Differential distribution of KChIPs mRNAs in adult mouse brain

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Abstract

The K⁺ channel interacting proteins (KChIPs1–4) interact with and modulate activity and trafficking of Kv4 potassium channels. We report here the distribution of KChIPs in adult mouse brain. KChIP1 was expressed in a subpopulation of neurons widely distributed in brain and enriched in Purkinje cells of the cerebellum and in the reticular thalamic and medial habenular nuclei. KChIP2 transcripts were highly expressed in layer IV of the cerebral cortex and in striatum and hippocampus, but expressed at low levels in cerebellum. KChIP3 transcripts were detected primarily in the layer V and deep layer VI of the cerebral cortex, the hippocampus, and the entire cerebellum. KChIP4 was highly expressed by neurons in layers II–IV of cortex and in hippocampus, thalamus and the Purkinje cell layer of the cerebellum. Collectively, different KChIPs appear to be expressed by selected neuronal populations in different brain regions where expression of Kv4.2 and Kv4.3 have been reported. These findings support the likelihood of functional interactions between KChIPs and Kv4 K⁺ channels in brain.

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Theme: Excitable membranes and synaptic transmission

Topic: Potassium channel structure, function, and expression

Keywords: KChIPs; Kv4; Expression pattern

1. Introduction

Neuronal calcium sensor (NCS) proteins play important roles in modulating neuronal function [7]. More than 20 NCS proteins have been identified from various species. These proteins share high homolog within their C-terminal frequenin domain with diversified N-termini. Another structural characteristics of NCS proteins is containing four EF-hand calcium binding motifs in each protein. Potassium channel interacting proteins (KChIPs), including KChIP1–4, are a newly identified subfamily of NCS proteins found only in mammalian cells [3,8,10,19]. KChIP proteins interact with voltage-gated potassium channels (A-type) and Alzheimer’s disease-associated presenilin-2 [3,8,19]. KChIP3 also binds to a downstream regulatory element (DRE) and functions as a transcriptional repressor [10]. Consistent with a function in modulating potassium channels, deletion of KChIP2 from the mouse genome results in a complete loss of calcium dependent transient outward potassium current and confers susceptibility to ventricular tachycardia [16]. Reduced Kv4 channel activity has also been observed in KChIP3-null mice [17]. KChIP3 deficient mice show both reduced responses in models of acute pain through a transcriptional mechanism and decreased amyloid β production via down-regulating γ-secretase activity [11,17]. Thus, individual KChIPs may regulate multiple neuronal functions whereas all KChIPs modulate Kv4 K⁺ channel activity.

Most reported studies of KChIPs focus on their modulation of voltage-gated Kv4 K⁺ channel activity. In the nervous system, Kv4 K⁺ channels prevent backpropagating action potentials, help establish slow repetitive spike firing, and contribute to spike repolarization and signal...
amplification. In the heart, these channels mainly function to shape the repolarization phase of the action potential. KChIPs have been shown to modulate Kv4 K⁺ channels through multiple mechanisms, including regulating cell surface expression, slowing rapid inactivation and accelerating recovery from inactivation [3,4,12,14,19,29]. Northern blot hybridization indicates that KChIPs are predominantly expressed in brain, with KChIP2 seen also in heart [3,16,19]. Studies using the polymerase chain reaction (PCR) detect KChIP mRNAs in tissues other than brain, although expression levels of KChIPs in these tissues are apparently low [2,20]. Immunofluorescent staining of brain sections identifies KChIP3 protein in what are likely to be neurons in the CA1–CA3 regions of hippocampus and the granule cell layer of cerebellum of adult mouse [13]. Detailed distribution of KChIPs in adult brain remains unknown.

To determine the localization of specific KChIPs in brain in order to shed light on their potential function, we determined the expression pattern of KChIPs in mice brain. We report here differential expression of different KChIPs in selected neuronal populations and brain regions.

