Short Communication

Immunohistochemical Distribution of DSIP Immunoreactivity in the Human Hypothalamus during the First Postnatal Year. A Preliminary Report.

(DSIP / immunofluorescence / hypothalamus / infant / development)

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Abstract. The distribution of DSIP-IR cell bodies and fibers was investigated in the normal human hypothalamus during the first postnatal year using the indirect immunofluorescence technique. The analysis of the immunohistochemical patterns obtained in the seven cases analyzed showed regional differences in the localization of cell bodies and fibers. Immunoreactive perikarya were relatively few, and were mostly scattered throughout the anterior and the mediobasal hypothalamus. DSIP-IR fibers and terminal-like structures were observed throughout the rostro-caudal extent of the hypothalamic region. In the present study, we noticed qualitative changes in the density of DSIP immunoreactivity in several hypothalamic structures such as the preoptic area and the median eminence with respect to age. These postnatal differences observed for DSIP could be related to neuronal maturation processes occurring at this period in the central nervous system as well as other physiological processes controlling the evolution of DSIP concentrations. These data are compatible with the proposed role of the neuropeptide in the regulation of many postnatal physiological functions.

The brains of mammals are not mature at birth, in particular in humans (Swaab, 1995; Ulfig et al., 2000). Brain growth and development are influenced by the hormonal state, in which the hypothalamus plays the major regulatory role. It has been established that hypothalamic peptide variations are regulated by hormonal feed-back, with the maturation of this latter also being dependent upon the whole functional maturation of the brain (Swaab, 1999). The control of pituitary hormones secretion does not reach the complete functional state before the postnatal period. Their serum concentrations are more elevated in neonates than in older infants (Kopp et al., 1992; Swaab, 1995). This suggests a postnatal setup of the regulatory system and of the control of hormonal secretion. Furthermore, correlation has been established between temporary changes of hormonal concentrations and the alteration of the peptidergic hypothalamic factors. For this, we previously studied the postnatal distribution of many peptides and/or their receptors in the hypothalamus in human, and marked age-related changes have been evidenced (Najimi et al., 1989; 1990; 1991 a, b; Kopp et al., 1992; Sarrieau et al., 1994). Many other peptidergic factors have been reported to be involved in the control of hormonal secretion and release, such as the nonapeptide delta sleep-inducing peptide (DSIP). Evidence that DSIP is present in the central nervous system was provided in earlier studies by biochemical investigations (Graf and Kastin, 1986). They revealed in laboratory animals and human brains that the hypothalamic region exhibits the highest concentrations of this neuropeptide.

As far as humans are concerned, much less is known about the localization and distribution of this factor, particularly in the hypothalamus. Our current knowledge of its distribution is only based on studies performed in adults principally (Vallet et al., 1990). Thus, the aim of the present study was to establish a detailed investigation of DSIP-immunoreactive (DSIP-IR) neurons in the human hypothalamus during the first postnatal year.

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Abbreviations: DSIP – delta sleep-inducing peptide, DSIP-IR – DSIP-immunoreactive, PBS – phosphate-buffered saline, pmd – post-mortem delay.

Material and Methods

The hypothalami examined in this investigation were obtained from seven infant human brains (Edouard Herriot and Lyon Sud hospitals; Lyon, France). They were devoid of neurological and neuroendocrinological disorders before demise (Table 1). The fixation was achieved after autopsy by perfusion through the carotis interna of 1.5 l of 4% paraformaldehyde (0.1 M phosphate-buffered saline (PBS), pH 7.4) in approximately 30 min. A block of brain including the hypothalamus was excised, frozen in liquid nitrogen and cut with a cryostat into 20-µm thick coronal sections. The sections were then mounted on chromalun-gelatin-coated slides and stored at –20°C.

For the immunohistochemistry, the indirect immunofluorescence technique (Coons et al., 1958) was used. The mounted hypothalamic sections were incubated with DSIP-antiserum at working dilution 1 : 1000 overnight at 20°C. The incubation medium also contained 0.3% Triton X-100, 1.0% normal sheep serum and 0.1% sodium azide. After incubation, the sections were washed with PBS and incubated with IgG goat anti-rat fluorescein isothiocyanate (Miles, Ltd. Co., Paris, France) at the dilution 1 : 100 during 1 h, rinsed in PBS and mounted in a phosphate buffer/glycerine mixture (1 : 3). The anti-DSIP serum used was produced in rat by conjugating the peptide (Bachem, Bubendorf, Switzerland) with thyroglobulin and glutaraldehyde. Its characteristics have been described in detail previously (Charnay et al., 1989).

