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DETECTION OF GROWTH HORMONE RECEPTOR mRNA IN AN OVINE CHOROID PLEXUS EPITHELIUM CELL LINE

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The expression of growth hormone receptor (GHR) mRNA in an ovine choroid plexus cell line (SCP) was studied. RNA isolated from SCP cells was subjected to reverse transcription followed by PCR using a set of primers, designed on the basis of the ovine liver GHR sequence. A specific product with expected size of 1204 bp was obtained and the nucleotide sequence was found to be identical to that of ovine liver GHR. When the PCR product was used as a probe for Northern blot analysis, a transcript of 4.4 kb was detected in mRNA isolated from the SCP cells. This is the first report demonstrating the presence of mRNA for GHR in the choroid plexus.

Growth hormone (GH) is a polypeptide secreted from the pituitary gland and is well-known for its function to promote growth and a number of metabolic actions. Recent findings suggest that GH may also play a functional role in the central nervous system (CNS). For instance, GH-therapy in adults with GH deficiency was shown to affect the mental state as well as the levels of various hormones and transmitter substances in the cerebrospinal fluid (CSF) (1-5). Subcutaneously injected GH results in enhanced levels of GH in the CSF (2, 4, 5), suggesting that the hormone is able to pass the blood-brain barrier (BBB). The mechanism underlying this transport of GH from blood to CSF as well as the CNS effects are not yet known. However, during the past decade a bulk of evidence for the presence of GH receptors in the CNS have been reported. Specific binding sites for growth hormone have been identified in several CNS areas such as pituitary, choroid plexus, hippocampus and hypothalamus (6-9). While GH receptors in the hypothalamus and pituitary have been suggested to play a role in the regulation of GH secretion (8), the physiological relevance of binding sites in choroid plexus and hippocampus is

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poorly understood. It was recently suggested that the GH binding units in choroid plexus may be involved in a receptor-mediated transport of the hormone over the BBB into the CNS (7). To further examine this hypothesis it would be necessary to get increased knowledge about the chemical nature and structure of GH binding site/receptor. GHR is a member of the GH/prolactin/cytokine receptor superfamily and consists of a polypeptide with a single transmembrane domain (10, 11). "Soluble" or "binding protein" forms of these receptors have also been characterized and consist only of the extracellular domain of the full-length receptor. For GHR, a circulating growth hormone-binding protein (GHBP) has been characterized in rat (12), mouse (13) and human (14). In rat and mouse GHBP is suggested to be generated by alternative RNA splicing (12, 15). In contrast, the GHBP in human is reported to be generated by post-translational processing of the GHR (16). In the present study we have applied reverse transcription-polymerase chain reaction (RT-PCR) and Northern blot analysis to investigate the presence of GH receptor mRNA in an ovine choroid plexus cell line (SCP).

MATERIALS AND METHODS

Cell culture: The ovine choroid plexus epithelium cell line, SCP was obtained from ATCC (Rockville, Maryland, U.S.A.). The cells were grown as monolayer cultures in minimum essential medium (MEM) supplemented with Earle's BSS, non-essential amino acids, 100 U/ml penicillin, 100 µg/ml of streptomycin sulphate and 10% fetal calf serum (GibcoBRL) at 37°C in a humified atmosphere of 5% CO_2. The cells were passaged twice a week.

RNA extraction: The SCP cells were harvested and total RNA was extracted using a guanidinum thiocyanate-phenol-chloroform method (17). Briefly, 1-2 x 10^7 cells were homogenized in 6 M guanidinum thiocyanate containing 50 mM Tris-HCl, pH 7.5, 0.32 M EDTA, 2% N-lauroyl-sarcosine and 70 mM ß-mercaptoethanol. After homogenization, a phenol-chloroform extraction was carried out. The aqueous phase was collected and RNA was precipitated with isopropanol. The pellet was washed in 70% ethanol and dissolved in water. RNA concentration was determined at 260 nm. Poly(A)$^+$ RNA, for Northern blot analysis, was isolated from SCP cells by oligo(dT)-cellulose chromatography (QuickPrep Micro mRNA Purification Kit, Pharmacia).

Reverse transcription - polymerase chain reaction (RT-PCR): Complementary DNA was generated from the total RNA preparation using random hexamer primers. 5 µg of total RNA was reversed transcribed in a final volume of 20 µl for 60 minutes at 37°C. The reaction mixture contained 1 x RT buffer (50 mM Tris-HCl, pH 8.3, 75 mM KCl, 3 mM $MgCl_2$), 10 mM DTT, 0.5 mM of each dNTP, 0.5 µg random primers and 400 units M-MLV reverse transcriptase (GibcoBRL). An aliquot (2 µl) of the RT reaction mixture was amplified in a final volume of 25 µl. The reaction mixture included 1 x Stoffel buffer (10 mM Tris-HCl, pH 8.3, and 10 mM KCl), 3.75 mM $MgCl_2$, 0.25 mM of each dNTP, 25 pmol of each primer and 5 units AmpliTaq DNA polymerase (Stoffel fragment, Perkin Elmer Cetus). The PCR primers used were 5'-GGTCTAGAATGGAAAGAATGCCCCGATTA (corresponding to nucleotide 222 to 245 of the ovine liver GHR extracellular domain) and 5'-GGGAGCTCGTCGCTTA CCTGGGCATAAAA (corresponding to nucleotide 1390 to 1410 of the ovine liver