2. Materials and methods

2.1. Materials

HeLa cells were purchased from ATCC and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. C57BL6 mice were purchased from Charles River Laboratory. cDNAs encoding mouse KChIP1 (accession no. AY171234), KChIP2 (accession no. NM145704), and KChIP4 (accession no. NM019789) were cloned from a yeast two-hybrid screen using Adenomatous Polyposis Coli (APC) cDNA as a bait (not shown). cDNAs encoding mouse KChIP3 (accession no. NM145704) were kindly provided by Dr. Michael Meyer [26]. All cDNAs cover the entire coding sequence of KChIPs and include both 5’ - and 3’-untranslated regions. The lengths of probes are 1093 bp (KChIP1), 1008 bp (KChIP2), 728 bp (KChIP3), and 900 bp (KChIP4).

2.2. Transfection and immunoblotting

Transfection was performed using LipofectAmine 2000 reagent (Invitrogen) according to the manufacturer’s instructions. Cells were collected 48 h after transfection. Immunoblotting was done as previously described [28]. Briefly, cells or mouse brain tissues were lysed in SDS sample buffer followed by determination of protein concentration. Proteins were separated on 4–20% Tris–glycine gels (Invitrogen), electrotransferred onto PVDF membranes (Millipore), immunoblotted with respective antibodies and detected by ECL (Amersham).

2.3. In situ hybridization

2.3.1. Probe preparation

cDNAs encoding KChIP1–4 in pBluescript were linearized using appropriate restriction enzymes and purified with a Qiaquick PCR kit (Qiagen). Digoxigenin-labeled probes were synthesized using a DIG RNA Labeling Kit (Roche) and purified using a G50 Quick Spin column (Roche).

2.3.2. Brain tissue sections

C57BL6 mice were anesthetized by inhalation of isoflurane and fixed by transcardiac perfusion with 4% paraformaldehyde in PBS. Brains were removed, further fixed at 4 °C overnight, and immersed in 30% sucrose/PBS at 4°C overnight. Specimens were mounted in OCT. Serial sections (45 μm thick) were generated with a cryostat and used fresh.

2.3.3. In situ hybridization

Mouse brain sections were fixed in 4% paraformaldehyde for 20 min, washed in PBS twice with each for 5 min, and treated with 1 μ/ml proteinase K (50 mM Tris–HCl, pH 7.5 and 5 mM EDTA) for 20 min. After washing in PBS for 5 min, sections were fixed again for 15 min, treated with 0.25% acetic anhydride in 0.1 M triethanolamine for 10 min, and washed in PBS for 5 min. Prehybridization was performed in prehybridization buffer (50% formamide, 5× SSC, 0.3 mg/ml yeast tRNA, 100 µg/ml heparin, 1× Denhardt’s solution, 0.1% Tween, 0.1% CHAPS and 5 mM EDTA) at 60 °C for 2 h, followed by hybridization with 1 µg/ml digoxigenin-labeled probes in prehybridization buffer at 60 °C overnight. After hybridization, sections were washed in 2× SSC at 60 °C for 15 min, treated with 1 µg/ml RNase at 37 °C for 30 min, washed twice in 0.2× SSC at 60 °C for 30 min each, and washed three times in PBT (0.1% Triton X-100 and 2 mg/ml BSA in PBS) at room temperature for 20 min each. Both antisense and sense (for control) probes of each KChIP were used for hybridization.

For detection, sections were blocked in blocking buffer (5% goat serum in PBT) at room temperature for 1 h, followed by reaction with alkaline phosphatase-conjugated
anti-digoxigenin Fab fragments in blocking buffer (1:2000; Roche) at 4 °C overnight. Unbound Fab fragments were removed by washing three times in PBT buffer for 20 min each. Sections were developed by incubation in AP buffer (100 mM Tris–HCl, pH 9.5, 50 mM MgCl₂, 100 mM NaCl, 0.1% Tween-20) containing nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolylphosphate substrate (NBT/BCIP) at room temperate until the appropriate color was seen.

