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Dendritic A-type potassium channel subunit expression in CA1 hippocampal interneurons

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Abstract

Voltage-gated potassium (Kv) channels are important and diverse determinants of neuronal excitability and exhibit specific expression patterns throughout the brain. Among Kv channels, Kv4 channels are major determinants of somatodendritic A-type current and are essential in controlling the amplitude of backpropagating action potentials (BAPs) into neuronal dendrites. BAPs have been well studied in a variety of neurons, and have been recently described in hippocampal and cortical interneurons, a heterogeneous population of GABAergic inhibitory cells that regulate activity of principal cells and neuronal networks. We used well-characterized mouse monoclonal antibodies against the Kv4.3 and KChIP1 subunits of A-type Kv channels, and antibodies against different interneuron markers in single- and double-label immunohistochemistry experiments to analyze the expression patterns of Kv4.3 and KChIP1 in hippocampal CA1 neurons. Immunohistochemistry was performed on 40 μ m rat brain sections using nickel-enhanced diaminobenzidine staining or multiple-label immunofluorescence. Our results show that Kv4.3 and KChIP1 component subunits of A-type channels are co-localized in the soma and dendrites of a large number of GABAergic hippocampal interneurons. These subunits co-localize extensively but not completely with markers defining the four major interneuron subpopulations tested (parvalbumin, calbindin, calretinin, and somatostatin). These results suggest that CA1 hippocampal interneurons can be divided in two groups according to the expression of Kv4.3/KChIP1 channel subunits. Antibodies against Kv4.3 and KChIP1 represent an important new tool for identifying a subpopulation of hippocampal interneurons with unique dendritic A-type channel complement and ability to control BAPs.

Keywords

ion channel; hippocampus; immunohistochemistry; GABA; parvalbumin; somatostatin; calbindin; calretinin

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Neuronal dendrites are complex processing elements that actively integrate electrical and chemical activity in space and time (Johnston et al., 1996). The presence of backpropagating action potentials (BAPs) has been demonstrated on the dendrites of many different types of neurons in the central nervous system (Kim and Connors, 1993; Stuart and Sakmann, 1994b; Hausser et al., 1995; Magee and Johnston, 1995; Spruston et al., 1995; Bischofberger and Jonas, 1997; Williams and Stuart, 2000a, b). BAPs have been proposed to play crucial roles in short- and long-term changes in synaptic efficacy, in local synaptic feedback, and in the stabilization of nascent synapses (Waters et al., 2005). The active properties of dendrites have been extensively investigated in cortical and hippocampal pyramidal cells in which the importance of dendritic voltage-gated channels selective for Na^+ , Ca^{2+} and K^+ has been defined (Stuart and Sakmann, 1994a; Magee and Johnston, 1995; Hoffman et al., 1997; Bekkers, 2000; Korngreen and Sakmann, 2000). Among the voltage-gated potassium or Kv channels, A-type channels are crucial for the control of the amplitude of BAPs (Johnston et al., 1999; Migliore et al., 1999; Johnston et al., 2000).

Kv channels comprise four pore-forming and voltage-sensing α subunits that assemble into homo- or hetero-tetramers, often associated with auxiliary subunits (Trimmer and Rhodes, 2004). Of the different mammalian Kv4 α subunits (Kv4.1, Kv4.2, Kv4.3), Kv4.2 is the α subunit responsible for comprising the channels that underlie the somatodendritic A-current in most pyramidal neurons (Chen et al., 2006). Certain pyramidal neurons express Kv4.2 together with pore-forming Kv4.3 subunits (Serodio and Rudy, 1998; Rhodes et al., 2004) suggesting the presence of heterotetrameric Kv4 channels in these cells. In contrast, Kv4.3 mRNA was uniquely observed in a large number of hippocampal interneurons by *in situ* hybridization (Serodio and Rudy, 1998), and hippocampal interneurons display strong Kv4.3 immunoreactivity (Rhodes et al., 2004). Native neuronal Kv4 channels also have auxiliary subunits that regulate their biophysical properties and expression (Jerng et al., 2004). Among these auxiliary subunits, KChIPs (An et al., 2000) are Ca^{2+} binding proteins that bind to the cytoplasmic N-terminal domain of Kv4 α subunits (An et al., 2000; Holmqvist et al., 2002). Kv4.2 in pyramidal neurons exists in association with auxiliary KChIP2, KChIP3 and KChIP4 subunits, while KChIP1 is present with Kv4.3 in hippocampal interneurons (Rhodes et al., 2004).

