Antibody-based Validation of CNS Ion Channel Drug Targets

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Target validation and selection are critical early steps in the drug discovery process as they trigger substantial activity and investment to identify potential drug candidates. Antibodies play a crucial role in target selection as they provide powerful tools to explore the distribution and subcellular location of candidate molecules and can also be used to explore function. For studies of ion channel targets, antibodies are indispensable as they allow for direct exploration of the subunit composition of channel complexes in native tissues using biochemical and anatomical methods. This perspective summarizes the application of antibodies to target characterization and validation within the context of a CNS therapeutic discovery program targeting voltage-gated K^+ channels. We also discuss a strategy for the development, characterization, and validation of high-quality antibody reagents to support key activities in drug discovery.

Ion Channels/Drug Discovery/Immunohistochemistry/Brain/Epilepsy

Target selection is perhaps the most critical step in early drug discovery. This seminal event—deciding which target to pursue, its level of "validation" for a specific medical condition and whether to pursue it with a small or large molecule, agonist or antagonist, etc.—triggers a number of downstream activities requiring substantial investments of time, personnel, and financial resources. The processes and events leading up to ion channel target selection are complex and vary widely across the pharmaceutical/biotechnology industries and academia. However, once an ion channel target has been selected, the approaches for its rigorous characterization are common, requiring a systematic, multidisciplinary effort spanning genetics, anatomy, biochemistry, cell biology, and electrophysiology. Although all candidate drug targets require detailed characterization in order to be pursued effectively, multisubunit ion channels are among the most challenging and, owing to the array of cell types, diversity of channel phenotypes and highly polarized neuronal architecture, CNS channels are particularly daunting. The aim of this Perspective is to describe some of the challenges to ion channel target characterization and selection for drug discovery, based on experiences within CNS drug discovery programs focused on neuronal voltage-gated K^+ (Kv) channels. A cornerstone of these programs was the development of specific, extensively characterized antibody reagents that could be used to generate detailed information on the distribution, subcellular localization, and molecular composition of individual channel complexes, as a basis for target selection and to help identify potential therapeutic applications for subtype-selective channel modulators (Rhodes et al., 1996, 1997, 2004; Bowlby et al., 2005).

Targeting Kv channels in the CNS is an extraordinarily challenging endeavor. In the ideal paradigm, one could select a Kv channel for drug discovery based on knowledge of its biophysical and pharmacological properties, association with specific neurotransmitter systems and mode of dysregulation in CNS disease. This information, coupled with an understanding of the channel's subunit composition, would be used to construct a cell line expressing the component subunits that would be used to identify and characterize molecules that modulate channel activity. Knowledge of the channel's anatomical distribution would guide selection of in vitro and in vivo pharmacology models, which would then be used to explore the consequences of channel modulation and further elucidate potential therapeutic utility. The anatomical and in vivo data might also reveal potential adverse consequences associated with modulating the target channel's activity.

Nevertheless, the process is rarely this simple. Although it is possible to use conventional electrophysiological recording techniques to characterize the basic biophysical and pharmacological properties of many neuronal Kv channels, the combinatorial assembly of voltage-sensing and pore-forming Kv α subunits, and their associated cytoplasmic Kvβ subunits, leads to formation of an incredibly diverse array of channel types (Jan and Jan, 1997). This heteromultimeric assembly of subunits makes it extremely challenging to define native Kv currents in terms of component subunits that can then be expressed in heterologous cells to support

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Abbreviations used in this paper: BKα, BK channel α subunit; KChIP, Kv channel interacting protein; Kv, voltage-gated potassium.

