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Short communications

Differential distribution of calcineurin Aα isoenzyme mRNA's in rat brain

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Summary. Specific antisense oligonucleotide probes for the α isoforms of the catalytic subunit (A-subunit) of calcineurin were prepared and the distribution of Aα1 and Aα2 mRNA's has been studied in rat brain using in situ hybridization histochemistry. Clear regional differences have been observed for the Aα1 and Aα2 isoforms. The predominant form, Aα1, was found to be preferentially expressed in the caudate putamen, the pyramidal cell layer of the hippocampus, specific cortical cell layers, the cerebellar granular cell layer and some other brain areas. On the other hand, the Aα2 isoform, although being generally less abundant than Aα1, gave an intense autoradiography signal in the dentate gyrus of the hippocampus and was the major transcript in the amygdala, the superior and the inferior colliculus, the central gray matter and the reticular formation. These regional differences might reflect specific functions exerted by the two alternatively spliced isoenzymes in the CNS and opens the perspective of interfering with defined calcineurin-dependent signal transduction pathways using isoform-specific compounds.

Key words: Calineurin – In situ hybridization – Oligonucleotide probes – Protein serine phosphatase

Introduction

Calcineurin is a calcium/calmodulin dependent serine/threonine-specific protein phosphatase which is abundantly expressed in the brain. The phosphatase is heterodimeric and consists of a catalytic (A) and a regulatory (B) subunit (Klee et al. 1979).

Much attention has been devoted to calcineurin since it appeared that this enzyme plays a key role in certain calcium-dependent cellular processes such as the transcriptional activation of the interleukin 2 (IL-2) gene in helper T-cells (for review see Liu 1993). Immunosuppressants like cyclosporin A and fujimycin (FK-506) have been shown to inhibit calcineurin activity when associated with their respective intracellular binding proteins, the cyclophilins and the fujimycin binding proteins (FKBP) (Liu et al. 1991). Targeted modulation of calcineurin activity through specific compounds might therefore prove to become a useful pharmacological approach for interfering with calcineurin-dependent signal transduction pathways.

Two isoforms of the catalytic subunit Aα and Aβ have been cloned (Ito et al. 1989; Kuno et al. 1989). In addition, subtypes (α1, α2, β1, β2 and β3) of both isoforms, which result from alternative splicing, have been described (Guerini and Klee 1989; Kincaid et al. 1990). The differential mRNA distribution of the general Aα and Aβ isoforms has been studied in rat brain (Takaishi et al. 1991). Currently, no information is available concerning the function and distribution of the alternatively spliced subtypes of the Aα and Aβ isoforms.

In this study we have investigated the specific mRNA distribution of the Aα1 and Aα2 subtypes of the catalytic A subunit of calcineurin in rat brain.

Methods

Specific oligonucleotide probes for the different calcineurin Aα isoenzymes were synthesized on a 380B Applied Biosystems DNA synthesizer. The probe for the general catalytic isoform Aα was complementary to nucleotides 1710–1754 of calcinerurin Aα sequence (Ito et al. 1989; Takaishi et al. 1991). Aα1 and Aα2 probes were complementary to nucleotides 1550–1589 of the rat Aα1 sequence (Ito et al. 1989, Genbank access number D90035) and 1591–1630 of the rat Aα2 sequence (Miyamoto 1991, Genbank access number X57115), respectively.

Four male Wistar rats (Madorin, Fullinsdorf, Switzerland) were killed by decapitation, the brains were quickly removed and immediately frozen on dry ice. Twenty μm cryostat sections were used for in situ hybridization histochemistry which was performed as described previously (Massieu et al. 1992). Using terminal transferase (Boehringer Mannheim, FRG), all the probes were labeled at their 3′-end with ^{32}P-α-dATP (3000 Ci/mmol, New England Nuclear, Hertfordshire, UK) to similar specific activities ($1.8–2.0\times10^4$ Ci/mmol). After hybridization, sections were exposed to β-Max film (Amersham, Buckinghamshire, UK) at −70 °C. Sections hybridized with different probes were exposed

