The CD26-Related Dipeptidyl Aminopeptidase-like Protein DPPX Is a Critical Component of Neuronal A-Type K$^+$ Channels

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Summary

Subthreshold-activating somatodendritic A-type potassium channels have fundamental roles in neuronal signaling and plasticity which depend on their unique cellular localization, voltage dependence, and kinetic properties. Some of the components of A-type K$^+$ channels have been identified; however, these do not reproduce the properties of the native channels, indicating that key molecular factors have yet to be unveiled. We purified A-type K$^+$ channel complexes from rat brain membranes and found that DPPX, a protein of unknown function that is structurally related to the dipeptidyl aminopeptidase and cell adhesion protein CD26, is a novel component of A-type K$^+$ channels. DPPX associates with the channels' pore-forming subunits, facilitates their trafficking and membrane targeting, reconstitutes the properties of the native channels in heterologous expression systems, and is coexpressed with the pore-forming subunits in the somatodendritic compartment of CNS neurons.

Introduction

Transient, subthreshold-activating, somatodendritic A-type K$^+$ currents (I$_{SA}$s) have fundamental roles in neuronal function. The unique properties of the underlying channels, including rapid, transient activation in the subthreshold range of membrane potentials, fast inactivation, and fast recovery from inactivation, cause delayed excitation, can influence spike repolarization, and contribute to the regulation of the frequency of repetitive firing (Baxter and Byrne, 1991; Connor and Stevens, 1971; Hille, 2001; Liss et al., 2001; Llinas, 1988; Rudy, 1988). The importance of the somatodendritic I$_{SA}$ in regulating firing frequency was recently highlighted by the demonstration that the pacemaker frequency of individual dopaminergic neurons in the *substantia nigra*, and hence their levels of dopamine release, is directly correlated with the density of this current (Liss et al., 2001). I$_{SA}$s also have important roles in signal processing in dendrites, including the temporal regulation of action potential back propagation, the integration of synaptic inputs, the filtering of fast synaptic potentials, and the induction of long-term potentiation (Hoffman et al., 1997; Johnston et al., 2000; Schoppa and Westbrook, 1999; Watanabe et al., 2002).

The functional importance of the I$_{SA}$ and the potential clinical importance of A-type K$^+$ channels as targets for drug design for disorders such as Parkinson's disease and epilepsy emphasize the significance of understanding the molecular composition of native I$_{SA}$-generating channels. Studies on I$_{SA}$-like currents expressed in *Xenopus* oocytes injected with different rat brain mRNA size fractions led to the suggestion that the underlying channels consist of pore-forming subunits and modulatory accessory proteins (Rudy et al., 1988). It is now known that Kv4 proteins are the principal, or pore-forming, subunits of the channels mediating most of the fast somatodendritic I$_{SA}$ in neurons (Baldwin et al., 1991; Johns et al., 1997; Malin and Nerbonne, 2000; Liss et al., 2001; Serodio et al., 1994, 1996; Tkatch et al., 2000; Tsunoda and Salkoff, 1995). In addition, a family of Ca^{2+} binding proteins called KChIPs was recently identified (using yeast-two hybrid screens) that facilitate the expression and modify the properties of Kv4 channels in heterologous expression systems and are likely to be components of native channels mediating I$_{SA}$s (An et al., 2000; Kuo et al., 2001).

However, additional key components must exist, since the properties of the I$_{SA}$ in neurons cannot be reproduced in heterologous expression systems by either Kv4 subunits alone or in combination with KChIP proteins (Nadal et al., 2001). In particular, KChIPs considerably slow down the time course of Kv4 channels (An et al., 2000; Nakamura et al., 2001a), whereas, in many neuronal populations, the I$_{SA}$ is *faster*, not *slower*, than the current generated by channels containing only Kv4 proteins (Nadal et al., 2001). These differences are important, since the gating kinetics of A-type K$^+$ channels is fundamental to their special roles in neuronal excitability (Connor and Stevens, 1971; Rush and Rinzel, 1995) and are not due to differences in expression environment (i.e., neurons versus *Xenopus* oocytes or transfected mammalian cells), as demonstrated by the observations of Shibata et al. (Shibata et al., 2000), who found that the currents expressed in cerebellar granule cells following transfection of Kv4.2 cDNAs were *slower* than the native I$_{SA}$ *in the same cells*. The discrepancy between native and heterologously expressed currents could be explained by the existence of unidentified molecular factors that modulate the activity of the pore-forming

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subunits. This hypothesis is supported by experiments showing that a 4–7 Kb cerebellar mRNA fraction accelerated the kinetics of Kv4 channels when coinjected with Kv4 cRNAs into *Xenopus* oocytes (Nadal et al., 2001). These observations led to the suggestion that transcripts in 4–7 Kb mRNA encode a factor termed "KAF" (for $\underline{K}^+$ channel $\underline{a}$ccelerating $\underline{f}$actor), perhaps a novel channel-associated protein or an enzyme that modulates Kv4 channel properties.

The channels mediating the I_{SA} in neurons have not been characterized biochemically, an approach that has been extremely useful in identifying key components of other channel types (Isom et al., 1995; Krapivinsky et al., 1995). To define the molecular components of neuronal I_{SA} channels and attempt to identify proteins that allow the reconstitution of channels with native properties, we purified neuronal Kv4 channels and associated proteins. Channel complexes consisted of three main types of protein: Kv4, KChIP, and an unidentified protein of 115 kDa. The 115 kDa protein was purified and sequenced by tandem mass spectrometry. This led to the discovery of a novel channel-associated protein, the CD26-related protein DPPX, a protein of intriguing structure but unknown function (Wada et al., 1992; de Lecea et al., 1994; Kin et al., 2001). Functional reconstitution experiments in *Xenopus* oocytes demonstrated that DPPX (also known as DPP6 or BSPL) corresponds to KAF and is necessary to reproduce the fast kinetics of native I_{SA} channels. DPPX facilitates the intracellular trafficking of Kv4 proteins and drastically increases the surface expression of Kv4 channels. It has functional effects on the channels that are of considerable physiological significance. DPPX is expressed in neuronal populations that also express Kv4 proteins and in similar somatodendritic subcellular patterns, indicating that this novel associated protein is an important component of the channels mediating the I_{SA} in many neurons. Moreover, given the homology to CD26, DPPX may endow Kv4 channels with roles in cell adhesion and interactions with components of the extracellular matrix.

Results

Immunopurification of Kv4 K$^+$ Channels from Rat Cerebellar Membranes

Biochemical methods were used to characterize the molecular components of native I_{SA} channels and to try to identify new modulatory factors. Antibodies against Kv4.2 proteins crosslinked to protein-A Sepharose 4B beads were used to immunoprecipitate Kv4 channel complexes from nondenaturing detergent extracts of rat cerebellar membranes. After extensive washing, the immunopurified complexes were eluted, and their components were separated by SDS-PAGE. The resulting gel was silver stained (Figure 1A, lane labeled "Kv4.2-Ab beads"). The results from this purification were compared to those from a parallel extraction using protein-A Sepharose beads crosslinked to preimmune sera (Figure 1A, lane labeled "control beads") and to those from an extraction using protein-A Sepharose beads crosslinked to Kv4.2 antibodies carried out in the presence of an excess of immunogenic peptide (Figure 1A, lane labeled "+peptide"). There are three prominent bands in the

Kv4.2-Ab beads lane that are not seen in the control beads lane and are nearly completely suppressed when the Kv4.2 Ab-beads are preincubated with an excess of antigenic peptide: a band of ∼70 kDa (labeled "Kv4.2" in Figure 1A), a small band of ∼30 kDa (labeled "KChIP" in Figure 1A), and a band of ∼115 kDa (labeled "p115" in Figure 1A). The band of ∼70 kDa corresponds in size to Kv4 polypeptides and reacts with Kv4.2 antibodies in an immunoblot from a gel run in parallel (Figure 1B). The identification of this band as Kv4.2 was confirmed by sequencing by tandem mass spectrometry (MS/MS). The ∼30 kDa band has the size expected of KChIP proteins and reacts with a pan-KChIP antisera in immunoblots (Figure 1C). Sequencing by MS/MS confirmed the identification of this band as KChIP proteins.

DPPX Proteins Are Components of Native Kv4 Channels

The 115 kDa band is as prominent as the Kv4.2 and KChIP bands, suggesting that it may correspond to an important Kv4 channel associated protein. To investigate the functional significance of the 115 kDa polypeptides, the band was purified, trypsin digested, and the resulting peptides subjected to MS microsequencing. Several peptides were sequenced and were shown to correspond to a previously identified protein known as dipeptidyl aminopeptidase-like protein (DPPX), of which two alternative spliced isoforms (DPPX-S and DPPX-L) have been characterized (Figure 2A). (The existence of a third embryonic alternative spliced version has been reported, but this isoform has not been characterized [Hough et al., 1998]). DPPX-S and DPPX-L are integral membrane glycoproteins with one putative transmembrane domain, a long C-terminal extracellular domain, and a short divergent intracellular amino-terminal sequence (Figure 2; de Lecea et al., 1994; Kin et al., 2001; Wada et al., 1992). DPPX was identified in 1992, but its function has remained obscure. It is related to DPPIV or CD26 (33% identity and ∼50% similarity), a dipeptidyl-aminopeptidase expressed in brain tissue only at trace levels and probably not in neurons, but with important roles in T cell activation, metabolism of peptide hormones, and cell adhesion (reviewed in De Meester et al., 1999; Gorrell et al., 2001; Hildebrandt et al., 2000). However, DPPX lacks enzymatic activity, even when the consensus active site sequence present in functional dipeptidyl proteases has been restored by mutagenesis (Kin et al., 2001; see Figure 2A).

Antibodies against DPPX labeled the p115 band observed in immunoprecipitates of Kv4.2 channel complexes, confirming the association of DPPX and Kv4.2 proteins in brain tissue (Figure 1D). Moreover, antibodies against DPPX coimmunoprecipitate Kv4.2 (Figure 3A).