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drug screening. Adding further complexity is the observation that some Kv channels are targeted to distal dendrites, some are clustered along myelinated axons, and others are concentrated at nerve terminals (Trimmer and Rhodes, 2004)—all locations that are challenging to access by conventional electrophysiological recording and therefore difficult to study at a biophysical or pharmacological level. Finally, in cases where the native current can be characterized, it is not currently possible to recapitulate the subunit composition or stoichiometry of the native channel in a heterologous expression system. In combination, these challenges force a compromise between what we know about the native channel and what we can practically achieve in a cell-culture system. Nevertheless, for the purposes of screening and lead identification and drug discovery, knowledge of anatomical distribution and subunit coassociation, based on anatomical observations and coimmunoprecipitation analyses, provides a good place to begin the process.

When we began our work on the pharmacology of K^+ channels in the early 1990's, little was known about the physiology, biochemistry, and pharmacology of the native proteins. Initial reports identifying cDNAs and characterizing the conductance properties of Kv channel α subunits of mammalian Kv channels had just appeared (e.g., Chandy et al., 1990) and efforts were underway in many laboratories to correlate the expression of mRNAs encoding individual subunits with currents that had been recorded from cultured neurons or brain slices (e.g., Koch et al., 1997). However, as these data emerged it became clear that there was not a 1:1 correlation between the properties of Kv channel α subunits expressed individually in heterologous cells and the properties of currents recorded in native systems. In addition, because neurons are highly polarized cells, it was quickly appreciated that expression of mRNA at the soma was not informative as to the subcellular location of the encoded protein and therefore could not provide insight into the potential role of the channel in neuronal physiology. As individual neurons were shown to express many distinct Kv subunits, it also became clear that mRNA localization had limited value as a tool to correlate subunit expression with Kv channel currents. Reagents to definitively identify protein subunits and determine their abundance and distribution were required.

Presented with this challenge, we decided to use a comprehensive immunological and neuroanatomical approach that relied on an intensive effort to generate and characterize subunit-specific mouse monoclonal and rabbit polyclonal antibodies (hereafter referred to as mAbs and pAbs, respectively) targeting individual channel subunits. We used these antibodies as tools to elucidate the subunit composition of Kv channels, reveal their subcellular distribution, and determine their association with specific neurotransmitter systems in mammalian brain. Although antibodies can play a critical role in all aspects of target validation for drug discovery, they are uniquely suited for studies of Kv channels owing to their potential for specificity, ability to work across a range of assay platforms, and the potential that the antibody reagents themselves may serve as pharmacological tools to characterize channel activity. However, the use of poorly characterized antibodies that do not have the requisite specificity for the target antigen or have not been fully validated for use in the intended application (e.g., immunohistochemistry, immunoprecipitation) has led to perpetuation of conflicting and at times erroneous information. Although we recently documented steps that investigators can use to validate antibody reagents as tools to neuroscience discovery (Rhodes and Trimmer, 2006), some of those points are worth restating here.

The generation and validation of antibodies with the desired efficacy and specificity has many parallels to the drug discovery process. The process involves selecting a specific immunogen (i.e., the molecular entity used to immunize the source animal for antibody production). In the post-genomic era, these generally comprise synthetic peptides or recombinant protein fragments, as opposed to subcellular fractions or proteins purified from native tissue. The design of immunogen, whether it be a synthetic peptide or recombinant protein, greatly impacts the downstream in vivo events that lead to the generation of a humoral immune response and the characteristics of antibody-producing cells and antibodies, and the subsequent screening and purification steps required before the antibody reagents can be applied to target validation studies. While the design of immunogens is guided by considerations of the complexities of MHC-mediated antigen presentation and other immunological considerations, an overriding consideration is whether the sequence used is likely to generate an antibody specific for the target. For neuronal ion channel subunits, this can be a challenge as they are typically members of a large family of highly related polypeptides. The goal is to find a sequence that satisfies general considerations as to its potential to be on the solvent-accessible surface of the target protein's three-dimensional structure, not present on other ion channel family members, and if possible, on any other protein in the mammalian proteome. For pAb production, this can usually be accomplished with a synthetic peptide, whose optimum size for immunogenicity and ease of synthesis ranges between 15 and 22 amino acids. Recombinant protein fragments are generally larger, given the practical difficulty of working with small cDNA fragments needed to generate peptide-sized recombinant proteins. The larger recombinant proteins typically generate a more robust and varied humoral immune response, yielding antibodies that work well across many

applications, but are more difficult to design for specificity of the resultant antibodies against the target of choice without potential for cross-reactivity against related family members. If financially and technically feasible, it is advantageous to undertake both approaches in parallel.