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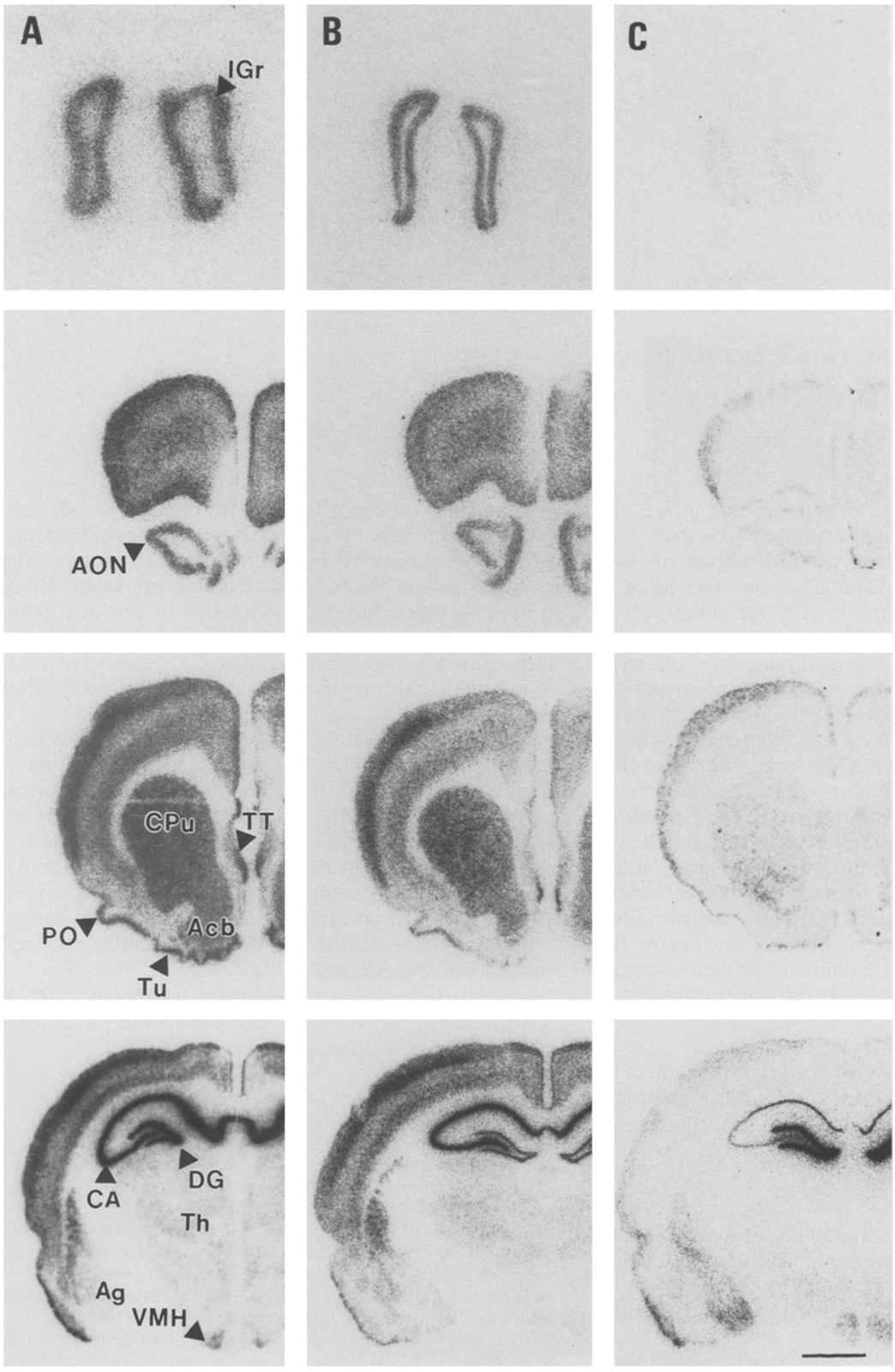

