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Ontogeny of Somatostatin Gene Expression in Rat Diencephalon

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Key Words. Somatostatin • Messenger RNA • Ontogeny • Brain, rat

Abstract. Somatostatin gene expression is first detectable, using in situ hybridization, on the 14th fetal day in rat diencephalon. Other structures expressing somatostatin messenger RNA include the anterior basal periventricular nucleus, amygdalo-hippocampal complex, dorsolateral thalamus and distinct areas in the parieto-frontal cortex. Semiquantitative analysis reveals that somatostatin synthesis increases progressively throughout the last third of fetal life and onto postnatal life.

Introduction

Somatostatin is a relatively abundant neuropeptide with a unique distribution in the brain of humans, rodents and other mammals [1-5]. Extrahypothalamic somatostatin has been implicated in, among other functions, neural growth and plasticity [1, 6].

The ontogeny of immunoreactive somatostatin in the rat brain has been described in cortical neurons [7] as well as in the hippocampus and hypothalamus [8-10]. Somatostatin messenger RNA (SS-mRNA) has been detected as early as the 7th fetal day in whole rat brain [11]. Using northern blot analysis, SS-mRNA was detected on the 14th and 16th fetal days in rat diencephalon and hypothalamus, respectively [12, 13].

This study was designed to investigate the ontogeny of somatostatin gene expression in structures of the rat diencephalon in the detail afforded by in situ hybridization histochemistry. A specific question addressed was: is there a perinatal decrease in somatostatin gene expression as has been described for corticotropin releasing hormone [14, 15]?

Materials and Methods

Tissue Preparation

Time-pregnant Sprague-Dawley-derived rats were obtained from Zivic-Miller (Zelienople, Pa.) at least 2 days prior to sacrifice. Rats were kept on a 12-hour light/dark cycle (lights on 7 a.m.-7 p.m.), and given access to unlimited lab chow and water. Pregnancy was dated by the presence of a vaginal plug (day 0). Gestation in these rats lasts for 21 days. Fetal brains were obtained on the 14th, 16th, 17th and 21st (last) fetal day, and on the 1st, 4th and 100th postnatal days. Brains were obtained between 8.30 and 9.30 a.m. Prenatally, pregnant rats were anesthetized with CO₂, fetuses were quickly dissected and heads were removed onto powdered dry ice. Postnatally, pups were decapitated, and brains removed onto dry ice. Brains were stored at −80°C.

Brains were cut into 20-μm coronal slices in a cryostat (IEC, Mass.) and mounted on gelatin-coated slides. Brain regions were identified by established landmarks [16, 17]. Sequential slices were cut from the anterior commissure/septum through the caudal hypothalamus.

Hybridization Histochemistry

Prior to in situ hybridization (ISH), slides were brought to room temperature, air-dried and fixed for 20 min in fresh 4% buffered PBS-paraformaldehyde. Slides were dehydrated through increasing ethanol concentrations, rehydrated, exposed for 8 min to 0.5% acetic anhydride-0.1 M triethanolamine (pH = 8), then dehydrated through 100% ethanol.

ISH was modified from Young et al. [18]. Prehybridization for 1 h in hybridization buffer lacking dextran (0.2 ml/slice) was followed by a 20-hour ISH at 37°C. Hybridization buffer consisted of: 50% for-
Fig. 1. Coronal slices of the diencephalon of rats on the 14th fetal day; (F14) (a), F16 (b), F21 (c) and 100th postnatal day (d), subjected to ISH with a labelled oligodeoxynucleotide complementary to SS-mRNA. Bar = 1 mm. AH = Amygdalo/hippocampal complex; hpv = periventricular nucleus; LAT = lateral ventricle; DLT = dorsolateral thalamus; PFC = parieto-frontal cortex; III = 3rd ventricle.

