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DIFFERENTIAL DISTRIBUTION OF MESSENGER RNAs FOR CATHEPSINS B, L AND S IN ADULT RAT BRAIN: AN *IN SITU* HYBRIDIZATION STUDY

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Abstract—The cysteine lysosomal proteases comprise a large family of highly conserved enzymes which are essential for intracellular protein turnover. These proteases are very efficient in their ability to degrade components of the extracellular matrix, and have been implicated in processes of cell growth, malignant transformation and inflammation. There is also a growing body of evidence for their involvement in the metabolism of the amyloid precursor protein. The production of insoluble βA4 amyloid peptide is thought to be one of the key events that lead to the development of Alzheimer's pathology.

To see the physiological role these enzymes play in the brain, we studied the relative abundance and distribution of the messenger RNAs for three lysosomal cysteine proteases, cathepsins B and L and cathepsin S, by *in situ* hybridization histochemistry in rat brain. All three enzymes are capable of degrading components of the extracellular matrix but they have different substrate preferences and resistances to neutral pH. We found that the mRNAs for cathepsins B, L, and S have different expression patterns in brain. Cathepsin B mRNA shows the highest level of expression. It has a wide distribution, and is preferentially expressed in neurons. The expression patterns of cathepsin B and cathepsin L mRNA overlap in many brain regions; in some areas they complement each other. Cathepsin B and L mRNAs are highly expressed in the choroid plexus, a structure that is instrumental in brain development. Both transcripts are also abundant in the neuropeptide synthesizing hypothalamic nuclei. Cathepsin S mRNA has wide expression pattern throughout brain, in grey and white matter. A great number of cells that express cathepsin S have microglial morphology.

Regions that are known to contain the highest amounts of the amyloid precursor protein express highest levels of cathepsin B and cathepsin L mRNA. Also, all three transcripts are highly represented in regions that are most prone to degeneration in Alzheimer's disease. These results suggest a role for these lysosomal hydrolases released from degenerating cells in the development of Alzheimer's pathology.

The mammalian lysosomal cysteine proteases, also known as cathepsins, are members of the papain family of cysteine proteases. It is a large group of enzymes with remarkable homology between species throughout the plant and animal kingdom.[50] The members of this family are mainly implicated in intracellular protein turnover.[8,26] Due to the ability of these enzymes to degrade constituents of the extracellular matrix, they have also been implicated in processes of cell growth, tumor invasiveness and inflammation.[44] Although the biochemistry of different members of this family is fairly well studied, their physiological role and the regulatory mechanisms that govern their action in normal and pathological conditions are poorly understood.

Cathepsin B and cathepsin L are two prominent members of the papain family with broad substrate specificity. Their expression is highly elevated in cells that have undergone malignant transformation.[36] Rat cathepsin B is coded for by a 2.2 kb mRNA in the tissues examined.[38] It is a multivalent enzyme that can act as dipeptidyl carboxypeptidase and as an endopeptidase; as an endopeptidase it has preference for basic amino acids at the P2 position.[4] Cathepsin B is capable of retaining a small but significant percentage of its activity at neutral pH.[4] Besides tumor invasiveness, cathepsin B has been implicated in inflammation, and bone resorption.[19]

Cathepsin L, also known as "major excreted protein" is a very potent collagenolytic cysteine lysosomal protease.[31] It is an endopeptidase of broad specificity with preference for hydrophobic amino acids at the P2 and P3 positions. It is quickly inactivated at neutral pH.[28] In several rat tissues (liver, kidney, testis), there are two transcripts for cathepsin L: a major transcript of about 1.7 kb and a minor transcript of 2.3 kb, which differ between each other in the lengths of their 3' untranslated region.[20] Growth factors, and tumor promoters greatly elevate the levels of cathepsin L mRNA and cause secretion of large amounts of the mature

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enzyme and its precursor form. Its expression is also upregulated during tissue remodeling.[19,25]

Recently, we isolated the full-length cDNA for rat cathepsin S, as well as partial cDNA for four putative novel cysteine proteases from rat brain.[35] Rat cathepsin S is coded for by a 1.4 kb mRNA. It is an endopeptidase of broad substrate specificity that has preference for small neutral amino acids at the P2 and P3 positions.[35] An intriguing feature of the rat and bovine cathepsin S homologs is their ability to retain up to 80% of activity at neutral pH.[9,10] This property makes cathepsin S a very suitabale candidate for an extracellular as well as an intracellular protease. A human variant, cloned from alveolar macrophages, was shown to have elastinolytic activity.[41]

Little is known about the expression pattern of these three proteases in brain. The expression of cathepsin B has been studied immunohistochemically in rat and human brain.[6] Little is known about the protein expression of cathepsin L in rat brain, and there is only one report on the regional distribution of the mRNA for cathepsin S in rat brain.[35]

