

Review

Role of kallikrein enzymes in the central nervous system

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Abstract

Kallikreins are a subgroup of the serine protease family of enzymes. Until recently, it was thought that the human kallikrein gene family includes only three members. Over the past 3 years, the human kallikrein gene locus on chromosome 19q13.4 has been characterized. This family includes 15 members for which new nomenclature has been established. A number of kallikreins are expressed in the central nervous system (CNS). Experimental evidence has shown that at least two kallikreins, *KLK6* and *KLK8*, have potential functions in the CNS. *KLK8* (neuropsin) is highly expressed in brain tissues and may play a role in brain development, plasticity and response to stress. Of particular interest is the possible involvement of kallikreins in the pathogenesis of Alzheimer's disease (AD). *KLK6* (zyme/protease M/neurosin) seems to be down regulated in serum and tissues of Alzheimer's disease patients and may be involved in amyloid metabolism.

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1. Introduction

1.1. The human kallikrein gene family

The term “kallikrein” was introduced in the 1930s to describe proteolytic enzymes that can release small vasoactive peptides from high molecular weight precursors. There are two categories of kallikrein enzymes. Plasma kallikrein is encoded by a single gene

on chromosome 4. This enzyme, which has serine protease activity, releases the vasoactive peptide bradykinin from a high molecular weight precursor synthesized in the liver [1]. The human tissue kallikreins are a family of genes localized on chromosome 19, which also encode for serine protease enzymes. In this review, we will focus only on the human tissue kallikrein gene family; plasma kallikrein will not be discussed further.

Based on the original definition of kallikreins, which is based on the kininogenase activity of these enzymes, only pancreatic/renal kallikrein (*KLK1* gene; hK1 protein) fulfills this criterion. Until a few years ago, two other enzymes, human glandular kallikrein 2 (*KLK2* gene; hK2 protein) and human kallikrein 3 (*KLK3* gene; hK3 or PSA protein), were also classified as members of the human tissue kallikrein gene family,

Abbreviations: KLK, human kallikrein gene; hK, human kallikrein protein; AD, Alzheimer's disease; tPA, tissue plasminogen activator; PSA, prostate specific antigen; APP, amyloid precursor protein; MSP, myelencephalon-specific protease.

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based on a number of significant homologies and similarities with pancreatic/renal kallikrein. More recently, other genes, encoding for similar enzymes (see below), are also classified as members of the human kallikrein gene family. This classification is not based on the functional definition of kallikreins, but rather on structural criteria and gene location. Based on the latter definition, the number of genes that are included in the human tissue kallikrein gene family, originally thought to be much smaller than similar families found in rodents, is now increased to 15. The size of the family in humans is comparable to homologous families found in rat and mouse [2–8]. The official names of all kallikrein genes and proteins as well as a description of the human tissue kallikrein gene locus on chromosome 19q13.4 have been recently reviewed [9,10]. All known kallikrein genes map within an approximately 300-kb region. The lengths of the genes, the distances between them, as well as the direction of transcription, have now been accurately defined [9,11,12]. Telomeric from the last kallikrein gene identified (*KLK14*), we cloned a gene that belongs to the Siglec multigene family [13,14]. This finding suggests that this genomic region defines the end of the kallikrein gene family and the beginning of another family (the Siglec family of genes) [15]. Centromeric from the *KLK1* gene, another novel gene is located, namely ‘testicular acid phosphatase’ (*ACPT*), which is not a serine protease and appears to indicate the end of the kallikrein gene family from this end [16].

The genomic organization of each one of these kallikrein genes is very similar and is described in detail elsewhere [2,3,17]. In short, all genes encode for putative secreted serine proteases and have five coding exons of similar lengths. All genes share significant sequence homologies at both the DNA and amino acid level and many of them are regulated by steroid hormones. Despite these similarities, the tissue expression of these genes is different. Some genes are expressed in very few tissues, while others are abundantly expressed in many tissues. Detailed tissue expression data can be found in recent reviews [2,4].