Fig. 1A–C. Regional distribution of the calcineurin Aα isoforms as determined by in situ hybridization in coronal sections of rat brain. **A** (*left column*) General isoform Aα. **B** (*middle column*) Alternatively spliced isoform Aα1. **C** (*right column*) Alternatively spliced isoform Aα2. Scale bar = 1.5 mm. Abbreviations: *Acb*, nucleus accumbens; *Ag*, amygdala; *AON*, anterior olfactory nucleus; *CA*, pyramidal cell layer of Ammon's horn; *CG*, central gray matter, *CPu*, caudate putamen; *DG*, granular cell layer of dentate gyrus; *DR*, dorsal raphe nucleus; *Ent*, entorhinal cortex; *Gr*, granular cell layer of cerebellum; *IC*, inferior colliculus; *IGr*, internal granular layer of olfactor bulb; *LG*, lateral geniculate nucleus; *Pn*, pontine nuclei; *PO*, primary olfactory cortex; *Rt*, reticular formation; *Rtg*, reticulotegmental nuclei; *SC*, superior colliculus; *Th*, thalamus; *Tu*, olfactory tubercle; *TT*, taenia tecta; *VMH*, ventromedial hypothalamic nucleus

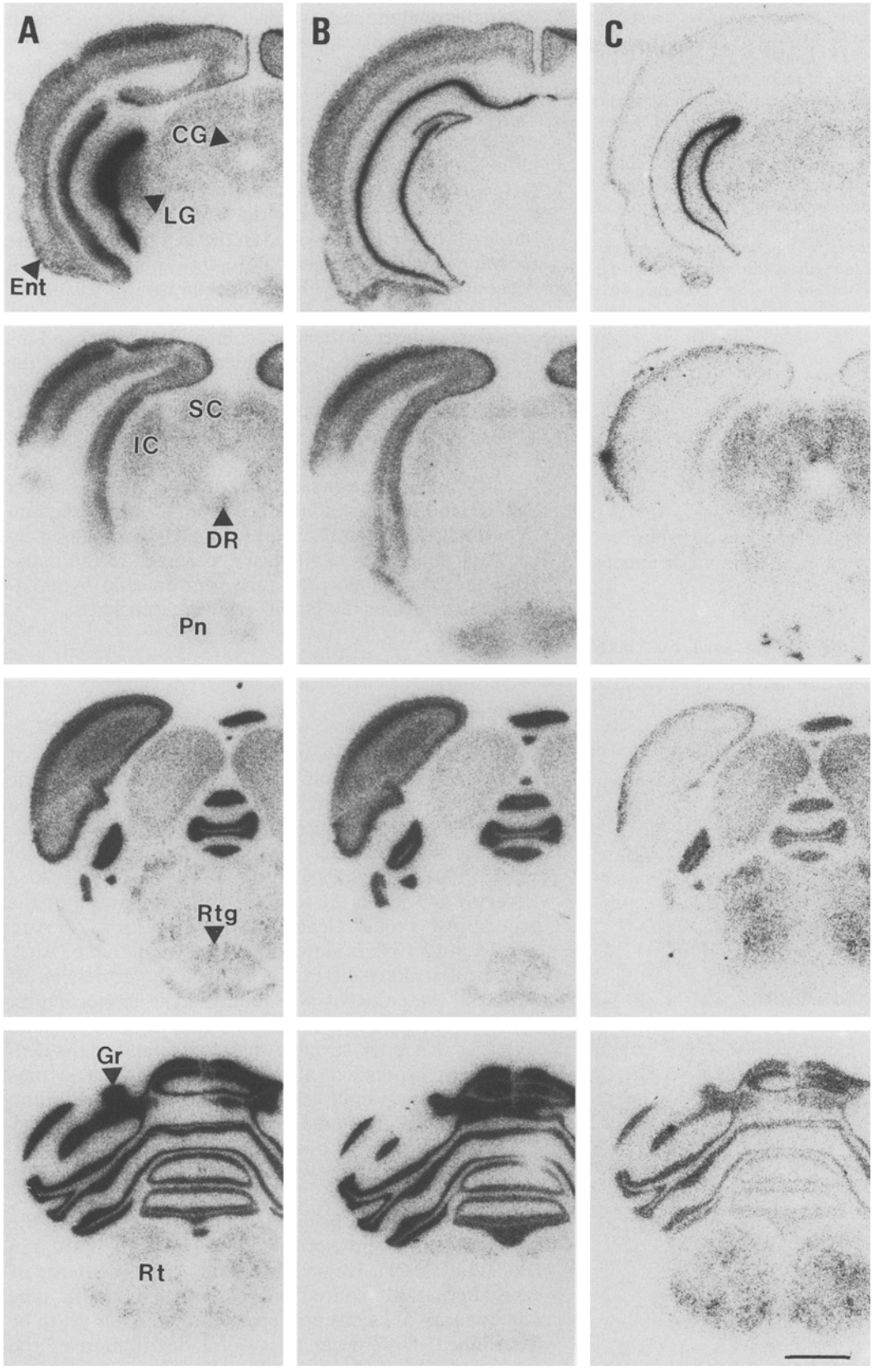

Fig. 1

for the same periods of time (5 days) to allow optimal comparison of autoradiographic signal intensities. The specificity of the hybridization signal of the two probes was verified by adding an excess (100-fold) of the same unlabelled probe to the hybridization buffer which abolished the signal. Treatment of the tissue sections with 30 µg/ml RNase prior to hybridization for 20 min at 37 °C resulted in the complete disappearance of the autoradiographic signals. After exposition to films, the sections were stained with 0.02% toluidine blue for histological controls.

