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Short Report

Developmental spatiotemporal expression of Alzheimer βAPP isoforms in the chick embryo

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ABSTRACT The ß-amyloid precursor proteins (ßAPPs) are a group of glycoproteins that result from the alternative splicing of a single primary transcript. They contain in their sequences the ß-amyloid peptide (ßA4), the principal component of the cerebral amyloid plaques found in patients with Alzheimer's disease. Using antibodies recognizing different regions of the major ßAPPs, we have analysed by immunohistochemistry their spatiotemporal expression during chick embryogenesis. We have observed that the ßAPP isoforms containing the Kunitz protease inhibitor domain (ßAPP-KPI) have a more widespread distribution than the isoforms lacking this domain. Also, differential patterns of expression between both types of isoforms were detected in many embryonic tissues, including the floor and roof plates of the neural tube, and sites of active axogenesis in the dorsal diencephalon and mesencephalon. An antibody that recognizes the C-terminal sequence of ßA4 did not display immunoreaction until a relatively late phase of embryogenesis, the 8 day of incubation. These observations suggest that the chick embryo may be a simple and useful model for the analysis of ßAPP processing and function.

In the present study we have analyzed by immunohistochemistry the expression and distribution of the Alzheimer ßAPP isoforms during development of the chick embryo. Three antibodies against different regions of the major ßAPPs were used (see Fig. 1): anti-KPI rabbit AB5302 (Chemicon), a polyclonal antibody directed against the Kunitz-type serine protease inhibitor domain of ßAPP; anti-ßA42 rabbit AB5078P (Chemicon), a polyclonal antibody generated against the 6-amino acid C-terminal sequence of the human ß-amyloid peptide 1-42; and mouse anti-643-695/Jonas (Boehringer), a monoclonal antibody against the C-terminal end of ßAPP. Antibodies from the Developmental Studies Hybridoma Bank for additional markers as HNF3ß, Nkx2.2, Islet, Lim2, PAX7, NCAM, L1 and NAPA73 were also used in order to identify those structures where ßAPP or ßA4 overlap expression of a particular marker. The antigen retrieval (microwave treatment) method and ABC procedure were applied to either paraffin or cryostat sections of chick embryos collected on several fixatives throughout the 3 to 9 days of incubation.

The results are summarized in the Table 1. It is noteworthy that the signal obtained with the anti-KPI antibody displayed a more widespread distribution than those observed with the other antibodies, despite the fact that it is designed against a region of ßAPP contained only in those isoforms bearing the KPI domain. This observation may be due to the fact that the anti-KPI antibody recognizes a more accessible (the extracellular) region of ßAPP and, in addition, that it may detect both the unprocessed and the secreted forms of ßAPP after its proteolytic cleavage by either alpha- or beta-secretase (see the last review by Selkoe, 2001). The results also show that there is a developmental temporal and spatial regulation of ßAPP isoforms in many embryonic tissues of the chick, reflecting a probable differential regulation of the diverse mRNAs coming from the unique primary transcript and in the proteolytic processing of the diverse ßAPPs, a fact already noted in many adult and embryonic tissues of the different species analyzed, including man. Another salient result is the absence of an evident immunoreactivity for the antibody anti-ßA42 until the 8th or 9th day of incubation, the latest phase of embryogenesis analyzed in this study. It was first detected in the adrenal gland, dorsal root ganglia and motor neurons (Fig. 2).

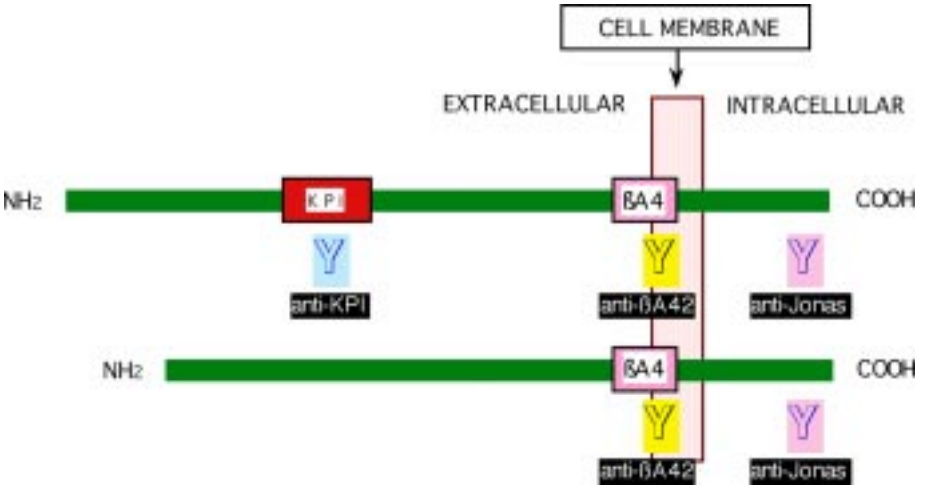

Fig. 1. Schematic diagrams of the major βAPP isoforms and the three antibodies used in the study.