Hippocampal interneurons are a heterogeneous population of GABAergic inhibitory cells that regulate complex interactions among principal cells including population oscillations, epileptic synchronization and plasticity (Freund and Buzsaki, 1996). Despite their importance, only recently have the functional properties of their dendrites been investigated, presumably due to the thinness of their dendrites relative to those on pyramidal cells, and their unpredictable locations within the brain (Goldberg et al., 2003). Recent studies have shown that the dendrites of different types of interneurons vary in intrinsic excitability, synaptic conductances, Ca^{2+} handling and the learning rules they implement (Goldberg and Yuste, 2005). In mouse neocortical fast spiking and irregular spiking interneurons, BAP-evoked calcium transients are proximally restricted (Goldberg et al., 2003), and rat neocortical bitufted interneurons of layer 2/3 exhibit distance dependent attenuation of calcium responses (Kaiser et al., 2001). In contrast, in rat hippocampal oriens-alveus interneurons BAPs were present along the length of the dendrites and did not exhibit attenuation (Martina et al., 2000) and in rat CA1 stratum radiatum interneurons BAPs were associated with increased calcium transients to up to 150-160 μm from the soma (Rozsa et al., 2004).

The distinct characteristics of BAPs in different interneurons are presumably the consequence of differences in the expression of voltage-gated ion channels. Dendritic A-type Kv4 channels are especially crucial to the control of the amplitude and propagation of BAPs in pyramidal neurons and A-type potassium currents have also been implicated in the spatial control of BAP in mouse neocortical interneurons (Goldberg et al., 2003). As such, we investigated the

distribution of Kv4.3 and KChIP1 subunits of dendritic A-type channels, focusing on interneurons in the CA1 subfield of adult rat hippocampus, which have been the focus of recent studies of dendritic A-type channels (Martina et al., 2000; Lien et al., 2002; Bourdeau et al., 2007).

Experimental procedures

Antibodies

Monoclonal antibodies against Kv4.3 and KChIP1 were generated as described previously (Rhodes et al., 2004) and are available from NeuroMab (www.neuromab.org). Commercially available antibodies against different interneuron markers were employed as detailed in Table I.

Tissue preparation

All animal use procedures were in strict accordance with the Guide for the Care and Use of Laboratory Animals described by the National Institutes of Health. Nine adult Sprague Dawley (Taconic) rats were anesthetized with 60 mg/Kg pentobarbital (intraperitoneally) and briefly perfused with ice-cold saline solution followed by a slow perfusion of 4% paraformaldehyde in phosphate-buffered solution. Sagittal brain sections (each 40 μ m thick) were prepared as previously described (Rhodes et al., 1997).

Immunoperoxidase staining of brain sections

For diaminobenzidine/nickel ammonium sulfate staining, free-floating sections were permeabilized with 0.1% Triton X-100 in Tris-buffered saline (TBS), blocked in 5% horse serum in TBS (vehicle), and incubated overnight in vehicle containing K55/7 (anti-KChIP1) or K75/30 (anti-Kv4.3) pure monoclonal antibody (Table 1). The sections were then incubated in biotinylated horse anti-mouse secondary antibody (Vector Laboratories, Burlingame, CA) for 1 hour. Detection of the antibody-antigen complexes was accomplished using the ABC Elite peroxidase reaction kit (Vector Laboratories, Burlingame CA) and visualized using nickel-enhanced diaminobenzidine procedure.

Multiple-label immunofluorescence staining of brain sections

Free-floating sections were blocked with a 10% goat serum solution in phosphate buffer containing 0.3% Triton X-100 (vehicle), then incubated overnight at 4°C in vehicle containing combinations of primary antibodies, either rabbit polyclonal-mouse monoclonal, or mouse monoclonal antibodies of different IgG isotypes (Table 1). Sections were incubated in species- or isotype-specific Alexa-conjugated secondary antibodies (Molecular Probes-Invitrogen, Carlsbad, CA) as described elsewhere (Strassle et al., 2005). For the double labeling of Kv4.3 and axons in Fig. 2A, we used the tyramide signal amplification (TSA) method to achieve immunolabeling with two antibodies from the same species (Teramoto et al., 1998; Nakamura and Uchihara, 2004). Sections were first incubated overnight with 50 ng/ml of K75/41 at which concentration this monoclonal antibody is not detected by Alexa-conjugated secondary antibodies (not shown). The K75/41 signal was detected with a biotinylated anti-mouse secondary antibody using a TSA kit (Invitrogen). Sections were then washed, and incubated with the pan-axonal marker antibody for five days at 4°C to facilitate antibody penetration into the tissue. The pan-axonal antibody was detected with the Alexa-conjugated secondary antibody as described above.

Imaging/data analysis

Images were recorded with a digital CCD camera installed on a Axiovert-200M Zeiss microscope, with a Zeiss Fluor 10x/0.50 numerical aperture lens and a 63x, 1.3 numerical

aperture lens, using the software Axiovision. Recorded CCD pictures of sub-regions were manually tiled, to create images of the entire hippocampus or of the CA1 subfield. Higher magnification pictures were obtained using a 63x/1.3 numerical aperture lens and a Carl Zeiss ApoTome. Only minor corrections of brightness and contrast were performed.

The counting method used was semi-quantitative because a subjective judgment had to be made regarding the threshold for counting a cell as positive for a specific staining. In each case *n* refers to individual sections, each derived from a separate individual. All data are presented as mean $\pm$ s.d.

Parvalbumin (PV): *n*=5; average PV-IR cells/sections = 61.1 ± 12.3 ; average KChIP1-IR cells/section = 142.6 ± 8.9 . Average total cells counted per section = 166 ± 9 .