Data from autoradiograms were analyzed using a microcomputer image device system (Imaging Research, St. Catharines, Ontario, Canada) as follows: for the Aα autoradiogram, the optic density (O.D.) assessed in the most densely labelled region (hippocampus) was arbitrarily taken as 100%, while the O.D. in regions with no significant signal (white matter) was considered as background. This O.D. range was divided into four signal intensity levels according to which the regions were ranked.

Results

In Fig. 1, the regional CNS distribution of mRNA of total calcineurin Aα catalytic subunit and of the alternatively spliced Aα1- and Aα2 isoforms is shown. The relative intensity of mRNA hybrization signals in the brain areas of interest are listed in Table 1. The distribution of Aα1 and Aα2 mRNA showed clear regional differences.

Table 1. Relative intensities of the Aα, Aα1 and Aα2 mRNA hybridization signals in various rat brain areas

Brain structure	Aα	Aα1	Aα2
Olfactory bulb (internal granular layer)	+ + +	+ + +	+/−
Anterior olfactory nucleus	+ + +	+ + +	+/−
Primary olfactory cortex	+ + + +	+ + +	+
Olfactory tubercle	+ + + +	+ + +	+
Amygdaloid nuclei	+ +	+/−	+ +
Caudate putamen	+ + +	+ + +	+/−
N. accumbens	+ + +	+ +	+
Entorhinal cortex	+ +	+	+
Taenia tecta	+ + + +	+ + + +	−
Hippocampal pyramidal cell layer	+ + + +	+ + +	+
Hippocampal granular cell layer of dentate gyrus	+ + + +	+	+ + +
Cortical layer I	+/−	+/−	−
II	+ + +	+ +	+
III	+ + +	+ + +	−
IV	+	+	−
V	+ + +	+ + +	−
VI	+ + +	+ + +	−
Thalamic nuclei	+	+	+/−
Ventromedial hypothalamic nucleus	+ +	+	+
Lateral geniculate nucleus	+ +	+	+
Pontine nuclei	+ +	+ +	−
Superior colliculus	+ +	+/−	+ +
Inferior colliculus	+ +	+/−	+ +
Central gray matter	+ +	−	+ +
Dorsal raphe nucleus	+ +	+/−	+ +
Reticulotegmental nuclei	+ +	+ +	−
Reticular formation	+ +	−	+ +
Cerebellar granular cell layer	+ + + +	+ + +	+

+ + + +, very high; + + +, high; + +, moderate; +, low signal intensities

Hybridization experiments were performed three times. Signal intensities were evaluated as described in the Methods section