The cysteine lysosomal proteases have been implicated in the normal and pathogenic metabolism of the amyloid precursor protein.[16,17,22,42] The processing of amyloid precursor protein, which gives rise to the insoluble βA4 amyloid peptide that is found in the core of the mature senile plaques, is one focus in the investigation of the pathogenesis of Alzheimer's disease. There seem to be at least two processing pathways for the amyloid precursor protein.[11,16,22,43] There is evidence that the nonsecretory pathway of amyloid precursor protein degradation is mediated by cysteine proteases from an acidic intracellular compartment.[30] When cells that endogenously express high amounts of the precursor protein or have been transfected with the corresponding cDNA are treated with inhibitors of cysteine lysosomal proteases, there is accumulation of a 22,000 mol. wt amyloidogenic C-terminal peptide that is normally assumed to be a short-lived processing intermediate.[30]

High amounts of enzymatically active cathepsin B and L have been detected extracellularly in the senile plaques in the brains of Alzheimer's patients.[13,14] Degenerating neurons appear to be the main source of these enzymes. Precursor molecules have been found in many of the plaques; therefore, cathepsins may generate the βA4 insoluble peptide locally, contributing to the maturation of the plaques.

Because of the strong implications of the involvement of these three proteases in normal and abnormal protein turnover, and tissue remodeling due to growth and trauma, we established a comparative map of the distribution of their mRNAs in rat brain. We examined the relative levels of the mRNAs for cathepsins B, L, and S in rat brain by northern analysis and in situ hybridization histochemistry.

EXPERIMENTAL PROCEDURES

Northern analysis

Tissue extraction, RNA preparation and northern analysis of total rat brain RNA were performed as previously reported.[35] ^{32}P-labeled cDNA probes for rat cathepsins B, L, and S were generated by random priming using as a template ~500 bp fragments of their coding sequences subcloned in the pGEM3Zf+ as reported.[35] Each probe was hybridized to 30 μg of total brain RNA. For quantitative analysis of the results, the blots were reprobed with a random primer labeled cDNA probe against cyclophylin (1B15), a constitutively expressed protein.

The autoradiograms were densitized as described.[35] Briefly, an image analysis system, consisting of a video camera (Cohu), digitized board (PC vision Plus), and JAVA software (Jandel Scientific) were used. Typically two or three exposures of each membrane were quantified, and the results divided by the exposure time. Only the values which were in the linear range of the film and the camera, as determined by standard curves, were used.

In situ hybridization

Probe synthesis. The portions of rat cathepsins B, L, and S cDNAs (570, 495 and 502 bp long, respectively) used to produce cDNA probes for the northern analysis were chosen to generate cRNA probes. In this region the homology among the three fragments is as follows: 40% identity between cathepsin B and L, 45% identity between cathepsin B and S and 65% identity between cathepsin L and S. The fragments were subcloned in the Sma I site of the pGEM3Zf+ vector to enable synthesis of antisense and sense riboprobes from the SP6 or T7 promotor. ^{35}S-labeled cRNA probes with high specific activity were synthesized using standard in vitro transcription protocols.[37]

Tissue preparation and hybridization. Brains were quickly removed from 10-week-old male Sprague–Dawley rats (Taconic, Germantown, NY) and frozen in isopentane at −30°C. Fifteen-micrometer-thick coronal sections were mounted on L-polylysine-coated slides and processed for in situ hybridization according to previously published protocols.[49] Adjacent sections were hybridized with ^{35}S-labeled cRNA probes for cathepsins B, L, and S of similar specific activities. Hybridization was performed overnight at 50°C. After eliminating the excess of unbound cRNA probes by treatment with RNase A (200 μg/ml) the sections were washed in 0.1 × standard saline citrate at 65°C for 1 h. The tissue was then dehydrated through graded alcohols (50–100%), air dried and exposed to Kodak XAR-5 X-ray film. To enable analysis of the signal on a cellular level, the slides were dipped in NTB-2 or L4 photographic emulsion and stored at 4°C for one to three weeks before developing. The developed emulsion dipped sections were counterstained with 0.05% Cresyl Violet (pH 4.0), and analysed with a Leitz microscope. Ten comparative analyses were performed with sections from all the examined areas using brains from five animals.

In situ hybridization controls. Two types of controls were used to ensure specificity of the hybridization signal: a sense strand control and a RNase A pretreatment control. For the sense strand controls, pairs of adjacent sections were divided in two sets. One set was hybridized to the antisense cRNA probes for cathepsins B, L, and S, and the other was hybridized to the corresponding sense probes. For the RNase pretreatment control, pairs of adjacent sections were divided in two sets: adjacent sections from the first set were hybridized to antisense radiolabeled cRNA probes for cathepsins B, L, and S and processed according to the above protocol. The sections from the second set were treated with RNase A (200 μg/ml) before the proteinase K treatment, and further processed identically. No signal higher than background was seen with any of the sense probes used, or

when the tissue was treated with RNase A before hybridization to the antisense probes.

