 14, the GH-IR in the brain was restricted to specific tissues and cells. For instance, immunoreactive cells were present in the molecular and pyramidal layers of the cerebral cortex, in the gray matter of the cerebellum, in the choroid plexus, and in the walls of the ventricles.

In summary, GH- and GHR-like proteins are abundant in neural tissues of the chick during the first third of incubation, becoming discretely localized to specific tissues and cells during later incubation. The localization of GH and GHR in these tissues, prior to the ontogeny of plasma GH, suggests autocrine or paracrine roles for GH during early embryogenesis.

Introduction

It is now well established that the brain is an extrapituitary site of growth hormone (GH) gene expression (see Harvey et al. 1993 for review). Immunological studies have identified GH-like proteins in homogenates of the whole brain, the telencephalon, diencephalon, mesencephalon, metencephalon and myelencephalon, at concentrations greater than those in blood (Hojvat et al. 1982a,b, 1986). The GH concentration is greatest in the amygdala, hippocampus and hypothalamus, although it is <1% of that in the pituitary gland. Immunocytochemical studies in rats have also shown that GH is present in perikarya, fibers and axon terminals in amygdaloid and hypothalamic nuclei (Lechan et al. 1981, 1983). GH immunoreactivity is also present in preoptic hypothalamic cells in the brain of fish (Hansen & Hansen 1982; Wright 1986). In birds, GH immunoreactivity has recently been demonstrated in perikarya and fibers in the hippocampus, medial and lateral septum, median eminence and hypothalamus (paraventricular, periventricular, inferior and infundibular nuclei) of turkeys and ring doves (Ramesh et al. 2000). The cellular distribution of GH within the brain during embryonic or fetal development is, nevertheless, still largely unknown.

Immunoreactive GH has been measured in extracts of fetal rat brain, in which the GH concentration peaks during gestation and declines towards term, contrary to the ontogenic increase in pituitary GH content (Hojvat et al. 1982b). The appearance of GH in the rat brain, on the tenth day of gestation, occurs prior to that in the pituitary gland, on the twelfth day (Hojvat et al. 1982b). The abundance and/or cellular distribution of GH within the brain is thus likely to change during ontogeny. This possibility was therefore assessed in the present study, in which the localization of GH in the brain and other neural tissues has been determined in chick embryos before and after the differentiation of pituitary somatotrophs.
Materials and Methods

Tissues

Fertile White Leghorn chicken eggs from the University of Alberta Poultry Unit were incubated at 37.5 °C in humidified air (Hamburger & Hamilton 1951). The eggs were turned one quarter of a revolution each day during the 21-day incubation period. Whole chick embryos at embryonic day (ED) 3, 6 and 7 were dissected into phosphate-buffered saline (PBS, pH 7.4) prior to fixation. Older embryos, at 14 and 19 days of incubation, were killed by decapitation and the heads were similarly collected into PBS. These ages were selected to determine the ontogenic appearance of neural GH. The animal handling was approved by the Health Science Animal Welfare Committee of Alberta Poultry Unit were incubated at 37 °C for 21 days. Fertile White Leghorn chicken eggs from the University of Alberta were used in all experiments.

Immunocytochemistry

Materials and Methods

Tissues

Fertile White Leghorn chicken eggs from the University of Alberta Poultry Unit were incubated at 37.5 °C in humidified air (Hamburger & Hamilton 1951). The eggs were turned one quarter of a revolution each day during the 21-day incubation period. Whole chick embryos at embryonic day (ED) 3, 6 and 7 were dissected into phosphate-buffered saline (PBS, pH 7.4) prior to fixation. Older embryos, at 14 and 19 days of incubation, were killed by decapitation and the heads were similarly collected into PBS. These ages were selected to determine the ontogenic appearance of neural GH. The animal handling was approved by the Health Science Animal Welfare Committee of Alberta Poultry Unit were incubated at 37 °C for 21 days. Fertile White Leghorn chicken eggs from the University of Alberta were used in all experiments.