Somatostatin (SO): *n*=4; average SO-IR cells/sections = 54.7 ± 8.7 ; average KChIP1-IR cells/section = 167.3 ± 7.4 . Average total cells counted per section = 192 ± 11 .

Calbindin (CB): *n*=4; average CB-IR cells/sections = 49.5 ± 12.1 ; average KChIP1-IR cells/section = 177.7 ± 15.7 . Average total cells counted per section = 191 ± 17 .

Calretinin (CR): *n*=5; average CR-IR cells/sections = 101 ± 15.6 ; average KChIP1-IR cells/section = 178.4 ± 20.7 . Average total cells counted per section = 219 ± 24 .

GABA: *n*=3; average GABA-IR cells/sections = 207 ± 1 (strong GABA-IR clearly visible at 10x magnification = 153.7 ± 9.5); average KChIP1-IR cells/section = 169 ± 8.7 . Average total cells counted per section = 207 ± 1 .

Results

Kv4.3 and KChIP1 are colocalized in CA1 hippocampal interneurons

To determine the cellular expression of Kv4.3 and KChIP1 in CA1 hippocampal neurons, we stained adult rat hippocampal sections with anti-Kv4.3 (K75/30, K75/41) or anti-KChIP1 (K55/7, K55/29) monoclonal antibodies (Table 1). As described previously (Serodio and Rudy, 1998; Rhodes et al., 2004), among the different Kv4 α and KChIP auxiliary subunits, high levels of Kv4.3 and KChIP1 were observed in interneurons of the hippocampal region. As shown in Fig. 1, immunoperoxidase staining revealed dense immunoreactivity for Kv4.3 in the molecular layer of the dentate gyrus and in the strata oriens, radiatum and lacunosum moleculare of CA3 corresponding to the dendritic region of pyramidal cells. In addition Kv4.3 was clearly visible on the soma and dendrites of a large number of cells scattered throughout the different hippocampal fields and layers (Fig. 1A). KChIP1 immunoreactivity was found predominantly, if not completely, in the somatodendritic region of a large number of cells heterogeneous in shape and size, also distributed throughout the whole hippocampus (Fig. 1B).

Since immunoperoxidase staining for Kv4.3 and KChIP1 in interneurons of the CA1 subfield of the hippocampus appeared very similar, we next assessed the extent of their cellular and subcellular overlap in this region using double-label immunofluorescence staining of single sections. These studies confirmed that the similar patterns of staining observed in DAB-stained sections was due to co-expression of Kv4.3 and KChIP1 in the same subpopulation of neurons. Double-labeled immunofluorescence staining of 40 μ m sagittal rat sections revealed a virtually complete colocalization of Kv4.3 and KChIP1 in a large number of neurons of different size and shape scattered throughout all layers of CA1 (Fig. 1C-E).

The majority of these neurons presented comparable immunofluorescence signal for both Kv4.3 and KChIP1 with the only difference being that Kv4.3 was relatively enriched in the cell membrane while KChIP1 was also present in the cytoplasm (Fig. 1F). A few interneurons

exhibited differences in the intensity of signal for one or the other subunit with Kv4.3 > KChIP1 in certain cells (Fig. 1H-J) and the opposite in other cells (Fig. 1G-H). There was no apparent correlation of these staining patterns with any specific morphological or spatially-restricted subpopulation of neurons.

We had generated two anti-KChIP1 mouse monoclonal antibodies (K55/7 and K55/29) of distinct immunoglobulin isotype (IgG1 and IgG2a, respectively) that can be effectively employed in multiple-label immunofluorescence experiments. Given the precise overlap in the cellular and, for the most part, subcellular staining patterns of Kv4.3 and KChIP1 in CA1, we thereafter used anti-KChIP1 monoclonal antibodies to mark Kv4.3/KChIP1 channels for the remainder of the studies described below. Control double-labeling experiments using K55/7 and K55/29 revealed the expected complete overlap at the cellular and subcellular level (not shown).

Somatodendritic expression of KChIP1 in CA1 GABAergic interneurons

To demonstrate that Kv4.3 and KChIP1 were expressed exclusively on the soma and dendrites we double-stained hippocampal sections with the combination of either anti-Kv4.3 (K75/41) or anti-KChIP1 (K55/29) and anti-pan-axonal marker neurofilament or anti-dendritic marker MAP2 monoclonal antibodies (Table 1). In interneurons the Kv4.3 (and KChIP1, not shown) signal was distinct from that of the axonal marker (Fig. 2A). KChIP1 (and Kv4.3, not shown) signal overlapped extensively with that of MAP2 (Fig. 2B). KChIP1 immunoreactive cells were distributed in all layers of CA1 with higher presence in the pyramidal cell layer. Their heterogeneous location, size and shape was clearly distinct from the homogeneous location and morphology of CA1 pyramidal cells.