3. Results

3.1. Distribution of KChIP proteins in adult mouse brain

For immunodetection of specific KChIP proteins in brain we obtained mono- and polyclonal antibodies against KChIP1, KChIP2, KChIP3, and KChIP4 from a variety of sources (see Materials and methods). Because KChIPs share more than 60% homology within their frequinin domain, we verified the specificity of these antibodies by transfecting HeLa cells with cDNAs encoding individual KChIPs and performing Western blots with the antibodies on cell lysates. The results indicate that specific KChIPs were detected only by their respective antibodies (Fig. 1A), demonstrating the specificity of the four antibodies against specific KChIP proteins.

To determine the expression of individual KChIPs, lysates made from various tissues of adult mouse were immunoblotted with the anti-KChIP antibodies. KChIP proteins were primarily detected in brain. In brain, KChIP proteins were differentially expressed among the subregions analyzed (Fig. 1B). Although KChIP1 and KChIP4 proteins were detected in most regions analyzed, KChIP1 protein was abundant in cerebellum, thalamus, and hypothalamus, and KChIP4 protein was expressed at a relatively high level in cerebellum and Shalamus. Both KChIP1 and KChIP4 proteins were expressed at low levels in the striatum. High levels of KChIP2 protein were detected in cerebral cortex, striatum and hippocampus, while KChIP2 protein was low in the hypothalamus. In contrast to KChIP1 and KChIP4, little KChIP2 was detected in cerebellum. KChIP3 was detected in most brain regions with the lowest expression seen in the hypothalamus. These results indicate that KChIP proteins are differentially expressed in specific regions of adult mouse brain. High level of KChIP2 transcripts is reported in heart tissue [3,16]. A weak band was detected in the heart by anti-KChIP2 antibody in this study (Fig. 1B). The low KChIP2 protein level detected in heart may be resulted by fast degradation of KChIP2 mRNA or protein. Nevertheless, we cannot exclude the possibility that the anti-KChIP2 antibody used in this study recognizes only the minor KChIP2 isoform because multiple splicing KChIP2 isoforms are found in heart [16]. Little KChIP protein was seen in lung.

3.2. Distribution of KChIP transcripts in adult mouse brain

To determine the expression pattern of KChIP mRNAs in brain, in situ hybridization with probes specific for individual KChIPs was performed on adult mouse brain sections. mRNAs encoding all KChIPs were seen in almost all brain regions with which the expression level of individual KChIPs varied among different regions of brain (Fig. 2; Table 1). Detailed description of the expression of KChIP transcripts in specific brain regions is provided below. To summarize, KChIP1 transcripts were distributed in all brain regions and enriched in cerebellar Purkinje cells and some thalamic nuclei. KChIP1 transcripts were particularly abundant in the medial habenular nucleus and reticular thalamic nucleus. Consistent with the immunoblotting results, KChIP2 mRNA was not detected in cerebellum but highly expressed in hippocampal CA regions, the dentate gyrus,

![Fig. 1. Expression of KChIPs in mouse tissues. (A) Characterization of KChIP antibodies. Cell lysates expressing control plasmid (C) and myc-tagged KChIP1 (lane 2), KChIP2 (lane 3), KChIP3 (lane 4), and KChIP4 (lane 5) were immunoblotted with antibodies to KChIP1, KChIP2, KChIP3, and KChIP4. The same membrane was detected with anti-tubulin antibody to show equal loading (Tub). Molecular weight markers are shown on the left side of the figure. (B) Expression of KChIPs in mouse tissues. Lysates made from adult mouse brain (Brain), heart (Heart), lung (Lung), and different brain areas, including cortex (Cx), cerebellum (Ce), hippocampus (Hipp), striatum (Str), thalamus (Tha), and hypothalamus (Htha), were immunoblotted with antibodies specifically recognizing KChIPs. Antibodies used are labeled on the right side of the figure. The same membrane was detected with anti-tubulin antibody to show equal loading (Tub). Molecular weight markers are shown on the left side of the figure.](image-url)
striatum, and in the amygdaloid and lateral septal nuclei. KChIP3 transcripts were largely detected in cortex, hippocampus, thalamus, and amygdala, while KChIP4 was highly expressed in cerebellar Purkinje cells, thalamus, and hypothalamus (Fig. 2). In control experiments, little hybridization signal was detected in sections hybridized with sense probes (not shown).