RESULTS

Northern analysis using rat brain RNA shows that cathepsin B mRNA has the highest level of expression (about two-fold more than cathepsin S and about seven-fold more than cathepsin L mRNA) in rat brain (Fig. 1a). In agreement with previous reports, cathepsin B and S are expressed as single transcripts of 2.2 and 1.4 kb, respectively. Two messages were detected for cathepsin L; a major transcript of about 1.7 kb and a minor transcript of about 2.3 kb visible upon longer exposure of the blot (Fig. 1a, lower panel). The minor transcript might correspond to a 2.3-kb transcript found in rat testis, kidney and liver, that was shown to differ from the major transcript in the length of the 3′ untranslated region.[20]
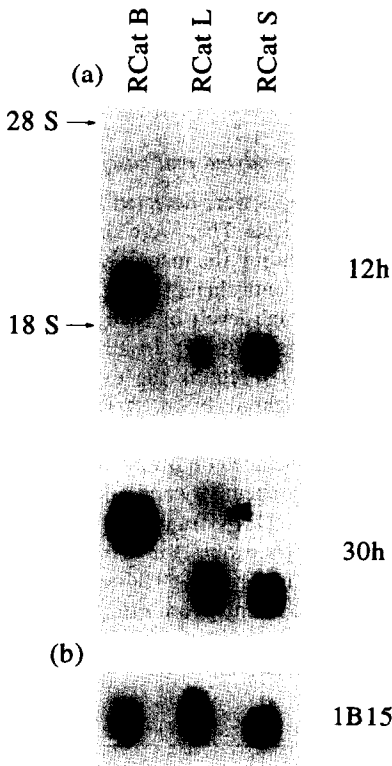

Fig. 1. Northern analysis of the relative abundance of cathepsins B, L, and S in rat brain. (a) Upper panel: approximately 30 μg of total RNA was hybridized to radiolabeled rat cathepsin B (RCat B), rat cathepsin L (RCat L), or rat cathepsin s (RCat S) of similar specific activities. Preparation of RNA, northern blotting, and hybridization conditions are described in Experimental Procedures. The blots were exposed to film for 12 h at −70°C with an intensifying screen. The positions of the 28S and 18S RNA were visualized by ethydium bromide staining. (a) Lower panel: longer (30 h) exposure of the blots described above shows a minor transcript of ∼2.3 kb for cathepsin L. (b) The same blots were probed with cyclophilin (1B15), a constitutively expressed mRNA.

In situ *hybridization*

Telencephalon

In the olfactory bulbs cathepsins B, L, and S mRNAs have remarkably different expression patterns (Fig. 2a–c). Cathepsin B mRNA is most abundant in the mitral cell layer (Fig 2a, 3a). It is also expressed in the periglomerular cells of the glomerular layer, as well as in the tufted cells (Fig. 3a). The mRNA for cathepsin L is expressed preferentially in the internal granular cell layer (Fig. 2b). In contrast, the message for cathepsin S is relatively highly expressed in a number of cells from all layers of the olfactory bulbs, including the olfactory nerve layer (Figs 2c, 3h).

In the cortical fields the expression of these messages is different both in terms of quantity and regional distribution. Cathepsin B and L mRNAs shows highest expression in the frontal, piriform and entorhinal cortex, (Fig. 2d, e, j, k). Cathepsin B mRNA is more abundant both in frontal and entorhinal cortex. Except for layer I, cathepsin B and L are expressed in all cortical layers. Cathepsin B mRNA is especially highly expressed in cortical neurons. Cathepsin S mRNA shows a rather homogeneous distribution in all cortical fields and in all cortical laminae. The expression is somewhat higher in the retrosplenial and entorhinal cortex (Fig. 2l). From the examination of emulsion dipped sections it seems that this is due to higher expression in individual cells in these areas rather than a greater number of cells expressing this message (not shown). This message also can not be detected in the piriform cortex (Fig. 2f). In the caudate putamen the mRNA for cathepsin S is observed in a relatively small number of small size cells. There is no significant expression of cathepsin B mRNA in the caudate–putamen, while moderate amounts of cathepsin L mRNA are present in both neurons and glia. Unlike cathepsin L mRNA, cathepsin S and cathepsin B transcripts are relatively highly expressed in the ventral pallidum (Fig. 2d, f). The analysis of emulsion dipped sections revealed high levels of cathepsin B and cathepsin L mRNA in the neurons from the nucleus basalis of Meinert (Fig. 3c). The transcripts of cathepsin B and L are also abundant in the tenia tecta, indusium griseum, neurons from the diagonal band of Broca, the lateral and medial septal nuclei. In the medial septum the expression of cathepsin B mRNA is higher. Cathepsin B is also highly expressed in the bed nucleus where no significant amounts of cathepsin L or S mRNA could be found. Cathepsins B and S are expressed in high levels in the amygdala.