At least some Kv4 channels in cerebellum probably contain DPPX and kChIP proteins in the same channel complex, since antibodies against KChIP proteins immunoprecipitate DPPX (Figure 3B) and antibodies to DPPX immunoprecipitate KChIPs (Figure 3A).

DPPX Is the Missing Factor Necessary to Reconstitute the Properties of Native I$_{SA}$ Channels

To investigate the functional effects of the association of Kv4 pore-forming subunits with DPPX, we characterized
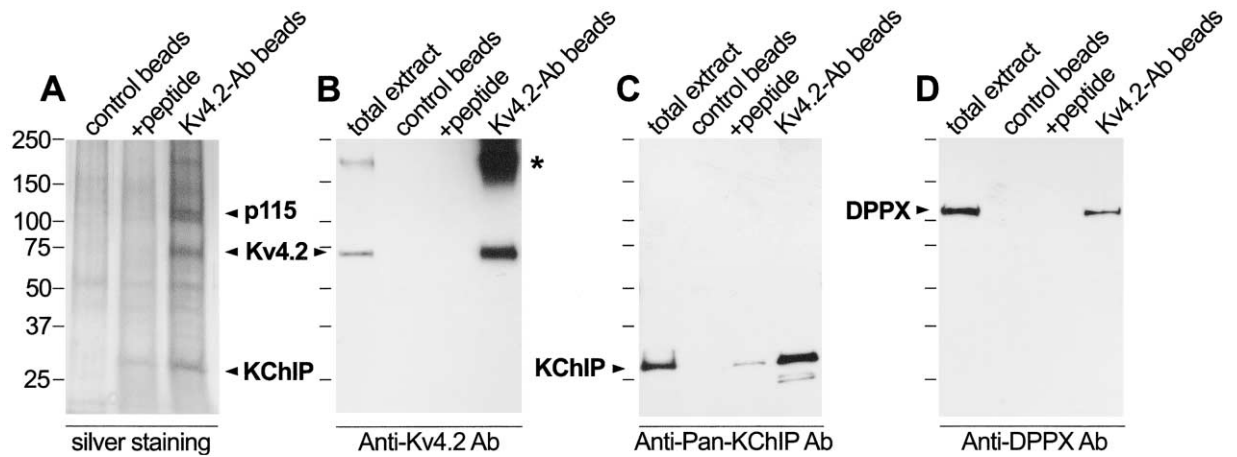
Figure 1. Immunoprecipitation of Kv4.2 Channel Complexes with Kv4.2 Antibodies Precipitates Kv4.2, KChIP, and 115 kDa Polypeptides

(A) Silver-stained SDS-PAGE gel of polypeptides immunoprecipitated from cerebellar membrane extracts with antibodies against Kv4.2 proteins crosslinked to protein-A Sepharose beads (lane labeled "Kv4.2-Ab beads"), or beads crosslinked to Kv4.2 antibodies in the presence of an excess of immunogenic peptide (lane labeled "+peptide"), or beads crosslinked to preimmune sera (lane labeled "control beads").

(B–D) Immunoblots of an identical gel to that shown in (A) run in parallel, transferred to nitrocellulose membranes, and probed sequentially with antibodies to Kv4.2 (B), KChIP1-4 (C), and DPPX proteins (D). A lane containing a sample of the extract used for immunoprecipitation was also run on this gel (lane labeled "total extract"). *, Aggregated Kv4.2 protein.

the currents generated by coexpressing Kv4 subunits and DPPX in heterologous expression systems. *Xenopus* oocytes coinjected with Kv4.2 and DPPX-S cRNAs had A-type K⁺ currents that were up to 25 times larger than those produced by Kv4.2 alone, depending on the ratio of Kv4.2:DPPX-S cRNAs (Figures 4A–4C). A similar effect was observed in CHO cells transfected with Kv4.2 and DPPX-S cDNAs. In fact, Kv4.2 channels were difficult to express in mammalian cells, as previously reported (An et al., 2000; Nakamura et al., 2001b). Most
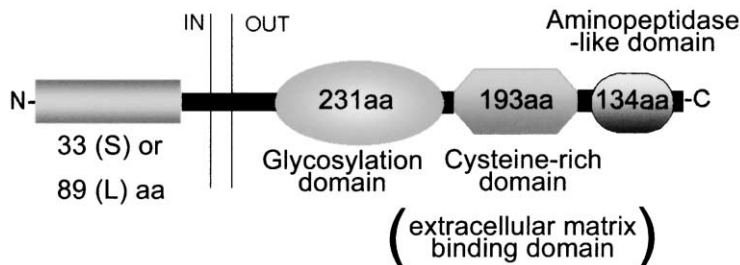

Figure 2. Structure of DPPX

(A) Amino acid sequences of the two DPPX isoforms, DPPX-S and DPPX-L, in rat. DPPX-S, a 115 kDa brain-specific protein (Kin et al., 2001), is the predominant isoform. While DPPX-L is only detected in brain RNA by RT-PCR, transcripts encoding DPPX-S are detected in Northern blots and are widely distributed in brain tissue (Wada et al., 1992). Peptide sequences obtained by MS/MS are shown in bold italics. The mutated active site sequence of serine proteases (G-X-S-X-G) is underlined. In DPPX, the catalytic serine is substituted by aspartate.

(B) Schematic diagram of DPPX based on the structure of CD26 (DPPIV). CD26 has a cysteine-rich domain that binds adenosine deaminase and components of the extracellular matrix.
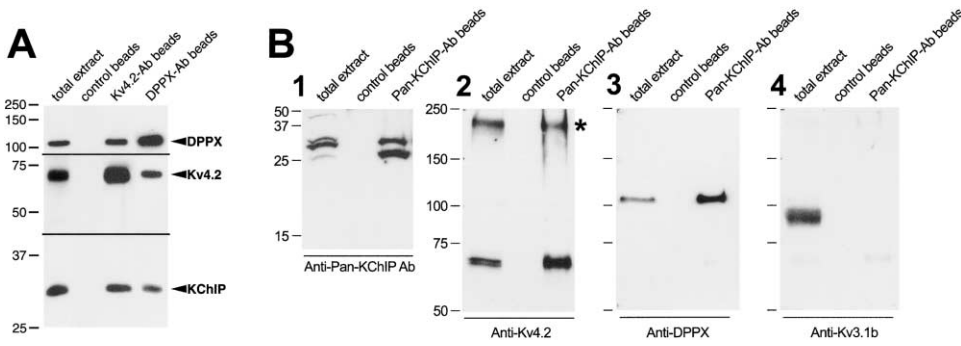
Figure 3. Interactions between Kv4.2, DPPX, and KChIP Proteins in Rat Cerebellar Membranes

(A) Coimmunoprecipitation of Kv4.2, DPPX, and KChIP proteins. Antibodies to DPPX or Kv4.2 crosslinked to protein A-Sepharose 4B beads (DPPX-Ab beads or Kv4.2-Ab beads, respectively) were used to immunoprecipitate extracts of cerebellar membranes treated with a reversible crosslinking reagent prior to solubilization as described. The immunoprecipitation products were collected, crosslinked proteins were dissociated with 2-mercaptoethanol, and the components were separated by 10% SDS-PAGE. A lane containing a sample of the extract used for immunoprecipitation was also run on this gel (lane labeled "total extract"). Proteins were transferred to a nitrocellulose filter, which was cut into three sections and each probed with the antibodies indicated on the right.

(B) KChIP proteins associate with Kv4.2 and DPPX proteins but not with Kv3.1b K^+ channel subunits in rat cerebellar membranes. A pan-KChIP antisera crosslinked to protein A-Separose 4B beads was used to immunoprecipitate a crosslinked extract as above. The immunoprecipitation products were collected, crosslinked proteins were dissociated with 2-mercaptoethanol, and the components were separated by 18% (1) and 7% (2–4) SDS-PAGE. Proteins were transferred to nitrocellulose filters and probed with the antibodies indicated underneath each immunoblot. *, Aggregated Kv4.2 proteins.
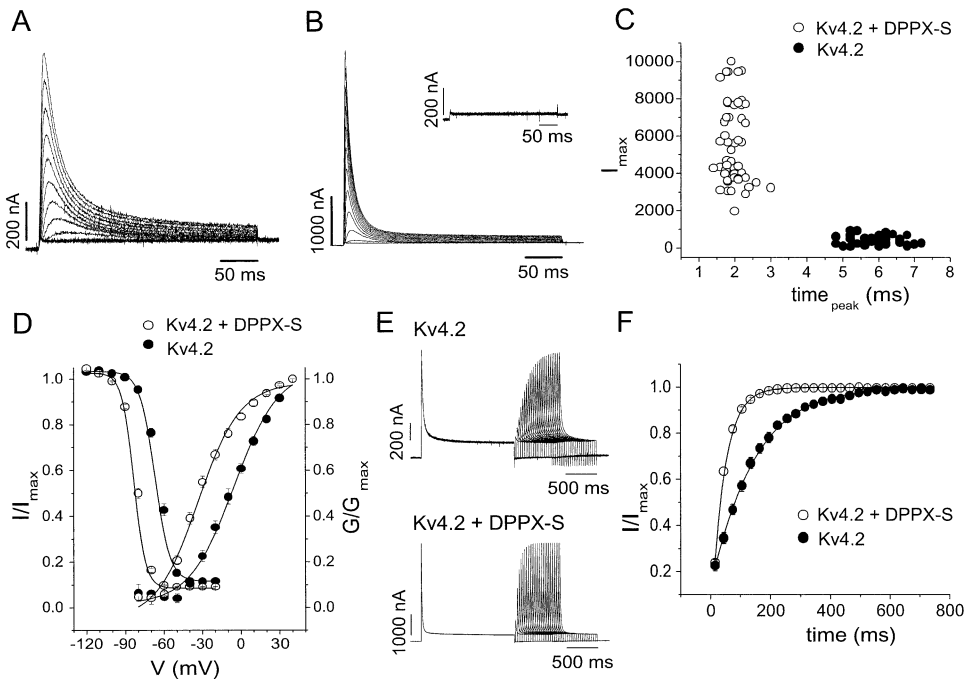

Figure 4. Effects of DPPX-S on Kv4.2-Mediated A-Type K^+ Currents in *Xenopus* Oocytes

(A and B) A-type K^+ currents recorded in representative *Xenopus* oocytes injected with Kv4.2 cRNA alone (A) or Kv4.2 and DPPX-S cRNAs at a 1:1 ratio (B). The inset in (B) shows the currents recorded in an oocyte injected with DPPX-S cRNA alone.