3.3. Cerebral cortex

All KChIP transcripts were detected in the cerebral cortex. However, transcripts were differentially distributed in different cortical layers. KChIP1 transcripts were expressed in neurons in all layers of the cerebral cortex. KChIP2 transcripts were particularly rich in layer IV with relative lower levels seen in layer VI. KChIP3 hybridization signals in layers II, III, and V were weaker than those seen in layer VI. In contrast to KChIP2, KChIP3 hybridization signals were stronger in layers II, III, and V than those in layers IV and VI. Interestingly, KChIP3 was highly expressed in the deep layer VI of the cerebral cortex. KChIP4 transcripts were detected in layers II–V and were more abundant in layers II, III, and V. Nevertheless, the expression pattern of KChIP4 in cerebral cortex was different from that of KChIP1. Few KChIP2, KChIP3, and KChIP4 transcripts were detected in layer I (Fig. 3A).

3.4. Hippocampal formation

KChIP1 transcripts were evenly distributed throughout the hippocampal region where they are likely expressed in
interneurons. KChIP2–4 transcripts were detected mainly in the pyramidal cell layer of hippocampal CA regions and in the dentate gyrus. The level of KChIP3–4 transcripts in the dentate gyrus was higher than that seen in hippocampal CA regions. In hippocampus, KChIP2 hybridization signals were particularly high in the CA3 region. KChIP3 and KChIP4 transcripts were also abundant in the subiculum region. By contrast, KChIP1 and KChIP2 signals were detected in only a few neurons in the subiculum (Fig. 3B).

3.5. Thalamus

KChIP1 signals were generally low and evenly distributed in most of thalamus. Nevertheless, KChIP1 transcripts were abundant in the paraventricular thalamic nucleus and intermediodorsal thalamic nucleus relative to the rest of the thalamus. KChIP1 hybridization signals were particularly enriched in the reticular thalamic nucleus, where GABAergic interneurons are abundant. KChIP3–4 transcripts were highly expressed and evenly distributed in the thalamus. Consistent with the immunoblotting results, KChIP2 transcript levels were low in the thalamus (Fig. 3C).

3.6. Hypothalamus

KChIP1 transcripts were abundant and uniformly distributed in hypothalamus and enriched in some regions. Strong KChIP1 hybridization was seen in the dorsomedial ventromedial nucleus. Similar to expression patterns seen in the thalamus, KChIP3 and KChIP4 transcripts were detected at moderate levels and evenly distributed in the hypothalamus, while little KChIP2 mRNA was seen (Fig. 3D).

3.7. Amygdala

All KChIPs were highly expressed in the amygdala. Neurons expressing KChIP1 were evenly distributed in this structure. Hybridization signals for KChIP2–4 were particularly strong in the piriform cortex. Some enrichment of KChIP2–4 transcripts was also seen in lateral and basolateral amygdaloid nuclei (Fig. 3E).

3.8. Striatum

Individual neurons in the striatum were unevenly labeled with the KChIP1 probe. Consistent with the immunoblotting results, KChIP2 transcripts were abundant in striatum where hybridization signals were gradually reduced from the dorsal (high) to ventral (low) sides. KChIP3 showed an expression pattern similar to KChIP2 but was much less abundant than KChIP2. Little KChIP4 expression was seen in striatum, consistent with results of the immunoblotting analysis (Fig. 3F).

3.9. Cerebellum

KChIP1 transcripts were detected exclusively in cerebellar Purkinje cells. Few KChIP2 transcripts were seen in agreement with the immunoblotting analysis. By contrast, KChIP3 transcripts were detected in the molecular layer, the granular layer, Purkinje cells, and the cerebellar nucleus. KChIP4 mRNA was detected in Purkinje cells and the cerebellar nucleus (Fig. 3G).

