DPP10 Modulates Kv4-mediated A-type Potassium Channels*

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A new member of a family of proteins characterized by structural similarity to dipeptidyl peptidase (DPP) IV known as DPP10 was recently identified and linked to asthma susceptibility; however, the cellular functions of DPP10 are thus far unknown. DPP10 is highly homologous to subfamily member DPPX, which we previously reported as a modulator of Kv4-mediated A-type potassium channels (Nadal, M. S., Ozaita, A., Amarillo, Y., Vega-Saenz de Miera, E., Ma, Y., Mo, W., Goldberg, E. M., Misumi, Y., Ikehara, Y., Neubert, T. A., and Rudy, B. (2003) Neuron. 37, 449–461). We studied the ability of DPP10 protein to modulate the properties of Kv4.2 channels in heterologous expression systems. We found DPP10 activity to be nearly identical to DPPX activity and significantly different from DPPIV activity. DPPX and DPP10 facilitated Kv4.2 protein trafficking to the cell membrane, increased A-type current magnitude, and modified the voltage dependence and kinetic properties of the current such that they resembled the properties of A-type currents recorded in neurons in the central nervous system. Using in situ hybridization, we found DPP10 to be prominently expressed in brain neuronal populations that also express Kv4 subunits. Furthermore, DPP10 was detected in immunoprecipitated Kv4.2 channel complexes from rat brain membranes, confirming the association of DPP10 protein with native Kv4.2 channels. These experiments suggest that DPP10 contributes to the molecular composition of A-type currents in the central nervous system. To dissect the structural determinants of these integral accessory proteins, we constructed chimeras of DPPX, DPP10, and DPPIV lacking the extracellular domain. Chimeras of DPPX and DPP10, but not DPPIV, were able to modulate the properties of Kv4.2 channels, highlighting the importance of the intracellular and transmembrane domains in this activity.

We recently identified DPP10, a new member of a family of proteins characterized by structural similarity to dipeptidyl peptidase (DPP) IV (1). DPPIV (also known as CD26) is a multifunctional protein. It is a membrane-bound enzyme belonging to the S9B prolyl oligopeptidase class of serine proteases. Its exopeptidase activity has great physiological importance in the metabolism of peptide hormones and is currently being investigated as a target for the treatment of type II diabetes. DPPIV has important functions also in cell adhesion, cellular trafficking, and regulation of T cell activation, which are mediated by functional domains distinct from the catalytic domain (2–5). DPP10 is the most studied member of a growing class of interesting molecules with diverse activities.

DPP10 is prominently expressed in the brain as well as adrenal glands and trachea, but its functions remain to be discovered. The human DPP10 gene was recently identified as a candidate for susceptibility to asthma, a common disease of the airways involving atopic inflammation and hyper-responsiveness to various agents (6). Consistent with this report, independent mouse genome screens have linked airway hyper-responsiveness in mice to a region homologous to the location of DPP10 in humans (7, 8). Insight into how DPP10 relates to human physiology and disease is hindered by the lack of understanding of its cellular functions. In this study, we attempt to ascribe a function to this potentially important protein.

Within the DPPIV-like class of proteins, DPP10 is most closely related to DPPX (also known as DPP6). In DPPX, an aspartic residue replaces the serine of the catalytic triad, rendering the protein inactive against substrates cleaved by DPPIV and other S9B prolyl oligopeptidases. The catalytic serine residue is also mutated in DPP10; accordingly, DPP10 was found to lack DPP activity (1). These observations suggest that DPPX and DPP10 may not act as enzymes in vivo, whereas peptidase activity is a defining property of the DPPIV-like class of proteins.

We recently found that DPPX is associated with the pore-forming subunits of Kv4-mediated A-type K+ channels and modulates the cellular trafficking, membrane targeting, and functional properties of these channels (9). Given the sequence similarities between DPP10 and DPPX and in an attempt to understand the functions of DPP10, we investigated its ability to modulate Kv4-mediated A-type K+ channels. We found striking similarities to DPPX in the ability of DPP10 to traffic Kv4.2 proteins to the membrane and to modulate the functional properties of Kv4.2 channels. In contrast, DPPIV had more modest effects on Kv4.2 channels. We constructed chimeras lacking the extracellular portion of the protein, including the entire catalytic domain. These DPPX and DPP10 chimeras displayed significant Kv4 modulatory activity, strongly sug-
suggesting that this function is not mediated by enzymatic activity.