To demonstrate that the KChIP1-positive cells in CA1 were hippocampal GABAergic interneurons, we performed multiple-label immunofluorescence (Fig. 2C-D) for anti-KChIP1 (K55/7), and a rabbit polyclonal anti-GABA antibody as a marker for hippocampal GABAergic interneurons (Table I). KChIP1 immunoreactive cells represented the vast majority of the GABAergic-IR neurons detected at 10x magnification in the CA1 subfield ($75.2 \pm 3.5\%$, $n=3$). Small and statistically insignificant differences between the individual laminae of CA1 were obtained in the overall number of GABA-positive neurons that were also KChIP1-positive. The highest level of correspondence was found in stratum radiatum, where $78.4 \pm 3.8\%$ ($n=3$) of the GABA-positive cells were also KChIP1-positive. Other laminae gave lower but not statistically significantly different values (all $n=3$) in the number of GABA-positive neurons that were also KChIP1-positive: stratum pyramidale $78.1 \pm 6.1\%$ (see Fig. 2C); stratum lacunosum-moleculare $75.1 \pm 2.4\%$ (see Fig. 2D); and stratum oriens $70.8 \pm 5.6\%$. Thus, the overlap of staining for KChIP1 and GABA was extensive and did not significantly differ between the different laminae of hippocampal CA1.

Interestingly, throughout CA1 only $68.4 \pm 4.8\%$ ($n=3$) of KChIP1-positive neurons were also strongly immunoreactive for GABA staining (Fig. 2C-D). Small and statistically insignificant ($n=3$) differences were obtained between CA1 laminae for the number of KChIP1-positive neurons that were also GABA-positive: stratum radiatum: $75.3 \pm 3.5\%$; stratum oriens: $74.8 \pm 1.4\%$; stratum lacunosum-moleculare: $66.0 \pm 5.8\%$; and stratum pyramidale $60.6 \pm 12.8\%$. The other 30% of KChIP1-positive cells presented a weak anti-GABA signal that was possible to visualize only at higher magnification with the use of a confocal microscope (Fig. 2D, cell on right). As described previously (Freund and Buzsaki, 1996), there exist minor subpopulations of GABAergic interneurons that stain only weakly or not at all with anti-GABA antibodies and the optimal preservation of GABA staining requires a special fixation protocol (Sloviter et al., 2001) that was not applied here.

KChIP1 expression among four major hippocampal GABAergic interneuron subpopulations

To better characterize the subpopulation of interneurons expressing Kv4.3 and KChIP1 we next examined the extent of cellular colocalization of KChIP1 with four immunohistochemical markers (Table 1) defining distinct subpopulations of GABAergic interneurons that together encompass the majority of hippocampal GABAergic interneurons: the Ca²⁺-binding proteins parvalbumin (PV), calbindin (CB) and calretinin (CR), and the neuropeptide somatostatin (SO).

We first verified that the reagents used here gave staining patterns similar to those described in the literature (Baimbridge et al., 1982; Celio, 1986; Kosaka et al., 1987; Sloviter and Nilaver, 1987; Sloviter, 1989; Sequier et al., 1990; Resibois and Rogers, 1992) and as reviewed (Freund and Buzsaki, 1996). These experiments yielded staining consistent with that described previously, and will not be shown but briefly described to provide background information. PV-positive cells were concentrated in the strata pyramidale and oriens (see Fig. 3C). Their somata were relatively large and their dendrites were mostly radially oriented and varicose. SO-positive interneurons in CA1 were found primarily confined to the stratum oriens and alveus, with only a small number of SO-positive cells in the stratum pyramidale and even fewer in stratum radiatum and lacunosum moleculare (see Fig. 4C). CB staining in the hippocampus was very strong in the mossy fibers, and in CA1 CB staining was present in both principal cells (somata in the first 2-3 superficial rows of pyramidal cells) and in the somata and dendrites of GABAergic interneurons (see Fig. 5C). CB-positive interneurons were most abundant in the distal part of stratum radiatum but were also present in lower numbers in strata oriens, pyramidale and lacunosum-moleculare. The number of CB-immunoreactive interneurons was higher toward the subicular end of CA1. Immunostaining for CR revealed a large number of interneurons in all layers and subfields of the hippocampus and of the dentate gyrus (see Fig. 6C).

KChIP1 immunoreactivity was present in interneurons in each of these different subpopulations, as detailed below and in Tables II and III.

Parvalbumin—In CA1, KChIP1 expression was detected in ≈60% of the PV-positive interneurons (Fig. 3; Table II). Higher overlap between KChIP1 and PV staining was observed in stratum pyramidale (≈70%) than in stratum oriens, where only half of the PV-positive interneurons were also KChIP1-positive (Table II). In strata radiatum and lacunosum-moleculare, virtually no PV-positive interneuron could be seen, as described above. Note that KChIP1 staining was present in some interneurons with strong PV staining and also in some interneurons with lower levels of PV staining (Fig. 3). Overall, ≈25% of the total KChIP1-positive interneurons in CA1 were contributed by the PV-positive subset of GABAergic interneurons (Table III). PV-positive interneurons in strata pyramidale and oriens contributed exclusively to this total, as very few interneurons in strata radiatum and lacunosum-moleculare were PV positive. There was no obvious correlation between cells exhibiting higher and lower levels of KChIP1 staining and staining for PV.