A similar consideration holds for the decision to make pAbs versus mAbs. A phenomenal antibody of either kind is invaluable, poor versions of either can be problematic to the point of being virtually useless, or even worse, incredibly misleading. That said, there are a number of factors that can go into the choice of whether an intended project should be via the pAb or mAb route, although initiating both in parallel, as we did in our studies of Kv channels in CNS, greatly increases the probability of the ultimate goal, having a reliable reagent in hand for use in target validation. As far as the considerations, pAbs are present in antiserum obtained from the immunized animal and generally need to be purified from the serum before use. This can be a significant impediment to obtaining a monospecific pAb that is simply not a factor for mAbs. The method of choice for purifying pAbs is affinity purification against the immunogen, as this allows for separation of specific antibodies, which represent a minority of the total immunoglobulins in serum, from the excess of nonspecific yet biochemically-similar immunoglobulin molecules present. For mAbs the monoclonal immunoglobulin is the only or predominant immunoglobulin in the preparation and can be effectively purified by standard protein biochemistry procedures (e.g., ammonium sulfate precipitation, ion exchange, or size exclusion chromatography). Note that these steps are often employed as preliminary steps in affinity-based purification of pAbs. In the case of pAbs raised against recombinant protein immunogens, the affinity purification itself may require more than one step, as the immunogenic sequence may have segments specific to the desired target and others representing sequences conserved in other family members. A two-step affinity approach can lead to a selective enrichment of the former and depletion of the latter and yield a specific antibody preparation. An alternative approach is to use the larger recombinant fragment for immunization, and a smaller fragment (recombinant or synthetic) representing the isoform-specific region for affinity purification. While similar considerations are involved in choosing immunogens for mAb projects, there is substantially more flexibility in immunogen design/choice, as downstream selection by strategic screening can identify and eliminate off-target or cross-reacting mAbs.

Another consideration when choosing pAb versus mAb projects is the potential advantage of having a heterogeneous mixture of monospecific antibodies in pAb preparations. The presence of a diverse repertoire of monospecific antibodies with different characteristics can yield a single "all-purpose" reagent that can be applied to diverse immunohistochemical and biochemical procedures. While it is possible to obtain an all-purpose mAb, there are numerous examples of mAbs whose utility is restricted to a subset of (or even a single) applications, for example mAbs that work for immunoblotting or immunoprecipitation but not for immunohistochemistry. Within the heterogeneous collection of antibodies in a pAb preparation may include those that have restricted utility, but the presence of a diverse repertoire can allow for coverage of all intended applications.

An effective strategy for obtaining all-purpose mAbs is to start with a large pool (e.g., 50–100) of positive mAbs that have been identified as binding to the immunogen, for example in an ELISA assay. This increases the likelihood that within this pool will be a mAb that fills all needs. Generating large pools of positive mAbs is generally a reflection of the effectiveness of the immunization procedure. Our experience from ≈200 mAb projects is that recombinant protein fragments generate a more robust immune response than do synthetic peptides, and this yields a larger pool of ELISA-positive clones that can then enter the subsequent validation process. This is especially crucial for projects aimed at obtaining reliable mAbs for biochemistry and immunohistochemistry in native tissue. While most of the ELISA-positive clones will work fine in recognizing the overexpressed target protein in heterologous expression systems (e.g., transfected cells), the number that exhibit efficacy and monospecificity in native tissues is generally much lower. An effective multistep screening strategy takes advantage of the high levels of expression of the target protein in transfected cells versus low or absent expression in untransfected cells in immunocytochemical and immunoblot assays as an initial confirmation that ELISA-positive clones recognize the target protein of interest. However, there is no substitute for rigorous and comprehensive assays in the relevant native tissue expressing the endogenous target, as this is where antibody-based target validation will be pursued.