In order to simplify communication, an international group of scientists working in the field has established uniform nomenclature for the kallikrein genes and their encoded proteins. “KLK” is used to describe a kallikrein gene and “hK”, the correspond-

ing protein product [18]. In this manuscript, the official nomenclature for these genes and proteins is used throughout.

1.2. Kallikrein gene expression in the central nervous system (CNS)

At the mRNA level, all kallikrein genes, except *KLK3*, *13* and *15* were reported to be expressed at variable concentrations in the CNS, using Northern blotting and RT-PCR analysis. *KLK6-12* and *KLK14* are expressed at relatively higher amounts in the CNS, compared to other kallikreins.

KLK6 (previously known as *zyme/protease M/neurosin/PRSS 9*) was independently cloned by three research groups. Yamashiro et al. [19], using Northern blot analysis, found highest expression levels of the gene in the brain and named it “neurosin”. Similar results were reported by Little et al. [20] who named the gene “zyme” and provided evidence about its possible involvement in Alzheimer’s disease (AD, discussed below). The rat and human orthologs of the same gene were independently cloned by Scarisbrick et al. [21] and it was named Myelencephalon specific protease. By RT-PCR, *KLK6* mRNA was also found to be expressed at high levels in the spinal cord and cerebellum [22]. We also observed relative abundance of hK6 protein in CSF [23]. Okui et al. [24], using monoclonal antibodies, N-terminal sequencing and enzymatic activity studies, have shown that hK6 in CSF occurs mainly as inactive pro-enzyme. Northern blot analysis showed that *KLK6* is most abundantly expressed in the spinal cord, hippocampus, substantia nigra, and basal ganglia [25]. Scarisbrick et al. [21] reported that *KLK6* is expressed in different brain areas including brain stem, spinal cord, substantia nigra and hippocampus.

Yoshida et al. [26] cloned the cDNA of the human *KLK8* (*neuropsin*) gene from hippocampus, and Mitsui et al. [11] reported that *KLK8* occurs in two isoforms of 260 and 305 amino acids in length, with type 2 carrying an insertion of 135 bp in the second coding exon. Type 2 neuropsin was shown to be preferentially expressed in human adult brain and hippocampus, although both types were expressed at comparable amounts in fetal brain. Dot blot hybridization showed that *KLK8* is expressed in various regions of the adult human brain, including the hippo-

campus, cerebral cortex, amygdala, cerebellum and frontal lobe [11].

We found highest levels of *KLK5* mRNA expression in the adult brain and breast tissues and lower levels in the cerebellum and spinal cord [5]. More recently, we detected low levels of hK5 protein in adult cerebellum, spinal cord and cerebrospinal fluid, while higher levels were found in fetal cerebellum and spinal cord (our data, submitted for publication). *KLK14* mRNA was detectable at high levels in spinal cord, cerebellum and fetal brain and at lower levels in adult brain [14].

Yoshida et al. [27] cloned the human *KLK11* gene (previously known as trypsin-like serine protease or hippostasin) from hippocampus cDNA, using PCR approaches. By RT-PCR, the gene has higher expression in the cerebellum [28]. More recently, Mitsui et al. [29] reported the existence of two alternatively spliced forms of *KLK11*, brain-specific and prostate-specific. In situ hybridization revealed that the brain type is preferentially expressed by the pyramidal neurons of the human hippocampus, while no glial cells in any portion of the human brain showed any signal [29]. The production of brain-specific isoforms represents a mechanism by which the specificity of the proteolytic function could be achieved. Recent reports indicated the presence of brain-specific isoforms of certain serine proteases, including kallikreins. In addition to *KLK8* and *KLK11* discussed in this review, a novel isoform of granzyme K is expressed specifically in the mouse brain, and a brain-specific isoform of the mouse kallikrein gene hippostasin was also recently cloned [30].

