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SEXUAL DIMORPHISM OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IN THE RAT INTERPEDUNCULAR NUCLEUS

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The intensity of immunostaining for the glial fibrillary acidic protein (GFAP) is outstandingly high in the interpeduncular nucleus. This nucleus was compared in males and females for its GFAP immunoreaction. Immunohistochemistry was carried out on free floating vibratome slices and evaluated by surface densitometry.

While in males the reactions were similar, females showed individual variations. Since the interpeduncular nucleus is a hormonally inactive brain area where gonadal hormones do not induce plastic synaptic changes, it is concluded that concerning this astroglial marker a sexual dimorphism exists also outside the "endocrine brain".

Keywords: GFAP – interpeduncular nucleus – sexual dimorphism – rat

INTRODUCTION

Astrocytes are known to undergo changes in a number of experimental and pathological situations (for references see [13]). It has also been established that different hormonal states have an influence on astroglia mainly in the neurohormonal regulatory centres of the hypothalamus. At these sites, the astroglial marker glial fibrillary acidic protein (GFAP) has been shown to alter with changing hormone levels [1, 10, 16, 17]. Sex steroids were found particularly effective to elicit alterations in both the amount and immunoreactivity of GFAP (for references see [3]). Immunohistochemical and *in situ* hybridization studies of the rat hypothalamus also suggest that the expression of this astroglial marker is more intense in males than in females [1]. While the hypothalamic expression of GFAP has been claimed to be hormone-sensitive [11], on the basis of investigations in the arcuate nucleus of the rat hypothalamus [15, 19], hormone-induced changes in GFAP-immunoreactivity (GFAP-ir) were

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interpreted as accompanying plastic changes of synapses. Thus, the question whether sex steroids have a direct effect on astroglia [11] or an astroglial reaction is coupled to the hormone-induced plasticity of synapses [4, 15] is still pending.

In previous studies on the regional distribution of GFAP-ir throughout the brain [20] an outstandingly high immunoreactivity was found in the interpeduncular nucleus (IPN). To our present knowledge, this nucleus does not participate in any hormonal regulatory mechanism, nor does it respond with plastic neuronal changes to different hormonal states (unpublished observation). Therefore, the IPN might be a suitable territory to test if there exists a genuine sexual dimorphism for GFAP in a "non-endocrine" region of the brain. To this end we performed the GFAP immunostaining of the IPN in age-matched males and females.

MATERIALS AND METHODS

Three-months-old albino rats (4 males and 6 females) were transcardially perfused under deep Nembutal anesthesia with a mixture of paraformaldehyde, picric acid (4% each) and 0.3% glutaraldehyde in 0.1 M phosphate buffer. Brains were removed from the skull and kept in the same fixative for 2 h. After an overnight soaking in 0.1 M phosphate-buffered saline (PBS), 60 μm vibratome sections were cut from the midbrain in the coronal plane, and rinsed in three changes of PBS. Free-floating sections were processed. They were first treated for 10 min with 1% sodium-borohydride to eliminate free aldehyde radicals, and then for 2 h with 10% normal goat serum to inhibit non-specific immunoreactivities, both treatments being performed at room temperature. Following repeated rinses in PBS, sections were incubated at 4 °C for 36 h with a monoclonal anti-GFAP serum (Boehringer) applied in 1 : 400 dilution. Incubation was terminated by rinsing in three changes of PBS. As second antibody, biotinylated rabbit anti-mouse IgG (RAM) was used at a dilution of 1 : 100 for 5 h, followed by the avidin-biotin-peroxidase complex (Vectastain, dilution 1 : 100). The immune reaction was visualized with 3,3′-diaminobenzidine tetrahydrochloride (DAB). Sections were mounted on a glass slide and coverslipped for light microscopy. Some preparations were processed for resin embedding and semithin sectioning. Semithin sections (0.5–1.5 μm) were stained with 1% toluidine blue. For the description of section planes and microscopically distinct areas, the distances from the bregma are given in mm, the symbol '–' indicates the caudal direction.

Owing to the 60 μm thickness of vibratome sections in which immunoreaction is restricted to a 10–15 μm surface zone, it was of special importance to compare images taken at identical focus depths. This was achieved by focusing consistently on the GFAP-immunostained perivascular glia at the upper surface of the section.

The intensity of immunostaining was evaluated by surface densitometry. The microscopic images were focused on the upper section surface, and digitized into 8-bit grey-level images. A 250×250 μm area was measured in the central part of the IPN. GFAP-ir elements were extracted from the background by thresholding using the NIH Image Software 1.6.1.v.

Control incubations were carried out either with the omission of the primary antiserum, the sections being processed with the peroxidase conjugate only, or with preabsorbed antisera. In these cases no immunostaining occurred.

RESULTS

In semithin preparations of the IPN (Fig. 1), astrocytes could readily be identified on the basis of their typical pale nuclei and the aggregation of chromatin adjacent the nuclear envelope [14]. No difference was seen between males and females in the histology, distribution and number per unit area of astrocytes.