Immunocytochemistry

Tissues were fixed in freshly prepared paraformaldehyde (4% w/v) (Sigma, Mississauga, Ontario, Canada) or Bouin’s fixative overnight at 4 °C. Tissues were then dehydrated in a graded series of alcohol (50%, 30–60 min; 70%, 30–60 min; 95%, 30–120 min) and cleared with Hemo-de (Fisher Scientific, Edmonton, Alberta, Canada) for 30 min. Tissues were then infiltrated with paraffin wax for 24–48 h at 60 °C, under normal atmospheric pressure. Serial transverse (4–8 μm) sections were then taken using a microtome and were mounted onto charged slides (Fisher Scientific). Immunocytochemical staining was performed with commercial reagents (Vector Laboratories, Burlingame, CA, USA; Sigma) using the avidin-biotin-peroxidase (ABC) (Hsu et al. 1981) or the alkaline phosphatase, anti-alkaline phosphatase (APAAP) (Sternberger 1979) method. Sections were incubated with specific polyclonal antiserum raised in rabbits against native chicken (c) GH (αcGH-1: Harvey & Scanes 1977) or recombinant cGH (αcGH-2: Porter et al. 1995). Both of these antibodies were diluted 1:4000 in PBS or in 1–5% normal goat serum (NGS) overnight, at room temperature. A mouse monoclonal antiserum raised against glycosylated chicken growth hormone (1H7: Berghman et al. 1987) was also used at 1:1000, diluted in NGS or PBS. After incubation, the slides were washed three times for 15 min in PBS. Sections were then incubated for 1 h at room temperature in biotinylated goat anti-rabbit immunoglobulin G (IgG) (Sigma, 1:500) or in a biotinylated anti-mouse IgG (Sigma, 1:500). The slides were then washed in PBS and incubated in ABC reagent for 1 h at room temperature and washed in PBS. Staining was visualized using the chromogen substrate diaminobenzidine tetrahydrochloride (DAB) (Sigma), which resulted in a brown coloration. In some instances, nickel chloride (Sigma) was added to the DAB, resulting in a black precipitate. The specificity of staining was determined by preabsorbing the GH antiserum with recombinant cGH (Amgen, Thousand Oaks, CA, USA; 1 mg/ml) for 1 h prior to section incubation. Non-specific staining was determined by replacing GH antiserum with pre-immune rabbit serum (NRS). Other controls included the omission of the secondary antibody and the replacement of the primary antibody with PBS.

The APAAP technique involved the use of a secondary antibody conjugated with alkaline-phosphatase (Sigma). After incubation with the secondary antibody, the sections were washed in PBS and incubated with 10% APAAP, for 1 h at room temperature. The sections were then washed and Fast Red TR/Naphth As-MX (Sigma) was applied to tissue sections until optimum color development.

GH receptor (GHR) immunoreactivity was detected using a polyclonal antibody (at a concentration of 1:400) raised in rabbits against recombinant chicken GH binding protein (rcGHBP) (Huang et al. 1993, Hull et al. 1996), using the ABC technique, as detailed above. The specificity of staining was demonstrated by replacement of the primary antibody with non-immune rabbit serum (NRS) or PBS. In previous studies (Hull et al. 1996), the specificity of staining was also established by the ability of excess rcGHBP or homogenates of COS cells expressing cGHBP to completely block its immunostaining. Since cGHBP is the extracellular domain of the cGHR, the primary antibody recognizes GHR/GHBP immunoreactivity.

Neural structures were identified according to Romanoff (1960), and Kuenzel & van Tienhoven (1982).