Somatostatin—KChIP1 was detected in roughly half of the CA1 interneuron subpopulation defined by staining for the neuropeptide SO (Fig. 4, Table II). The majority of these KChIP1- and SO-positive cells had medium to low levels of staining for KChIP1 and in the majority of these cells the SO signal was strong. KChIP1 staining was observed in a higher percentage of SO-positive cells in strata pyramidale and radiatum than in stratum oriens (Table II). However, it should be noted that the number of SO-staining interneurons in strata pyramidale and radiatum is very low relative to stratum oriens. In stratum lacunosum-moleculare virtually no SO-positive interneurons could be visualized. Overall, ≈18% of the total KChIP1-positive interneurons in CA1 were characterized by the presence of somatic somatostatin staining

(Table III). About half of KChIP1-positive interneurons in stratum oriens also stained for SO (Table III). In strata pyramidale and radiatum, where few SO-positive interneurons are found, the contribution of this subpopulation to the KChIP1-positive population was much lower and in stratum lacunosum-moleculare it was negligible (Table III). In general the KChIP1-expressing interneurons that stained for SO were smaller in size than the KChIP1-positive cells stained for PV.

Calbindin—KChIP1 immunoreactivity was observed in $\approx 70\%$ of the CA1 interneurons exhibiting CB staining (Fig. 5; Table II). Slightly higher but statistically insignificant differences in the number of KChIP1-positive cells within the CB-positive population in stratum radiatum relative to strata oriens and lacunosum-moleculare were observed (Table II). In stratum pyramidale it was difficult to distinguish between CB-positive principal cells and interneurons since principal cells in the first few superficial rows of the pyramidal cell layer also expressed CB. Only cells exhibiting higher CB immunoreactivity and a morphology and localization distinct from the very homogeneous principal cells were considered here as being CB-positive interneurons. KChIP1 was predominantly expressed in the subset of CB-positive cells presenting a higher intensity of CB staining. As was found for the other markers, $\approx 20\%$ of the total KChIP-positive population of interneurons was contributed by CB-positive cells (Table III). The contribution of this subfamily to the KChIP1-expressing interneuron subpopulation in CA1 was higher in stratum oriens, followed by stratum lacunosum moleculare, stratum radiatum, and stratum pyramidale (Table III).

Calretinin—KChIP1 expression was detected in approximately half of the interneurons defined by staining for CR (Fig. 6, Table II). KChIP1 was found in $\approx 70\%$ of the CR-positive interneurons in the stratum oriens, $\approx 60\%$ in the strata pyramidale and radiatum (Table II). In the stratum lacunosum-moleculare fewer CR-positive interneurons were present and the overlap with KChIP1-positive cells was only $\approx 30\%$. KChIP1-positive cells generally corresponded to neurons with low levels of CR staining. Again, $\approx 30\%$ of all KChIP1 immunoreactive interneurons in CA1 were contributed by the CR-positive GABAergic subpopulation. The contribution of CR-positive interneurons to the KChIP1-positive pool was higher in the strata pyramidale than in stratum oriens or stratum radiatum (Table III). In stratum lacunosum-moleculare the contribution of the CR subfamily to the group of KChIP1-staining interneurons was very low.

Discussion

Our studies here demonstrate the cellular colocalization of Kv4.3 and KChIP1 in the somatodendritic compartment of a large group of GABAergic interneurons in the CA1 subfield of the adult rat hippocampus. Our double-label immunofluorescence staining analysis revealed that KChIP1 is expressed in each of the major subpopulations of hippocampal interneurons defined by their expression of the Ca^{2+} -binding proteins PV, CB and CR, and the neuropeptide SO. KChIP1 was found in 63.4% ($\pm 9.3\%$) of PV-positive, 54.5% ($\pm 2.3\%$) of SO-positive, 71.8% ($\pm 8.3\%$) of CB-positive and 59.7% ($\pm 4.4\%$) of CR-positive interneurons found in hippocampal CA1.

The major role for KChIPs in brain neurons appears to be as auxiliary subunits of Kv4 channels (Rhodes et al., 2004). KChIPs have profound effects on Kv4 channel trafficking (An et al., 2000; Shibata et al., 2003; Hasdemir et al., 2005) and gating (An et al., 2000). This is further exemplified by the effects of genetic ablation of Kv4.2 expression in knockout mice, which depresses/eliminates expression of Kv4.2-associated KChIPs (Menegola and Trimmer, 2006). As such it was somewhat surprising that despite the virtually complete cellular colocalization of Kv4.3 and KChIP1, we did observe small differences in their precise subcellular localization and in relative staining intensity. In addition to the plasma membrane-