Moreover, in situ hybridization showed that DPP10 is expressed in neuronal populations also known to express Kv4 proteins (10, 11), and DPP10 protein was found to colocalize with Kv4.2 channel complexes from brain membranes, further suggesting that the in vitro effects likely occur in native cells of the central nervous system. Together, these results distinguish DPPX and DPP10 as a functional DPP subfamily able to effectively traffic and modulate Kv4 channels, a unique property within the larger DPP-like class of proteins. This function of DPP10 on potassium currents of excitatory membranes is the first demonstrated activity of DPP10 and could be important in determining asthma susceptibility.

**EXPERIMENTAL PROCEDURES**

**Preparation of Chinese Hamster Ovary (CHO) Cells Expressing Kv4.2 and DPP Proteins**—CHO-K1 cells (American Type Culture Collection, Manassas, VA) were plated at 70% confluence. CHO cells were cotransfected with the open reading frame of DPPX using the FuGENE 6 method (Roche Diagnostics) as described by the manufacturer. In other experiments, DPPX, DPP10, or DPPIV cDNA was cotransfected along with green fluorescent protein and Kv4.2. Note that, in this study, DPPX refers to the isoform previously identified as DPPX-s (9).

**Immunocytochemistry**—CHO cells were transfected as described above. In some experiments, we utilized a hemagglutinin (HA)-tagged Kv4.2 construct instead of Kv4.2 cDNA. At 48 h post-transfection, the cells were washed with phosphate-buffered saline (PBS; 140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, and 1.8 mM KH2PO4) and fixed for 20 min at 37 °C in 4% paraformaldehyde and 4% sucrose. After washing with PBS, cells were permeabilized with 0.1% Triton X-100 in PBS for 10 min at room temperature and washed again with PBS. The sample was incubated with 2% bovine serum albumin, 5% normal goat serum, and 0.2% gelatin in PBS (blocking solution) at 37 °C for 1 h. Cells were incubated with mouse anti-HA monoclonal antibody (0.1 ng/μl; Roche Diagnostics) in blocking solution at 4 °C overnight to detect HA-tagged Kv4.2 or with rabbit anti-Kv4.2 polyclonal antibody (1:1000 dilution; Sigma) to detect Kv4.2. After washing with PBS, cells were incubated with Cy3-conjugated goat anti-mouse or anti-rabbit IgG (Jackson Immunoresearch Laboratories, Inc., West Grove, PA) in PBS for 1 h. Cells were washed with PBS and then coverslipped. To visualize the fluorescence, we used a Zeiss LSM510 Meta laser scanning confocal microscope with a 30-milliwatt argon laser.

**Electrophysiological Analysis**—Whole cell currents were obtained at room temperature with the whole cell configuration of the patch clamp technique in tissue culture dishes on the stage of an inverted microscope with a 320× objective (Zeiss). Cells were transfected with Kv4.2 cDNA and incubated with 1 mg/ml N-hydroxysofuscinimidobiotin (Pierce) diluted in the same buffer for 1 h at 4 °C. Afterward, cells were rinsed twice with 0.1 M glycine in PBS containing 1 mM MgCl2 and 0.1 mM CaCl2, and perfused for 20 min in the same buffer containing 2 mM CaCl2 and 2 mM MgCl2. Finally, cells were lysed in TTNE buffer (50 mM Tris (pH 7.4), 1% Triton X-100, 150 mM NaCl, 1 mM EDTA) containing 10% glycerol, 2 μg/ml leupeptin, 2 μg/ml pepstatin A, 2 μg/ml aprotinin, and 100 μg/ml phenylmethysulfonyl fluoride and solubilized for 30 min at 4 °C, and samples were centrifuged at 13,000 × g to obtain the solubilized fraction. The biotinylated proteins were recovered from the solubilized cell lysates by incubation with 50 μl of packed immobilized streptavidin beads (Sigma). Biotinylated proteins were eluted from the beads in Laemmli sample buffer and analyzed by SDS-PAGE and Western blotting as described previously (13). The blot was incubated with anti-Kv4.2 or anti-HA primary antibody, followed by horseradish peroxydase-conjugated secondary antibody (Promega), and developed with enhanced chemiluminescent reagent (Pierce).

