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Localization of aldolase C mRNA in brain cells

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The expression of aldolase C and aldolase A mRNA was assessed by Northern blot hybridization using RNAs purified from cultured rat and mouse brain neurons and astroglial cells. Neurons were found to contain about 4-fold more aldolase C mRNA and about twice as much aldolase A mRNA than astroglia. Analysis of the cellular localization of aldolase C mRNA by in situ hybridization to brain slices showed a predominantly neuronal labeling with an irregular distribution. A strong signal was observed in Purkinje cell somata and a weaker signal in subpopulations of neurons in cerebral cortex, striatum, hippocampus, hypothalamic nuclei and primary olfactory cortex.

Aldolase C; Brain cell; Hybridization in situ

1. INTRODUCTION

Fructose 1,6-diphosphate aldolase (EC 4.1.2.13) occurs in mammalian tissues in three different molecular forms: aldolase A, present in muscle and most other organs, aldolase B found in liver and kidney, and aldolase C characteristic of nerve tissue. During ontogeny, expression of the aldolase C gene is quite ubiquitous.

In adult rat brain, the aldolase C subunit appears as part of a five-member set of hybrid A–C isoenzymes: there is a homotetramer aldolase C4, three heterotetramers C3A, C2A2, CA3 and a homotetramer aldolase A4 [1]. Until now, few immunohistochemical studies have investigated the cellular distribution of aldolase C within the nervous system [2,3].

Our laboratory has cloned the genes coding for aldolases A, B and C [4–6]. We are in possession of specific cDNA probes which offer the possibility of assessing the expression of isoaldolases in brain by Northern blotting and in situ hybridization. The probes were generated mainly from the 3′ untranslated region of rat aldolases A, B and C and allow specific detection of the corresponding aldolase mRNA in rat brain.

The aim of the present study was to determine in which type of cell aldolase C is synthesized. Is the mRNA expressed only in neuronal cells (as in the case of γ-enolase) [7], only in glial cells (as for creatine kinase BB isoenzyme) [8], or in both neurons and glia? One immunohistochemical study [3] has suggested that aldolase C protein is expressed in both cell types.

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2. MATERIALS AND METHODS

2.1. *Cell cultures*

Cells were derived from brains of random-bred Sprague-Dawley rats or Swiss mice. Cultures of neuronal cells were set up from single cell suspensions of fetal brains at 15–16 days of gestation as previously described [9,10] and were used at 14 days in vitro. The cultures were composed predominantly of neurons (>95%). The residual 1–5% of cells were GFAP+ astrocytes. 95% pure astroglial cell cultures were prepared from newborn cerebral hemispheres as described in [11]. The GFAP+ cells were identified as type-1 astrocytes [12].

RNA purification and Northern blots were performed as reported in [6]. For aldolase A, the probe consisted of a 360 bp fragment covering 175 bp of the 3′ coding sequence and 185 bp of the 3′ non-coding sequence. For aldolase C the probe consisted of the r.Al.C_2 insert [6].

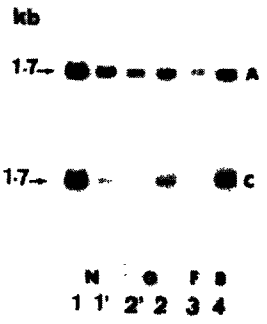

Fig. 1. Northern blot analysis of aldolase C and aldolase A mRNAs. 10 μg of total RNAs were electrophoresed in a 1.2% (w/v) formaldehyde agarose gel, blotted onto a nylon filter, then hybridized in (A) with a rat aldolase A DNA probe and in C with r.Al.C2 probe. (1) Rat and 1′ mouse primary cultures of total brain neurons; (2) rat and 2′ mouse secondary cultures of astroglial cells; (3) cultured rat fibroblasts; (4) adult rat brain. The arrow indicates the size (in kb) of the aldolase C mRNA.

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Preparation of tissue sections for in situ hybridization was performed as described in [13], using adult male Wistar rats, weighing 200–250 g. Sense and antisense aldolase C single-stranded cDNA probes were prepared from cDNA fragments subcloned into the M13 mp10 bacteriophage. The sense probe (Dde 23) consisted of a Dde fragment, spanning from nt 1391 to 1546 [14], totally included in the 3′ noncoding region. The antisense probe consisted of the r.Al.C2 insert [6]. The probes were prepared by primer extension using single-stranded recombinant M13 DNA as template, as described by De Keyzer et al. [15], with [^{35}S]dATP as the radioactive nucleotide.

In situ hybridization was performed according to the protocol of Bloch et al. [13]. The controls included the use of a sense probe (Dde 23), or of a cDNA probe unrelated to aldolase C such as preproenkephalin A (PPA) [16].