Table 1
Quantitative estimates of the relative distributions of KChIP transcripts in selected regions of the adult mouse brain as determined by in situ hybridization

<table>
<thead>
<tr>
<th>Brain region</th>
<th>KChIP1</th>
<th>KChIP2</th>
<th>KChIP3</th>
<th>KChIP4</th>
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<tbody>
<tr>
<td>Cerebral cortex</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Layer I</td>
<td>++</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Layer II</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Layer III</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>Layer IV</td>
<td>++</td>
<td>+++</td>
<td>/–</td>
<td>+</td>
</tr>
<tr>
<td>Layer V</td>
<td>++</td>
<td>+</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Layer VI</td>
<td>++</td>
<td>+/–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Deep layer VI</td>
<td>++</td>
<td>/–</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>Piriform cortex</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Hippocampal formation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA1</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>CA2</td>
<td>+</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>CA3</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Dentate gyrus</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Subiculum</td>
<td>+</td>
<td>+/–</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Habenula</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Thalamus</td>
<td></td>
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<tr>
<td>Reticular thalamic nu.</td>
<td>++++</td>
<td>++</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ventromed. thalamic nu.</td>
<td>+/-</td>
<td>–</td>
<td>++</td>
<td>+++</td>
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<tr>
<td>Ventrolateral thalamic nu.</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Laterodorsal thalamic nu.</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Mediodorsal thalamic nu.</td>
<td>+</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Central medial thalamic nu.</td>
<td>+++</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Hypothalamus</td>
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<td></td>
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<tr>
<td>Dorsomed. ventromed. nu.</td>
<td>++++</td>
<td>/–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Dorsomed.</td>
<td>++</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>hypothalamic nu.</td>
<td>/–</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ventromed.</td>
<td>++</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>hypothalamic nu.</td>
<td>/–</td>
<td>–</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granule cell layer</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>–</td>
</tr>
<tr>
<td>Molecular layer</td>
<td>–</td>
<td>–</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>Purkinje cell layer</td>
<td>++++</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Deep cerebellar nu.</td>
<td>–</td>
<td>–</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Amygdala</td>
<td>++</td>
<td>++++</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>Caudate putamen</td>
<td>+</td>
<td>+++</td>
<td>++</td>
<td>/–</td>
</tr>
<tr>
<td>(Striatum)</td>
<td></td>
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<tr>
<td>Septal complex</td>
<td></td>
<td></td>
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<tr>
<td>Medial septal nu.</td>
<td>++</td>
<td>+</td>
<td>–</td>
<td>++</td>
</tr>
<tr>
<td>Lateral septal nu.</td>
<td>–</td>
<td>+++</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Symbols reflect quantitative estimates of the levels of hybridization signals between different regions for the same probe and between different probes in the same region: ++++, very high; +++, high; +, moderate; +, low; –, not above background. Note: nu, nucleus; Dorsomed: dorsomedial; Ventromed: ventromedial.
3.10. Septal complex

KChIP1 and KChIP4 transcripts were abundant in the medial septal nuclei. KChIP2 mRNA was very abundant in the lateral septal nucleus. Little KChIP3 mRNA was detected in the septal complex (Fig. 3H).

4. Discussion

The expression pattern of specific KChIPs in adult mouse brain was investigated by both immunoblotting and in situ hybridization. The results show that KChIPs are widely expressed in the adult brain. Individual KChIPs are, however, differentially distributed in certain neuronal populations of different brain regions, and neurons or regions expressing more than one KChIP are also observed.

The differential distribution of KChIPs in brain detected by in situ hybridization suggests that the probes used in this study are specific to individual KChIPs. KChIP2 probe detected little mRNA in cerebellum where KChIP1, 3, and 4 were expressed, suggesting that KChIP2 probe did not cross-hybridize with the other three KChIPs (Fig. 3G). Likewise, Little hybridization signal was found in septal complex with KChIP3 probe while expression of KChIP1, 2, and 4 was found in the region (Fig. 3H). The results indicate that KChIP3 probe is specific for KChIP3. Moreover, little KChIP4 hybridization signal was found in striatum where specific expression of KChIP1–3 was found demonstrating the specificity of KChIP4 probe (Fig. 3F). We have also found that KChIP1 probe does not cross-hybridize with the KChIP2–4 in brain of KChIP1 null mouse despite expression of KChIP2–4 is not altered by ablation of KChIP1 (not shown).