**Immunopurification of Kv4.2 Channel Complexes**—Nonadenovirally transfected extracts of brain membranes were used to amorphous-DNA beads were prepared as described previously (9), except that whole brain rather than only cerebellum was used. In some experiments, extracts from membranes treated with a membrane-permeable cross-linker were used as described previously (9). Membrane extracts were immunopurified to Kv4.2 channel complexes as described previously (9). Briefly, after preclearing the membrane extracts with protein A-Sepharose 4B beads (Amersham Biosciences), the supernatant was incubated overnight at 4 °C with protein A-Sepharose 4B beads linked to anti-Kv4.2 antibody with dimethyl pimelimidate-2HCl (Pierce). The complexes were washed four times by centrifugation/resuspension with TTNE buffer, and the bound Kv4.2 complexes were eluted from the beads by adding sample buffer containing 2.5% 2-mercaptoethanol (which dissociates cross-linked complexes), 1 mM EDTA, 1.5% SDS, and 10% glycerol in PBS (pH 6.7). Eluted proteins were transferred by SDS-PAGE (7.5% Criterion gel, Bio-Rad). The gels were either silver-stained or used for immunoblotting.

**Immunoblot Analysis**—Immunoblots prepared as described previously (9) were incubated at 4 °C for 14 h with anti-Kv4.2 polyclonal antibody (1:1000 dilution), anti-DPPX polyclonal antibody (1:1000 dilution) (14), or anti-DPP10 polyclonal antibody COO-12 (1:250 dilution; a generous gift of Dr. William Cookson). Bound antibodies were detected by chemiluminescence using an ECL detection kit (Pierce).

**Mass Spectrometry and Protein Identification**—Silver-stained protein bands were excised from SDS-polyacrylamide gels under a tissue culture hood to minimize contamination, destained, and digested with trypsin as described previously (9). The peptides were extracted and analyzed by mass spectrometry as described previously (9) in the New York University Protein Analysis Facility.

**Nonradioactive in Situ Hybridization**—Nonradioactive in situ hybridization was conducted as described previously (15). 40-μm sections were taken from 10-week-old C57/BL6 mice. Floating sections were prehybridized at 60 °C for 2 h and then hybridized at the same temperature for 15 h. Post-hybridization washes were conducted at 65 °C. Detection of digoxigenin labeling proceeded for 7–13 h. Images were acquired with an Olympus PD 290 microscope equipped with a Magnafire digital camera.

**Statistics**—The two-population (independent) t test (Origin Version 6.1) was used for statistical comparisons. Traces of inactivating currents were fit to second-order exponentials using the standard exponential algorithm of Clampfit Version 8.2, constraining parameters to positive values. For correlation analysis, linear regressions with confidence intervals were determined using Origin Version 6.1 software.
**RESULTS**

**DPP10 Increases the Surface Expression of Kv4.2 Proteins**—When Kv4.2 proteins are expressed alone in mammalian cells, they produce minimal expression of functional potassium channels presumably due to retention of the channel proteins in the endoplasmic reticulum (9, 16). Coexpression of Kv4.2 with DPPX has been reported to rescue endoplasmic reticulum retention and to generate a large increase in potassium conductance in heterologous cells (9). We investigated whether DPP10 is also able to facilitate the trafficking of Kv4 proteins to the plasma membrane.

CHO cells were transiently transfected with Kv4.2 cDNA alone or with DPPX, DPP10, or DPPIV cDNA and analyzed for Kv4.2 expression 48 h later. Fig. 1A shows that, in CHO cells transfected with Kv4.2 cDNA alone, there was minimal surface expression of Kv4.2 protein, with most detected protein residing in the endoplasmic reticulum/Golgi complex. A similar distribution of Kv4.2 protein was observed when Kv4.2 was coexpressed with DPPIV (Fig. 1B), suggesting that DPPIV does not efficiently traffic Kv4.2 proteins to the membrane. As reported previously (9) and replicated in Fig. 1C, DPPX promoted a robust trafficking of Kv4.2 protein to the plasma membrane. Like DPPX, DPP10 facilitated surface expression of Kv4.2 protein (Fig. 1D). These results suggest that, like DPPX, DPP10 may be used endogenously to regulate the amount of Kv4.2 protein expressed at the cell surface, thereby regulating the excitability of the cell membrane.