Unlike transfected cells where expression can be manipulated by the investigator, and specificity easily confirmed by comparing transfected versus untransfected cells, validation of specificity in native tissue, especially those with the complexity of mammalian brain, can be difficult. As we reviewed previously (Rhodes and Trimmer, 2006), an effective approach is to compare patterns of mRNA expression derived from in situ hybridization analyses with the immunohistochemical staining pattern obtained using antibody preparations. This can yield a pattern of cellular expression of a particular target mRNA that can be used to validate cellular specificity of antibody immunoreactivity. The brain offers unique challenges when applying this approach, as axonally localized proteins can be found at sites distant from the

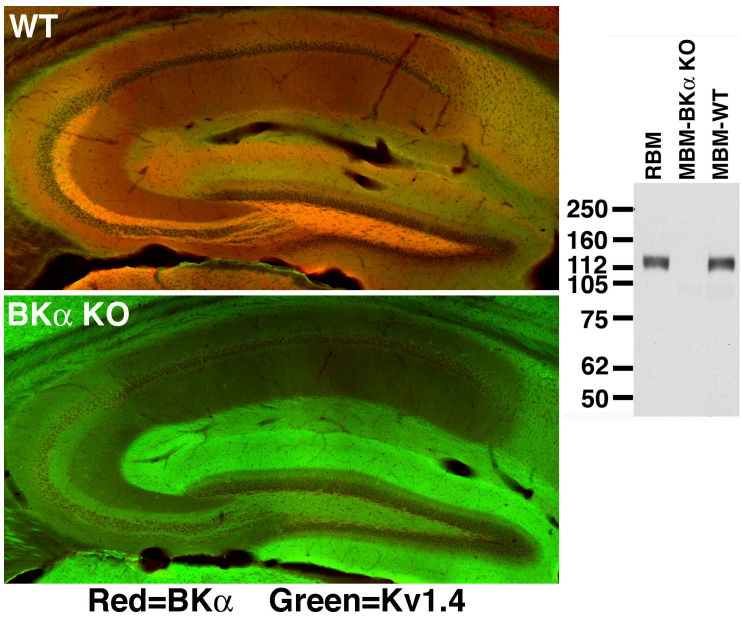

Figure 1. (Left panels) Double immunofluorescence staining of brain sections from wild-type and BKα knockout mice. Image shows the hippocampal field stained with mouse monoclonal anti-BKα L6/60 and anti-Kv1.4 K13/31 antibodies. Isotype-specific secondary antibodies were used to visualize bound monoclonal antibodies. Right panel shows an immunoblot performed with L6/60 on crude brain membranes from rats (RBM) and from wild-type (MBM-WT) and BKα knockout (MBM-BKα KO) mice.