It is well documented that each KChIPs has multiple splicing variants [6,14,16,27]. The splicing modification of KChIPs happens generally at the 5′-end of the gene resulting in distinct N-termini. The probes used in this study covers the coding sequence and 3′-UTR. Therefore, these probes likely detect most of, if not all, splicing variants of the KChIPs. It remains to determine the expression pattern of various splicing variants of individual KChIPs.

A primary activity of KChIPs is to modulate voltage-gated Kv4 potassium channels (A-type) [3,19]. In mammals, Kv4 channels, including Kv4.1, Kv4.2, and Kv4.3, are encoded by three individual genes [5,21,23]. Kv4.2 and Kv4.3 expression is high in the heart and brain while Kv4.1 is apparently expressed at low levels in these two organs [15,25]. Mammalian Kv4 channels regulate firing frequency, spike initiation, and waveform in neurons [9,18,24]. Results reported here show co-distribution of KChIPs with Kv4.2 and Kv4.3 transcripts. Nevertheless, the interaction between individual KChIPs and different Kv4 channels may vary in different brain regions.
regions. For example, KChIP1 is highly expressed by
eurons in the reticular thalamic nucleus where only
Kv4.2 transcripts are reported. Similarly, KChIP2 and
KChIP3 are coexpressed with Kv4.2 by neurons in the
striatum and granule cells of the cerebellum, respectively.
Together, co-expression of KChIPs and Kv4 in adult brain
supports previous reports on interaction of KChIPs with
Kv4 channels in vivo. The specificity of interaction
between individual KChIPs and a particular type of Kv4
channel remains to be investigated biochemically. More-
over, results from this study suggest a potential redu-
dancy of KChIPs in certain neuronal populations. Multiple
KChIPs, including KChIP1, KChIP3, and KChIP4, are
expressed in cerebellar Purkinje neurons. Consistent with
this hypothesis, deletion of the KChIP1 gene does not alter
expression of the three other KChIPs in brain (Xiong
and Zhang, unpublished results).

KChIP3 plays important roles in pain modulation
through a kappa-opioid receptor-mediated pathway [10,11].
KChIP3 transcripts co-localize partially with kappa-opioid
receptors in layer V and deep layer VI of the cerebral
cortex, and in thalamus and striatum [22]. KChIP3 is also
co-distributed with dynorphin in layer V of the cortex, the
dentate gyrus, and the habenula [1], which is consistent
with a reported function of KChIP3 in pain modulation
[10,11,22].

KChIP1 is expressed by a widely distributed subpopu-
ation of neurons in the brain but not in cerebellum. The
unique localization and distribution of KChIP1 neurons
suggest that they are interneurons. Consistent with this
hypothesis, expression of KChIP1 is high in the reticular
thalamic nucleus and cerebellar Purkinje cells where
parvalbumin-positive GABAergic interneurons are located.
These observations indicate that KChIP1 participates in
modulating inhibitory neuronal function. One exception is
that KChIP1 transcripts are also detected in the medial
habenular nucleus where cholinergic neurons are abundant.
Our findings suggest that individual KChIP may be
expressed in specific neuronal subtypes based on anatomy
or neurotransmitter phenotype.

Together, the study demonstrates co-distribution of
KChIPs and Kv4 voltage-gated channels in adult mouse
brain and supports a role for KChIPs in modulating Kv4 K+
channels in vivo. The differential localization of specific
KChIPs and the observation of a certain degree of over-
lapping expression in what are apparently the same cells
(i.e. KChIP1, 3, and 4 in Purkinje neurons) indicate the
complexity of KChIP functions.

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