**DPP10 Modulates Kv4.2 Channels**—The same population of cells analyzed by immunofluorescence was used for electrophysiology. CHO cells transfected only with Kv4.2 were often fragile, producing unstable whole cell electrophysiological recordings, perhaps due to bulky endoplasmic reticulum retention of proteins. In more healthy cells, Kv4.2 currents tended to be very small. Fig. 2A shows records from a CHO cell transiently transfected with Kv4.2 cDNA. This particular cell had unusual large currents compared with other cells transfected with Kv4.2 alone. However, the kinetics and voltage dependence of the currents in this cell were similar to those in other cells expressing Kv4.2 alone. Fig. 2 (B–D) shows representative recordings of cells cotransfected with Kv4.2 cDNA and DPPX, DPP10, or DPP10 cDNA, respectively. The peak currents with DPPX and DPP10 were significantly larger than those produced by Kv4.2 expression alone, yielding 6.7 and 5.9 times more current upon depolarization to 48 mV (Fig. 2E). In contrast to the large effects of DPPX and DPP10 on current levels, DPPIV coexpression produced only small increases in Kv4.2 currents (Fig. 2E). These observations are consistent with the results of Fig. 1, in which DPPX and DPP10, but not DPPIV, induced effective trafficking of Kv4.2 protein from the endoplasmic reticulum to the cell surface.

DPP10 and DPPX also had similar effects in modulating the kinetic properties and voltage dependence of Kv4.2 channels. Time to peak of A-type K⁺ currents produced by Kv4.2 when expressed alone was 11.4 ms at 48 mV for the example illustrated in the Fig. 2A and averaged 11.8 ± 3.7 ms (n = 6). Coexpression with DPPX or DPP10 produced a large reduction in time to peak: 3.8 and 2.2 ms at 48 mV for DPPX and DPP10, respectively, for the examples shown in Fig. 2 (C and D) and an average of 3.8 ± 0.4 ms (n = 7) and 3.5 ± 0.9 ms (n = 6), respectively.
A key feature of the effects of DPPX on Kv4 channels is the acceleration of the rate of macroscopic inactivation of the transient A-type current during a depolarizing step (9). This is an important effect of this protein because the native A-type K⁺ current in neurons inactivates at faster rates than the currents expressed by Kv4 proteins alone (9, 17). DPP10 produced an even stronger effect than DPPX on inactivation rates (Fig. 2). The average τ₀.₅ (time to achieve half-inactivation) when depolarized to 18 mV was 32.4 ± 6.7 ms for Kv4.2 plus DPPX (n = 7) and 17.1 ± 4.7 ms for Kv4.2 plus DPP10 (n = 5), both significantly faster than Kv4.2 alone at 61.5 ± 23 ms (n = 7).

To further characterize the difference in inactivation rates of the currents expressed by Kv4.2 subunits in the presence of DPPX or DPP10 protein, we fitted the decline of the transient Kv4.2-mediated current with exponential functions. A double exponential was required to fit the decline of the currents in the presence of DPPX and DPP10, as reported previously for Kv4-mediated currents with DPPX in *Xenopus* oocytes and for the transient K⁺ current in many neurons (9, 17). In comparing DPP10 with DPPX, we found that the fast time constant was significantly different (18.8 ± 4.9 ms for DPP10 (n = 5) and 35.0 ± 5.9 ms for DPPX (n = 7), p < 0.001). Differences in the slow time constant and contributions of each component did not reach statistical significance, suggesting that the difference in inactivation rates in the presence of DPP10 or DPPX is due mainly to changes in the rate of the process(es) underlying the fast time constant of inactivation. However, the mechanism responsible for this difference in inactivation rates is currently unknown.

DPPX and DPP10 also drastically increased the rate of recovery from inactivation. For the representative examples shown in Fig. 3, the τ of recovery at −112 mV decreased from 220 ms for the currents produced by Kv4.2 alone (Fig. 3A) to 41 and 23 ms for cells expressing Kv4.2 with DPPX (Fig. 3C) or DPP10 (Fig. 3D), respectively. On average, DPP10 and DPPX produced a similar acceleration of the recovery from inactivation (τ₀.₅,DPP10 = 55 ± 26 ms (n = 7) and τ₀.₅,DPPX = 43 ± 17 ms (n = 7), p = 0.36; τ₀.₅,Kv4.2 = 299 ± 98 ms (n = 7), p < 0.0001 compared with τ₀.₅,Kv4.2) (Fig. 3E).