associated pools of Kv4.3 and associated KChIP1, there exists in some cells a separate pool of cytoplasmic KChIP1. While this presumably represents KChIP1 in excess of that able to co-assemble with Kv4.3, it could also represent a pool whose function is independent of that of an auxiliary subunit of Kv4 channels, for example in binding of presenilin (Buxbaum, 2004). There were additional variations in the relative levels of Kv4.3 and KChIP1 immunoreactivity between CA1 interneurons that did not obviously correlate to the localization of the cell or its morphology. The variability in the relative levels of Kv4.3 and KChIP1 could presumably be due to the presence of non-Kv4-associated KChIPs (as discussed above), KChIPs associated with other Kv4 subunits (i.e. Kv4.1 or Kv4.2), or the expression of other KChIP subtypes. While *in situ* hybridization (Serodio and Rudy, 1998) suggests a minimal participation of Kv4.1 and Kv4.2 in interneurons, this lack of these subunits in interneurons has not been confirmed with immunohistochemistry, due to a lack of anti-Kv4.1 antibodies, and the strong staining of Kv4.2 (and KChIP2) in CA1 pyramidal cell dendrites that obscures the staining in interneurons. Kv4.2 staining has been observed in interneurons in mouse visual cortex (Burkhalter et al., 2006). However, KChIP3 could be detected weakly in the somata of a small number of interneurons (not shown). Thus, other Kv4:KChIP subunit combinations may exist in CA1 interneurons outside of the predominant Kv4.3:KChIP1 channel complexes.

KChIP1 appears to be particularly suited to A-type channels in interneurons. Unlike Kv4.3, which is also expressed in dentate granule cells and CA3 pyramidal neurons (Serodio and Rudy, 1998; Rhodes et al., 2004), KChIP1 expression is restricted to interneurons. Not all GABAergic inhibitory interneurons express Kv4.3 and KChIP1, such that we found that only 75% of GABA-positive cells express KChIP1. This value may be an underestimate due to problems with reliable detection of GABA immunoreactivity (Sloviter and Nilaver, 1987; Miettinen et al., 1992; Freund and Buzsaki, 1996). Why KChIP1 would be specifically suited to a specific subpopulation of interneurons is not clear from studies of KChIP function in heterologous cells, where KChIPs exhibit similar properties (Jerng et al., 2004). The generation of KChIP1 knockout mice would be an important contribution to our understanding of the specific role of KChIP1 in hippocampal interneuron function.

Our data show that within the CA1 interneuron population there is only a partial overlap of Kv4.3 and KChIP1 expression with each of the four major immunohistochemically-defined interneuron subpopulations. This suggests that within each of these classes there may exist heterogeneity in dendritic A-type Kv channel function due to cell-specific differences in Kv4.3 and KChIP1 expression. The contribution of other Kv4 α subunits to A-currents in hippocampal interneurons seems negligible (Serodio and Rudy, 1998; Lien et al., 2002), and a major contribution to the somatodendritic A-current by Kv1.4 subunits or other members of the Kv1 family with inactivation conferred by association with their auxiliary Kv β subunit can probably also be excluded (Rhodes et al., 1997). At least for the interneurons of the stratum oriens-alveus the contribution of inactivating Kv3 channels to A-type current is small and their properties are distinct from the predominant A-current phenotype (Zhang and McBain, 1995a). As such we would predict that the profile of Kv4.3/KChIP1 expression in hippocampal interneurons would correlate with somatodendritic A-type channel density and as such differences in neuronal function.

These expression patterns of Kv4.3/KChIP1 channel subunits are distinct from those described for Kv3 family Kv channel subunits, which correlate precisely with Ca²⁺-binding protein expression and firing frequency. Previous studies on interneuron expression of Kv3.1b and Kv3.2 showed that these subunits were expressed in virtually all PV-positive cells, some SO-positive cells, and no CB- or CR- positive cells (Du et al., 1996; Tansey et al., 2002), corresponding to the subpopulation of interneurons that exhibit high frequency firing. The expression patterns of Kv4.3/KChIP1 channels predicts a role distinct from regulation of firing frequency. The presence of A-current has been described for interneurons localized in different

layers of CA1. In stratum oriens-alveus interneurons A-type currents correlate with spike repolarization phenotypes (Zhang and McBain, 1995b), and are presumed to be based on Kv4.3 (Lien et al., 2002). The presence of two groups of hippocampal CA1 interneurons that differed by their presence or absence of A-currents was also observed in strata radiatum and lacunosum moleculare of guinea pigs (Fan and Wong, 1996), where the presence of A-type current correlated with a polygonal shaped soma and thick primary dendritic processes. A clear correlation of Kv4.3/KChIP1 staining to morphology was not observed in our studies, perhaps due to species differences. A-type channels in CA1 stratum lacunosum-moleculare interneurons, presumably formed by Kv4.3 (Bourdeau et al., 2007), are crucial to the generation of membrane potential oscillations and theta frequency activity (Chapman and Lacaille, 1999).

In conclusion, Kv4.3 and KChIP1 were found colocalized in the soma and along the dendrites of a large population of GABAergic interneurons with no preference for a specific immunohistochemical or functional group. As Kv4.3/KChIP1 expression defines this new subpopulation of hippocampal interneurons, antibodies against Kv4.3 and KChIP1 represent an important new tool for further studies of this class of interneurons. Expression of Kv4.3/KChIP1 channels should also confer upon these cells unique dendritic electrical properties including regulation of BAPs and theta oscillations.