3. RESULTS

3.1. *Northern blot analysis*

Fig. 1C shows that both astroglia and neurons contain aldolase C mRNA. As the probe used is derived from rat but contains a coding segment that cross-hybridizes with mouse RNA [6], the hybridization signal with rat cell extracts is considerably higher than with mouse. In both cases, however, the relative abundance of aldolase C mRNA is at least 4 times greater in neurons than in astroglia.

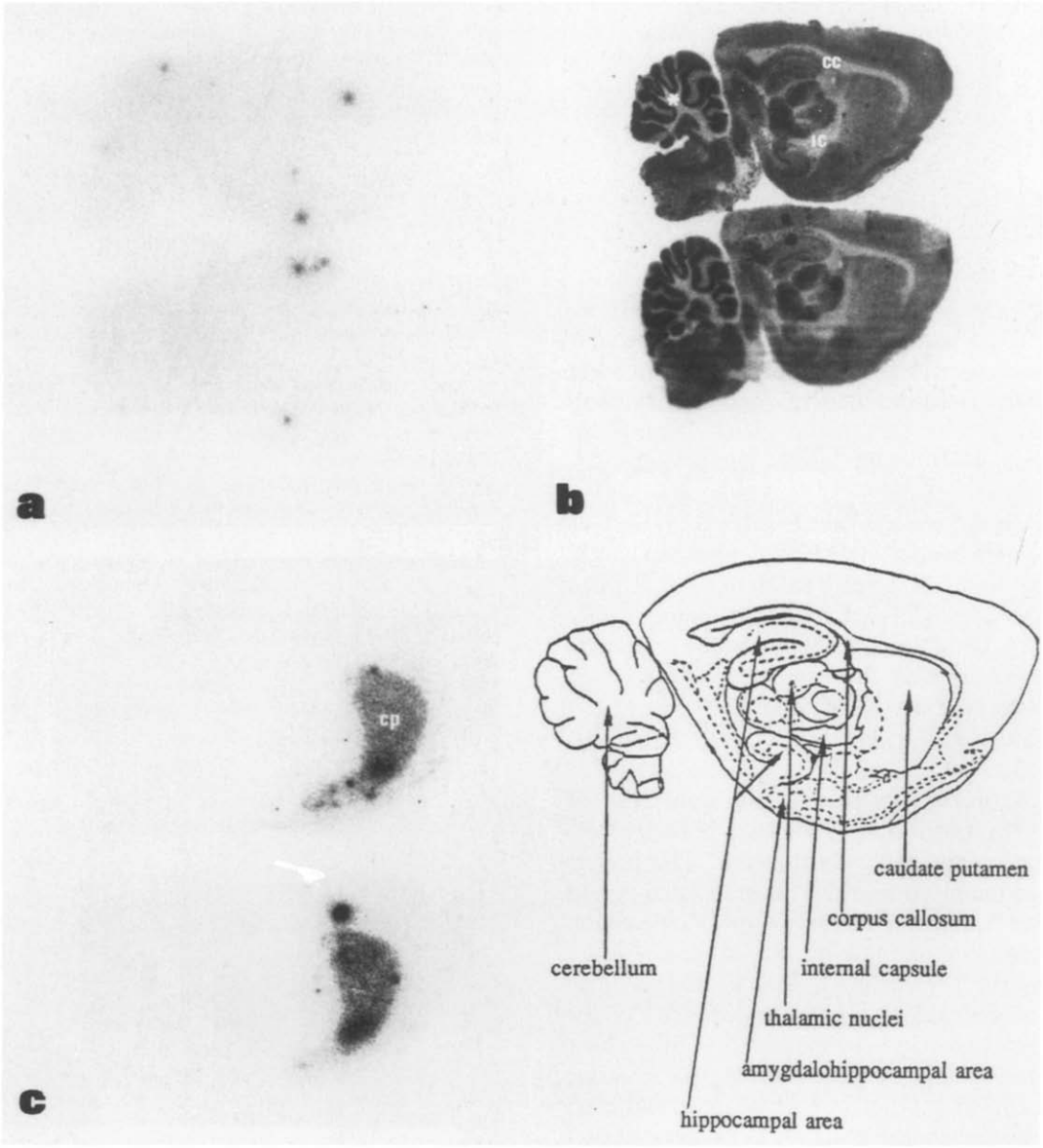

Fig. 2. Views of X-ray film obtained after 1 day exposure (a,b) or 2 days exposure (c) of rat brain sagittal sections incubated with different ^{35}S-labeled probes. (a) Sagittal sections were hybridized with the aldolase C Dde 23 sense probe. The sections show no labeling. (b) Adjacent sagittal sections were hybridized with aldolase C (r.Al.C2) antisense probe. The major gray matter areas of the brain are more or less intensely labeled (dark areas). The labeling of white matter in the cerebellum (*), the corpus callosum (CC) and the internal capsule (IC) is only slightly above background. (c) Sagittal sections were hybridized with a preproenkephalin A probe. Intense labeling is confined to the caudate putamen (CP); other areas are faintly labeled or unlabeled.