cell bodies that contain the mRNA. However, knowledge of brain anatomy and neuroanatomical connections is required to evaluate whether the mRNA expression patterns and immunostaining are consistent with one another. More recently, knockout and transgenic animals, typically mice, have emerged as an invaluable tool for validation of antibody specificity in native tissue. When available, a comparison of immunoreactivity in knockout and wild-type tissue by biochemical and immunohistochemical approaches can allow for definitive conclusions as to the specificity of a given antibody signal. An example of such an analysis is shown in Fig. 1, where sections from wild-type and BK channel α subunit (BKα) knockout mice (Meredith et al., 2004) were stained with anti-BKα (L6/60, red; Misonou et al., 2006) and anti-Kv1.4 (K13/31, green; Bekele-Arcuri et al., 1996) mAbs. The specificity of L6/60 staining to BKα in the hippocampus is evident by the lack of appreciable red signal in the section from the knockout animal. Fig. 1 also shows representative immunoblot staining using L6/60 against crude brain membranes prepared from rats, and from knockout and wild-type mice. Such experiments can provide the investigator with a high level of confidence in the specificity of a given antibody in native tissue. Similar approaches can be used for validation of antibody specificity in other applications (e.g., immunoprecipitation, immunoelectron microscopy). Knockdown approaches using siRNA can provide a similar confirmation. One caveat to the unfettered use of tissues from knockout animals for validation of antibody specificity is highlighted by our unexpected finding that genetic deletion of one subunit of a multisubunit ion channel complex led to a dramatic down-regulation of the expression of other subunits in the complex. Staining of brain sections from Kv4.2−/− mice revealed that immunoreactivity for the Kv channel interacting protein (KChIP) accessory subunits was dramatically reduced in regions where they associate with Kv4.2, but not where they associate with Kv4.3 (Menegola and Trimmer, 2006). For example, in hippocampi of Kv4.2−/− mice KChIP2 was down-regulated dramatically in CA1 neurons, where it is normally found coassociated with Kv4.2. We have not found such effects in the Kv1.1 knockout mouse (Wenzel et al., 2007). In general, incorporation of knockout mice into antibody validation procedures is a definitive method of demonstrating specificity. However, as with other aspects of the drug discovery process, there remains a need to use this as only one (albeit compelling) step in a comprehensive and systematic multipronged validation process (Rhodes and Trimmer, 2006).

Together, such a systematic approach to the generation and validation of antibody reagents can provide an investigator with a solid foundation to target validation in drug discovery. However, the reality is that drug discovery efforts are often undertaken by groups without experience and expertise in these approaches, and where establishing such expertise is not deemed to be a justified investment in time, and in financial and human resources. In this case commercial antibody companies offer an attractive source of antibodies against a wide array of potential drug targets. Such companies can have huge antibody catalogs (one recently advertised antibodies against 31,000 different targets!) such that it is likely that an antibody against your intended drug discovery target exists in the commercial sphere. Moreover, these reagents are available to the end user without any intellectual property strings attached, which may not be the case with antibodies from other sources (e.g., academic laboratories). However, as with

all transactions, the phrase caveat emptor applies. While it is the obligation of the supplier to make an honest effort to validate any commercial antibody to the best of their ability, and to make absolutely clear to the end user what validation was performed, it is unreasonable to expect that a antibody company could possibly validate their products against anything but a small number of standard applications and preparations. That said, these should include determination of specificity against the endogenously expressed target in native tissues, and not simply assays against overexpressed exogenous target in heterologous cells. In this case companies focused on a single target area (e.g., neuroscience) may be better able to provide more extensive validation in specific tissue type or application than a company with a broader focus.

To what extent the individual end-user should pursue additional validation is a matter of much discussion. It is unreasonable that after paying the high mark up that is typical of commercial antibody suppliers that an investigator should have to spend additional funds and effort on "quality control" experiments that should have been done during antibody generation and validation, and before distribution. That said, should a reliable antibody be necessary for key experiments aimed at evaluating a target for a long-term drug discovery program, the financial repercussions could be enormous. The same care and attention to detail that goes into arguably more familiar steps in the subsequent preclinical (pharmacology, medicinal chemistry, pharmacokinetics, drug metabolism, cytochrome P450 interactions, etc.) and clinical (safety, efficacy, etc.) drug discovery process also apply to antibody-based target validation. Our perspective is that having in-house expertise in antibody-based target validation, including generation of reliable antibody reagents and their effective use in immunohistochemical and biochemical analyses of the expression, localization, and coassociation of target ion channel subunits, is a justified investment as it represents a crucial first step in an overall drug discovery process that may in total last many years and costs millions of dollars.