Results

Immunocytochemistry

At ED 3, the neural tube develops anteriorly to give rise to the brain. In birds, the divisions of the brain, anteriorly to posteriorly, are the telencephalon (which develops into the cerebral hemispheres), the diencephalon or thalamus and hypothalamus, the mesencephalon or mid-brain, the metencephalon or cerebellum, and the myelencephalon or medulla oblongata. At ED 3, all of these divisions are present and the lumen of each division is continuous with the others. At ED 3, all the major divisions of the brain were GH immunoreactive. Fig. 1A shows that the telencephalon is strongly immunoreactive, with GH immunoreactivity in all cells lining the lumen. This staining is present in cytoplasmic and nuclear or perinuclear compartments of most cells. The specificity of this staining is indicated by its loss after the primary antisera had been preincubated with recombinant cGH (Fig. 1B). The replacement of the antisera with NRS similarly failed to stain adjacent sections (data not shown). The otic vesicle, which develops into the ear, was also strongly GH immunoreactive (Fig. 1C). This immunoreactivity was again lost following preabsorption of the primary antisera.
Figure 1 GH immunoreactivity in neural tissues of the ED 3 embryo. (A) Transverse section through the telencephalon (Te). The wall of the telencephalon is strongly stained for GH. The olfactory pit (Op) is also GH immunoreactive and is of a similar staining intensity to the telencephalon. The amnion (a), mesoderm (M), surrounding the brain and epidermis (ep) of the head are also immunoreactive. T, teleocoel. Bar = 120 μm. (B) GH immunoreactivity is reduced in the telencephalon, olfactory pit, mesoderm and epidermis when the primary antibody (αGH1) is preabsorbed with recombinant cGH. Note the higher background contrast necessary for visualization of the tissue section. (C) The otic vesicle (Ot) and associated nerve ganglia show strong GH immunoreactivity. IX, 9th cranial nerve (glossopharyngeal), VII & VIII, 7th and 8th cranial nerves which are closely associated with each other. Bar = 60 μm. (D) Preabsorption of the primary antibody abolishes staining; this section shows the same area as (C). (E) The semilunar nerve ganglion or Vth cranial nerve (V) is strongly GH immunoreactive. Bar = 60 μm. (F) The neural tube (Nt) towards the posterior of the embryo and the notochord (N) are strongly GH immunoreactive. Bar = 60 μm.
with recombinant cGH (Fig. 1D). Several cranial ganglia were also GH immunoreactive. The Vth (semilunar) nerve, VIIth (facial) nerve, VIIIth (acoustic) nerve and the IXth (glossopharyngeal) nerve ganglia were all strongly GH immunoreactive (Fig. 1E). The neural tube, posteriorly, was also GH immunoreactive, with an intensity of staining comparable with the anterior neural tube (Fig. 1F). Identical results were observed when αcGH-2 or 1H7 was used as the primary antibody (data not shown).

The widespread distribution of GH immunoreactivity in the neural tissues of the ED 3 embryos was mirrored by the cellular and subcellular distribution of GHR immunoreactivity (Fig. 2). The mesencephalon (Fig. 2A) is characteristic of the staining of other brain regions. Strong GHR staining was seen in the wall of the mesencephalon and the surrounding mesodermal tissue. The otic vesicle (Fig. 2B) and the associated ganglia (the facial/acoustic ganglia) were also strongly GHR immunoreactive. The optic vesicle, which develops into the eye, had intense GHR immunoreactivity (Fig. 2C). The Vth cranial ganglia and the surrounding mesoderm similarly contained intense GHR immunoreactivity (Fig. 2D). The replacement of the primary antisera with NRS or PBS did not result in any staining (data not shown).

In ED 6 embryos, the neural retina of the eye was strongly GH immunoreactive (not shown). The epithelial and lens fiber cells were intensely stained for GH immunoreactivity (Fig. 3A). This staining was lost following the preabsorption of the primary antibody with recombinant cGH (Fig. 3B). The cytoplasm of the cortical lens fiber cells was less immunoreactive than the nuclei (Fig. 3A).

By 7 days of incubation (stage 31), GH immunoreactivity was still abundantly present in the optic cup (Fig. 4A). The neural retina was intensely and evenly stained for
GH (Fig. 4B), although the mesodermal layer lying above the pigmented retina was much less immunoreactive, with only a few cells staining for GH. The epidermis of the head was also GH immunoreactive (Fig. 4B). The GH immunoreactivity in the eye neural retina was primarily nuclear (Fig. 4D) and was lost following preabsorption of the antibody with recombinant cGH (Fig. 4C).

The diencephalon and Rathke’s pouch of the ED 7 embryo were intensely stained for GH-like proteins, particularly in nuclear or perinuclear compartments (Fig. 4E), whereas only a few scattered cells in the surrounding mesoderm were lightly stained (Fig. 4E and Fig. 5A). The otic vesicle and cells in the acoustic (VIIth and VIIIth nerves) and semilunar (Vth) ganglia were also strongly immunoreactive (Fig. 5B).

