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ANP Ontogeny in Rat Developing in the Lateral Choroid Plexus

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Abstract: A immunohistochemical study was carried out to verify whether ANP is present in the lateral ventricle plexus in adult and embryos rats. The results evidenced the ANP presence in ependymal cells of the plexus in adult rat and also in embryo at 16° day of foetal life suggesting the important role of ANP also in the development and maturation of brain.

Key words: ANP, ventricle plexus, ontogeny, rat development, brain

INTRODUCTION

The Choroid Plexuses (CPs) are able to synthesize neuropeptides, as vasopressin and ANP.

Regarding to ANP, many years ago the ANP (Atrial Natriuretic Peptide) presence was demonstrated in the Cerebrospinal Fluid (CSF) in different vertebrate species as frog, rat, dog and man. In particular, it was shown that ANP was synthesized in the human brain because in cerebrospinal fluid ANP was eluted at the position of a low molecular weight and no high molecular weight form could be detected, but in the plasma low and high molecular forms were found to be present, thus it is possible that ANP does not come from the heart (Marumo *et al*., 1987). Also in dog it was demonstrated that in CSF the ANP does not come from blood, but it originates from brain (Marumo *et al*., 1988).

Furthermore, also in frog it was demonstrated that the CP is an important target organ for the ANP and that ANP, by binding with its receptors, is involved in the process of osmoregulation and the control of the cerebrospinal production (Tei *et al*., 1998).

ANP was found in adult rat brain, but few are the studies carried out in rat during the foetal life; we previously studied the ANP-ontogeny in the hypothalamic supraoptic (Farina Lipari *et al*., 2005) and suprachiasmatic (Farina Lipari *et al*., 2007) nuclei and considering the important action of the ANP in the regulation of the body fluids, we consider to be interesting to study the ANP ontogeny in the lateral plexuses in developing rat brain to establish at which developmental stage ANP appears; moreover, we want to compare it with the developmental stages in which ANP appears in the hypothalamic supraoptic and suprachiasmatic nuclei.

Furthermore, since we early studied in rat hypothalamic nuclei the ontogeny of vasopressin (Farina Lipari *et al*., 1993) and since vasopressin and ANP play an antagonist role we want compare the developmental stages of vasopressin and ANP.

MATERIALS AND METHODS

Four pregnant female Wistar rats were bred and housed in conditions of the constant temperature and humidity and 12:12 h light-dark cycle and given free access to food and water ad libitum. The rats at 16° day of gestation and at 18° day of gestation life were deeply anaesthetized with pentobarbital. The 16° and 18° day old embryos were fixed in toto by immersion in Bouin's fluid. Brains were removed, fixed in the same fixative, dehydrated in graded alcohols and embedded in paraffin.

Six-seven micra serial sections were prepared and stained with hematoxylin-eosin or immunohistochemical method. The sections were ANP-immunostained and detected with the avidin-strepavidin system. Endogen peroxidase was blocked by incubation with PBS buffer containing 0.3% hydrogen peroxide for 5-10 min at room temperature. Following blockage of the non specific binding by normal goat serum, the sections were treated with ANP (Ser-Tyr) polyclonal antiserum raised in rabbit (Chemicon) at serial dilution (1/500,1/600,1/800) in 0.05 M PBS buffer, pH 7.2 for 12 h at 4°C; subsequently were washed in PBS buffer (three times, each 3 min). The reaction was revealed with the avidin-biotin complex or the streptavidin system (Ylem); binding was demonstrated by amino-ethyl-carbazole as substrate. The negative controls were those in which the primary antiserum was omitted.