Aα1, the predominant isoform, was highly expressed in the internal granular layer of the olfactory bulb, the anterior olfactory nucleus, the caudate putamen, the nucleus accumbens, the cortical layers II, III, V and VI, and the granule cell layer of the dentate gyrus of the hippocampus. Extremely high levels of Aα1 mRNA were observed in the primary olfactory cortex, the olfactory tubercle, the taenia tecta, the pyramidal cell layer of the hippocampus and the granular cell layer of the cerebellum. Moderate to low levels of Aα1 were found in the cortical layer IV, the reticulotegmental nuclei and the pontine nuclei. Although globally less abundant than the Aα1 isoform, Aα2 showed a very strong hybridization signal in the granular cell layer of the hippocampal dentate gyrus. Furthermore, Aα2 was the preferential transcript in the amygdala, the superior and the inferior colliculus, the central gray matter, the dorsal raphe and in various nuclei of the reticular formation. Relatively high levels of Aα2 mRNA were detected in the hippocampal pyramidal cell layer, whereas the levels of Aα2 mRNA in the cortical layer II and in the granular cell layer of the cerebellum were moderate. Signals of approximately equal intensity for Aα1 and Aα2 mRNA's were observed in the thalamus, the entorhinal cortex, the ventromedial hypothalamic nucleus and the lateral geniculate nucleus.

Discussion

In this study we have investigated the distribution of mRNA of the Aα1 and Aα2 isoforms of the catalytic subunit of calcineurin. In situ hybridization with a nonselective Aα oligonucleotide probe yielded a distribution very similar to that previously reported by Takaishi et al. (1991). The distribution of the Aα1 and Aα2 isoforms add up to the total distribution pattern of the general Aα probe. Generally, the expression of Aα1 mRNA in rat CNS is more pronounced than that of Aα2. The most striking difference between the expression of Aα1 and Aα2 mRNA was found in the hippocampus. Whereas the Aα1 hybridization signal in the pyramidal cell layer was much stronger than that found in the dentate gyrus, expression of Aα2 mRNA was most pronounced in the granule cell layer of the dentate gyrus and a weaker signal was found in the pyramidal cell layer. Furthermore, a series of brain structures preferentially expressed either Aα1 or Aα2 mRNA (see Fig. 1 and Table 1).

Two-dimensional gel electrophoresis has revealed the presence of different isoforms of calcineurin in rat brain (Polli et al. 1991). However, the exact correspondence of each alternatively spliced mRNA with the specific type and quantity of calcineurin protein in brain has yet to be determined. Differences in isoform distribution of the calcineurin Aα isoforms might reflect specific neuronal functions performed by these isoenzymes. The 10 amino acid insert which distinguishes Aα1 from Aα2 is positioned 10 amino acids N-terminal to the autoinhibitory domain of calcineurin (Hashimoto et al. 1990) and may therefore significantly affect activity.

A high degree of co-localization of FKBP and calcineurin in rat brain indicates that a putative complex of these two proteins plays a role in the normal brain (Steiner et al. 1992). A number of different cellular proteins are dephosphorylated by calcineurin. One of the best characterized substrates of calcineurin is DARPP-32, a cytosolic cAMP-sensitive protein which is involved in the intracellular signalling of dopamine (King et al. 1984; Halpain et al. 1990). Since the mRNA's of both the Aα1 and Aα2 isoforms, but also of calcineurin Aβ (Takaishi et al. 1991), has been found in striatal regions, it would be interesting to investigate which of the calcineurin isoforms dephosphorylates DARPP-32 most efficiently.

A key role of calcineurin in the regulation of ion channel and receptor activity has recently been investigated in several studies. Calcineurin dephosphorylates the L-type voltage-dependent calcium channel, thereby reducing its activity (Armstrong 1989). In addition, desensitization of various ligand-gates ion channels, such as that of capsaicin and presumably also that of $GABA_A$ receptors, is calcium dependent and mediated by calcineurin (Yeats et al. 1992; Chen et al. 1990). Desensitization of various G-protein coupled receptors such as muscarinic M2 and metabotropic glutamate receptors seems to regulated by calcineurin (Sakuta et al. 1991; Catania et al. 1991). It remains to be determined wether this different functional effects can be ascribed to one of the alternatively spliced isoenzymes. Characterization of the calcineurin isoforms might be critical in understanding the toxic side effects associated with the therapeutic uses of cyclosporin and FK-506.

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