In the immunohistochemical controls, we first omitted the specific antiserum or substituted it by non-immune serum (which gave no positive reaction); second, each antiserum used was absorbed by the homologous conjugated and non-conjugated DSIP (peptide concentration 20 µM) and produced the same results, irrespective of its being preabsorbed or not by other peptides tested (e.g. somatostatin, neurotensin, vasoactive intestinal polypeptide, beta-endorphin, cholecystokinin and substance P). In addition, no immunoreactive neuronal DSIP elements were observed in the absence of the primary antiserum.

Results

DSIP-containing neuronal elements were not homogeneously distributed throughout

Table 1. Source of brain tissues

Brain	Sex	Age	Post-mortem delay (h)	Cause of death
A	F	28 d	4	necrotizing enterocolitis
B	M	30 d	6	diaphragmatic hernia with pulmonary hypoplasia
C	M	40 d	10	purpura fulminans
D	M	60 d	12	septicemia
E	F	120 d	10	pulmonary hemorrhage
F	M	150 d	10	gastric lesions
G	M	270 d	12	enterocolitis

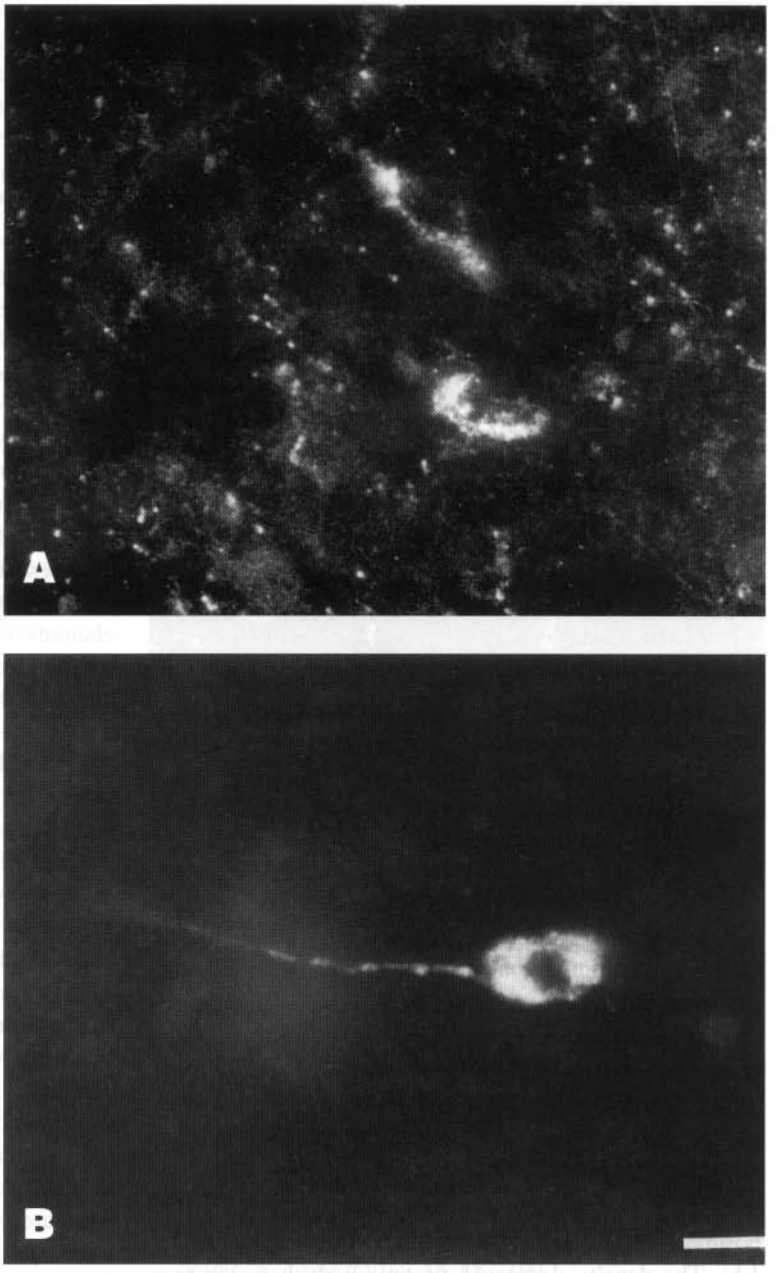

Fig. 1. DSIP-immunoreactive cell bodies and fibers in the preoptic area of an infant aged 30 days (A) and an infant aged 120 days (B). The staining is present in the cytoplasm of cell bodies and in varicosities in fibers. Scale bar: 25 µm.