(C) Peak current at +40mV (I_{max}) and time-to-peak ($time_{peak}$) for the currents recorded in oocytes injected with Kv4.2 cRNA alone or Kv4.2 plus DPPX-S cRNAs at a 1:1 ratio.

(D) Normalized conductance-voltage (G/G_{max}) and steady-state inactivation (I/I_{max}) curves for A-type currents recorded in oocytes injected with Kv4.2 cRNA alone or Kv4.2 plus DPPX-S cRNAs at a 1:1 ratio (mean ± SEM; n = 22). The curves were fitted with Boltzmann functions (see Table 1).

(E) Recovery from inactivation of the A-type currents in oocytes expressing Kv4.2 or Kv4.2 plus DPPX-S cRNAs (at a 1:1 ratio). Shown are the currents recorded during test pulses to 50mV following a test pulse to the same voltage separated by increasing time intervals at −110mV.

(F) Time course of the recovery from inactivation of the A-type currents expressed by Kv4.2 or Kv4.2 plus DPPX-S cRNAs (1:1 ratio) at −110mV (mean ± SEM; n =22). The curves were fitted with a single exponential, and the results are tabulated in Table 1.

transfected cells (~60%) had no detectable currents, and these were small (<200 pA at +40mV, average 86 pA; n = 9) in cells with measurable current. Cotransfection with DPPX-S resulted in the efficient expression of Kv4.2 channels with currents in the order of ~2 nA (n = 7).

DPPX-S also had large effects on the kinetics and voltage dependence of Kv4.2 currents in *Xenopus* oocytes and CHO cells. The effects appeared to be similar in the two preparations and were characterized in *Xenopus* oocytes, where their magnitude could be easily controlled by injecting different ratios of DPPX-S to Kv4.2 cRNAs. The effects of DPPX-S on the kinetics and voltage dependence of the Kv4.2 currents in oocytes injected with a Kv4.2:DPPX-S cRNA ratio of 1:1 (corresponding to an ~2:1 molar cRNA ratio) that produced moderate effects on current magnitude are illustrated in Figure 4. DPPX-S increased the rate of inactivation of Kv4.2 currents (Figures 4A and 4B; Table 1), considerably decreased the time for the currents to reach a maximum (time to peak; Figures 4A–4C; Table 1), and produced a 28mV negative shift in the conductance-voltage relation and a 16mV negative shift in the voltage dependence of steady-state inactivation (Figure 4D; Table 1).

DPPX-S also increased the rate of recovery from inactivation (Figures 4E and 4F; Table 1), an effect of considerable interest, since fast recovery from inactivation is a distinguishing property of Kv4-mediated A-type K$^+$ channels. DPPX-S when injected alone did not induce any current (see inset in Figure 4B).

DPPX-S had similar effects on the currents produced by cRNAs for Kv4.3, the other *Kv4* gene prominently expressed in brain (Serodio and Rudy, 1998) (data shown in Table 1). These effects of DPPX-S are specific to Kv4 proteins, since coexpression of DPPX-S with Kv1.4 proteins, which also produce fast inactivating K$^+$ channels, had no effects on the expressed currents (data not shown). DPPX-L, the minor isoform in brain, has not been systematically studied but appeared to have qualitatively similar effects on Kv4.2 currents as those described for DPPX-S.

In contrast to the accelerating effect of DPPX, KChIPs slowed down the kinetics of inactivation considerably (Table 1), as previously reported (An et al., 2000; Nakamura et al., 2001a). Coexpression of Kv4.2 with DPPX-S and KChIP1 in the same oocytes produced currents with intermediary properties (Table 1), which depended on the relative concentrations of injected KChIP1 and DPPX-S cRNAs. For example, the inactivation $t_{1/2}$ was 11.3 ± 0.95 ms (n = 9) in oocytes injected with a 1:1:1.5 Kv4.2:KChIP1:DPPX-S cRNA molar ratio (5, 2.5, and 15 ng/oocyte, respectively) (Table 1) and 22 ± 4 ms (n = 8) in oocytes injected with twice the amount of KChIP1 cRNA.

The effects of DPPX-S on Kv4 channels in *Xenopus* oocytes are summarized and compared to the properties of native I$_{SA}$s in Table 1. The properties of the currents expressed in oocytes coinjected with Kv4.2 and DPPX-S are remarkably similar to those of the I$_{SA}$ recorded in a number of CNS neurons that prominently express Kv4.2. In particular, the kinetics of the native currents is faster than that of the currents expressed by Kv4.2 proteins alone, as previously noted (Nadal et al., 2001). The faster kinetics of the native channels is reproduced in oocytes by coexpression with DPPX and stands

in contrast to the significant slow down of the inactivation time course produced by KChIPs. The currents expressed by Kv4.3 are slower than those produced by Kv4.2, and although DPPX also accelerates Kv4.3 currents, they remain slower than those expressed by Kv4.2 + DPPX. In addition, the midpoint of the steady-state inactivation of Kv4.3 currents (with or without DPPX) tends to occur at more positive potentials than that of Kv4.2 currents (Table 1). Interestingly, similar trends (Table 1) are observed in neuronal populations that express predominantly (if not only) Kv4.3 (Serodio and Rudy, 1998). These results strongly suggest that the associated DPPX proteins are KAF, the missing Kv4 channel-modifying factor necessary to reconstitute the currents observed in neurons.

To further confirm that DPPX is responsible for KAF activity, we carried out hybrid arrest experiments utilizing antisense oligonucleotides directed to the DPPX peptides identified by microsequencing of p115. Incubation of the 4–7 Kb mRNA fraction of cerebellar mRNA with antisense oligonucleotides (but not control sense oligonucleotides) arrested the ability of this mRNA fraction to modify Kv4.2 channels expressed in *Xenopus* oocytes (Figure 5). All the effects of this fraction on Kv4.2 channels were absent in antisense-treated 4–7 Kb mRNA. Together these results leave little doubt that DPPX corresponds to KAF and that *all* the KAF activity in 4–7 Kb cerebellar mRNA is mediated by DPPX.

DPPX Proteins Regulate the Trafficking of Kv4 Proteins

Channel accessory subunits may increase the efficiency of surface channel expression by facilitating the trafficking of channel complexes from the endoplasmic reticulum (ER), through a number of mechanisms, such as masking ER retention motifs in channel proteins or assisting the proper folding of pore-forming subunits (Margeta-Mitrovic et al., 2000; Zerangue et al., 1999). Kv4 proteins have an arginine-rich region in the C-terminal area following the last membrane-spanning domain. This region has several sequences that resemble the ER retention motifs RKR and RXR identified in ATP-sensitive K$^+$ channels and GABA$_B$ receptor subunits (Margeta-Mitrovic et al., 2000; Zerangue et al., 1999). These sequences may explain why Kv4 proteins are largely retained in the ER when transfected alone into mammalian cells (An et al., 2000; Nakamura et al., 2001b). To investigate whether an effect on trafficking may mediate the large increase in current magnitude produced by DPPX, we studied the subcellular localization of Kv4.2 proteins in CHO cells transfected with Kv4.2 with or without DPPX cDNAs. When expressed alone, Kv4.2 proteins were concentrated within the perinuclear endoplasmic reticulum, as previously reported (An et al., 2000; Nakamura et al., 2001b), but when coexpressed with DPPX-S (Figures 6A and 6B) or DPPX-L (data not shown), Kv4.2 proteins were redistributed to the cell surface. To quantify the effects of DPPX on the surface expression of Kv4.2 proteins in transfected cells, the external surface of the cells was biotinylated, the cells were solubilized, and Kv4.2 proteins were quantified in immunoblots following precipitation with Sepharose beads crosslinked to avidin. To normalize for

Table 1. Voltage Dependence and Kinetic Properties of Kv4-Mediated A-Type Currents in *Xenopus* Oocytes and Native I_{SA}s in Neurons