In ED 14 embryos, the brain was still very GH immunoreactive (Fig. 6), although the immunoreactivity was not widespread and was restricted to specific tissues and cells. For instance, most cells in the choroid plexus (a circumventricular organ) stained particularly strongly for GH (Fig. 6A), as did cells in the pineal stalk and pineal gland (data not shown). This staining was lost when the primary antibody was preabsorbed with recombinant cGH (Fig. 6B). Within the cerebral cortex, many cells had GH immunoreactivity within the molecular and pyramidal layers (Fig. 6C). This staining was again lost following preabsorption of the primary antibody with recombinant cGH (Fig. 6D). Because of their position and morphology, it was presumed that many of the immunoreactive cells in the gray matter of the cerebral cortex were probably astrocytes (Fig. 7A). Numerous smaller cells were also strongly stained for GH (Fig. 7B). This staining was lost following the preabsorption of the primary antibody with recombinant cGH (Fig. 7C). Large pyramidal neurons were also intensely stained for GH (Fig. 7C). Large cells from the pyramidal neuron layer showed very dense nuclear GH immunoreactivity and lesser cytoplasmic staining (Fig. 7D). Nerve tract fibres located in the white matter of the cerebral cortex also contained GH immunoreactivity (Fig. 7E).

At ED 19 (stage 45), the ependymal cells lining the ventricles were immunoreactive, including the subtrochlear organ, which was intensely stained for GH (Fig. 8B). Preincubation of the primary GH antibody with recombinant cGH resulted in a complete loss of staining (data not shown).

Within the cerebellum of the ED 19 embryo, the cells of the gray matter were intensely stained for GH, using αGH1 as the primary antibody. Identical results were seen when αcGH-2 was used as the primary antibody (data not shown). The Purkinje cells, located between the gray and molecular layers of the cerebellum, were particularly GH immunoreactive (Fig. 8A). The molecular layer and the white matter did not appear to be as strongly stained (Fig. 8A). Preincubation of the primary antibody with recombinant cGH completely abolished staining in the cerebellum (data not shown).

Discussion

The possibility that GH may be present in neural tissues of the chick embryo was first indicated by its presence in the neural tube (spinal cord) of ED3 embryos (Harvey et al. 2000b), as confirmed in the present study (Fig. 1F). The results of these studies also show, for the first time, that GH immunoreactivity is present in discrete cells in the developing brain, and is present in otic and optic tissues.

www.endocrinology.org
Figure 4 GH immunoreactivity in the ED 7 eye. (A) Transverse section through the head of a 7-day embryo showing part of the developing eye. The neural retina (nr) is very intensely stained but the mesoderm (m) which lies above the neural retina is much less immunoreactive, suggesting that staining is specific. The head epidermis (ep) was also strongly immunoreactive. a, amnion. (B) High power magnification of the neural retina which lies below the pigmented retina (pr). Notice that scattered cells within the mesoderm (m) were lightly stained (arrows). (C) The immunoreactive staining was completely abolished when the primary antibody (αGH1) was preincubated with recombinant cGH. (D) Higher magnification of the neural retina, showing nuclear staining (arrow). (E) GH immunoreactivity in Rathke’s pouch (R) and diencephalon (D) of the ED 7 chick embryo showing intense nuclear staining (large arrow). Scattered cells in the surrounding mesenchyme are also immunoreactive (small arrows). Bars = 50 μm.
In the brain, GH immunoreactivity is clearly abundant and widespread during the first third of incubation, becoming discretely localized in specific neuronal cells in the last third of incubation. Although GH immunoreactivity is found in many cells in early embryogenesis, it is specifically located in the perikarya of pyramidal neurons within the cerebral cortex and in Purkinje cells of the cerebellum in ED 19 embryos. The distribution of GH immunoreactivity in the embryonic brain prior to hatch therefore differs from its predominantly hypothalamic location in adult turkeys and ring doves (Ramesh et al. 2000). This could reflect a species difference or difference due to ontogeny.