Using RT-PCR, we have previously shown that *KLK9* is expressed at relatively high levels in the cerebellum and spinal cord, and at lower levels in the adult brain [12]. Liu et al. [31] showed no expression of the *KLK10* (*NES1*) gene in the brain by Northern analysis, but Luo et al. [32] demonstrated presence of low levels of hK10 protein in the CSF and in brain tissue cytosolic extracts (our unpublished data).

No detectable expression of *KLK7* mRNA was found in the fetal or adult brain by Northern blotting [33]; however, using the more sensitive RT-PCR technique, *KLK7* mRNA was found to be expressed in the brain, spinal cord and cerebellum [34]. Low levels of expression of *KLK12* were found in adult but not fetal brain [35].

Melegos et al. [36] reported hK3 (PSA) presence in the CSF and provided preliminary evidence that CSF hK3 is of brain origin. hK3 expression was also reported in two neuroblastoma cell lines [37].

1.3. Biological functions of kallikreins

Three of the 15 kallikreins have been assigned to a specific biological function. hK1 exerts its biological activity mainly through the release of lysyl-bradykinin (kallidin) from low molecular weight kininogen [1]. However, the diverse expression pattern of hK1 has led to the suggestion that the functional role of this enzyme may be specific to different cell types [38,39]. Apart from its kininogenase activity, hK1 has been implicated in the processing of growth factors and peptide hormones in light of its presence in pituitary, pancreas and other tissues [40]. As summarized by Bhoola et al. [38], hK1 has been shown to cleave pro-insulin, low-density lipoprotein, the precursor of atrial natriuretic factor, prorenin, vasoactive intestinal peptide, pro-collagenase and angiotensinogen.

The physiological function of hK2 protein has been examined only recently. Seminal plasma hK2 was found to be able to cleave seminogelin I and seminogelin II but at different cleavage sites and with a lower efficiency than PSA [41]. Furthermore, a role of hK2 in regulating growth factors, through IGFBP-3 proteolysis, has been suggested. Recently, hK2 was found to activate the zymogen or single chain form of urokinase-type plasminogen activator (uPA) in vitro [42].

Since hK3 is present at very high levels in seminal plasma, most studies have focused on its biological activity within this fluid. Lilja [43] has shown that PSA rapidly hydrolyzes both seminogelin I and seminogelin II, as well as fibronectin, resulting in liquefaction of the seminal plasma clot after ejaculation. Several other potential substrates for PSA have been identified, including IGFBP-3 [44], TGF- β [45], basement membrane, parathyroid hormone-related peptide and plasminogen [46]. The physiological relevance of these findings is still not clear.

1.4. Potential physiological and pathological roles of kallikreins in the central nervous system

Since the kallikrein family in humans was characterized only recently, most of our knowledge about the

potential functions of kallikreins in brain tissues was generated from studies in mouse and rat animal models and from the fact that kallikreins are serine proteases.

Recent studies have revealed important roles of secreted serine proteases in the brain. For example, tissue plasminogen activator (tPA) is expressed in the hippocampus [47], and concentrated at the growth cones by binding to cell surface receptors [48]. Disruption of the tPA gene leads to resistance to neuronal degeneration and reduced susceptibility to pharmacologically induced seizures [49]. Thrombin was also reported to cause neurite retraction [50], in addition to regulation of several cellular activities of neurons and astrocytes in various developmental phenomena in the brain [51].

A recent report described cloning of the mouse ortholog of the *KLK6* gene and localization of its expression in mature oligodendrocytes [52]. Developmentally, the mRNA was expressed 2–7 days after maturation, suggesting that it has a role in the processes occurring after oligodendrocyte maturation such as myelination or turn over of proteins in the myelin [52].

