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McAB 300 ANTIBODY AGAINST CALBINDIN D-28K IS A GLIAL MARKER IN THE TELEOST BRAIN

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INTRODUCTION

Calbindin D-28k (CaBP28k) is a cytosolic water soluble calcium-binding protein, which is located in all areas of the central nervous system where it labels specific populations of neurons[3]. In a previous work on the distribution of calcium-binding proteins in the teleost cerebellum[1], we observed that after immunostaining with the monoclonal antibody 300 (McAB 300) against CaBP28k[3], Purkinje cells were not stained, contrary to what is observed in other phylogenetic groups[3,4]. However, abundant cells with other specific morphological features and distributions were labeled. These immunopositive cells seem to correspond to glial cells. This observation is surprising since CaBP28k-immunoreactivity is considered, and widely used, as an excellent neuronal marker demonstrating specific neuronal subpopulations throughout the brain[2,3].

The goals of this work are: 1) to describe McAB 300 staining in different brain areas; 2) to compare this staining with other monoclonal and polyclonal antibody-stainings against CaBP28k, and 3) to compare the labeling for McAB 300 in the fish brain with the stainings for typical glial markers such as glial fibrillary acidic protein (GFAP) and vimentin.

METHODS

Eight rainbow trouts (*Oncorhynchus mykiss*), six tenches (*Tinca tinca*) and four Wistar rats were used. After anesthesia, the animals were perfused with 4% paraformaldehyde and 15% saturated picric acid in 0.1 M phosphate buffer, pH 7.2 (PB). Half of the brains were included in paraffin, sectioned at 9 µm and mounted on slides. The rest were cut at 30 µm using a cryostat. Alternated sections were incubated in primary antiserum (McAB 300) diluted 1:1000, 1:2500 and 1:5000 in PB containing 10% normal horse serum, followed by the avidin-biotin-peroxidase method as has been previously described, including the same controls of the immunostaining procedure[1].

In addition, the remaining sections were stained with monoclonal antibodies 292 and 318 against CaBP28k[3], with a polyclonal antibody (R8701) against CaBP28k[2] all diluted 1:1000, and with antibodies against GFAP (clone G-A-5) and vimentin (clone V9), from Boehringer Mannheim, diluted 1:400.

The monoclonal antibodies against CaBP28k used, have been produced in mice against