An example of this approach involving CNS ion channel drug targets comes from our studies of Kv channels. With well-validated antibody reagents in hand (Bekele-Arcuri et al., 1996), we were able to first perform an extensive series of immunohistochemical studies aimed at localizing individual Kv channel subunits to specific cell types and neuroanatomical circuits within the mammalian brain, with the goal of selecting specific channel types/complexes for our drug discovery programs. Initially, we focused our attention on Kv1 channel subunits largely because selective pharmacological tools (e.g., the dendrotoxins) were already available to study this ion channel family. Pharmacological evidence indicated that application of α-dendrotoxin to block Kv1 channels in hippocampal slice preparations facilitated action potential propagation and neurotransmitter release (Halliwell et al., 1986). These observations suggested that selective blockers of Kv1 channels might have therapeutic application in disorders characterized by impaired action potential propagation, such as multiple sclerosis, or reduced neurotransmitter release, such as Alzheimer's disease. However, we were also interested in Kv1 channels because of evidence that dramatically reduced activity of these subunits, by genetic mutation/deletion of individual subunits (Smart et al., 1998) or by application of high toxin concentrations, evoked seizure activity (Bagetta et al., 1992). This latter result indicated that "openers" or activators of Kv1 channels might have utility as anti-epileptic or perhaps even anxiolytic agents. However, at the outset of this work we did not know which of the dendrotoxin-sensitive Kv1 channels to target or which neurotransmitter systems might be affected through their modulation.

Upon generation of subunit-specific antibodies for Kv1 α and Kvβ subunits, we were able to use single-and multiple-label immunohistochemical methods to determine that these subunits are predominantly localized along axons, where they are concentrated at nodes of Ranvier and in the preterminal axonal segments and axon terminals of glutamatergic and GABAergic pathways within the CNS (Rhodes et al., 1995, 1996, 1997). In these locations, Kv1 channels are exquisitely positioned to regulate action potential propagation and neurotransmitter release. By using a strategy combining immunohistochemistry, immunoprecipitation, and selective neurotoxin lesions, we demonstrated that channels containing Kv1.1, Kv1.2, Kv1.6, and Kvβ2 were concentrated at nodes of Ranvier and were also present along unmyelinated axons and at axon terminals in many brain regions. Within the hippocampal formation, channels containing Kv1.1, 1.2, Kv1.4, and Kvβ1 subunits were concentrated along axons and in terminal fields of the perforant path, mossy fiber pathway, and Schaffer collateral pathways, where they presumably regulate glutamatergic transmission within the trisynaptic hippocampal circuitry (Monaghan et al., 2001). In biochemical analyses employing coimmunoprecipitation of associated channel subunits and subsequent analyses by immunoblotting, we determined that in the sites where immunohistochemical staining for Kv1 α and Kvβ subunits overlapped, the individual subunits were coassociated in heteromultimeric channel complexes (Rhodes et al., 1995, 1996, 1997). Although these and other studies provided evidence for a 1:1 association of Kv1 α and Kvβ subunits within Kv1 channel complexes (e.g., channels are $\alpha_4\beta_4$ hetero-octamers), these studies would not allow for a determination of the precise stoichiometry of the individual Kv1 α or Kvβ subunits within the channel complexes. Nevertheless, by comparing the distribution and coassociation of individual Kv1

subunits in vivo with the results of electrophysiological studies that examined the currents produced by coexpression of individual subunits in heterologous cells, we predicted that the currents formed by Kv1.1/Kv1.2/Kvβ2 subunits, in the absence of Kv1.4 or Kvβ1, would have a delayed rectifier phenotype and high sensitivity to peptide toxins including α-dendrotoxin (Trimmer and Rhodes, 2004). These channel complexes would be interesting targets for channel blockers where the goal is to facilitate conduction of action potentials and neurotransmitter release. Channels containing Kv1.4 and/or Kvβ1 in addition to other Kv1-family α subunits would show less sensitivity to α-dendrotoxin and would have a rapidly inactivating phenotype. Based on their high densities within excitatory limbic circuits, we reasoned that activators or "openers" of these channels might dampen excitability within these pathways and therefore have potential therapeutic utility in treating epilepsy or anxiety disorders. Although a comprehensive discussion of the subsequent drug discovery strategy and outcome is well beyond the scope of this perspective, by expressing combinations of Kv1 α and Kvβ subunits in heterologous cells (CHO cell lines, *Xenopus* oocytes, or yeast), we were able to screen for small molecule modulators of Kv1 channels and successfully identify blockers of delayed-rectifier type Kv1 channels, and compounds that slowed inactivation conferred by the Kvβ1 (Zhang et al., 2004). This latter class of compounds, termed "disinactivators," showed intriguing in vivo pharmacological properties, having antiepileptic activity in rodent seizure models and anxiolytic activity in rodent models of behavioral conflict (Zhang et al., 2004).