Crystal structure and enzymatic data indicated that hK6 is the human ortholog of the myelencephalon-specific protease (MSP) [53], which is a member of the rat kallikrein gene family. This enzyme is abundantly expressed in the rodent central nervous system, with tissue-specific expression in the spinal cord and medulla oblongata [21]. MSP was shown to be up regulated in response to glutamate receptor-mediated cytotoxic injury [21]. Scarisbrick et al. [54] have recently investigated the role of hK6 in CNS demyelination. Excess hK6 resulted in a dramatic loss of processes from differentiated oligodendrocytes. They further demonstrated that myelin basic protein and myelin oligodendrocyte glycoprotein can serve as hK6 substrates. Two potential homologues of the same gene have been identified in the mouse genome, the brain skin serine protease (BSSP) and brain serine protease (BSP) [55,56].

Petraki et al. [57], using monoclonal and polyclonal antibodies, localized human hK6 protein in many tissues, including the central nervous system. Strong and diffuse positivity was observed in the epithelium of the choroid plexus. In the cerebellum, the antigen was expressed in the Purkinje cells and the stellate (basket cells), but not in the granular cells. Nerve cells

showed weak immunostaining in the entire CNS as well. Staining of the peripheral nerves was also intense [57].

In rodents, mouse neuropsin is one of the well-studied kallikreins. It is mainly expressed in the mouse hippocampal pyramidal neurons. Studies have shown that it is involved in hippocampal plasticity. Intraventricular injection of neuropsin monoclonal antibodies was shown to reduce the epileptic pattern and inhibit the progression of kindling [58]. In addition, electrical stimulation to hippocampus or amygdala was generated to alterative expression of neuropsin in brain [59,60]. Oxidative stress significantly reduced neuropsin expression and 30 days of treatment was needed to restore this level [61]. The human neuropsin ortholog, *KLK8*, has 73% amino acid identity with the mouse neuropsin gene, with conservation of the key amino acid residues essential for activity, including the cysteine residues [26]. If this similarity extends to functional attributes, we can assume that the human and rodent proteins have a similar function in the CNS.

Reports indicate that neuropsin might be involved in synaptogenesis, neural development [62], regulation in long-term potentiation (LTP) [63] and seizures in kindled brain [60]. Hirata et al. [64] have recently shown that neuropsin knock-out mice have marked abnormalities in the synapses and neurons in the CA1 subfield of the hippocampus and suggested that it is involved in connectivity of group CA1 synapses and consequently in hippocampal networking. Davies et al. [65] reported that loss of neuropsin predisposes to global seizure activity. Suzuki et al. [62] examined the ontogeny of mouse neuropsin expression in the brain and concluded that the widespread localization and the change of expression pattern during embryonic development are suggestive of neuropsin being a multifunctional protein that is involved in development, neural plasticity and cerebrospinal fluid production. Recently, endogenous neuropsin was found to be extracellularly localized in neuronal cell bodies and their neurites in mouse hippocampal cultures and is able to enhance neurite projection from soma. It was thus suggested that neuropsin is involved in neurite outgrowth and fasciculations during development of the nervous system [66]. Experimental evidence has also shown that neuropsin has a regulatory effect on Schafer-collateral long-term potentiation in the mouse hippocampus [63].

1.5. Kallikreins in Alzheimer's disease

Alzheimer's disease (AD) is the major cause of dementia in the elderly. Although rare genetic forms of AD exist, most patients are classified as having sporadic AD. The two major pathological lesions characteristic of Alzheimer's disease are the aggregated β -amyloid peptides, which are generated from breakdown of the amyloid precursor protein (APP) by proteases and the neurofibrillary tangles. Serine proteases and their inhibitors have been implicated in neurodegenerative disorders such as AD [67,68].