The GH immunoreactivity in neural tissues of the chick embryo is likely to reflect the distribution of GH rather than GH-like proteins, since it was detected by three different chicken GH antibodies, was ontogenically restricted to specific cells and could be competitively blocked by preabsorption of the primary antibodies with recombinant GH. Furthermore, the distribution of GH immunoreactivity in the embryo was very different from the distribution of prolactin, luteinizing hormone, and thyrotropin immunoreactivity (Murphy et al. 2000). Peripheral tissues of early chick embryos have also been shown to react similarly to the same GH antibodies, and this reactivity is associated with proteins in extracts of the head and body that are identical in size to pituitary GH (Harvey et al. 2000a,b). The GH immunoreactivity in neural tissues of the chick embryo are thus elastic to reflect the expression of the GH gene, since GH mRNA has been detected by RT-PCR in the heads, bodies and eyes of early chick embryos (Harvey et al. 2000b). Moreover, although neural GH in the rat brain differs from pituitary GH in amino acid sequence (Hojvat et al. 1982a), neural GH in adult chickens is identical to pituitary GH in nucleotide sequence (Render et al. 1995). This immunoreactivity is unlikely to reflect the sequestration of pituitary GH, since it occurs prior to the ontogeny of morphologically identifiable pituitary somatotrophs (at day 12–14; Malamed et al. 1993) and pituitary cells that secrete GH (at day 16; Porter 1997).

GH immunoreactive cells were abundantly present in Rathke’s pouch at ED 7 (Fig. 4E and Fig. 5A), but it is significant that most of the reactivity was associated with nuclear or perinuclear compartments. In contrast, GH immunoreactivity in somatotrophs identified by their morphological characteristics is restricted to cytoplasmic secretory granules and is present in only a small population of pituitary cells (Malamed et al. 1993, Porter 1997). The abundant GH immunoreactive cells in Rathke’s pouch (the primordial pituitary) thus differ from the fully differentiated pituitary somatotrophs that appear in the last third of incubation.

The nuclear or perinuclear localization of GH in many neural cells of the chick embryo is consistent with its localization in many peripheral tissues (Harvey et al. 2000b). GH immunoreactivity has also been demonstrated in nuclear compartments in mammalian studies (e.g. Rezvani et al. 1973, Bonfàcino et al. 1983). A nuclear localization of GH within GH-producing cells may occur if it escapes packaging in the Golgi following its synthesis, and this is particularly likely in rapidly growing or differentiating embryonic cells, as occurs in rapidly proliferating or neoplastic cells (Möhl et al. 1996, Lincoln et al. 1998). A nuclear or perinuclear localization of GH may also reflect the subcellular distribution of the GHR, since it is well

![Figure 5](https://www.endocrinology.org)
established that membrane-bound GH is rapidly internalized to nuclear compartments in target cells (e.g. Lobie et al. 1991, 1994a,b, Fraser & Harvey 1992).

The early ontogeny of GH in neural tissues of the chick embryo is consistent with the ontogeny of GH in the rat brain prior to pituitary somatotroph differentiation (Hojvat et al. 1982b) and the appearance of GH mRNA in the head of embryonic trout prior to pituitary differentiation (Yang et al. 1999). This early ontogeny may reflect an earlier expression of Pit-1, since Pit-1 is detectable in the brains of ED 2 chick embryos and Pit-1 is the transcription factor that induces the transcription of the pituitary GH gene (Harvey et al. 2000a). Indeed, Pit-1 is not present in the brains of adult rats (Emanuelle et al. 1992), but is widespread in the brains of fetal rats (He et al. 1989), prior to the appearance of GH immunoreactive proteins (Hojvat et al. 1982b).

Within the embryonic chick brain, GH immunoreactivity was particularly abundant in the choroid plexus, as in the brains of developing rats (Hojvat et al. 1982a, Lechan et al. 1983) and the brains of adult turkeys and ring doves (Ramesh et al. 2000). It is therefore likely that GH is produced in this tissue, although GH also binds to this circumventricular organ (CVO) in rats (van Houten 1980, Garcia-Aragon et al. 1992), rabbits (Mangurian et al. 1994), sheep (Thornwall et al. 1995) and man (Lai et al. 1991) to facilitate two-way GH transport through the blood–brain barrier. It is therefore of interest that GH immunoreactivity was also abundant in another CVO, the pineal gland (data not shown), as also observed in adult turkeys (Ramesh et al. 2000). Proteins with GH-like immunoreactivity and physiochemical characteristics have similarly been identified in extracts of the ovine pineal gland (Noteborn et al. 1993). The intense GH