Cathepsin B mRNA is the most abundant of the three messages in the structures of the hippocampal formation. It is highly expressed in the CA1–3 fields of the Ammon's horn and in the dentate gyrus (Fig. 2g). The message is also abundant in cells from the oriens layer and radiatum layer as well as in the hilus. Cathepsin L mRNA is expressed in the same

structures to a lesser degree. Also, unlike cathepsin B mRNA its expression in the granular cells of the dentate gyrus overpowers its expression in the pyramidal cells of the CA1–3 fields (Fig. 2h). Cathepsin S mRNA could not be detected in the laminated structures of the hippocampus (Fig. 2i). Again it was detected in small number of diffusely distributed cells of small size. Cathepsin S transcript is present in glial cells of the corpus callosum (Fig. 2f). High levels of cathepsin L mRNA were observed in a fewer number of cells in this interhemispheric commissure, while the signal for cathepsin B mRNA was no greater than background.

Diencephalon

The expression of cathepsin B and cathepsin L mRNAs in the hypothalamus is confined to specific nuclei. The messages localize to the ventromedial, dorsomedial, paraventricular, supraoptic, suprachiasmatic and arcuate nucleus. The paraventricular and supraoptic nucleus have the highest levels of both messages (Fig. 3b, f). The message for cathepsin S could not be detected in significant levels in any of these nuclei.

Cathepsin S mRNA is expressed in a small number of cells in the habenular complex, while cathepsin B and L are highly expressed in the median habenular body (Fig. 2g–i). All three messages are widely expressed in different thalamic nuclei; however, cathepsin B mRNA shows the highest and mostly neuronal distribution.

Mesencephalon

The messages for cathepsin B and L co-localize in the areas of the mesencephalon examined. The areas with highest expression are the nucleus dorsal raphe, the pontine nuclei, the ventral nucleus of the lateral lemniscus, the red nucleus and the substantia nigra pars compacta (Fig. 2j, k). Cathepsin B mRNA is expressed to a greater extent in all these structures, being overwhelmingly abundant in the neurons of the nucleus dorsal raphe, the pontine nuclei, and the red nucleus. Both messages are present in relatively high levels in the central gray (Fig. 2j, k). In the mesencephalic structures cathepsin S mRNA shows

again a diffuse distribution pattern with very few exceptions such as central gray and substantia nigra pars reticulata (Figs 2l, 3i, j).

The primary sensory neurons and the motor neurons from the mesencephalic and motor nucleus of the trigeminal nerve stand out in the expression of cathepsin B mRNA. The message is also highly expressed in other cranial nerve nuclei (VI, VII). The mRNA for cathepsin L was also observed in these neurons although at lower levels. Relatively high levels of cathepsin B and L were detected in the neurons of the locus coeruleus and different groups of adrenergic neurons in the mesencephalon (not shown).

Cerebellum

In the cerebellum cathepsin B is most intensely expressed in the Purkinje cells (Fig. 3e). It is also abundant in the granular cell layer (Fig. 2m). The message could be seen in only a few cells in the molecular layer. Cathepsin L mRNA has a similar distribution pattern, but the levels of its expression are drastically lower (Fig. 3g). Neither of these messages was detected in the white matter. Cathepsin S mRNA is diffusely distributed in the molecular layer, granular layer and white matter of the cerebellum (Fig. 3k).

Choroid plexus, ependyma and vasculature

All three messages were detected in the ependymal lining of the ventricles. They were also detected in the astrocytes surrounding the macrovessels, and at very low levels in the endothelial lining. In the choroid plexus there are high levels of both cathepsin B and L transcripts (Figs 2g, h, 3g). In all these structures, cathepsin S mRNA retains the expression pattern that it has throughout brain: it is abundant in a relatively small number of cells (Fig. 2i).

Under the stringency conditions used in the *in situ* hybridization histochemistry and taking into account that none of the cDNA fragments chosen for synthesis of the cRNA probes for *in situ* hybridization histochemistry is more than 60% homologous to the corresponding fragments from other known cysteine cathepsins, it is highly likely that we detect specific hybridization signals. However, we cannot exclude

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Abbreviations used in Figs 2 and 3