In coronal sections of the midbrain of male rats GFAP immunoreactivity was pronounced throughout the entire IPN. In the rostral portion of the nucleus (–5.80),

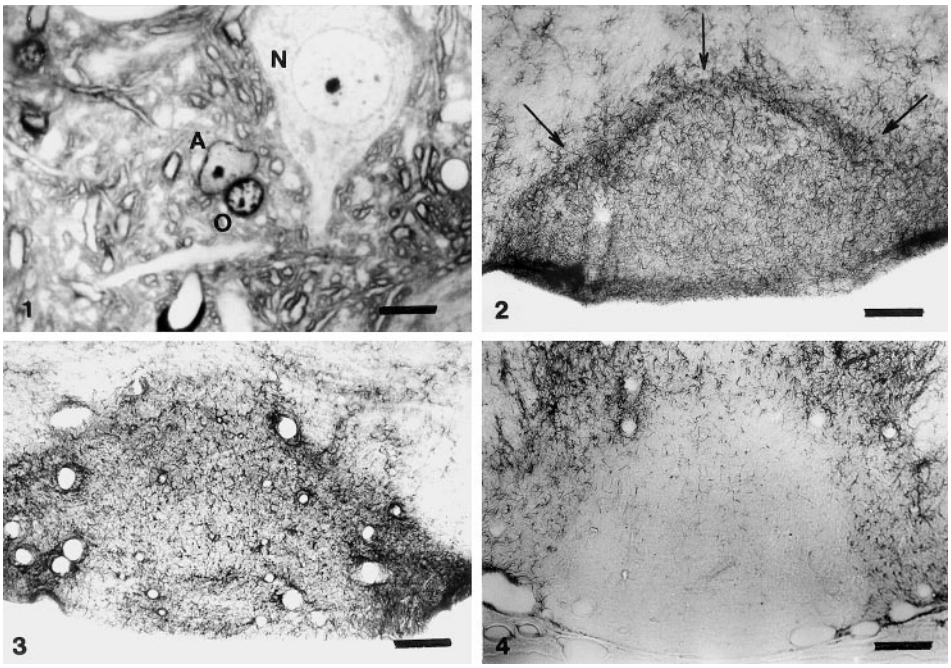

Fig. 1. Semithin section from the IPN showing a neuron (N), an astrocyte (A), and an oligodendrocyte (O). Each cell type is distinguishable on the basis of nuclear structure. Astrocytes have pale nuclei with chromatin accumulated at the nuclear membrane. Scale bar: 35 μm

Fig. 2. Coronal section through the mid-portion of the male IPN stained for GFAP-ir. An intense reaction is seen throughout but the periphery of the nucleus (arrows) is particularly intensely immunolabelled. Scale bar: 90 μm

Figs 3, 4. GFAP-ir in coronal sections through the mid-portions of IPNs from two age-matched females. In animal 1 (Fig. 3) the immunostaining is only slightly reduced as compared to that in the male and the reduction is confined to the central region of the nuclear core. In animal 2 (Fig. 4) the entire core of the nucleus is unstained and a marked decrease of staining is observed on the periphery. Scale bar: 90 μm

immunoreactivity was evenly distributed. In the mid-portion (–6.04), immunoreaction was more intense at the periphery than in the core of the nucleus (Fig. 2). Using the widely accepted terminology for the subdivisions of the IPN [9, 12], the more intensely reacted periphery included the lateral, dorsolateral and dorsomedial subnuclei. The core corresponded to the caudal and intermediate subnuclei. In sections between –6.30 and –6.72, the peripherally intense staining was only laterally observed including the lateral and dorsolateral subnuclei. The dorsomedial subnucleus was less heavily labeled, similarly to the core area. From the dorsolateral subnucleus, clusters of GFAP-immunostained astrocytes were detected to form a lateral extension of the peripheral population. The immunostaining of pericapillary astroglia processes was marked throughout the entire IPN.

Midbrain sections from female rats showed a considerably lower intensity of immunostaining as compared to similar sections from males. This applied to both core and periphery of the IPN. Unlike in males, however, in the females the intensity of GFAP-ir exhibited wide individual variations (Figs 3, 4). It is, however, necessary to note that intensity-range of staining in females was below the mean intensity observed in males. In terms of densitometric values, this meant 56.47 ± 11.63 ($n = 18$) and 48.0 ± 36.07 ($n = 18$) for males and females, respectively.

DISCUSSION

The IPN is an important relay nucleus of the limbic system which has no direct involvement in neurohormonal regulation. In view of the fact that no difference could be observed between males and females concerning size, distribution and packing density of astrocytes, the present findings indicate a sexual dimorphism caused by different levels of GFAP-ir in the intact male and female IPN. We could thus strengthen in a hormonally inactive brain area the argument that GFAP-ir is more intensely expressed in males than in females. Our findings also suggest that, at least in the IPN, this particular type of sexual dimorphism is genuine, i.e. not induced by synaptic or other reactive changes. In experimental and pathological degeneration of synapses, GFAP has been shown to increase substantially in reactive astrocytes [5, 13] and to be expressed in astrocytes of regions where it is normally not detectable [7, 8]. The evenly intense immunostaining in the male has been shown in another study to decrease upon castration without apparent structural reorganization of the nucleus [6]. Thus it is believed that in the IPN, the difference in GFAP-ir between males and females is a primary feature of astrocytes rather than their reaction to a loss of synapses. On the other hand, it is remarkable that while in males a consistently strong reaction was encountered, in females the GFAP-ir showed individual variations, although always within a range below the reaction intensity of males. This argues for additional sexual cycle-related fluctuations of GFAP-ir in the IPN of females. Data on the hormonal dependence of GFAP have been published for the hippocampus and cerebellum [2, 18] indicating that further brain areas might also be involved in hormone-related changes of GFAP.

It can be concluded that a genuine sexual dimorphism of astroglia exists in the IPN, an area not belonging to the "endocrine brain". GFAP-immunoreactivities are higher in males than in females, whereas fluctuations in the female GFAP-reactions may be sexual cycle-related. This assumption awaits experimental support.

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