DPPX was also found to shift the voltage dependence of Kv4.2 activation to hyperpolarizing potentials. A 28-mV hyperpolarizing shift was reported in *Xenopus* oocytes (9). We observed a 26.5-mV hyperpolarizing shift in the *V₅₀* (voltage yielding half-maximal conductance) of Kv4.2 channels with DPPX coexpression in CHO cells (Fig. 2F). DPP10 similarly shifted the voltage dependence of Kv4.2 channel activation 19.6 mV in the hyperpolarizing direction. Interestingly, there was no measurable effect of DPPIV on this parameter (Fig. 2F), which is of physiological significance given the importance of sub-threshold activation of A-type currents in neurons.

Interestingly, the differences in inactivation rates were the only parameter analyzed in which differences between DPPX and DPP10 reached statistical significance (p = 0.0014). To further analyze the relationship between kinetic parameters, we carried out a correlation analysis in which the kinetic values of individual experiments were plotted for each population. Fig. 4A shows that time to peak and the rate of recovery from inactivation are highly correlated parameters (R = 0.929) among all experiments as well as within each population. In turn, the rate of inactivation was highly correlated with the rate of recovery from inactivation (R = 0.932) (Fig. 4B) and time to peak (R = 0.936) (Fig. 4C) for Kv4.2 alone and for Kv4.2 with DPPX or DPP10. However, most of the experimental points for Kv4.2 plus DPPX are outside the 95% confidence limit of the correlation. For Kv4.2 plus DPPX, the relationship between the rate of inactivation and other kinetic parameters was shifted such that inactivation was slower than would be predicted by the effects of DPPX on time to peak and recovery from inactivation.

The results of Figs. 1–4 show that DPP10 increased Kv4 current magnitude and modulated the kinetic and voltage-dependent properties of Kv4.2 channels in a similar fashion to DPPX. For each parameter analyzed, the presence of DPPX or DPP10 modified the Kv4.2-mediated currents such that they more closely resembled the A-type current recorded in central nervous system neurons containing Kv4.2 (9). In contrast, DPPIV had only small effects on Kv4.2 currents (discussed further below). DPPX and DPP10 thereby define a functional subfamily among DPPIV-like molecules. Similar effects of DPP10 on the modulation of Kv4.2 channels were observed in *Xenopus* oocytes (data not shown).

DPP10 Is Closely Related to DPPX—The DPP10 amino acid sequence shares 51% identity with DPPX compared with 32% identity with DPPIV (1). Phylogenetic analysis showed that DPP10 and DPPX form an evolutionarily divergent subfamily within the extended DPPIV-like family (1). Considering the similarity in function between DPPX and DPP10, we used primary sequence comparisons and protein modeling to further
characterize the structural similarities between DPP10 and DPPX and to identify possible regional determinants of Kv4 modulatory activity. Fig. 5A shows an alignment of the primary sequences of the transmembrane domains and immediate intracellularly neighboring residues, or juxtamembrane region, of DPPX, DPP10, and DPPIV. Conservative replacements and identical amino acids are shaded. DPPX and DPP10 are remarkably similar in these domains: DPP10 shares 92% similarity with DPPX compared with 41% similarity between DPP10 and DPPIV.

Fig. 5 (B–D) depicts the backbone structure of the extracellular domains of DPPX, DPP10, and DPPIV. The structures of the extracellular domains of DPPX and DPP10 were modeled using the crystal structure of the extracellular domain of DPPIV as a template (Protein Data Bank code 1N1M) (18). In Fig. 5B, the backbone of the extracellular domain of DPPX is aligned with that of DPPIV, whereas in Fig. 5 (C and D), the extracellular domains of DPP10 and DPPX, respectively, are aligned with that of DPPIV. The color in these alignments reflects the degree of conservation at each residue, using the root mean square deviation and 20 orderly colors of the B-factor palette (blue, most conserved; red, least conserved). All three proteins show a similar folding pattern. However, when the backbones of DPPX and DPP10 are superimposed, there is so little deviation in the predicted structures that, throughout most of the alignment, there appears to be a single backbone. This is contrasted with the alignment of DPPIV or DPPX and DPPIV shown in Fig. 5 (C and D), respectively, where deviation is much more evident. The modeling predicts a root mean square deviation of 0.69 Å for the extracellular domains of DPP10 and DPPIV and 1.29 and 1.49 Å for the extracellular domains of DPP10 or DPPX and DPPIV, respectively.