Figure 6 GH immunoreactivity in the choroid plexus and the cerebral cortex of the ED 14 chick embryo. (A) The choroid plexus (Ch) is strongly immunoreactive. (B) Staining is abolished after preabsorption of the primary antibody (αGH1) with recombinant cGH. (C) Various layers of the gray matter of the cerebral cortex are reactive. At this stage of development, the cortex is not fully differentiated. The outer molecular layer (MI) is characterized by fewer cells and fibers than the inner pyramidal layer. (D) Preabsorption of αGH1 with recombinant cGH completely abolished staining in a similar section to (C). Bar= 50 μm.
immunoreactivity of these circumventricular organs is also likely to reflect receptor-bound ligand, since the choroid plexus has a greater abundance of GHRs than any other site in the central nervous system (Lai et al. 1991, Lobie et al. 1993). The ependymal cells lining the ventricular system of the rat brain also have intense GHR immunoreactivity (Lobie et al. 1993) and it is therefore not surprising that the subtrochlear organ in the chicken brain (comprised of modified ependymal cells) contained intense GH immunoreactivity (Fig. 8).

Although GH is abundantly present in neural tissues of the chick embryo, it is absent from the circulation of early

Figure 7 GH immunoreactivity in the cerebral cortex of the ED 14 chick embryo. (A) Immunoreactive cells from the gray matter of the cerebral cortex (possibly astrocytes). (B) Small and densely packed cells from the white matter showing intense reactivity (arrow). (C) Immunoreactive cell bodies of large pyramidal neurons (arrow), which sent their axons towards the white matter. (D) Dense nuclear labeling (arrow) in pyramidal cells. (E) Nerve tract fibers (arrow) from the white matter are immunoreactive. (F) A region similar to that shown in (B), showing that preabsorption of αGH1 with recombinant cGH completely abolished staining. Bar = 10 μm.
embryos (Harvey et al. 1979), indicating that it is not secreted from neural sites. Neural GH is therefore likely to act locally (Nyberg & Burman 1996). Indeed, the widespread distribution of GH within the brain of the early chick embryo mirrored the distribution of GHR immunoreactivity. Although the presence of GH binding sites in the central nervous system was first demonstrated in adult chickens (Attardo & Harvey 1990), the distribution of GHR immunoreactivity in the brain during ontogeny was surprising, since hepatic GHR mRNA was not detectable in chick embryos until ED 15 (Burnside & Cogburn 1992, Tanaka et al. 1996) and because hepatic membrane binding sites for GH were not detected until ED 14 (Vanderpooten et al. 1991, 1992). This may reflect the abundance of the GHR, since retinal ganglion cells in rats contain intense GHR immunoreactivity, whereas the inner plexiform layer of the retina has only moderate to weak GHR immunoreactivity (Lobie et al. 1993). The localization of GH and GHR in the optic and otic vesicles (Figs 1 and 2) indicates that GH may have roles in the development of the ear and the eye. This is, however, the first demonstration of GH in these tissues and its putative roles are therefore uncertain. It is, nevertheless, of interest that insulin-like growth factor-I (IGF-I) is also located in the neural retina of chicks, since IGF-I affects lens differentiation (Wride 1996) and is inducible by GH in other tissues (Radecki & Scanes 1997). It is also of interest that somatostatin, which regulates the release of pituitary GH (Harvey et al. 1993), is also present in the retina of embryonic and adult chickens (Ellis et al. 1983) and may thus be involved in the local production or release of GH within the eye.

In summary, the results of this study clearly establish that GH and GHR are present in the neural tissues of chick embryos prior to and after the differentiation of pituitary somatotrophs. It is therefore likely that GH participates in the growth and differentiation of the chick embryonic brain, cranial nerves, eye and ear. The colocalization of GH and GHR in these tissues during early embryogenesis, prior to the ontogeny of plasma GH, indicates that GH is likely to act as an autocrine or paracrine hormone in these extrapituitary sites.

Acknowledgements

The authors would like to thank Dr T E Porter, Dr J Burnside and Dr L R Berghman for the gift of antibodies, and the National Science and Engineering Research Council of Canada and the Medical Research Council of Canada for financial support.

References


Fraser RA & Harvey S 1992 Ubiquitous distribution of growth hormone receptor and/or binding proteins in adenohypophysial tissue. *Endocrinology* 130 3593–3600.


Hill DJ 1992 What is the role of growth hormone and related peptides in implantation and development of the embryo and foetus? *Hormone Research* 38 8–34.


Received 22 January 2001
Accepted 7 February 2001