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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]?

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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 CO_2, 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-

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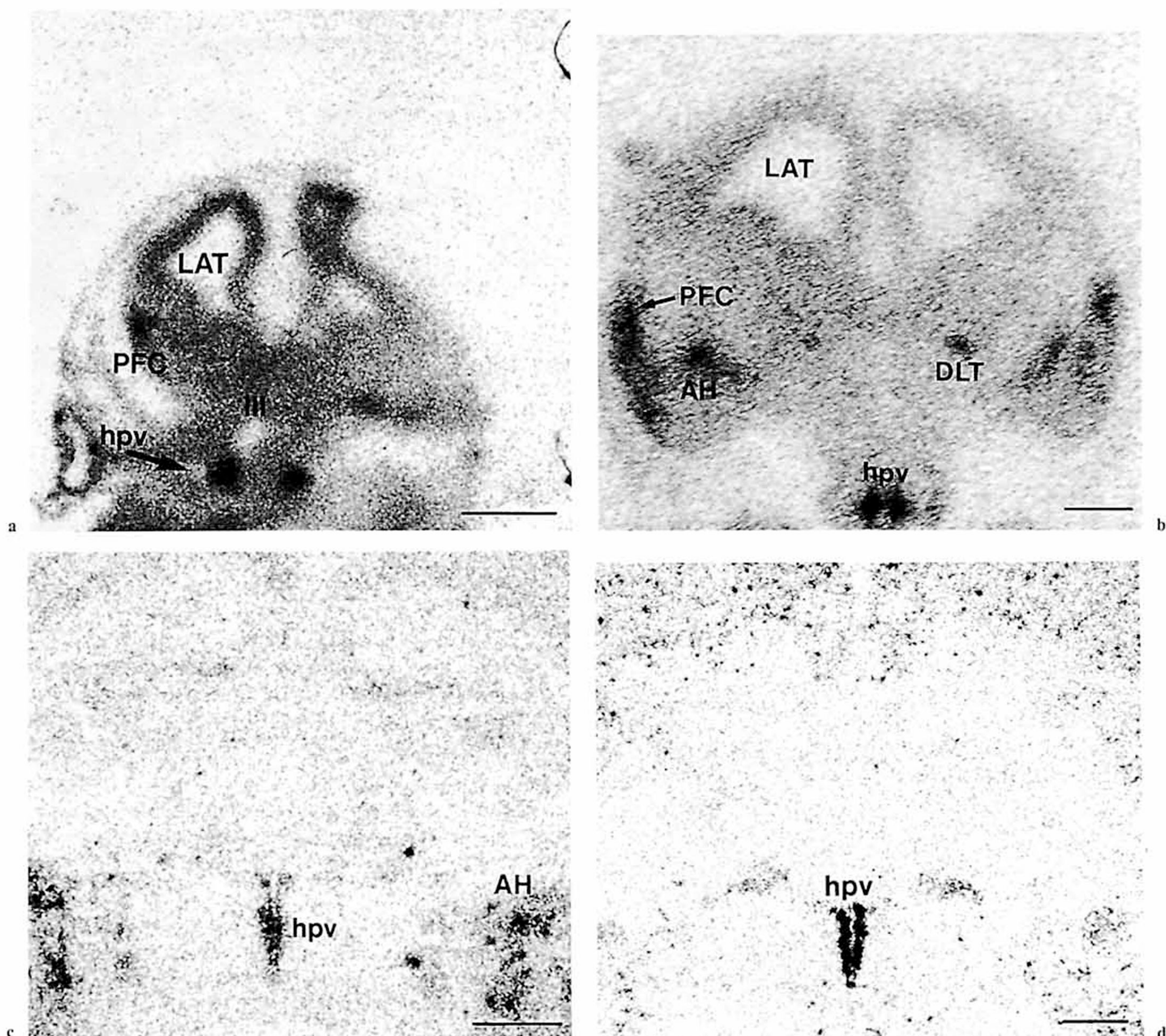

Fig. 1. Coronal slices of the diencephalon of rats on the 14th fetal day; (F14) (a), F16 (b), F21 (c) and 100th postnatal day (d), subjected to ISH with a labelled oligodeoxyonucleotide complementary to SS-mRNA. Bar = 1 mm. AH = Amygdalo/hippocampal complex; hpv = periventricular nucleus; LAT = lateral ventricle; DLT = dorsolateral thalamus; PFC = parieto-frontal cortex; III = 3rd ventricle.