| | Voltage Dependence | | | | | Kinetics (at Room Temperature) | | | References |
| | Activation | | | Inactivation | | | | | |
	V_{on}, mV	$V_{0.5}$, mV	k, mV	$V_{0.5}$, mV	k, mV	t_{peak}, ms	$t_{0.5}$, ms	τ_{rec}, ms	
Kv4.2- or Kv4.3-mediated A-type currents in *Xenopus* oocytes									
Kv4.2 (n = 22)	−50 to −40	−5.3 ± 2.2	17.6 ± 2.1	−66.9 ± 0.5	5.8 ± 0.5	5.7 ± 1.1 (40mV)	27.4 ± 1.7 (40mV)	131.1 ± 5.6 (−110mV)	n
Kv4.2 + DPPX-S (2:1) (n = 26)	−60 to −50	−33.4 ± 2.1	16.5 ± 2.0	−83.3 ± 0.2	4.6 ± 0.2	2.0 ± 0.3 (40mV)	10.0 ± 0.8 (40mV)	41.1 ± 1.3 (−110mV)	n
Kv4.2 + KChIP1 (1:2) (n = 23)	−50	−17.2 ± 4.2	24.5 ± 5.5	−71.4 ± 0.2	5.9 ± 0.6	5.8 ± 0.3 (40mV)	54 ± 5.6 (40mV)	58.5 ± 4.0 (−110mV)	n
Kv4.2 + KChIP1 + DPPX-S (1:1:1.5) (n = 9)	−60 to −50	−19.7 ± 5.1	14.0 ± 5.0	−76.7 ± 0.6	4.5 ± 0.3	2.4 ± 0.3 (40mV)	11.3 ± 0.95 (40mV)	56.8 ± 4.2 (−110mV)	n
Kv4.3 (n=12)	−50	−15.4 ± 1.6	19.9 ± 1.8	−63.0 ± 0.2	6.3 ± 0.1	8.1 ± 0.6 (40mV)	46.4 ± 3.8 (40mV)	233.8 ± 5.6 (−110mV)	n
Kv4.3 + DPPX-S (2:1) (n = 13)	−60	−30.3 ± 1.9	16.3 ± 1.9	−71.4 ± 0.3	6.7 ± 0.1	4.9 ± 0.7 (40mV)	32.3 ± 4.5 (40mV)	75.4 ± 1.5 (−110mV)	n
Kv4.3 + KChIP1 (1:2) (n = 8)	−50	−17.9 ± 4.5	16.5 ± 5.0	−57.9 ± 0.3	5.8 ± 0.2	8.8 ± 0.3 (40mV)	69.0 ± 1.8 (40mV)	94.4 ± 11.1 (−110mV)	n
Neurons predominantly expressing Kv4.2									
Striatum	−60 to −50	−33 to −10	7.5 to 17.7	~−79	~8	~1–2 (25, 40mV)	~15–18 (25mV) and 7–11 (0mV)	τ_1: 8.7; τ_2: 109 $A_1/A_1 + A_2$: 0.75 (−95mV)	a, b
Trigeminal neurons	−60	−37	6.8	−72	5.8	2.1 (−25mV)	12.5 (−25mV)	45 (−95mV)	c
Cerebellar granule cells	−60 to −50	−20.5	13.5	~−76	~7.8	~1.3 (40mV)	7.0–8.4 (40mV) and ~13.1 (0mV)	40 (−90mV) and 35 (−100mV)	d, e
CA1 hippocampal pyramidal cells	−60	~−31	11.3	−83	7.5	~2 (−10mV)	17 (−10mV) and ~10.1 (50mV)	39 (−100mV) and 32 (−110mV)	f, g
Neurons expressing Kv4.2 and Kv4.3									
Thalamus (VB)	−60 to −70	−36	10.8	−74.7	6.3	~1–2 ms (40mV)	~20 (40mV)	31 (−110mV)	h
Piriform cortex*	−60	−22.3/−24.4	~11	−65.4/−55.5	6.15/6.4	~3.9 (−15mV)	20.6/12.5 (−15mV)	NA	i
Neurons predominantly expressing Kv4.3									
Hippocampal interneurons	−60	−18.6 to −6.2	~20	~−72	~8	1 (20%–80%) (40mV)	18–20 (30mV) and 28 (40mV)	134.6 (−90mV) and τ_1: 30, τ_2: 165 $A_1/A_1 + A_2$: 0.3 (−90mV)	j, k
Purkinje cells	−60	−38.5	5.4	−65	8.1	<9 (40mV)	65 (60mV)	NA	l
Substantia nigra*	−60	−27.1/−28.6	6.5/10.2	−64/−77	6.6/7.6	~2 (−40mV)	25.4/33.2 (−40mV)	30/172 (−80mV)	m

(continued)

Table 1. Continued

Current properties (averages ± SEM for the indicated number of cells) obtained in *Xenopus* oocytes injected with the indicated cRNAs (ratios expressed as molar ratios) are compared to published properties in different types of neurons. Activation: V_{on}, membrane potential at which significant currents first become apparent; $V_{1/2}$ and k, midpoint and slope, respectively, of the conductance-voltage curve fitted to a first-order Boltzmann function. Inactivation: $V_{1/2}$ and k, midpoint and slope of the steady-state inactivation curve. Kinetic parameters: t_{peak} is the time for the current to reach its maximum value at the indicated voltage. In one instance (one of the reports on hippocampal interneurons), the 20%–80% rise time was reported instead; $t_{1/2}$ is the time at which half of the current has inactivated at the indicated voltage. For published data, we converted reported time constants of inactivation to half-inactivation times in order to be able to compare the values obtained with single or double exponential fits. τ_{rec} is the time constant of recovery from inactivation at the indicated recovery voltage. When the values between different publications are similar, an average is shown; when they differ too much, the range of values is presented. All kinetic parameters are reported at room temperature. A Q_{10} of 2.8 (Huguenard et al., 1991) was used to calculate the values if the measurements were reported at a different temperature. Activation parameters are less reliable because the conductance-voltage curve of I_{SA}s often does not saturate in the range of potentials used to fit the curve to a Boltzmann function. NA, not available. *, For some parameters, two different values were reported. References: a, Surmeier et al., 1988, 1989; b, Tkatch et al., 2000; c, Hsiao and Chandler, 1995; d, Bardoni and Belluzzi, 1993; e, Cull-Candy et al., 1989; f, Numann et al., 1987; g, Klee et al., 1995; h, Huguenard et al., 1991; i, Banks et al., 1996; j, Zhang and McBain, 1995; k, Martina et al., 1998; l, Wang et al., 1991; m, Liss et al., 2001; n, this study.

differences in transfection efficiencies between different samples, the cells were cotransfected with GFP cDNA and GFP quantified in immunoblots. Cells cotransfected with Kv4.2 and DPPX-S cDNAs had 19.5 times more surface-exposed Kv4.2 protein than cells transfected with Kv4.2 alone (n = 3). This resulted from an ∼2-fold increase in the total Kv4.2 protein level and a 9- to 10-fold increase in the proportion of surface-exposed Kv4.2 protein. These effects appear to be specific to Kv4 proteins, since DPPX did not change the distribution of Kir6.2 proteins (Figures 6C and 6D), components of K_{ATP} channels that are also retained in the ER unless coexpressed with the Kir6-associated SUR subunits (Zerangue et al., 1999).

Colocalization of DPPX and Kv4 Proteins in the Somatodendritic Compartment of CNS Neurons

Immunohistochemistry of brain tissue was used to compare the patterns of expression of DPPX proteins with those of Kv4 subunits. DPPX proteins were widely expressed in rat brain, consistent with previous reports on DPPX mRNAs (de Lecea et al., 1994; Wada et al., 1992), and were particularly prominent in brain areas where Kv4 products are most strongly expressed (Maletic-Savatic et al., 1995; Serodio and Rudy, 1998; Sheng et al., 1992). This includes neuronal populations that express both Kv4.2 and Kv4.3, as in the granule cells of the cerebellar cortex (Figures 7C and 7E), the dentate gyrus (Figure 7G) and the olfactory bulb, and in thalamic relay neurons in several thalamic nuclei; it also includes neuronal populations that express mainly (or only) Kv4.2, such as CA1 pyramidal neurons in the hippocampus (Figures 7B and 7G), neurons in the pontine nucleus (Figure 7A), the olfactory tuberculus, and the striatum; as well as neurons expressing mainly (or only) Kv4.3, like Purkinje cells (inset in Figure 7C) and neurons in the substantia nigra compacta and in the periglomerular area in the olfactory bulb.

Kv4.2 proteins have been shown to have a characteristic somatodendritic pattern of expression in the hippocampus and granule cell layer of the cerebellar cortex (Sheng et al., 1992; Maletic-Savatic et al., 1995), consistent with electrophysiological studies demonstrating high levels of I_{SA} in dendrites (Hoffman et al., 1997; Johnston et al., 2000). DPPX had a similar subcellular distribution in these areas (Figures 7B–7H), although DPPX antibodies produced stronger staining of somas but weaker staining of dendrites, compared to antibodies to Kv4.2. It remains to be seen whether these differences in staining intensities reflect real differences in channel composition in somas and dendrites, which could explain differences in the electrophysiological properties of the somatic and dendritic I_{SA} (Hoffman et al., 1997), or whether they reflect differences in the processing and storage of both proteins.

Discussion

We have identified DPPX as a novel associated protein of Kv4-mediated neuronal A-type K^+ channels and have shown that this channel component is the additional molecular factor needed to explain the discrepancies observed between native channels and Kv4 channels

A

1

200 nA

50 ms

2

200 nA

50 ms

3

2,000 nA

50 ms

4

200 nA

50 ms

B

1

$V_{1/2}=-28.9\pm2.3$
$k=15.0\pm2.3$

G/G_{max}

$V_{1/2}=-5.2\pm2.2$
$k=21.5\pm2.2$

V (mV)

2

$V_{1/2}=-69.5\pm0.5$
$k=8.7\pm0.2$

I/I_{max}

$V_{1/2}=-84.6\pm0.2$
$k=5.7\pm0.1$

V (mV)

3

$=56.9\pm8.0$

I/I_{max}

$=162.9\pm39.0$

T (ms)