Intense labelling for ßAPP was found in embryonic sites of growing axons (evidenced by NAPA 73, NCAM and L1 immunoreactivity) as the dorsal diencephalon and mesencephalon (with anti-Jonas) or the floor plate (with anti-KPI), suggesting a possible functional role for ßAPP in axonal elongation and/or guidance during development. The immunoreaction with anti-KPI in the floor plate was seen in both the floor plate cells and in the subjacent axons crossing the commissural

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TABLE 1

SUMMARY OF THE EXPRESSION OF DIFFERENT βAPPs IN THE CHICK EMBRYO AS REVEALED BY DOMAIN-SPECIFIC ANTIBODIES

Days of incubation:	3		4		5		6		8 and 9	
anti-KPI	Posterior epithelium of the lens	+	Heart	+++	Spinal cord	+	CNS	+	Spinal cord motoneurons	++
	Heart ventricle	++	CNS	+	Motoneurons	++	Retina ganglion cells	++	DRG	++
			Floor and roof plates	+	Interneurons	++	Cranial ganglia	+	Skeletal myoblasts	++
			Retina	+	Floor and roof plates	+			Heart	+++
			Anterior epithelium of the lens	+	DRG	++				
			Cornea	+	Cardiac and skeletal myoblasts	+++				
			DRG	+						
			Cranial ganglia	+						
			DML of the dermamyotome	+						
			Mesonephros	+						
anti-Jonas	Ependymal layers of diencephalon and neural retina	+	Diencephalon	+	Mesonephros	+	CNS	+	Spinal cord motoneurons	+
	Adenohypophisis	+	Mesencephalon	+					Skeletal myoblasts	++
			DML of the dermamyotome	+						
			Anterior epithelium of the lens	+						
			DRG	+						
anti-ßA42	ND		ND		ND		ND		Spinal cord motoneurons	+
									DRG	+
									Adrenal gland	+

Immunoreactivity was semiquantitatively evaluated as: +, low; ++, high and +++, very high. CNS, central nervous system; DML, Dorsomedial lip; DRG, dorsal root ganglion; ND, not detected.

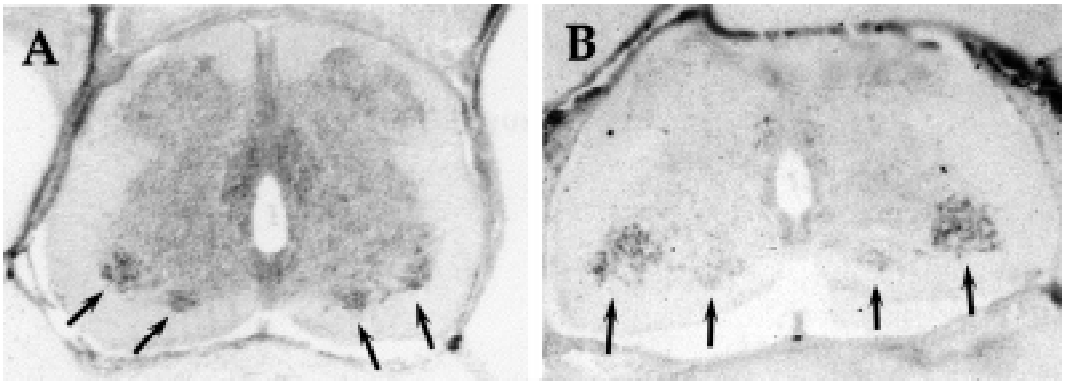

Fig. 2. Immunoreaction with anti-KPI (A) and anti-βA42 (B) antibodies in the spinal cord of 9-day embryos. *Note that motor neurons (arrows) of the lateral column are the most intensely marked cells with the anti-ßA42 antibody.*

midline. Intense *in situ* hybridization for ßAPP mRNA has been observed in the roof plate of the lumbar spinal cord by 4 and 5 days of incubation (Barnes *et al.,* 1998), a site where we have seen rich immunoreaction with the anti-KPI antibody.

Our laboratory has previously described a differentially and developmentally regulated expression of each of the four major ßAPP mRNAs in several tissues of the rat embryo, including the optic primordium (Sarasa *et al.,* 2000). Immunolocalization studies of ßAPP have been also performed during normal rat development and the eye is one of the early expression sites of ßAPP (Ohta *et al.,* 1993). In the chick embryo we have found labelling for ßAPPs, both containing and lacking the KPI domain, from E3 and with changing levels and places of expression as development proceeds.

In conclusion, the observations reported in this study are suggesting that the chick embryo may be a useful *in vivo* model for the analysis of the processing and developmental function of the diverse ßAPP molecules.

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