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Abbreviations

Kv, voltage-gated potassium; KChIP, potassium channel interacting protein; BAPs, back-propagating action potentials; TBS, Tris-buffered saline; PV, parvalbumin; SO, somatostatin; CB, calbindin; CR, calretinin.

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Fig. 1.

Kv4.3 and KChIP1 are colocalized in the CA1 subfield of the hippocampus. (A-B) immunoperoxidase staining of 40 μm sagittal rat hippocampal sections with anti-Kv4.3 (A; K75/30) and anti-KChIP1 (B; K55/7) monoclonal antibodies. The staining pattern of these A-type channel subunits in the CA1 subfield of hippocampus is similar and is localized in the soma and dendrites of a large group of heterogeneous cells scattered in all layers. Scale bar 200 μm . (C-E) double-label immunofluorescence staining of the CA1 subfield of hippocampus with anti-KChIP1 (E, green in C; K55/29) and anti-Kv4.3 (D, red in C; K75/41) monoclonal antibodies. The two subunits are virtually completely colocalized with only few cells presenting preferential immunofluorescence staining for one or the other subunit. Scale bar = 100 μm . (F-J) Higher magnification images showing the overlap of Kv4.3 (middle panels and red in top panels) and KChIP1 (bottom panels and green in top panels). Kv4.3 is associated with the plasma membrane and KChIP1 with the plasma membrane and cytoplasm. The majority of the cells exhibited comparable immunofluorescence signals for both subunits (F). A small subset of cells exhibited preferential staining for either Kv4.3 or KChIP1 (G-J),

including a small number of neurons with very weak KChIP1 staining (upper cell in I, cell on left in J). Scale bar = 20 μ m.

Fig. 2.

Kv4.3 and KChIP1 are localized in the somata and dendrites of CA1 GABAergic interneurons. (A) double-label immunostaining with anti-Kv4.3 (middle panel, red in left panel; K75/41) and pan-axonal (right panel, green in left panel) monoclonal antibodies. No colocalization was observed indicating the absence of axonal localization of Kv4.3. (B) double-label immunostaining with anti-KChIP1 (middle panel, red in left panel; K55/29) and anti-MAP2 (right panel, green in left panel) monoclonal antibodies. The staining was colocalized in KChIP1-positive cells confirming the somatodendritic distribution of KChIP1. (C-D) double-label immunostaining with mouse anti-KChIP1 (K55/7; middle panels, red in left panels) and rabbit anti-GABA (right panels, green in left panels) antibodies in strata pyramidale (C) and lacunosum-moleculare (D) of CA1. Note that $\approx 30\%$ of KChIP1-positive cells presented very weak GABA immunostaining, often possible to visualize only at higher magnification with the use of a confocal microscope as in the cell on the left in D. Scale bar = 20 μm .

Fig. 3. Expression of KChIP1 in the CA1 interneuron subpopulation expressing PV. (A-C) double-label immunofluorescence staining of the CA1 region of rat hippocampus with anti-KChIP1 (B, red in A; K55/29) and anti-PV (C, green in A) monoclonal antibodies. Scale bar = 100 μ m. (D) percentage of the PV-positive subpopulation also positive for KChIP1 (*= no PV-positive cells or only very few were present in the strata radiatum and lacunosum-moleculare). (E) percentage of the KChIP1-positive subpopulation also positive for PV. (F-I) higher magnification views of cells presenting overlapping PV (bottom panels and green in top panel) and KChIP1 (middle panels and red in top panel) staining in the strata pyramidale (F-H) and in the stratum oriens-alveus (I). In panels H and I cells immunoreactive exclusively for KChIP1 are also observed. Scale bar = 20 μ m.

Fig. 4.

Expression of KChIP1 in the CA1 interneuron subpopulation expressing SO. (A-C) double-label immunofluorescence staining with anti-KChIP1 (B, red in A; K55/29) and anti-SO (C, green in A) monoclonal antibodies. Scale bar = 100 μ m. (D) percentage of the SO-positive subpopulation also positive for KChIP1. (*=in the stratum lacunosum-moleculare no cells or only very few were SO-positive). (E) percentage of the KChIP1-positive subpopulation also positive for SO. F-I: higher magnification views of cells exhibiting overlapping SO (bottom panels and green in top panels) and KChIP1 (middle panels and red in top panels) staining in the strata oriens-alveus (F-G) and pyramidale (H), and at the border between strata pyramidale and oriens (I). In F a cell staining exclusively for SO is present, while in G and I cells staining exclusively for KChIP1 are present. Scale bar = 20 μ m.

Fig. 5.