4

$I_{max}(nA)$

$T_{peak}(ms)$

● Kv4.2
▲ Kv4.2 + 4-7 Kb[-Kv4s] + sense
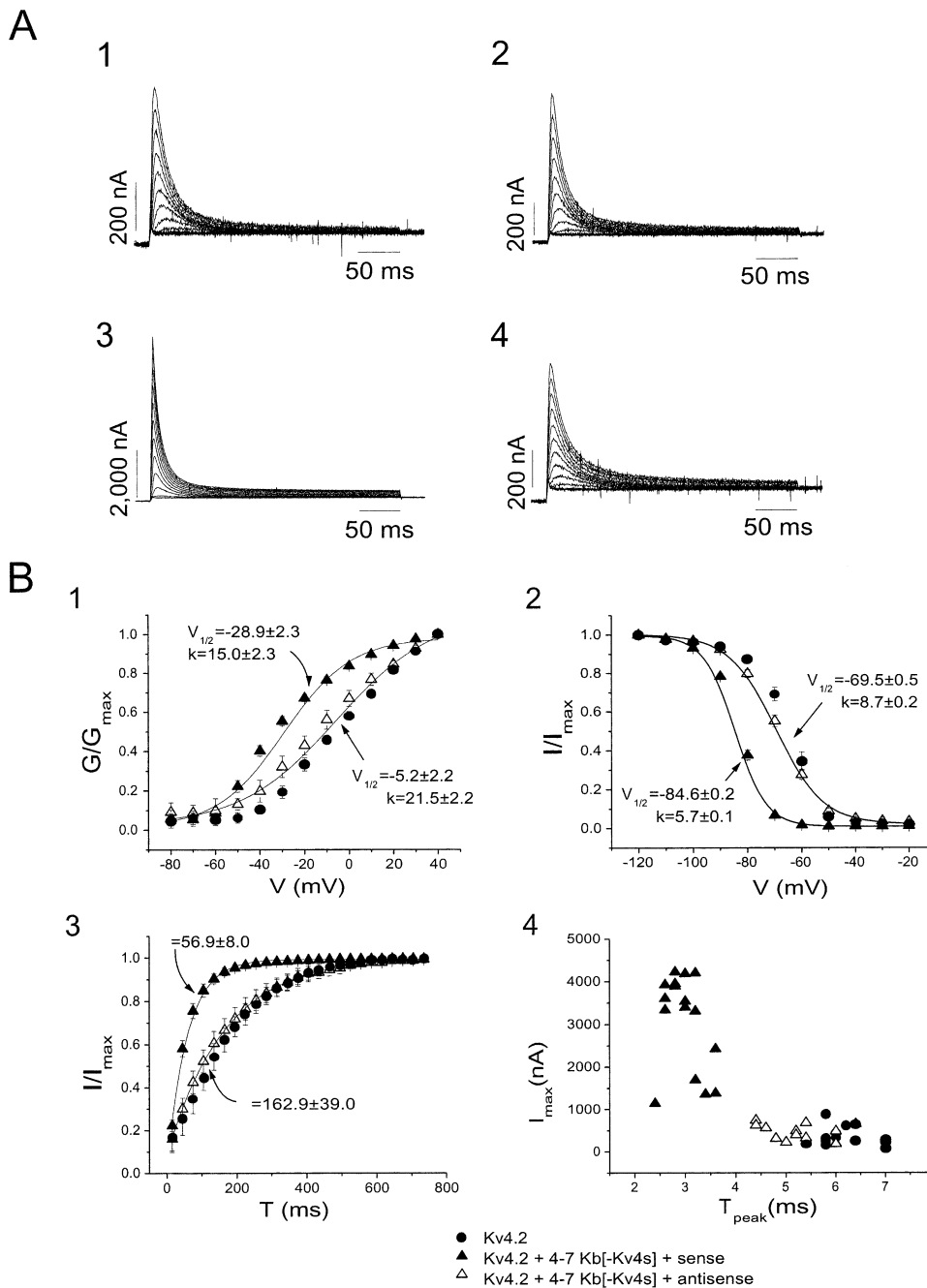△ Kv4.2 + 4-7 Kb[-Kv4s] + antisense

Figure 5. Hybrid Arrest of KAF Activity by Antisense Oligonucleotides to DPPX

(A) A-type currents in *Xenopus* oocytes injected with (1) Kv4.2 cRNA incubated with *sense* oligonucleotides to one of the peptide sequences obtained by MS/MS from the ~115 kDa band, (2) Kv4.2 cRNA incubated with *antisense* oligonucleotides, (3) a mixture of Kv4.2 cRNA and the KAF-containing 4–7 Kb cerebellar mRNA fraction pretreated with *sense* oligonucleotides, and (4) a mixture of Kv4.2 cRNA and the KAF-containing 4–7 Kb mRNA fraction pretreated with *antisense* oligonucleotides. The oligonucleotides had no effect on the currents expressed by Kv4.2 cRNA alone (compare panels 1 and 2), but antisense oligonucleotides blocked the KAF activity present in 4–7 Kb mRNA. The increase in current magnitude and the acceleration of current kinetics observed when Kv4.2 cRNA was coinjected with sense-treated 4–7 Kb mRNA (3) were arrested by antisense treatment (4).

(B) Conductance voltage relation (1), steady-state inactivation (2), and recovery from inactivation at –110mV (3) for 12 oocytes injected with Kv4.2 cRNA alone, Kv4.2 cRNA plus KAF-containing 4–7 Kb mRNA treated with *sense* oligonucleotides, or Kv4.2 cRNA plus KAF-containing 4–7 Kb mRNA treated with *antisense* oligonucleotides. (4) Peak current at +40mV (I_{max}) and time for the current to reach a maximum value (T_{peak}) for oocytes injected with Kv4.2 cRNA alone, Kv4.2 cRNA plus KAF-containing 4–7 Kb mRNA treated with *sense* oligonucleotides, and Kv4.2 cRNA plus KAF-containing 4–7 Kb mRNA treated with *antisense* oligonucleotides. Antisense treatment prevented the shifts in activation and inactivation, the acceleration of the rate of rise and the rate of recovery from inactivation, and the increase in current magnitude produced by 4–7 Kb mRNA.
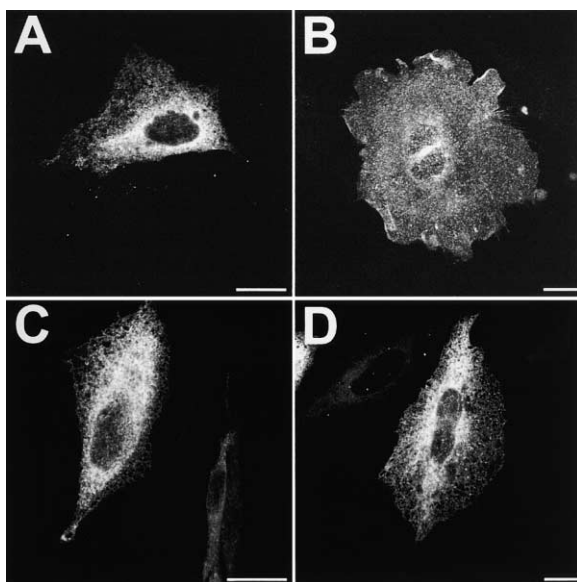
Figure 6. DPPX-S Promotes the Trafficking of Kv4.2 but Not Kir6.2 Subunits in CHO Cells

Cells were transiently transfected with Kv4.2 cDNA (A), with Kir6.2-Flag cDNA (C), and with combinations of Kv4.2 and DPPX-S (B) or Kir6.2-Flag and DPPX-S (D) cDNAs. Kv4.2 (A and B) and Kir6.2-Flag (C and D) were detected with anti-Kv4.2 and anti-Flag antibodies, respectively. Images were taken with a Leica confocal microscope. Note the perinuclear concentration of Kv4.2 and Kir6.2 proteins in cells transfected with the pore-forming subunits alone and the redistribution and increase in surface expression of Kv4.2 proteins when the cells were cotransfected with DPPX-S. However, there was no effect of DPPX-S on the distribution of Kir6.2 proteins. Scale bars, 20 μm.

expressed in heterologous expression systems. We have also shown that DPPX corresponds to KAF, the factor suggested to account for the effects of brain 4–7 Kb mRNA on Kv4 currents in *Xenopus* oocytes. The physiological significance of the effects of DPPX on Kv4 channel properties, such as activation and inactivation at subthreshold voltages and fast inactivation and reactivation, is highlighted by the importance of these properties on the regulation of firing frequency and dendritic function (Connor and Stevens, 1971; Johnston et al., 2000; Llinas, 1988; Rush and Rinzel, 1995).

The analysis of the polypeptide composition of Kv4 channels revealed that, in addition to Kv4 pore-forming subunits, the channel complexes include two types of associated proteins: KChIPs and DPPX. Although silver staining is not linear, the fact that the intensities of the DPPX and KChIP bands in Kv4 channel complexes are comparable to those of the pore-forming Kv4 proteins suggests that these are important elements of the channel complex. Furthermore, these associated proteins not only have major effects on the electrophysiological properties of the channels, but in fact they might be an absolute requirement for the expression of Kv4 channels in the neuronal membrane (Figure 6). This is becoming a common feature of several channels and receptors, with associated proteins having a critical role in the mechanisms that control the expression of appropriate numbers of properly assembled channel complexes.

In contrast to KChIP proteins, which slow down the inactivation of Kv4 channels several fold, DPPX increased the rate of channel inactivation (Table 1). This effect can explain why in neurons the I_{SA} is often faster than the currents expressed by Kv4 proteins (Nadal et al., 2001). The presence of DPPX proteins, which are glycoproteins (Kin et al., 2001), in Kv4 channel complexes may also explain why brain Kv4 channels bind to wheat-germ agglutinin (WGA) agarose (Sheng et al., 1993), in spite of the fact that Kv4.2 and Kv4.3 subunits lack N-glycosylation sites.

Kv4 channels are likely to be tetramers of Kv4 pore-forming subunits; however, the stoichiometry of the DPPX and KChIP associated proteins and whether Kv4 channels are always associated to both remain key questions for future studies. The existence of two pore-forming subunits (Kv4.2 and Kv4.3; Kv4.1 is not prominently expressed in adult rodent brain) and two different types of associated protein, each providing different voltage-dependent and kinetic properties to the channels, is likely to be a major factor in explaining the diversity of I_{SA}s in neurons (Rudy, 1988; Table 1).

DPPX Regulates the Trafficking and Surface Expression of Kv4 Channels

Our data show that DPPX specifically facilitates the trafficking of Kv4 proteins to the plasma membrane (Figure 6), which most likely explains the large increases in current amplitude obtained when Kv4 proteins were expressed with DPPX. KChIP proteins also promote the trafficking and surface expression of Kv4 proteins in transfected mammalian cells (An et al., 2000), although DPPX appears to be three to five times more effective than KChIPs in increasing current magnitude in *Xenopus* oocytes and CHO cells. Retention of components of multimeric protein complexes in the ER is thought to be part of a tightly controlled process to ensure the expression of appropriate numbers of correctly assembled complexes on the cell surface (Margeta-Mitrovic et al., 2000; Teasdale and Jackson, 1996). DPPX and KChIP associated proteins promote the trafficking and surface expression of Kv4 channels in a manner analogous to which SUR proteins associate with Kir6 subunits to promote the surface expression of K_{ATP} channels (Zerangue et al., 1999), and GB2 subunits promote the membrane trafficking and targeting of GABA$_B$ receptors upon forming heteromeric complexes with GB1 proteins (Margeta-Mitrovic et al., 2000). However, the mechanisms involved in the processing of Kv4 channels remain to be studied.