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cb	cerebellum	me5	mesencephalic trigeminal nucleus
cc	corpus callosum	MHb	median habenular body
cg	central gray	mi	mitral layer
cpu	caudate–putamen	ml	molecular layer
dr	dorsal raphe nucleus	ms	medial septum
ent	entorhinal cortex	on	olfactory nerve layer
e	ependymal and subependymal layer	pi	piriform cortex
ep	ependyma	pvn	paraventricular nucleus
gcl	glomerular cell layer	snr	substantia nigra reticulata
gl	gromerular layer	son	supraoptic nucleus
gr	granular layer	vp	ventral pallidum
ig	internal granular layer	wm	white matter
igr	internal granular layers	3V	third ventricle
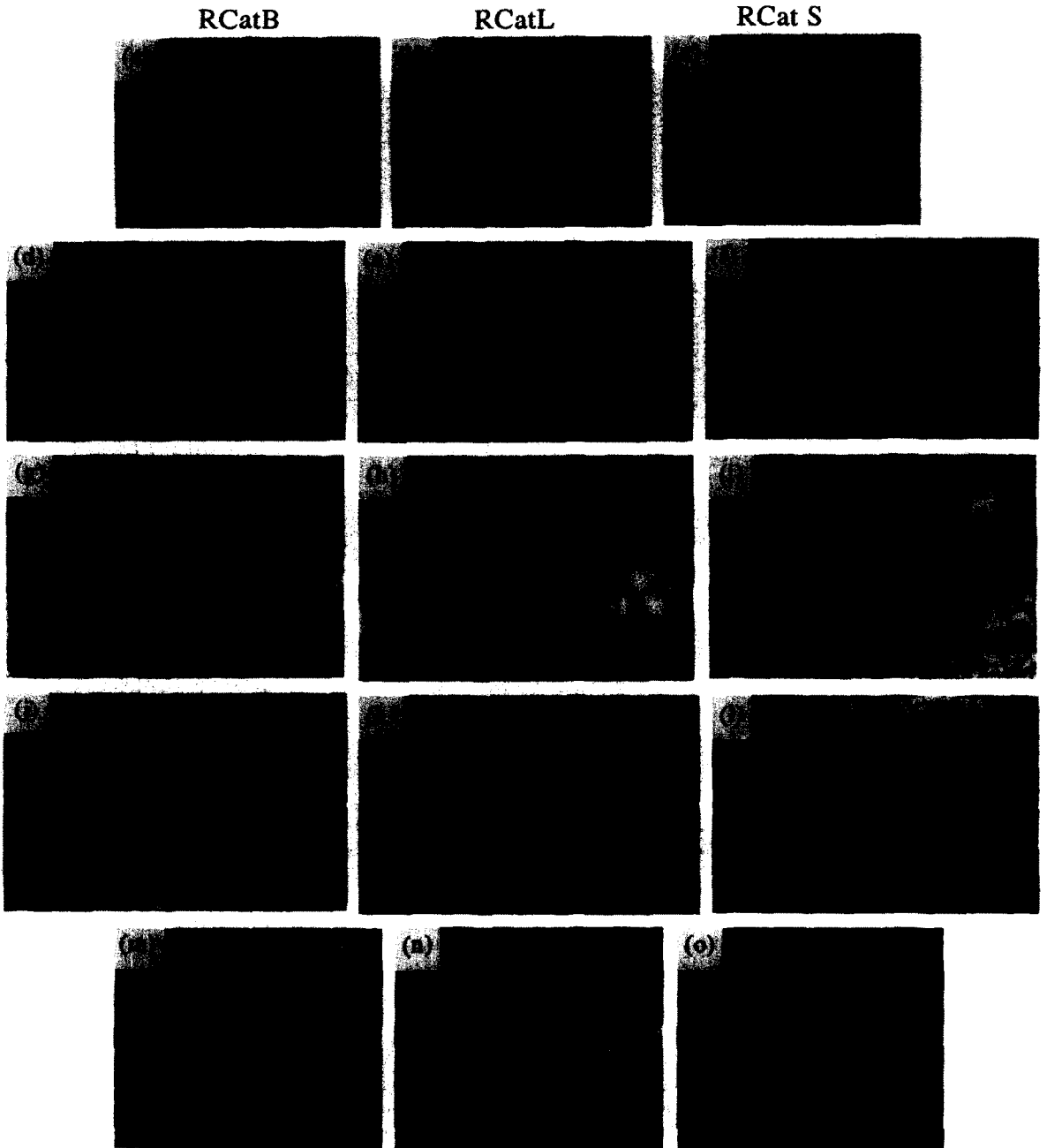
Fig. 2. The comparative distribution of cathepsins B, L, and S by *in situ* hybridization histochemistry. Darkfield autoradiograms of the distribution of cathepsin B (RCat B; a,d,g,j,m), cathepsin L (RCat L; b,e,h,k,n) and cathepsin S (RCat S; c,f,i,l,o) mRNAs in coronal sections of rat brain. a,b,c, olfactory bulb; note the abundance of cathepsin B mRNA in the mitral cell layer and its expression pattern complementary to cathepsin L. Also note the presence of cathepsin S mRNA in all layers, including the olfactory nerve layer. (d–f) Frontal cortex and septum; note the pronounced signal for cathepsin S in the corpus callosum and ventral pallidum. (g–i) Hippocampus; note the complementary expression of cathepsin B and L between the CA1–3 fields and dentate gyrus. The arrows in panel I point to lack of cathepsin S signal in the CA1–3 fields and dentate gyrus. (j–l) mesencephalon; (m–o) pons and cerebellum; note