So far, hK6 is the only kallikrein that was experimentally investigated in AD, although results are yet inconsistent. Little et al. provided the first evidence of the amyloidogenic activity of hK6. However, in co-transfection experiments, this enzyme was able to digest amyloid precursor protein 695 (APP 695). In the same report, hK6 was immunolocalized to the perivascular cells in monkey cortex and the microvessels and microglial cells of human AD brain samples. Another study showed that hK6 protein is localized at senile plaques and neurofilament tangles in AD brain, and at Lewy bodies in Parkinson's disease. In AD, staining of neurons with processes was rare in the damaged areas such as parietal cornices, and only nuclear staining was found in such cases. Also, the intensity of *KLK6* mRNA expression in the gray matter was decreased compared to normal controls. These results were obtained by both in situ hybridization and RT-PCR, and suggested a role for *KLK6* in degradation of β -amyloid protein [69].

The chromosomal locus 19q13.1–q13.3 is thought to be linked with familial Alzheimer's disease [70]. The apolipoprotein E gene, which is linked to Alzheimer's disease, maps to 19q13.2. Although the apoE ϵ 4 allele is strongly associated with Alzheimer's disease in late onset familial AD families, Yu et al. [71] used linkage analysis to demonstrate that the apoE region does not segregate with AD in a collection of late onset AD cases, suggesting that apoE is not the major locus. It is possible that the kallikrein locus may include a group of Alzheimer's disease-related protease genes.

We recently found that hK6 concentration is two-fold lower in Alzheimer's brain tissue extracts compared to normal and localized hK6 expression by

immunohistochemistry in the epithelial cells of the choroid plexus. However, CSF hK6 concentration was three fold higher in AD, compared to controls [72]. We also found that whole blood hK6 concentration in AD patients is higher than normal controls. These data suggest that hK6 might constitute a new biomarker for diagnosis and monitoring of AD. Using a sensitive immunoassay method, we have also recently reported presence of significantly lower amounts of hK6 in hippocampal and cerebral cortex extracts of Alzheimer's disease patients compared to normal subjects [73]. Another recent study examined the expression of all kallikrein genes in human cerebral cortex and hippocampus, two regions commonly involved in AD pathology, by RT-PCR and compared their expression between AD and control tissues [74]. *KLK1*, 4–8, 10, 11, 13 and 14 were found to be expressed in both cerebral cortex and hippocampus, while *KLK9* was expressed in the cortex but not the hippocampus and *KLK2*, 3, 12 and 15 were not expressed in either tissue. An 11.5-fold increase was demonstrated in *KLK8* mRNA levels in AD hippocampus compared to control [74].

Dihanich and Spiess [75] isolated an alternatively spliced form of *KLK15* (named ACO11) from Alzheimer's visual cortex mRNA, and found it, by PCR analysis, to be expressed in human hippocampus, visual cortex, glioma cells and rat cortex.

2. Conclusions

Recent evidence has shown that many kallikrein genes are expressed in relatively high levels in brain tissues. At least two kallikreins are linked to normal

Table 1
Research fronts for kallikreins in brain physiology and disorders

- Distribution in brain regions
- Production and secretion by specific cell types
- Mode of regulation
- Enzymatic specificity; candidate substrates
- Active vs. inactive precursors; mode of activation
- Overexpression/underexpression in disease states
- Regulation by inhibitors in brain tissues
- Amyloid formation or clearance
- Role in cerebrospinal fluid
- Interaction with other proteases involved in neurodegeneration
- Therapeutic targeting in CNS

brain physiology and development and show differential expression in response to brain stress and other pathologies. Extensive evidence from *KLK8* analysis in rodents supports that this enzyme should be further examined in relation to human CNS pathobiology. Several groups have shown a link between *KLK6* and Alzheimer's disease, although the possible mechanism of involvement is yet to be delineated. Other kallikreins need to be investigated for their possible roles in brain physiology and pathobiology. It is now clear that we are just beginning to examine these interesting enzymes and that there are numerous crucial questions that need clarification. In Table 1, we list some research fronts related to kallikrein research in the CNS.

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