A Function for DPPX

This study also led to the identification of a function for DPPX as a Kv4 channel-associated protein and key regulator of the processing, membrane expression, and function of I_{SA} channels. However, our results do not exclude the possibility that, as might be the case for KChIPs, DPPX proteins also have Kv4-unrelated functions (Cheng et al., 2002; Jo et al., 2001; Lilliehook et al., 2002). DPPX is a protein with interesting structural features (Figure 2). The homologous CD26 protein has a number of important functions mediated by its extracellular catalytic and cysteine-rich domains (De Meester
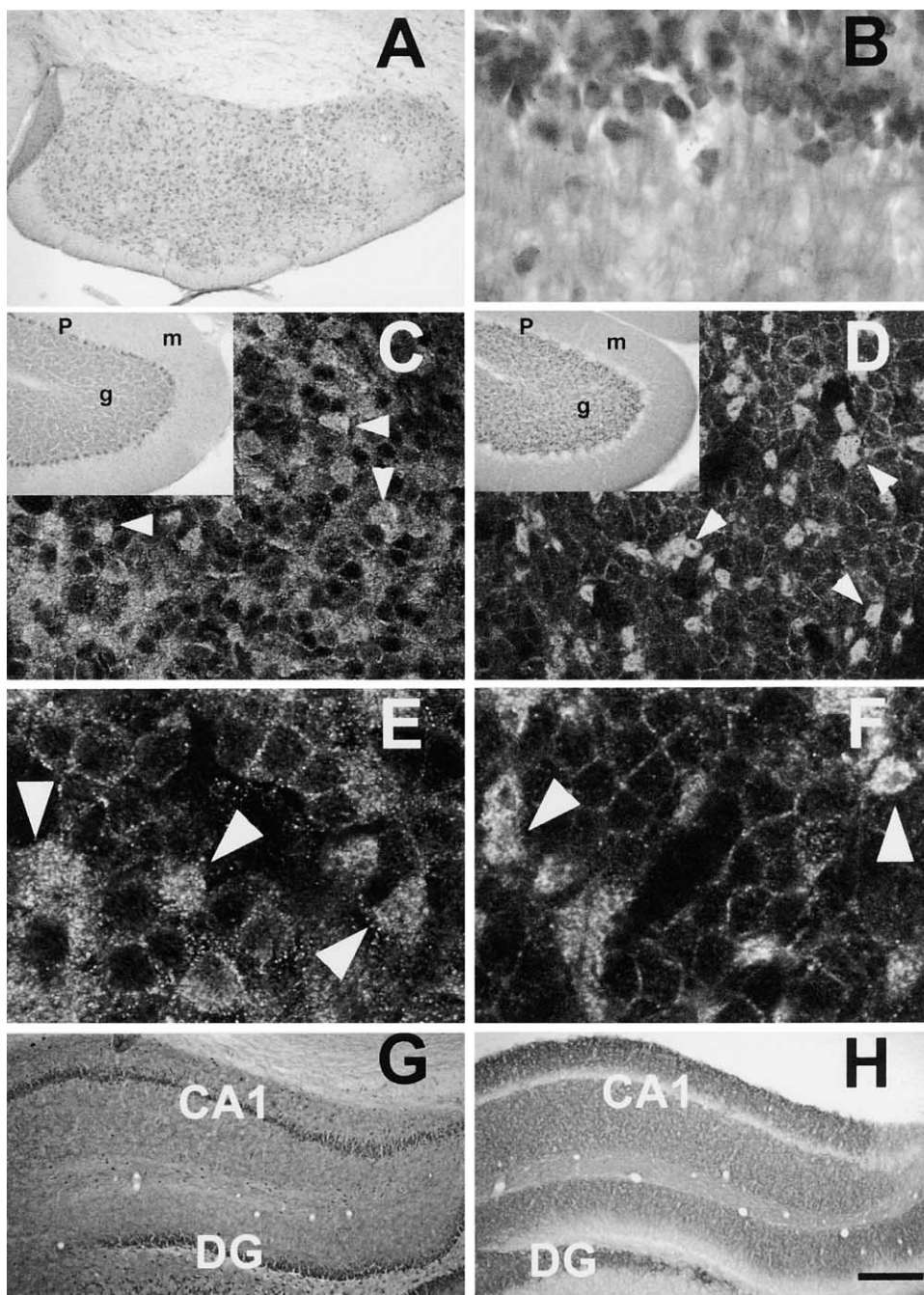
Figure 7. DPPX Is Expressed in Neuronal Populations Expressing Kv4.2 and Kv4.3 Proteins

Immunoperoxidase labeling of DPPX in the pontine nucleus (A) and in the soma and dendrites of CA1 pyramidal neurons in the hippocampus (B). (C–F) Confocal images of immunofluorescence labeling of DPPX (C and E) and Kv4.2 proteins (D and F) in the cerebellar cortex. Staining for both DPPX and Kv4.2 proteins outlines the soma of granule cells and the *glomeruli* containing the dendrites of these neurons (arrowheads). Low-magnification insets in (C) and (D) show that, in addition to labeling the granule cell layer, DPPX (but not Kv4.2) antibodies also stain Purkinje cells. These cells express Kv4.3 but not Kv4.2 mRNAs (Serodio and Rudy, 1998). (G and H) Immunoperoxidase detection of DPPX (G) and Kv4.2 proteins (H) in the hippocampus. The dendritic fields of CA1 pyramidal neurons and dentate granule cells are stained with the two antibodies. CA1, CA1 field of the hippocampus; DG, dentate gyrus; g, granule cell layer; m, molecular layer; P, Purkinje cell layer. Scale bar in (A), (G), and (H), 305 μm; (B), 30 μm; (C) and (D), 20 μm; insets in (C) and (D), 245 μm; (E) and (F), 7 μm.

et al., 1999; Hildebrandt et al., 2000; Gorrell et al., 2001). The cysteine-rich domain binds to components of the cell matrix and mediates roles in cell adhesion, cellular trafficking, and T cell activation. DPPX may confer tar-geting or cell adhesion properties to Kv4 channels through its homologous extracellular cysteine-rich do-main (Figure 2). This is reminiscent of the cell adhesion properties conferred upon Na$^+$ channels by the extracel-

lular immunoglobulin-like domain of their β subunits (Ratcliffe et al., 2001; Isom, 2002; Srinivasan et al., 1998). These properties could be important in determining the organization of Kv4 channels in the plasma membrane, which have been found to be concentrated in clusters at sites of synaptic contact in hypothalamic neurons (Alonso and Widmer, 1997). In another possible interesting scenario, cell-cell interactions or interactions with extracellular matrix components may modulate the function of A-type K⁺ channels. The inactive catalytic domain of DPPX may also play roles in protein-protein interactions, as it has been observed for the inactive guanylate kinase domain of CASK, a member of the family of membrane-associated guanylate kinases (MAGUKs) concentrated at neuronal synapses (Hsueh et al., 2000).

DPPX is now another example of enzyme-derived proteins that have lost their catalytic properties and have acquired novel functions. This growing list of proteins includes the Kvβ subunits, proteins that are essential components of voltage-gated K⁺ channels of the Kv1 subfamily and are nonenzymatic homologs of aldo-keto reductases (Campomanes et al., 2002; Gulbis et al., 1999; McCormack and McCormack, 1994). It also includes the already mentioned guanylate kinase domain of MAGUKs, such as PSD95 and CASK (Anderson, 1996).

While the intriguing ideas suggested here remain to be investigated, it is clear that the identification of DPPX as a Kv4-associated protein has opened the possibility of discovering new features of these important neuronal channels.

Experimental Procedures

Immunopurification of Kv4.2 Channel Complexes

Cerebellar tissue from P28-45 Sprague-Dawley rats was homogenized in 10 volumes of ice-cold sucrose buffer (0.32 M sucrose, 1 mM EDTA, 5 mM Tris·HCl, pH 7.4, and a cocktail of protease inhibitors: 10 μg ml^{-1} aprotinin, 10 μg ml^{-1} leupeptin, 10 μg ml^{-1} pepstatin, and 1 mM PMSF). The homogenate was centrifuged at 700 × g for 10 min; the pellet was washed once with 7 volumes of sucrose buffer, and the combined supernatants were centrifuged further at 27,000 × g for 40 min to yield a crude membrane pellet (P2). The pellet P2 was resuspended in TNE (50 mM Tris.HCL, pH 7.4, 150 mM NaCl, 1 mM EDTA, and protease inhibitors) and solubilized with 1% Triton X-100 (1 hr, 4°C). Insoluble material was removed by centrifugation at 20,000 × g for 30 min, and the supernatant was used for immunoprecipitation. The solubilized membrane extract (usually 4 mg of protein at 2 mg protein ml^{-1}) was precleared with protein A-Sepharose 4B beads (Amersham Pharmacia) or protein A-Sepharose 4B beads crosslinked to preimmune sera, for 2 hr at 4°C. After removing the beads by centrifugation at 20,000 × g for 30 s, the extract was incubated overnight at 4°C with protein A-Sepharose 4B beads crosslinked to Kv4.2 antibodies. The complexed beads were collected and washed four times by centrifugation/resuspension with extraction buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100). The bound Kv4.2 complexes were eluted from the beads by adding a sample buffer containing 2.5% 2-mercaptoethanol, 1 mM EDTA, 1.5% SDS, and 10% glycerol in 50 mM Tris buffer, pH 6.7, and the eluted proteins separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) according to Shevchenko et al. (1996). Immunoprecipitation of KChIP complexes utilized a Pan-KChIP antibody and used the same methods described above for Kv4 antibodies.

Antibodies were crosslinked to protein-A-Sepharose 4B beads with dimethyl pimelimidate·2HCl (DMP, Pierce). The association of Kv4.2 proteins with p115 and KChIP proteins was better preserved if interacting proteins were chemically crosslinked in the membrane preparation prior to detergent solubilization. For this purpose, the pellet P2 was washed twice with HEPES buffer (50 mM HEPES, pH 7.5, 150 mM KCl), resuspended (at a protein concentration of 1 mg ml^{-1}) in HEPES buffer containing 0.5 mg ml^{-1} of freshly prepared 3, 3′-dithiobispropionimidate (DTBP, Pierce), a thiol-cleavable crosslinking agent, and incubated at room temperature for 30 min. The preparation was centrifuged at 20,000 × g for 30 min, the pellet was washed with TNE and solubilized with 1% Triton X-100 in TNE, and the extract was immunoprecipitated, components extracted from the beads with sample buffer and separated on SDS-PAGE as described above. The 2-mercaptoethanol in the sample buffer dissociates the crosslinked complexes.