In subsequent studies in our laboratories, we took a similar approach but this time focused on Kv4 channels. At the time we initiated these studies, it was clear that Kv4 channels played an important role in regulating dendritic excitability in the CNS and that nonselective inhibitors of Kv4 channel activity modulated synaptic strength within the hippocampal formation and neocortex (Hoffman et al., 1997). We predicted that modulators (inhibitors) of these channels might facilitate long-term synaptic potentiation and provide a novel therapeutic approach to the treatment of Alzheimer's disease and other disorders characterized by age-related cognitive impairment. However, at the time we began this work, the subcellular distribution and subunit composition of native Kv4 channels was not well established. Using an approach similar to the one described above for Kv1 channels, we generated subunit-specific antibodies for Kv4 α subunits and also identified and characterized a family of accessory "β" subunits (KChIPs) for the Kv4 channel family (An et al., 2000). Using the same combination of immunohistochemistry, coimmunoprecipitation, and selective neurotoxin lesions, we determined that the Kv4.2 subunit is concentrated in the somatodendritic domain of many neuronal types, where it is found in the apical dendritic arbors of hippocampal and neocortical pyramidal cells, and in the dendrites of striatal projection neurons and cerebellar granule cells (Rhodes et al., 2004; Strassle et al., 2005). In these locations, Kv4.2 coassociates predominantly with KChIP2 and/or KChIP3. Although Kv4.3 is also concentrated in the somatodendritic domain of central neurons, its distribution only partially overlaps with Kv4.2. Rather, Kv4.3 immunoreactivity is concentrated in the apical dendritic arbors of specific populations of cortical and hippocampal pyramidal cells, and in the dendritic arbors of cortical, hippocampal, and striatal interneurons, where it colocalizes and coassociates with KChIP1 (Rhodes et al., 2004). Owing to the challenges associated with developing subunit-specific Kv channel blockers and the relative lack of selective pharmacological tools for evaluating Kv4 channels, we pursued a strategy to modulate the effects of KChIPs on Kv4 currents. As KChIPs enhance Kv4 channel activity by increasing surface expression, slowing inactivation and slowing the rate of recovery from inactivation, we reasoned that a KChIP modulator would effectively block or inhibit Kv4 channel activity. Although screening for small molecule inhibitors of a protein:protein interaction is challenging, this program led to the identification of molecules that bind to KChIP1 and modulate its effects on Kv4 channel activity (Bowlby et al., 2005). The therapeutic potential of these molecules is still under investigation.

Overall, the availability and use of high-quality antibody reagents adds tremendous value to the target validation process in drug discovery. For multisubunit, heteromeric ion channel targets in brain, these reagents are indispensable tools in the processes of target selection, characterization, and validation. Over the years, many of the assumptions we made about the therapeutic potential of subunit or subtype-specific channel modulators, based on anatomical localization and subunit coassociation of the target subunits as determined by immunohistochemistry and coimmunoprecipitation, were born out by the pharmacological tools that were identified through screening and subsequent characterization. The example set by this process of target identification and validation, based on a powerful combination of anatomical and immunological methods, has broad application within academic and industry drug discovery settings. The further application of these methods to studies of human brain tissue samples should facilitate development of more effective translational strategies in ion channel drug discovery.

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