The extracellular portion of DPPIV contains two highly conserved domains: a β-propeller and an α/β-hydrolase fold. The repeating β-sheets of the β-propeller are oriented diagonally in the superior field of Fig. 5 (B–D), whereas the hydrolase domain is inferior. The similarities between DPPX or DPP10 and DPPIV (Fig. 5, C and D, blue) are distributed throughout the extracellular domain. However, the similarities between DPPX and DPP10 (Fig. 5B) are stronger in the β-propeller compared with the hydrolase domain. This pattern of sequence conservation is especially intriguing when considering that both DPPX and DPP10 are mutated in their catalytic site and inactive against DPPIV-specific substrates. Together, the results of Fig. 5 show strong structural similarity between DPP10 and DPPIV in the juxtamembrane, transmembrane, and β-propeller domains.

Transmembrane and Juxtamembrane Domains of DPPX and DPP10 Modulate Kv4.2 Channels—To begin investigating the contribution of different structural regions of DPPX and DPP10 to potassium channel modulation, we created chimeric proteins with extracellular domains replaced by a series of Myc tags (XXMyc, 1010Myc, and IVIVMyc) (Fig. 6A). The chimeras had complete intracellular and transmembrane domains, yet lacked the entire hydrolase domain and β-propeller. To test whether the expression of the chimeric proteins was efficient and stable, we cotransfected CHO cells with Kv4.2 cDNA and the cDNA of one of the chimeras and then treated the cells for immunohistochemistry with anti-Myc antibody. All the chimeras described here produced significant and stable Myc staining, indicating that the protein was efficiently expressed in the cultured cells (data not shown).

The ability of the chimeras to traffic Kv4.2 protein to the membrane was tested by two methods. CHO cells were transiently transfected with Kv4.2 cDNA or HA-tagged Kv4.2 cDNA and a single DPP or chimera. Intact (nonpermeabilized) transfected cells were biotinylated to label surface protein. The biotin-tagged protein was isolated using streptavidin beads, separated by electrophoresis, and transferred to nitrocellulose paper, and the blot was treated with anti-Kv4.2 or anti-HA antibody, thereby detecting the Kv4.2 protein that effectively trafficked to the cell surface. As shown in Fig. 6B, XXMyc and 1010Myc dramatically increased detection of surface Kv4.2 protein, resembling the effect of DPPX. The same population of transfected cells was also analyzed by electrophysiological recording. Depolarizing steps to 48 mV induced large transient currents when XXMyc or 1010Myc was cotransfected with Kv4.2 cDNA (Fig. 6C), consistent with the results from the biotinylation experiment.
Chimeras of DPPX and DPP10 were also able to modulate the kinetic and voltage-dependent properties of Kv4.2 channels (Fig. 7). For each parameter analyzed, the effects of XXMyc and 1010Myc were in the same direction and usually of the similar magnitude compared with the effects of the original DPPs; XXMyc and 1010Myc coexpression with Kv4.2 resulted in faster current rise, inactivation, and recovery from inactivation and produced a shift in the voltage dependence of activation to hyperpolarized potentials. These results suggest that the juxtamembrane and transmembrane domains are important in the trafficking and modulation of Kv4.2 channels. Furthermore, as the chimeras lacked the entire hydrolase domain, these experiments strongly support the contention that the trafficking and modulation of Kv4 channels by DPPX and DPP10 are not mediated by enzymatic activity.

Despite the robust activity of XXMyc and 1010Myc, for all parameters, the average effects of these chimeras were smaller in magnitude than those of DPPX and DPP10 (Figs. 6C and 7). Although in many cases these differences did not reach statistical significance, the consistency of this trend suggests a role for the extracellular domain, perhaps in stabilizing the Kv4-expressing neuronal populations known to express Kv4 products (10), including neurons in the cerebellum, hippocampus, thalamus, olfactory bulb, neocortex, and specific brain-stem nuclei (Fig. 8A). Two probes against DPP10 (DPP10-A and DPP10-B; see “Experimental Procedures”) were constructed, and both produced similar patterns of expression.