mamide, 4 × SSC (1 × SSC is 0.15 *M* NaCl in 0.015 *M* sodium citrate, pH = 7), 0.5 g/ml sheared, single-stranded salmon sperm DNA, 25 μg/ml yeast tRNA, 100 m*M* DTT, 5 × Denhardt's solution and 10% dextran sulfate. Reaction volume was 0.03 ml/slice, under a coverslip in a humidity chamber. ISH was followed by serial washes at 37 °C: 4 × 15 min in 2 × SSC, then 30 min each in 1 × and 0.3 × SSC. The last two washes were at room temperature. Hybridized slices were dehydrated and apposed to film (Hyperfilm B-max, Amersham, Ill.) for 24 h and developed in 80% solution D-19 Kodak developer.

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-S^{35} (NEN, Wilmington, Del.) using terminal deoxynucleotidyl transferase (Bethesda Research Labs.). Specific activity of labelled probes was 5-8 × 10^8 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.

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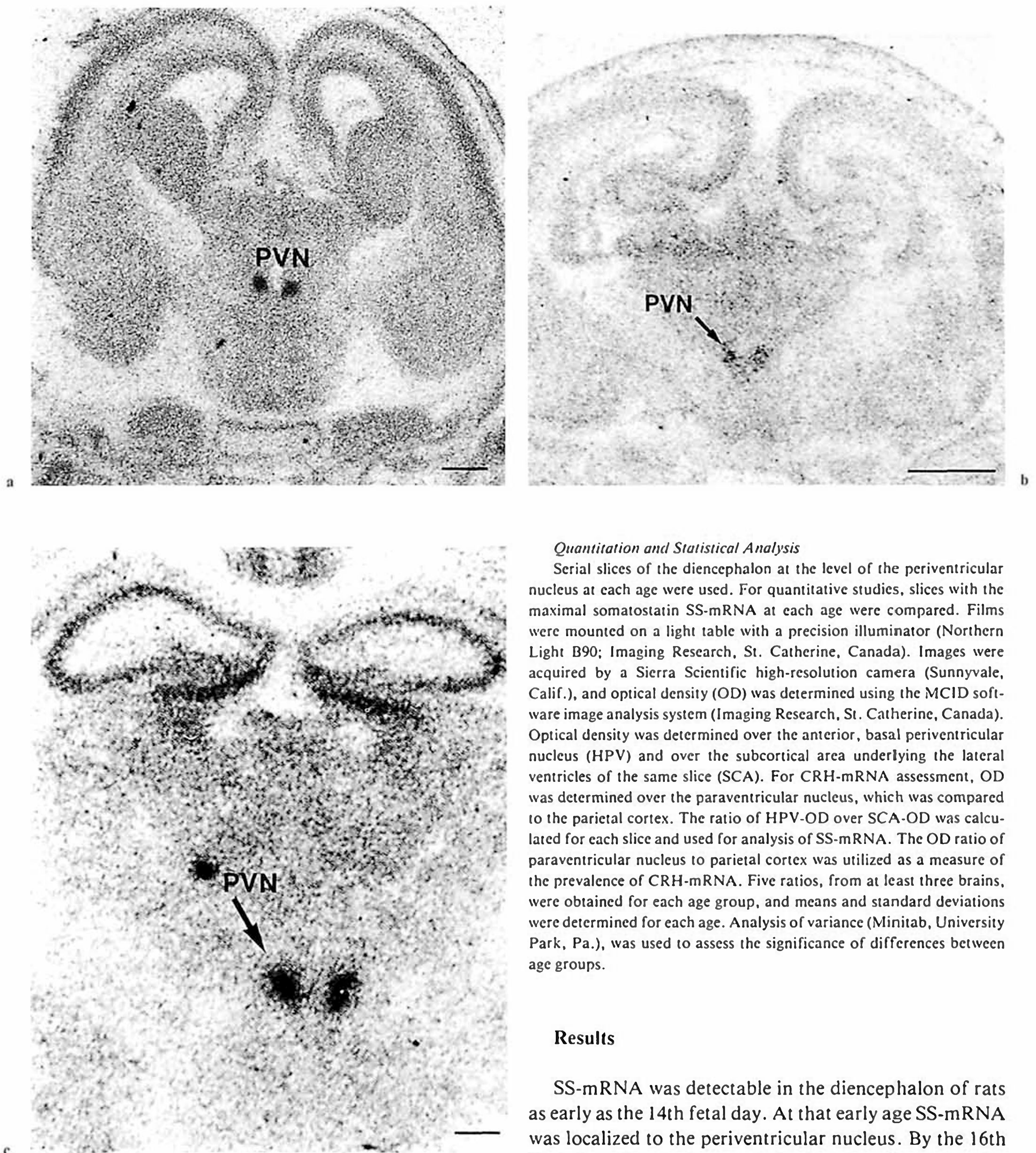