Expression of KChIP1 in the CA1 interneuron subpopulation expressing CB. (A-C) double-label immunofluorescence staining with mouse anti-KChIP1 (B, red in A; K55/7) and rabbit anti-CB (C, green in A) antibodies. Scale bar = 100 μ m. (D) percentage of the CB-positive subpopulation also positive for KChIP1. (E) percentage of the KChIP1-positive subpopulation also positive for CB. (F-I) higher magnification views of cells exhibiting overlapping CB (bottom panels and green in top panels) and KChIP1 (middle panels and red in top panels) staining in the at the border between strata pyramidale and oriens (F) and in strata lacunosum-moleculare (G) oriens-alveus (H) and radiatum (I). Scale bar 20 μ m.

Fig. 6. Expression of KChIP1 in the CA1 interneuron subpopulation expressing CR. (A-C) double-label immunofluorescence staining with mouse anti-KChIP1 (B, red in A; K55/7) and rabbit anti-CR (C, green in A) antibodies. Scale bar = 100 μ m. (D) percentage of the CR-positive subpopulation also positive for KChIP1. (E) percentage of the KChIP1-positive subpopulation also positive for CR. (F-I) higher magnification views of cells exhibiting overlapping CB (bottom panels and green in top panels) and KChIP1 (middle panels and red in top panels) staining in the strata pyramidale (F-H) and lacunosum-moleculare (I). The cells presenting both KChIP1 and CR staining were mostly weakly positive for CR in contrast with cells only positive for CR (H). Scale bar = 20 μ m.

Table I**Antibodies used in this study**

Name	Immunogen	Source	Form	[IgG]/Dilution
K75/30	GST-Kv4.3 (415-636)	Rhodes et al., 2004	Purified mouse mAb [*] IgG _{2A}	DAB 2 µg/ml
K75/41	GST-Kv4.3 (415-636)	Rhodes et al., 2004	Purified mouse mAb IgG ₁	IF 3.6 µg/ml
K55/7	GST-KChIP1 (full length)	Rhodes et al., 2004	Purified mouse mAb IgG ₁	IF 3.6 µg/ml DAB 0.36 µg/ml
K55/29	GST-KChIP1 (full length)	Rhodes et al., 2004	Purified mouse mAb IgG _{2A}	IF 4 µg/ml
Anti-GABA	GABA-BSA	Sigma A2052 lot 023K408	A-P Rabbit pAb	IF 50 ng/ml
Anti-CR	Recombinant rat CR	Chemicon AB5054 Lot 24040718	Rabbit pAb antiserum	IF 1:500
Anti-CB	Recombinant CB	Chemicon AB1778 Lot 24050344	A-P Rabbit pAb	IF 1:500
Anti-PV	Purified frog muscle PV	Sigma clone Parv-19, P3088 Lot 033K4846	Mouse mAb IgG ₁ ascites	IF 15 µg/ml
Anti-SO	SO peptide (14 a.a.)	Biomeda clone SOM-018, V1169	Purified mouse mAb IgG ₁	IF 7 µg/ml
Anti-CB	Purified bovine kidney CB-D-28K	Sigma, clone CB-955, C9848, Lot 113K4867	Mouse mAb IgG ₁ ascites	IF 15 µg/ml
MAP2	Purified bovine MAP2	Sigma, clone AP-20, M1406	Mouse mAb IgG ₁ ascites	IF 1:1000
Pan-axonal	Homogenized rat hypothalami	Covance, clone mixture, SMI-311R,	Mouse mAb IgG ₁ ascites	IF 1:1000

* mAb: monoclonal antibody; pAb-polyclonal antibody; A-P: affinity-purified

Table II
KChIP1 expression in specific interneuron subpopulations in hippocampal CA1

Laminae	Overlap/total PV	Overlap/total SO	Overlap/total CB	Overlap/total CR
Stratum oriens	52.0 (± 12.7)	46.1 (± 3.5)	73.0 (± 9.8)	71.5 (± 7.7)
Stratum pyramidale	71.6 (± 8.0)	82.9 (± 12.4)	69.7 (± 10.0)	62.8 (± 5.6)
Stratum radiatum	---	72.5(± 8.7)	76.8(± 18.9)	64.4 (± 10.8)
Stratum lacunosum-moleculare	---	---	71.5(± 9.2)	29.9 (± 11.8)
TOTAL	63.4 (± 9.3)	54.5 (± 2.3)	71.8 (± 8.3)	59.7 (± 4.4)

Table III
specific interneuron subpopulations in hippocampal CA1 expressing KChIP1

Laminae	PV [%]	SO [%]	CB [%]	CR [%]
Stratum oriens	38.1 (± 6.7)	41.9 (± 3.5)	30.3 (± 7.3)	32.9 (± 3.6)
Stratum pyramidale	40.7 (± 9.1)	10.4 (± 4.3)	12.4 (± 6.9)	47.6 (± 3.1)
Stratum radiatum	2.8 (± 4.9)	14.9 (± 7.9)	15.6 (± 2.2)	37.4 (± 11.0)
Stratum lacunosum-moleculare	0.7 (± 1.6)	0.9 (± 1.9)	21.6 (± 7.7)	10.0 (± 4.8)
TOTAL	25.9 (± 5.1)	17.8 (± 2.0)	20.0 (± 4.8)	33.7 (± 3.4)