A 39-base deoxynucleotide probe corresponding to the 3' coding region of the rat prosomatostatin mRNA [19], was generated using an Applied Biosystems (Foster City, Calif.) DNA Synthesizer. After purification, the probe was labelled on the 3' end [20] with dATP-S32 (NEN, Wilmington, Del.) using terminal deoxynucleotidyl transferase (Bethesda Research Labs.). Specific activity of labelled probes was 5-8 x 10⁶ dpm/µg. A 60-base deoxynucleotide probe corresponding to the 20 COOH-terminal amino acids of CRH [21] was similarly generated, labelled, and used as a specificity control.
Quantitation and Statistical Analysis

Serial slices of the diencephalon at the level of the periventricular nucleus at each age were used. For quantitative studies, slices with the maximal somatostatin SS-mRNA at each age were compared. Films were mounted on a light table with a precision illuminator (Northern Light B90; Imaging Research, St. Catherine, Canada). Images were acquired by a Sierra Scientific high-resolution camera (Sunnyvale, Calif.), and optical density (OD) was determined using the MCID software image analysis system (Imaging Research, St. Catherine, Canada). Optical density was determined over the anterior, basal periventricular nucleus (HPV) and over the subcortical area underlying the lateral ventricles of the same slice (SCA). For CRH-mRNA assessment, OD was determined over the paraventricular nucleus, which was compared to the parietal cortex. The ratio of HPV-OD over SCA-OD was calculated for each slice and used for analysis of SS-mRNA. The OD ratio of paraventricular nucleus to parietal cortex was utilized as a measure of the prevalence of CRH-mRNA. Five ratios, from at least three brains, were obtained for each age group, and means and standard deviations were determined for each age. Analysis of variance (Minitab, University Park, Pa.), was used to assess the significance of differences between age groups.

Results

SS-mRNA was detectable in the diencephalon of rats as early as the 14th fetal day. At that early age SS-mRNA was localized to the periventricular nucleus. By the 16th fetal day, sharply demarcated cell aggregates expressing somatostatin were evident in several regions (fig. 1). The anterior basal periventricular nucleus had the highest prevalence of peptide synthesis; SS-mRNA was also
abundant in the amygdalo-hippocampal complex, the
dorsolateral thalamus and distinct zones of the parieto­
frontal cortex. This distribution is markedly different
then that of CRH-mRNA, which is confined to the para­
ventricular nucleus of the hypothalamus at its onset on
the 17th fetal day (fig. 2). The distribution of somato­
statin gene expression did not change significantly during
the remainder of the fetal period in the rat. Quantitative
analysis of SS-mRNA in the periventricular nucleus (fig. 3)
suggests that the peptide is synthesized in progres­sively larger amounts from the onset of gene expression
through adulthood. That is in contradistinction to the
perinatal reduction in CRH gene expression, as seen in
figure 4.

Discussion

Somatostatin is widely distributed in the adult mam­
malian central nervous system. In some neurons of the
human and cat retina, it is transiently expressed during
development [22]. Moreover, somatostatin distribution
in the rat visual cortex is altered by early deafferentation
[6], but rat hypothalamic somatostatin distribution and
amount do not change with aging [23].

Hypothalamic somatostatin is a major determinant of
growth hormone secretion from the anterior pituitary.

Other putative roles, possibly related to differentia­tion
and neural plasticity are suggested by its localization to
structures other than those projecting to the median emi­
nence. The ontogeny of somatostatin gene expression in
anatomically defined subcortical and diencephalic struc­
tures has not been described.

We describe the onset and pattern of SS-mRNA pro­
duction in the periventricular nucleus and several limbic
structures of the developing rat, using ISH. This tech­
nique provides anatomic detail of the structures in­
volved. ISH afford enhanced sensitivity compared with
radioimmunoassay of the peptide [10], as well as avoid­
ing issues of antibody specificity to the somatostatin-14
and somatostatin-28 forms. While northern blot analysis
allows pooling of several animals, and can thus prove
more sensitive, it does not share the anatomical detail
provided by ISH [9, 11-13]. Our results show the onset
of SS-mRNA synthesis as early as the 14th fetal day.
Subsequently, SS-mRNA prevalence in the hypothala­
mic periventricular nucleus increases progressively to
adulthood. This pattern is in contradistinction to other
neuropeptides such as CRH (fig. 4): CRH gene expres­sion in the paraventricular nucleus decreases signifi­
cantly on the last day of gestation. The pattern of so­
matostatin gene expression during the late fetal and the
early postnatal life does not suggest its implication in
perinatal regulatory events.
References


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