GHR cytoplasmic domain). The PCR thermal profile used was: 94°C for 5 min followed by 40 cycles at 94°C for 45 sec, 60°C for 25 sec, 72 °C for 45 sec followed by a final 7 min extension at 72°C. One fifth of the PCR was analysed by agarose gel electrophoresis and ethidium bromide staining. The PCR product was cloned into the pCR3 plasmid vector (Eukaryotic TA Cloning Kit, Invitrogen) and sequenced by the dideoxy chain termination method (18) using a ^{T7}Sequencing Kit (Pharmacia).

Northern blot analysis: Five μg of poly(A)$^+$ RNA was electrophoresed through a 0.6% denaturating formaldehyde-agarose gel. The RNA in the gel was transferred to nylon filter in 10 X SSC (1.5 M NaCl, 0.15 M Na-citrate) and cross-linked under UV light. The membrane was prehybridized in RapidHyb buffer (Amersham) and hybridized to a ^{32}P-labeled probe, generated by random priming (rediprime, Amersham) of a 400 bp fragment (corresponding to the extracellular region of the GH receptor, nucleotides 222 to 622) derived from the PCR product obtained as above. The filter was washed twice in 2 x SSC at room temperature for 15 min, and twice in 0.5 x SSC at 65°C for 15 min. The filter was autoradiographed against Hyperfilm (Amersham) at -70°C for 4 days and then for 18 days.

RESULTS AND DISCUSSION

When RNA from the ovine choroid plexus cell line (SCP) was subjected to reverse transcription followed by PCR using GH receptor specific primers, a single product of the expected size (1204 bp) was obtained as revealed by agarose gel electrophoresis (fig. 1). Nucleotide sequence analysis showed that this PCR product is identical to the ovine GH receptor (data not shown). When poly(A)$^+$ RNA from the SCP cell line was subjected to Northern blot analysis using the obtained PCR product as

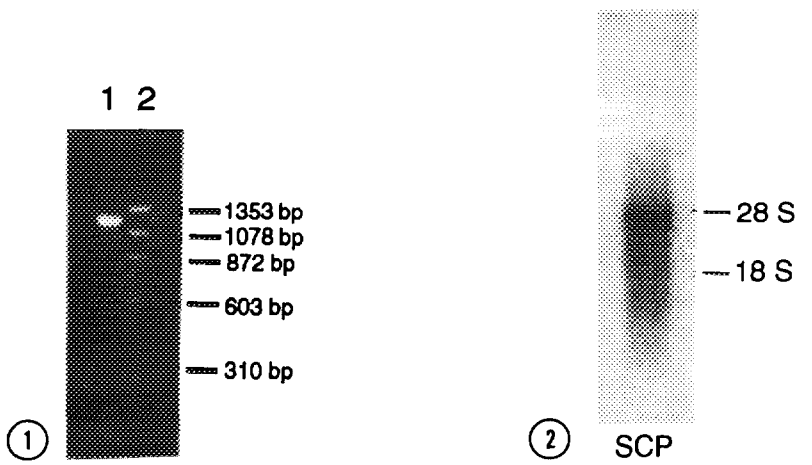

Fig. 1. Agarose gel electrophoresis analysis of growth hormone receptor RT-PCR product (1204 bp) from ovine choroid plexus cell line SCP (lane 1). Molecular weight marker ϕx174-Hae III fragments (lane 2).

Fig. 2. Northern blot analysis of GHR mRNA expression in SCP cells. Five μg of poly (A)$^+$ RNA was subjected to electrophoresis through an formaldehyde-agarose gel, blotted onto nylon membrane and hybridized to ^{32}P-labeled GHR-specific probe. Positions of 28 S and 18 S rRNA are indicated.

probe, a single major transcript (equal in size to that of 28 S rRNA) was detected after 4 days of exposure (fig. 2). This transcript corresponds to the 4.5 kb transcript detected in ovine liver (19). Smaller transcripts of 1.9 kb in ovine liver and 1.2 kb in mouse and rat liver have also been reported (12, 15). However, we could not detect any further transcripts in the ovine choroid plexus cell line by Northern blot analysis even after an 18 day exposure (data not shown). In rat and mouse the smaller transcripts code for the GH binding protein (GHBP) and are generated by the alternative splicing of the larger transcript (12, 15). However, in humans no smaller transcript has been detected and the GHBP is suggested to be derived posttranslationally from the GH receptor (16). Our results demonstrate that ovine choroid plexus epithelial cells (SCP) only contains the larger transcript, and suggest that either the GHBP is not present in these cells or is produced posttranslationally from the GH receptor, as is the case in humans.

In conclusion we have demonstrated that the RNA for GH receptor is expressed in choroid plexus epithelial cells. The physiological relevance of the GH receptors in this particular tissue as well as the molecular events that occur subsequent to GH binding are still not clarified and need futher investigation.

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