the high expression of cathepsin L in the choroid plexus of the fourth ventricle and third ventricle. mi, mitral layer; gl, gromerular layer; ig, internal granular layer; on, olfactory nerve layer; cc, corpus callosum; cpu, caudate–putamen; cx, cortex; ms, medial septum; ep, ependyma; vp, ventral pallidum; pi, piriform cortex; 3v, third ventricle; MHb, median habenular body; me5, mesencephalic trigeminal nucleus; dr, dorsal raphe nucleus; ent, entorhinal cortex; cb, cerebellum; ml, molecular layer; gr, granular layer. Exposure times: four days for cathepsin B, 10 days for cathepsin L and seven days for cathepsin S.
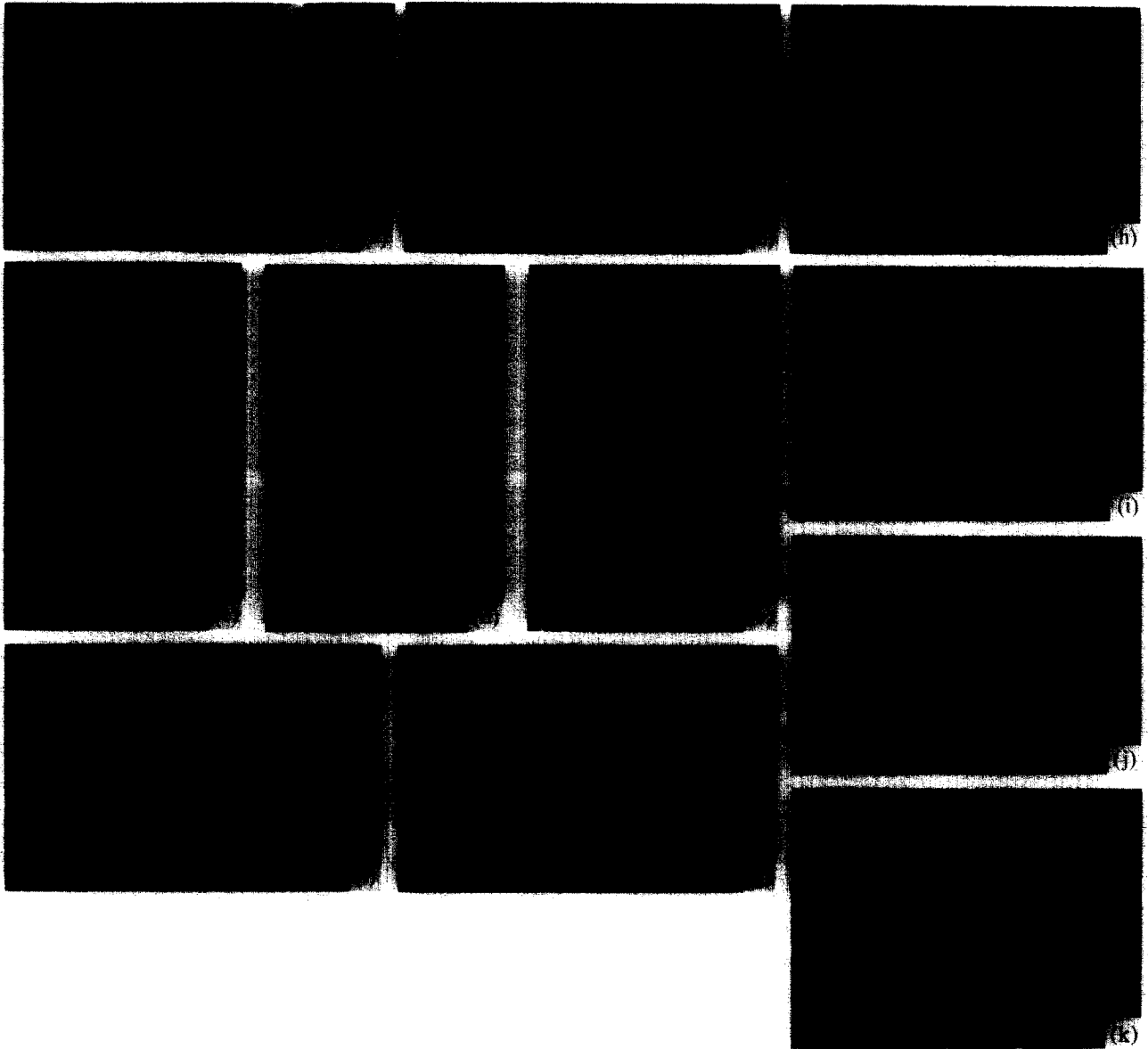
Fig. 3. Darkfield photomicrograph of emulsion-dipped sections showing the expression pattern of cathepsin B mRNA in cells from olfactory bulb (a), paraventricular hypothalamic nucleus (b), nucleus basalis of Meinert (c), mesencephalic trigeminal nucleus (d) and cerebellum (e). Arrowhead in A denotes tufted cells with high expression of cathepsin B mRNA. The expression of cathepsin L mRNA in supraoptic nucleus (F) and in choroid plexus (G). Arrowhead in F points to the astrocytes surrounding blood vessel. Arrows in G denotes purkinje cell layer. The expression of cathepsin S in the olfactory bulb (h), substantia nigra reticulata (i), central grey (j) and cerebellum (k). Dashed lines are used to outline structures as seen by light microscopy of the same counterstained sections. Exposure times: 10 days for cathepsin B, 21 days for cathepsin L and 15 days for cathepsin S. Magnifications: 20 × for all except 40 × for A.