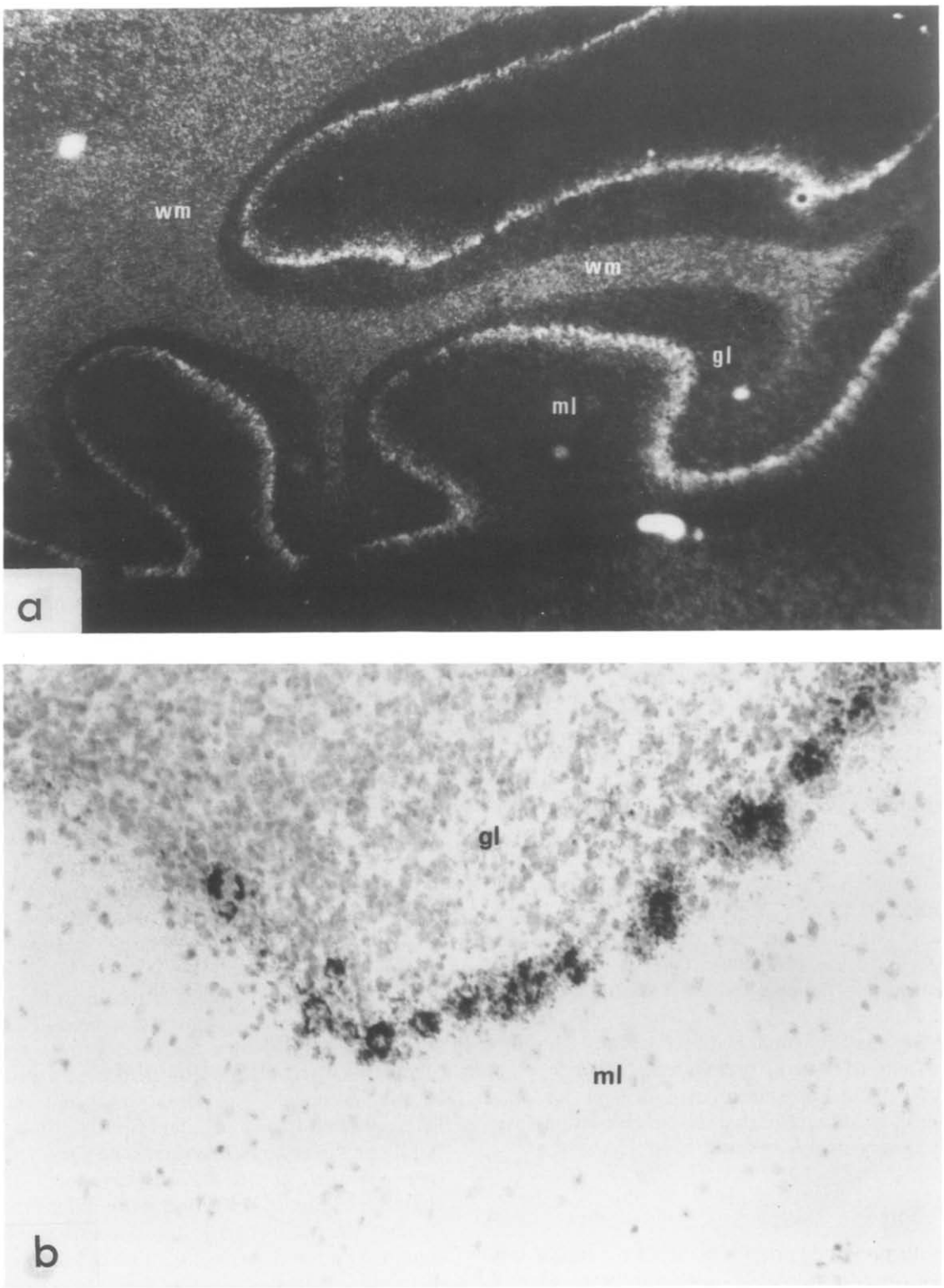

Fig. 3. Autoradiography of sagittal sections of rat cerebellum hybridized with the ^{35}S-labeled r.Al.C2 probe. The sections were exposed for 21 days on Ilford K5 emulsion and viewed under dark-field (a) or bright field (b) illumination after Toluidine blue staining. In (a), we note a heavily labeled layer of cells at the edge of the granule cell layer in the cerebellum. In contrast, the central axis of white matter (wm), the granule cell layer (gl) and the molecular layer (ml) are devoid of label (× 25). In (b), Purkinje cells show a high density of silver grains, indicative of a high aldolase C mRNA content (× 80).