DPPX and DPP10 expression appeared to overlap in certain Kv4-expressing neuronal populations, such as pyramidal cells of the neocortex, and in the globus pallidus (Fig. 8, B and C) as well as within the thalamus and olfactory bulb. Interestingly, in other neuronal populations where Kv4 channels are prominent and A-type potassium currents have been extensively studied (10, 11), neurons predominantly expressed either DPPX or DPP10. For example, DPPX expression was notable in the principal cells of the hippocampus, including CA1–CA3 pyramidal cells and granule cells of the dentate gyrus (Fig. 8, D and F). In the same population of neurons, DPP10 was weakly expressed (Fig. 8, E and G). In contrast, within the hippocampus, DPP10 was strongly expressed in GABAergic interneurons (Fig. 8, E and G). Similarly, DPPX was expressed in the neurons of the caudate/putamen and in cerebellar granule cells (Fig. 8, B and H), where DPP10 expression was weak (Fig. 8, C and I).
was strongly expressed, however, in cerebellar Purkinje cells (Fig. 8F), where DPPX expression was weak (Fig. 8H).

**DPP10 Associates with Kv4.2 Proteins in Brain Membranes**—Considering that DPP10 protein alters the functional properties of Kv4 channels in vitro and that DPP10 and Kv4 genes are expressed in overlapping neuronal populations, we next investigated whether DPP10 and Kv4 proteins associate in neuronal membranes. We used antibodies against Kv4.2 proteins cross-linked to protein A-Sepharose 4B beads to immunoprecipitate Kv4 channel complexes from nondenaturing detergent extracts of rat whole brain membranes. Immunopurified products were eluted from the beads, dissociated, and separated by SDS-PAGE.

Fig. 9 shows immunoblots of the proteins immunoprecipitated with anti-Kv4.2 antibody and stained for Kv4.2, DPPX, and DPP10. In addition to immunoprecipitating Kv4.2 (Fig. 9A) and DPPX (Fig. 9B), as previously observed with cerebellar membrane extracts (9), anti-Kv4.2 antibody also immunoprecipitated DPP10 from whole brain membrane extracts (Fig. 9C). This indicates that both DPP10 and DPPX are components of native Kv4 channel complexes in the brain. Kv4.2, DPPX, and DPP10 proteins were not recovered when the immunoprecipitation was conducted in the presence of excess antigenic peptide or with control beads lacking anti-Kv4.2 antibody (Fig. 9, A–C, lanes 3 and 4). Anti-DPP10 antibody, although useful for immunoblotting, was unsuccessful in immunoprecipitations, and therefore, we could not conduct the reciprocal experiment to demonstrate recovery of Kv4.2 protein from immunoprecipitates using anti-DPP10 antibody.

The presence of DPP10 proteins in Kv4.2 channel complexes immunopurified from whole brain membranes with anti-Kv4.2 antibody was also demonstrated by sequencing Kv4.2-associated proteins using tandem mass spectrometry. Fig. 9D shows a silver-stained gel of an immunopurified Kv4.2 sample. A peptide with the sequence ILAYDETTQK, corresponding to DPP10, was found in the indicated band. This band corresponds to the smaller and main band observed in immunoblots treated with anti-DPP10 antibody (Fig. 9C).

**DISCUSSION**

In this study, we have demonstrated that a recently cloned DPPIV-like protein known as DPP10 is able to modulate the activity of Kv4.2-mediated A-type K⁺ channels in mammalian cells and Xenopus oocytes. The effects of DPP10 were strikingly similar to those of the related protein DPPX, otherwise known as DPPIV. In contrast, DPPIV had much less pronounced effects on Kv4-mediated A-type currents. DPPIV and DPP10 compose a distinct phylogenetic subfamily among DPPIV-like proteins (1). Our results suggest that DPPX and DPP10 also compose a functional subfamily with the unique ability to effectively modulate Kv4-mediated currents.

DPP10 is prominently expressed in the brain (1, 6), notably present in neuronal populations that also express Kv4 proteins (Fig. 8), associates with native Kv4.2 channels in brain membranes (Fig. 9), and therefore likely contributes to the molecular composition of A-type currents in the central nervous system. Accordingly, DPP10 joins DPPIV and KChIPs as putative Kv4 channel-associated proteins. Subthreshold-activating, somatodendritic A-type K⁺ currents have fundamental...
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DPPX and DPP10 are associated with Kv4.3 channels. They are found in the subthreshold range of membrane potentials, fast inactivation, fast recovery from inactivation, and enrichment in somatodendritic neuronal membranes. DPP10 and DPPX are particularly interesting in that they modulate the kinetic properties and shift the voltage dependence of Kv4 channels such that the resultant current resembles more closely the A-type currents recorded in native neurons.