the possibility that the fragments used as probes cross react with the mRNAs from closely related unknown members of this family.

DISCUSSION

We examined the relative abundance and regional distribution of the mRNAs for rat cathepsins B, L, and S in rat brain by northern analysis and by *in situ* hybridization histochemistry. The results of the northern analysis show that cathepsin B is the most abundant of the three enzymes in rat brain. The detection of two transcripts for cathepsin L is in agreement with the finding by Erickson-Lawrence *et al.*[20] It is yet to be established whether the brain transcripts also differ only in the length of their 3′

untranslated region, and if there is tissue and/or cell-type specificity in their expression. We have also detected similar sized transcripts in rat pheochromocytoma (PC12) and rat glioma (C6) cell lines (unpublished observation). Since we used a segment from the coding sequence of cathepsin L that recognizes both transcripts as a probe in the *in situ* hybridization histochemistry, we were not able to distinguish between the distribution of these two messages.

The major finding of our comparative *in situ* hybridization study is that the mRNAs for cathepsins B, L, and S have different expression patterns in rat brain. Cathepsin B and L have wide but not homogeneous distribution. Cathepsin B mRNA is preferentially expressed in neurons, while cathepsin L mRNA is equally expressed in neuronal and nonneuronal cells. The distribution patterns of cathepsin B and cathepsin L mRNAs are largely similar, although there are areas (i.e. olfactory bulbs, hippocampus) where they are significantly different (Fig. 2a, b, g, h). The fact that cathepsin B mRNA is the most abundant of the three proteases, both in terms of wide distribution and levels of message in individual cells, might be due to its ability to function as a dipeptidyl carboxypeptidase and as an endopeptidase. Being such a versatile enzyme it might mark cells with high protein turnover rates.

Among the brain regions examined, cathepsin L mRNA shows the highest expression in the epithelial cells of the choroid plexus (Figs 2h, m, 3g). High levels of cathepsin B transcript are also present in this cerebospinal fluid forming structure (Fig. 2g, k). Interestingly, the choroid plexus is a major site for the production and secretion of cystatin C, a potent inhibitor of lysosomal cysteine proteases that can inhibit both cathepsin L and cathepsin B.[3] Comparative studies of the expression of cystatin C have revealed that the peak of its expression occurs early in development.[7,47] It has also been established that there is a downregulation of its expression in inflammatory pathological conditions. This suggests that the fine equilibrium between the synthesis and secretion of the cysteine proteases and cystatin C is a part of the processes of normal growth and development as well as part of the acute inflammatory response in brain. It is not known whether cathepsin B and L are normally present in the cerebrospinal fluid. Cathepsin L is readily inactivated at neutral pH, and therefore normally might not exist in an active circulating form. Nevertheless, in response to different stimuli it can be secreted to act locally in an acidified pericellular environment.[19]

Cathepsin B and cathepsin L are also expressed in the neuropeptide synthesizing nuclei of the hypothalamus: the supraoptic, paraventricular, suprachiasmatic and arcuate nuclei. It is not clear if these enzymes can produce active neuropeptides from inactive precursor molecules. There is evidence, mainly from electron-microscopic studies, that cathepsin B is involved in the production of bioactive molecules

from their precursors.[32,48] Cathepsin B has been found in the secretory vesicles from atrial and juxtaglomerular endocrine cells, and implicated to take part in the maturation of atrial natriuretic factor and renin, respectively.[32,48] Cathepsin H is another member of the papain family that is found to co-localize with precursor molecules in secretory granules from corticotrophic and melanotrophic cells from the pituitary and endocrine cells from pancreas.[46] Also, cathepsins B, L, and S have been implicated in the processing of thyroglobulin.[35,45]