The abundance of aldolase C mRNA in rat brain is approximately the same as in cultured rat neurons. Under the conditions of hybridization used, the aldolase C probe did not detect any mRNA species in RNA extracts from cultured rat fibroblasts. The results are consistent with the specificity of aldolase C mRNA expression in brain cells.

Hybridization of the same RNA preparations with an aldolase A probe (Fig. 1A) gave similar results, namely presence of aldolase A mRNA in astrocytic and neuronal cells, with a slightly stronger hybridization signal in neurons than in glial cells. As expected, aldolase A probe does hybridize with rat fibroblast RNA.

3.2. *In situ hybridization*

Hybridization of rat brain tissue sections with the control (sense) probe for aldolase C –Dde 23 – gave negative results (Fig. 2a). The second control probe consisting of a cDNA sequence coding preproenkephalin A (PPA) gave a selective labeling of the caudate-putamen brain area (Fig. 2c), as described previously [16]. A quite different pattern of labeling was observed when the sections were hybridized with aldolase C probe r.Al.C2: labeled areas were seen almost throughout the brain, as shown in Fig. 2b. The more heavily labeled areas were in hippocampus, thalamic nuclei, amygdaloid nuclei and cerebellum, the latter showing the most intense labeling. The white matter areas in the cerebrum, such as the corpus callosum, the internal capsule and the cerebellar white matter, seemed poorly labeled if at all. Analysis of histological sections treated for autoradiography with liquid emulsion confirmed and extended the X-ray film data described above. A more or less intense autoradiographic signal was revealed in some additional regions such as the cortex, striatum, hypothalamic nuclei and primary olfactor cortex (data not shown). The most intensely labeled cells were the Purkinje cells in the cerebellum (Fig. 3), with silver grains confined to the cell bodies. It should be noted that, at the single cell level, a considerable heterogeneity of labeling intensity could be observed in neighbouring nerve cells of several brain areas. Microscopoic analysis of cells in the white matter of cerebrum and cerebellum showed that the glial cell content of aldolase C mRNA is very low at best.

4. DISCUSSION

The results presented provide evidence for expression of the brain-specific aldolase C mRNA in both neurons and astroglial cells from rat and mouse brain. Northern blot hybridization experiments show that the level of aldolase C mRNA in cultured astroglia is significantly lower than in cultured neurons in which its level is somewhat higher than that in brain. The possibility of cross-hybridization of the aldolase C probe with aldolase A mRNA was excluded by the absence of labeling in the Northern blots of total mRNA from fibroblasts in which aldolase A mRNA is expressed. Hybridization experiments with probes specific for the latter showed that this isozyme as well is more abundant in neurons than in astrocytes; however, the difference is only about 2-fold. Since these are the two isoforms of aldolase expressed in brain, one may conclude that neuronal cells express a considerably higher level of aldolase mRNA than glial cells, which may be related to the high intensity of glycolytic metabolism in neurons.

In situ hybridization using rat brain sections confirmed and extended the results on the expression of aldolase C mRNA in different brain cell types by visualizing the labeling of individual cells in several brain areas. A high-power examination of the distribution of autoradiographic silver grains at the single cell level showed that aldolase C mRNA is predominantly associated with neurons and that its expression in different neuronal cell populations is quantitatively heterogeneous. The most striking example of that was found in cerebellum where the heavy labeling of Purkinje cells contrasts with faintly labeled or unlabeled granule cells on the one hand and cells in the molecular layer on the other hand.

Our results of aldolase C mRNA detection in isolated cells and brain slices are, as a whole, consistent with those obtained by Thompson et al. using an antibody directed against the C4 tetramers (i.e. composed of four aldolase C subunits [3]).

An interesting question is that of the biological significance of the isoenzymes. Aldolase B, the liver-specific isoenzyme, is indispensable for the hepatic and intestinal metabolism of fructose as it is the only aldolase form able to efficiently hydrolyse fructose 1 phosphate [17]. In contrast, the functional difference between aldolase A and aldolase C isoenzymes is not sufficiently obvious to explain why both are synthesized in brain and why their distribution within different structures of the central nervous system is heterogeneous. Whether this is relevant to peculiarities of the glycolytic metabolism in different brain cells is so far unknown. This situation is not unique and heterogeneity in the cellular distribution of another family of isoenzymes involved in the glycolytic pathway the enolases – has been described [18]. The characterization of the cell subpopulations that express strongly the brain-specific aldolase C isozyme is a first step in the attempt to shed light on the rationale for the restriction of these molecular forms that takes place in the course of evolution.

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