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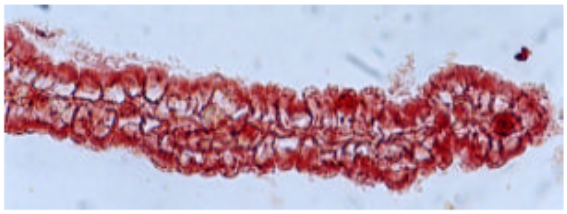

Fig. 1: Lateral choroid plexus of embryo at 16° day of foetal life. ANP immunopositivity is present in the ependymal cells

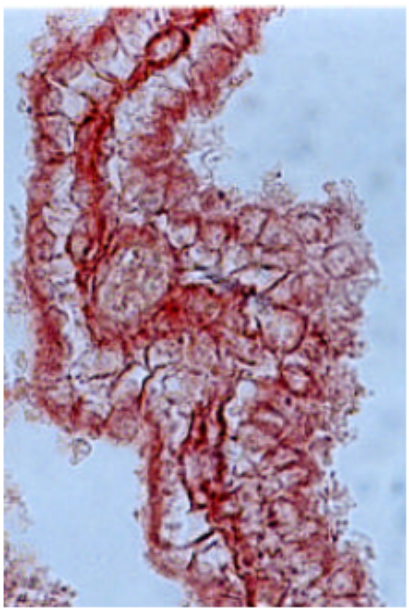

Fig. 2: Lateral choroid plexus of embryo at 18° day of foetal life. An intense ANP immunpositivity is present in the ependymal cells

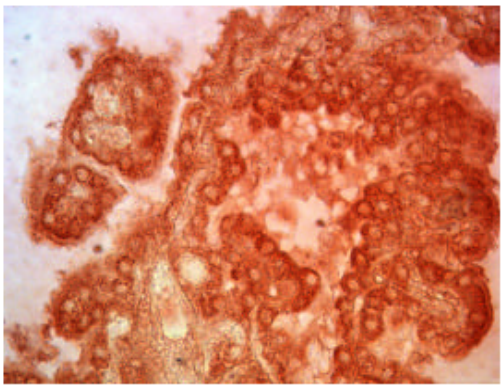

Fig. 3: Adult rat lateral choroid plexus. Many ANP-immunositive granules are visible

RESULTS

The lateral ventricles are lined with the ependyma and this epithelium continues into ependymal epithelium of CPs, which float into the ventricular cavity and are attached to tshe ependyma by a thin stalk. The ependyma of the CP is composed of a single layer of the cells. Within the core of the plexus there is a complex vascular network of relatively large venular-like capillaries.

The observations for ANP in the CPs of rat embryos at 16° and 18° day of the intrauterine life showed that the immunopositivity for ANP is present in embryo at 16° day (Fig. 1) and also in embryo at 18° day of intrauterine life (Fig. 2), the immunopositivite granules are more present in apical area of the ependymal cells .

The observations immunohistochemical for ANP in the CP of the adult rat evidenced an intense immuno-positivity in the epithelial cells and immunopositive granules are visible in whole cytoplasm (Fig. 3).

DISCUSSION

The ANP presence in the adult rat CPs has already been demonstrated (Chodoboski *et al*., 1997), but in this research we reported the results about the ANP ontogeny in lateral plexus of rat embryos to verify at that developmental stage ANP appears. The immohisto-chemical results showed that ANP is present in adult rat but showed that ANP appears precociously at 16° day of the foetal life and this immunohistochemical positivity increases at 18° day of foetal life. This is indicates that ANP is a peptide with important role in the brain development by its action on the regulation of the synthesis and composition of the CSF.

There is growing evidence that in the CPs the ependymal epithelium synthesizes and secretes vasopressin, since it has been shown that CP epithelia show intensive immunopositivity for vasopressin peptide (Chodoboski *et al*., 1997) near the apical membrane of the CP epithelia, which indicates that CP epithelium secrets ANP.

Indeed ANP has a antagonist role of the vasopressin and so we think it could be important to compared the developmental stage when the two peptides appear. In our previous study, it has been is reported that in the hypothalamic supraoptic nucleus the vasopressin appears at 16° day of the foetal life and successively at 18° day of fetal life in the hypothalamic paraventricular nucleus (Farina Lipari *et al*., 2001); thus by comparation of the results regarding the vasopressin and ANP, it is evident that both peptides appear at the same developmental

stage; this synchronism of the developmental stage suggests that the two peptides may interact between them and may regulate the volume and the electrolyte composition of CSF.

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