Immunoblot Analysis and Immunohistochemistry

Immunoblots prepared as previously described (Ozaita et al., 2002) were incubated with the appropriate polyclonal antibodies at a 1:1000 dilution at 4°C for 14 hr, and bound antibodies were detected using chemiluminescence with an ECL detection kit (Pierce, Rockford, IL). CHO-K1 cells were transiently transfected with Fugene (Roche Diagnostics, Indianapolis, IN) following the manufacturer's instructions and processed for immunofluorescence as described (Ozaita et al., 2002). The following primary antibodies were used: Kv4.2 antibodies (300 μg ml^{-1}; Nakamura et al., 2001b), anti-DPPX (3 mg ml^{-1}; Kin et al., 2001), Kv4.3 antibodies (Alomone, 300 μg ml^{-1}), Pan-KChIP antibody (antibodies that recognize KChIP1-4 to be described elsewhere, at 150 μg ml^{-1}); mouse anti-Flag (Kodak, 1:1000). Immunolocalization of DPPX and Kv4.2 proteins in rat brain used methods previously described for DAB immunohistochemistry and immunofluorescence (Ozaita et al., 2002).

Mass Spectrometry and Protein Identification

The silver-stained protein bands were excised from SDS-PAGE gel under a tissue culture hood to minimize contamination. After destaining with potassium ferricyanide and sodium thiosulfate (Gharahdaghi et al., 1999), the gel slices were cut into 1 mm³ pieces, reduced, carbamidomethylated, and digested with trypsin (Shevchenko et al., 1996). The peptides were extracted, dried under vacuum, and redissolved in 20 μl of 2% acetonitrile/0.1% formic acid in water. At first, samples were analyzed by direct infusion nano-ESI MS/MS using "medium" nanospray needles (Protana, Denmark). Later, data-dependent nanoLC-MS/MS analyses were carried out using a Waters CapLC (Milford, MA) and a Micromass Q-TOF (Manchester, UK) mass spectrometer along with a Picotip needle (New-Objective) and a home-made pre-column flow splitter [Y. Ma, X. Wang, W. Jiang, and T.A. Neubert, 2002, Proceedings of the 50th ASMS conference]. The analytical column was 75 μm I.D. × 15 cm, PepMap C18 (LC Packings). Mobile phase A was 2% acetonitrile/0.1% formic acid, while mobile phase B was 90% acetonitrile/0.1% formic acid. The gradient was 5%–60% B in 90 min. The flow rate was ~200 nL/min. Automatic switching between MS and MS/MS modes were controlled by MassLynx 3.5 (Micromass), dependent on both signal intensity and charge states from MS to MS/MS and on either signal intensity or time from MS/MS to MS. The MS/MS spectra were processed, and the deconvoluted MS/MS spectra were directly used to search, in batch, the NCBI nonredundant protein database using the Mascot search program (Matrix Science, UK).

Functional Expression in *Xenopus* Oocytes

Kv subunit and DPPX cDNAs were in vitro transcribed using T7 polymerase (Stratagene mCAP kit). Brain mRNA fractions or cRNAs were microinjected in *Xenopus laevis* oocytes for expression (Nadal et al., 2001; Serodio et al., 1994, 1996). Currents were recorded at room temperature and analyzed using two-microelectrode voltage clamp as described (Nadal et al., 2001; Serodio et al., 1994, 1996). A-type currents were obtained by subtracting the currents obtained during depolarizing pulses from −80mV to 40mV (in 10mV intervals) preceded by a 1 s prepulse to −40mV from the currents obtained during the same pulses preceded by a prepulse to −110mV (VH = −90mV; see Nadal et al. [2001]).

Antisense Hybrid Arrest

The KAF-containing Kv4-arrested 4–7 Kb mRNA fraction from rat cerebellum was prepared as described (Nadal et al., 2001). For hybrid arrest of KAF activity, the mRNA fraction was incubated with

antisense (or control sense) oligonucleotides following methods previously described (Nadal et al., 2001). The anti-DPPX oligonucleotide 5′-TTGGCTGGGAGGATGTAAGTAC-3′ used is complementary to a portion of the DYGGYLSTYILPAK peptide sequence identified by microsequencing of p115 (Figure 2A).

References

Alonso, G., and Widmer, H. (1997). Clustering of KV4.2 potassium channels in postsynaptic membrane of rat supraoptic neurons: an ultrastructural study. Neuroscience *77*, 617–621.

An, W.F., Bowlby, M.R., Betty, M., Cao, J., Ling, H.P., Mendoza, G., Hinson, J.W., Mattsson, K.I., Strassle, B.W., Trimmer, J.S., and Rhodes, K.J. (2000). Modulation of A-type potassium channels by a family of calcium sensors. Nature *403*, 553–556.

Anderson, J.M. (1996). Cell signalling: MAGUK magic. Curr. Biol. *6*, 382–384.

Baldwin, T.J., Tsaur, M.L., Lopez, G.A., Jan, Y.N., and Jan, L.Y. (1991). Characterization of a mammalian cDNA for an inactivating voltage-sensitive K+ channel. Neuron *7*, 471–483.

Banks, M.I., Haberly, L.B., and Jackson, M.B. (1996). Layer-specific properties of the transient K current (IA) in piriform cortex. J. Neurosci. *16*, 3862–3876.

Bardoni, R., and Belluzzi, O. (1993). Kinetic study and numerical reconstruction of A-type current in granule cells of rat cerebellar slices. J. Neurophysiol. *69*, 2222–2231.

Baxter, D.A., and Byrne, J.H. (1991). Ionic conductance mechanisms contributing to the electrophysiological properties of neurons. Curr. Opin. Neurobiol. *1*, 105–112.

Campomanes, C.R., Carroll, K.I., Manganas, L.N., Hershberger, M.E., Gong, B., Antonucci, D.E., Rhodes, K.J., and Trimmer, J.S. (2002). Kv beta subunit oxidoreductase activity and Kv1 potassium channel trafficking. J. Biol. Chem. *277*, 8298–8305.

Cheng, H.Y., Pitcher, G.M., Laviolette, S.R., Whishaw, I.Q., Tong, K.I., Kockeritz, L.K., Wada, T., Joza, N.A., Crackower, M., Goncalves, J., et al. (2002). DREAM is a critical transcriptional repressor for pain modulation. Cell *108*, 31–43.

Connor, J.A., and Stevens, C.F. (1971). Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma. J. Physiol. *213*, 31–53.

Cull-Candy, S.G., Marshall, C.G., and Ogden, D. (1989). Voltage-activated membrane currents in rat cerebellar granule neurones. J. Physiol. *414*, 179–199.

de Lecea, L., Soriano, E., Criado, J.R., Steffensen, S.C., Henriksen, S.J., and Sutcliffe, J.G. (1994). Transcripts encoding a neural membrane CD26 peptidase-like protein are stimulated by synaptic activity. Brain Res. Mol. Brain Res. *25*, 286–296.

De Meester, I., Korom, S., Van Damme, J., and Scharpe, S. (1999). CD26, let it cut or cut it down. Immunol. Today *20*, 367–375.

Gharahdaghi, F., Weinberg, C.R., Meagher, D.A., Imai, B.S., and Mische, S.M. (1999). Mass spectrometric identification of proteins from silver-stained polyacrylamide gel: a method for the removal of silver ions to enhance sensitivity. Electrophoresis *20*, 601–605.

Gorrell, M.D., Gysbers, V., and McCaughan, G.W. (2001). CD26: a multifunctional integral membrane and secreted protein of activated lymphocytes. Scand. J. Immunol. *54*, 249–264.

Gulbis, J.M., Mann, S., and MacKinnon, R. (1999). Structure of a voltage-dependent K+ channel beta subunit. Cell *97*, 943–952.

Hildebrandt, M., Reutter, W., Arck, P., Rose, M., and Klapp, B.F. (2000). A guardian angel: the involvement of dipeptidyl peptidase IV in psychoneuroendocrine function, nutrition and immune defence. Clin. Sci. (Lond.) *99*, 93–104.

Hille, B. (2001). Ion Channels of Excitable Membranes (Sunderland, MA: Sinauer Associates).

Hoffman, D.A., Magee, J.C., Colbert, C.M., and Johnston, D. (1997). K+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature *387*, 869–875.

Hough, R.B., Lengeling, A., Bedian, V., Lo, C., and Bucan, M. (1998). Rump white inversion in the mouse disrupts dipeptidyl aminopeptidase-like protein 6 and causes dysregulation of Kit expression. Proc. Natl. Acad. Sci. USA *95*, 13800–13805.

Hsiao, C.F., and Chandler, S.H. (1995). Characteristics of a fast transient outward current in guinea pig trigeminal motoneurons. Brain Res. *695*, 217–226.

Hsueh, Y.P., Wang, T.F., Yang, F.C., and Sheng, M. (2000). Nuclear translocation and transcription regulation by the membrane-associated guanylate kinase CASK/LIN-2. Nature *404*, 298–302.

Huguenard, J.R., Coulter, D.A., and Prince, D.A. (1991). A fast transient potassium current in thalamic relay neurons: kinetics of activation and inactivation. J. Neurophysiol. *66*, 1304–1315.

Isom, L.L. (2002). The role of sodium channels in cell adhesion. Front. Biosci. *7*, 12–23.

Isom, L.L., Ragsdale, D.S., De Jongh, K.S., Westenbroek, R.E., Reber, B.F., Scheuer, T., and Catterall, W.A. (1995). Structure and function of the beta 2 subunit of brain sodium channels, a transmembrane glycoprotein with a CAM motif. Cell *83*, 433–442.