Fig. 2. Coronal slices, at the level of the paraventricular nucleus, of rats on F17 (a), F21 (b), and on the 4th postnatal day (c), subjected to ISH with S^{35}-labelled probe for CRH-mRNA. Bar = 1 mm. PVN = Paraventricular nucleus.

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

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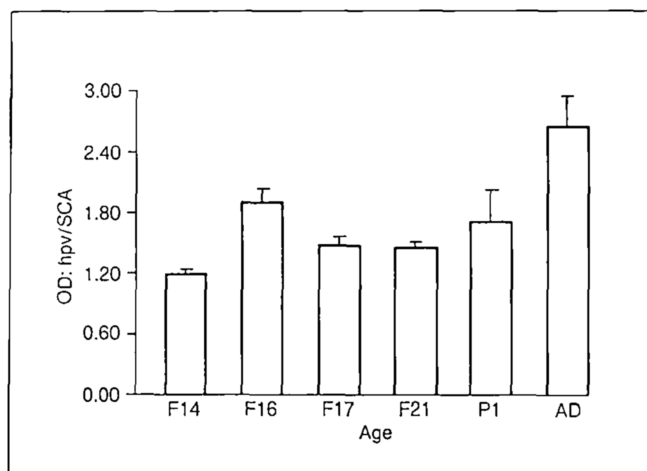

Fig. 3. Ontogeny of SS-mRNA in the periventricular nucleus of the rat (hpv). Results are expressed as the means and standard deviations of 5 ratios of OD: hpv/parietal subcortical area of the same slice (SCA). F14 etc. denote fetal days; P1 is the first postnatal day; AD = Adult. Value at F21 is not significantly different from those at F17 or P1.

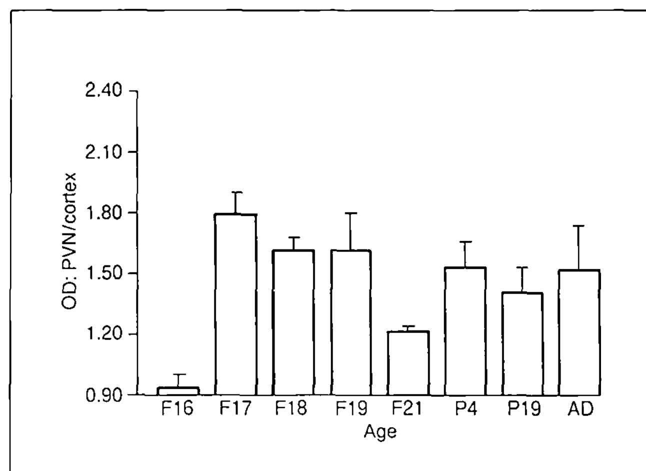

Fig. 4. Ontogeny of CRH gene expression in rat paraventricular nucleus (PVN). Results are expressed as means and standard deviations of 5 OD ratios: PVN/cortex. See text for details of semiquantitative analysis. F16 etc. denote fetal days; P4 etc. denote postnatal days; AD = adult. Value at F21 is significantly ($p < 0.01$) different from those at F19 and P4.

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 paraventricular nucleus of the hypothalamus at its onset on the 17th fetal day (fig. 2). The distribution of somatostatin 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 mammalian 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 eminence. The ontogeny of somatostatin gene expression in anatomically defined subcortical and diencephalic structures has not been described.

We describe the onset and pattern of SS-mRNA production in the periventricular nucleus and several limbic structures of the developing rat, using ISH. This technique provides anatomic detail of the structures involved. ISH afford enhanced sensitivity compared with radioimmunoassay of the peptide [10], as well as avoiding 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 hypothalamic 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 significantly on the last day of gestation. The pattern of somatostatin gene expression during the late fetal and the early postnatal life does not suggest its implication in perinatal regulatory events.

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