DPPX and DPP10 also facilitate the trafficking of Kv4.2 proteins to the plasma membrane. This effect likely contributes to the increase in A-type current magnitude observed when Kv4.2 is coexpressed with DPPX or DPP10. In addition, DPPX has been shown to increase the single channel conductance of Kv4 channels expressed in Xenopus oocytes (29). It is likely that this effect also contributes to increasing current density. It remains to be studied whether DPP10 also modulates Kv4 single channel conductance.

We compared the expression patterns of DPPX and DPP10 in the brain by in situ hybridization. Although expression seemed to overlap in some neuronal populations, other neuronal populations expressed predominantly DPPX or DPP10. This was most obvious in the principal cells of the hippocampus, in neurons in the caudate/putamen, and in cerebellar granule cells expressing predominantly DPPX compared with the GABAergic interneurons of the hippocampus and cerebellar Purkinje cells expressing mainly DPP10. This distribution is more interesting considering that Kv4.2 and Kv4.3 share a similar reciprocal distribution in these populations, with Kv4.2 present in the same regions as DPPX and Kv4.3 co-localizing with DPP10 (10, 11). This could explain why we did not detect DPP10 in immunopurified Kv4.2 channels from cerebellar membranes in our last study (9). Furthermore, these results raise the possibility that, in neurons expressing predominantly the Kv4.3 isoform, DPP10 and not DPPX is the main associated protein.

DPP10 and DPPX share nearly identical juxtamembrane and transmembrane sequences and a similar β-propeller structural motif. Interestingly, there is somewhat more divergence within the hydrolase domain (Fig. 5). Both DPPX and DPP10
have mutated catalytic sites, probably compromising the serine peptidase activity fundamental to the larger S9B prolyl oligopeptidase family (1, 14). The sequence divergence within the hydrolase domain may reflect an evolutionary drift of DPPIV-like proteins that no longer function as enzymes.

The chimeric proteins of DPPX and DPP10, consisting of only the DPP intracellular and transmembrane domains and six extracellular Myc tags, modulated Kv4 channels in a similar manner to the complete DPP proteins (Figs. 6C and 7). These chimeras lacked the entire catalytic domain, confirming that even if DPPX and DPP10 are enzymes, the ability to modulate Kv4 channels is not mediated by catalytic activity. The results also suggest that DPPX or DPP10 protein interacts with Kv4.2 subunits through the transmembrane and/or juxtamembrane domain and that these interactions within the membrane are important in modifying channel structure. Interestingly, the chimeric protein based on DPPIV (IVIVMyc) was inactive as a Kv4 modulatory protein. The efficacy of XXMyc and 1010Myc and the lack of activity of IVIVMyc argue for the importance of specific residues in the transmembrane and juxtamembrane domains in determining functional interactions with Kv4 proteins.

The activities of DPPX and DPP10 were greater in magnitude than those of XXMyc and 1010Myc, respectively, for every parameter analyzed. The lesser activity of all chimera compared with that of the original DPPs suggests that the extracellular domain also contributes to their association with Kv4 subunits. The extracellular domain may stabilize the transmembrane and intracellular protein-protein interactions. A role for the extracellular domain is also supported by the observation that DPPIV had modest effects on Kv4.2 channel function, all of which were lost upon removal of the extracellular domain.

DPP10 was recently identified as a candidate gene for asthma (6). The function of DPP10 in airway physiology and its dysfunction in asthma remain to be studied. However, in cells coexpressing DPP10 and Kv4.2, down-regulation or dysfunction of DPP10 would be predicted to decrease the magnitude of the A-type K+ current, thereby increasing the excitability of the cell membrane. DPP10 is expressed in the trachea at moderate levels (6), and A-type currents are present in many smooth muscle types (30). It will be of interest to investigate whether there are changes in A-type K+ currents in airway tissues from asthmatic patients.

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Note Added in Proof—While this paper was under review, Strop et al. (Strop, P., Bankovich, A. J., Hansen, K. C., Garcia, K. C., and Brunger, A. T. (2004) J. Mol. Biol. 343, 1055–1065) published a crystal structure for the extracellular domain of DPPX. This structure confirms the predictions of our model. In addition, Jerng et al. (Jerng, H. H., Qian, Y., and Pfaffinger, P. J. (2004) Biophys. J. 87, 2380–2396) published a paper on the effects of DPP10 proteins on Kv4 channels expressed in Xenopus oocytes. The effects reported in that study are qualitatively similar to those reported here in mammalian cells.

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