Jo, D.G., Kim, M.J., Choi, Y.H., Kim, I.K., Song, Y.H., Woo, H.N., Chung, C.W., and Jung, Y.K. (2001). Pro-apoptotic function of calsenilin/DREAM/KChIP3. FASEB J. *15*, 589–591.

Johns, D.C., Nuss, H.B., and Marban, E. (1997). Suppression of neuronal and cardiac transient outward currents by viral gene transfer of dominant-negative Kv4.2 constructs. J. Biol. Chem. *272*, 31598–31603.

Johnston, D., Hoffman, D.A., Magee, J.C., Poolos, N.P., Watanabe, S., Colbert, C.M., and Migliore, M. (2000). Dendritic potassium channels in hippocampal pyramidal neurons. J. Physiol. *525*, 75–81.

Kin, Y., Misumi, Y., and Ikehara, Y. (2001). Biosynthesis and characterization of the brain-specific membrane protein DPPX, a dipeptidyl peptidase IV-related protein. J. Biochem. (Tokyo) *129*, 289–295.

Klee, R., Ficker, E., and Heinemann, U. (1995). Comparison of voltage-dependent potassium currents in rat pyramidal neurons acutely isolated from hippocampal regions CA1 and CA3. J. Neurophysiol. *74*, 1982–1995.

Krapivinsky, G., Gordon, E.A., Wickman, K., Velimirovic, B., Krapivinsky, L., and Clapham, D.E. (1995). The G-protein-gated atrial K+ channel IKACh is a heteromultimer of two inwardly rectifying K(+)-channel proteins. Nature *374*, 135–141.

Kuo, H.C., Cheng, C.F., Clark, R.B., Lin, J.J., Lin, J.L., Hoshijima, M., Nguyen-Tran, V.T., Gu, Y., Ikeda, Y., Chu, P.H., et al. (2001). A defect in the Kv channel-interacting protein 2 (KChIP2) gene leads to a complete loss of I(to) and confers susceptibility to ventricular tachycardia. Cell *107*, 801–813.

Lilliehook, C., Chan, S., Choi, E.K., Zaidi, N.F., Wasco, W., Mattson, M.P., and Buxbaum, J.D. (2002). Calsenilin enhances apoptosis by altering endoplasmic reticulum calcium signaling. Mol. Cell. Neurosci. *19*, 552–559.

Liss, B., Franz, O., Sewing, S., Bruns, R., Neuhoff, H., and Roeper, J. (2001). Tuning pacemaker frequency of individual dopaminergic neurons by Kv4.3L and KChip3.1 transcription. EMBO J. *20*, 5715–5724.

Llinas, R.R. (1988). The intrinsic electrophysiological properties of mammalian neurons: insights into central nervous system function. Science *242*, 1654–1664.

Maletic-Savatic, M., Lenn, N.J., and Trimmer, J.S. (1995). Differential spatiotemporal expression of K+ channel polypeptides in rat hippo-

campal neurons developing in situ and in vitro. J. Neurosci. *15*, 3840–3851.

Malin, S.A., and Nerbonne, J.M. (2000). Elimination of the fast transient in superior cervical ganglion neurons with expression of KV4.2W362F: molecular dissection of IA. J. Neurosci. *20*, 5191–5199.

Margeta-Mitrovic, M., Jan, Y.N., and Jan, L.Y. (2000). A trafficking checkpoint controls GABA(B) receptor heterodimerization. Neuron *27*, 97–106.

Martina, M., Schultz, J.H., Ehmke, H., Monyer, H., and Jonas, P. (1998). Functional and molecular differences between voltage-gated K+ channels of fast-spiking interneurons and pyramidal neurons of rat hippocampus. J. Neurosci. *18*, 8111–8125.

McCormack, T., and McCormack, K. (1994). Shaker K+ channel beta subunits belong to an NAD(P)H-dependent oxidoreductase superfamily. Cell *79*, 1133–1135.

Nadal, M.S., Amarillo, Y., de Miera, E.V., and Rudy, B. (2001). Evidence for the presence of a novel Kv4-mediated A-type K(+) channel-modifying factor. J. Physiol. *537*, 801–809.

Nakamura, T.Y., Nandi, S., Pountney, D.J., Artman, M., Rudy, B., and Coetzee, W.A. (2001a). Different effects of the Ca(2+)-binding protein, KChIP1, on two Kv4 subfamily members, Kv4.1 and Kv4.2. FEBS Lett. *499*, 205–209.

Nakamura, T.Y., Pountney, D.J., Ozaita, A., Nandi, S., Ueda, S., Rudy, B., and Coetzee, W.A. (2001b). A role for frequenin, a Ca2+-binding protein, as a regulator of Kv4 K+-currents. Proc. Natl. Acad. Sci. USA *98*, 12808–12813.

Numann, R.E., Wadman, W.J., and Wong, R.K. (1987). Outward currents of single hippocampal cells obtained from the adult guinea-pig. J. Physiol. *393*, 331–353.

Ozaita, A., Martone, M.E., Ellisman, M.H., and Rudy, B. (2002). Differential subcellular localization of the two alternatively spliced isoforms of the kv3.1 potassium channel subunit in brain. J. Neurophysiol. *88*, 394–408.

Ratcliffe, C.F., Westenbroek, R.E., Curtis, R., and Catterall, W.A. (2001). Sodium channel beta1 and beta3 subunits associate with neurofascin through their extracellular immunoglobulin-like domain. J. Cell Biol. *154*, 427–434.

Rudy, B. (1988). Diversity and ubiquity of K channels. Neuroscience *25*, 729–749.

Rudy, B., Hoger, J.H., Lester, H.A., and Davidson, N. (1988). At least two mRNA species contribute to the properties of rat brain A-type potassium channels expressed in Xenopus oocytes. Neuron *1*, 649–658.

Rush, M.E., and Rinzel, J. (1995). The potassium A-current, low firing rates and rebound excitation in Hodgkin-Huxley models. Bull. Math. Biol. *57*, 899–929.

Schoppa, N.E., and Westbrook, G.L. (1999). Regulation of synaptic timing in the olfactory bulb by an A-type potassium current. Nat. Neurosci. *2*, 1106–1113.

Serodio, P., and Rudy, B. (1998). Differential expression of Kv4 K+ channel subunits mediating subthreshold transient K+ (A-type) currents in rat brain. J. Neurophysiol. *79*, 1081–1091.

Serodio, P., Kentros, C., and Rudy, B. (1994). Identification of molecular components of A-type channels activating at subthreshold potentials. J. Neurophysiol. *72*, 1516–1529.

Serodio, P., Vega-Saenz de Miera, E., and Rudy, B. (1996). Cloning of a novel component of A-type K+ channels operating at subthreshold potentials with unique expression in heart and brain. J. Neurophysiol. *75*, 2174–2179.

Sheng, M., Tsaur, M.L., Jan, Y.N., and Jan, L.Y. (1992). Subcellular segregation of two A-type K+ channel proteins in rat central neurons. Neuron *9*, 271–284.

Sheng, M., Liao, Y.J., Jan, Y.N., and Jan, L.Y. (1993). Presynaptic A-current based on heteromultimeric K+ channels detected in vivo. Nature *365*, 72–75.

Shevchenko, A., Wilm, M., Vorm, O., Jensen, O.N., Podtelejnikov, A.V., Neubauer, G., Mortensen, P., and Mann, M. (1996). A strategy for identifying gel-separated proteins in sequence databases by MS alone. Biochem. Soc. Trans. *24*, 893–896.

Shibata, R., Nakahira, K., Shibasaki, K., Wakazono, Y., Imoto, K., and Ikenaka, K. (2000). A-type K+ current mediated by the Kv4 channel regulates the generation of action potential in developing cerebellar granule cells. J. Neurosci. *20*, 4145–4155.

Srinivasan, J., Schachner, M., and Catterall, W.A. (1998). Interaction of voltage-gated sodium channels with the extracellular matrix molecules tenascin-C and tenascin-R. Proc. Natl. Acad. Sci. USA *95*, 15753–15757.

Surmeier, D.J., Bargas, J., and Kitai, S.T. (1988). Voltage-clamp analysis of a transient potassium current in rat neostriatal neurons. Brain Res. *473*, 187–192.

Surmeier, D.J., Bargas, J., and Kitai, S.T. (1989). Two types of A-current differing in voltage-dependence are expressed by neurons of the rat neostriatum. Neurosci. Lett. *103*, 331–337.

Teasdale, R.D., and Jackson, M.R. (1996). Signal-mediated sorting of membrane proteins between the endoplasmic reticulum and the golgi apparatus. Annu. Rev. Cell Dev. Biol. *12*, 27–54.

Tkatch, T., Baranauskas, G., and Surmeier, D.J. (2000). Kv4.2 mRNA abundance and A-type K(+) current amplitude are linearly related in basal ganglia and basal forebrain neurons. J. Neurosci. *20*, 579–588.

Tsunoda, S., and Salkoff, L. (1995). Genetic analysis of Drosophila neurons: Shal, Shaw, and Shab encode most embryonic potassium currents. J. Neurosci. *15*, 1741–1754.

Wada, K., Yokotani, N., Hunter, C., Doi, K., Wenthold, R.J., and Shimasaki, S. (1992). Differential expression of two distinct forms of mRNA encoding members of a dipeptidyl aminopeptidase family. Proc. Natl. Acad. Sci. USA *89*, 197–201.

Wang, Y., Strahlendorf, J.C., and Strahlendorf, H.K. (1991). A transient voltage-dependent outward potassium current in mammalian cerebellar Purkinje cells. Brain Res. *567*, 153–158.

Watanabe, S., Hoffman, D.A., Migliore, M., and Johnston, D. (2002). Dendritic K+ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons. Proc. Natl. Acad. Sci. USA *99*, 8366–8371.

Zerangue, N., Schwappach, B., Jan, Y.N., and Jan, L.Y. (1999). A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. Neuron *22*, 537–548.

Zhang, L., and McBain, C.J. (1995). Potassium conductances underlying repolarization and after-hyperpolarization in rat CA1 hippocampal interneurones. J. Physiol. *488*, 661–672.