Similar levels of cathepsin S mRNA were observed throughout the brain regions examined in gray and white matter. Its overall expression pattern, in a relatively small number of cells scattered throughout brain resembles the distribution of microglia.[33] Many of the cells that express cathepsin S have size and morphology typical for microglia. Also, microglia play the role of macrophages in brain and cathepsin S is known to be highly expressed in alveolar macrophages and in spleen.[29,41] Recently, the presence of cathepsins B and L activities were reported in cultured microglia by enzyme histochemistry.[2] Since cathepsin S is also capable of cleaving the substrates used in this study, some of the detected activity could be due to its presence in this cell type. Because of its unusual homogeneous pattern of expression cathepsin B might be associated with a particular functional state of different cells, rather than being involved in the general protein turnover and therefore being expressed in most cells from a particular structure.

This study shows a significant correlation between the distribution of cathepsins B, L, and S mRNAs and the distribution of the mRNA and protein for different isoforms of the amyloid precursor protein as reported by several other investigators.[12,27,34] Namely, evidence from immunohistochemistry and *in situ* hybridization histochemistry studies in rat and mouse brain shows that the highest levels of amyloid precursor protein transcripts and protein are in the mitral cells in the olfactory bulb, cerebral cortical neurons, neurons in the septum and the diagonal band of Broca, the pyramidal and granular cells in the hippocampus, neurons of the amygdala, the red nucleus, the cranial nerve nuclei, pontine nuclei and the Purkinje cells in the cerebellum.[12,27,34,40] The distribution of the amyloid precursor protein in rat brain matches for the most part its distribution in human brain.[1,15,24,27] All these regions contain exceptionally high levels of cathepsin B mRNA and significant levels of cathepsin L mRNA (Figs 2g, h, 3a, d). The structures of the basal forebrain, known to be prone to degeneration and dense plaque formation express significant levels of the transcripts for all three enzymes. Like the amyloid precursor protein transcripts, relatively low levels of cathepsins B, L, and S mRNA could be detected in the astrocytes surrounding the bigger blood vessels and very low expression was seen in the endothelial lining of the vessels.[12,34]

The metabolism of the amyloid precursor protein is a complex and unresolved phenomenon. Recent evidence shows that soluble βA4 is a product of normal processing of amyloid precursor protein and that the production of βA4 is affected by lysosomotropic agents but not by cysteine protease inhibitors.[39,42] Therefore, the insolubility of the βA4 might be a result of its excessive production and/or defect in its clearence. Nevertheless, the high degree of co-localization of this molecule with cathepsin B and L throughout brain and the evidence by other groups about the involvement of the lysosomal cysteine proteases in the processing of amyloid precursor protein, suggest a role for these enzymes in its processing.

Since high levels of expression of the amyloid precursor protein per se are not sufficient for plaque formation, it is interesting to speculate that deregulation of one or several enzymes involved in its normal processing or ectopic expression of enzymes that are not normally involved in the processing may result in change of the processing pattern of the amyloid precursor protein in terms of excessive production of the βA4 peptide or generation of fragments that have toxic effect on the neighboring neurons. This can elicit a vicious physiological circle where the dying neurons and activated astrocytes and microglia can then further release cathepsins that can generate more amyloid peptides from the native amyloid precursor protein molecules. In this respect the possible preferential expression of cathepsin S mRNA by microglia is particularly intriguing. Microglia are thought to contribute to the Alzheimer's pathology as mediators of the inflammatory response in brain.[21] Although the microglia have been found in the center of mature senile plaques and have been shown to produce different isoforms of the amyloid precursor protein in vitro, their role as a source of plaque amyloid is uncertain.[21,23] Microglia activated as a result of different trauma might secrete cathepsin S in the extracellular matrix where it can act on amyloid precursor protein molecules released from damaged neurons. The preference for neutral amino acids at the P2 and P3 positions and its resistance to neutral pH, make cathepsin S an ideal protease for the production of βA4 peptide from the amyloid precursor protein extracellularly. Alternatively, cathepsin S could act intracellularly on endogenous amyloid precursor protein molecules or those produced by damaged neurons and phagocytosed by microglia.

CONCLUSIONS

We have shown that Cathepsin B and L are widely but differentially expressed. Their distribution patterns overlap to a great extent, but there are areas where they are quite different. Cathepsin S mRNA shows an unusual distribution pattern, being expressed in a relatively small number of different cells in all the regions examined. There is a high degree of correlation between the distribution of cathepsins B & L mRNAs and the expression of the amyloid precursor protein reported by others.[12,27,34,40] Also, the morphology and the distribution of many cells that express cathepsin S mRNA is typical for microglia, cells that mediate the inflammatory response and take an active part in the establishment of the cytopathological features of Alzheimer's disease.

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