Protein Kinase D as Novel Therapeutic Target for Neurological Disease

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SUMMARY
Protein kinase D (PKD) is a novel kinase whose catalytic domain resembles calcium calmodulin kinase and its regulatory domains resemble protein kinase C. We have discovered that (1) PKD is dually regulated by the NMDA and metabotropic glutamate receptors (mGluRs). In turn, PKD regulates AMPAR recycling, especially the GluR2 subunit. (2) We have found that CNS-specific PKD1 deficient mice are viable and breed normally, but have a selective deficit in one form of synaptic plasticity known as long-term depression (LTD). LTD is believed to be important for AD and addiction. (3) It forms a pathway that mediates mGluR regulation of histone deacetylases and neuronal gene regulation. In particular, we found it is required to regulate a gene that is known to be critical for learning and memory and that is dysregulated in AD. (3) We have also discovered that PKD is abnormally activated in mouse models of Alzheimer’s disease (AD) and Huntington’s disease. (4) We have found novel small molecule inhibitors of PKD in neurons. We expect that PKD plays important roles in mediating activity-dependent adaptive responses in the nervous system. As such, it may be a therapeutic target for epilepsy, AD, and other neurological disorders.

APPLICATIONS
♦ Novel methods of treating neurological diseases and neurological manifestations of human CNS diseases, ranging from Alzheimer’s disease to epilepsy

ADVANTAGES
♦ The invention relates to the utility of PKD as a therapeutic target and the discovery of small molecules that inhibit PKD in neurons. Although PKD has been studied to some extent in lymphocytes and in myocytes, it is virtually unstudied in neurons. As such, safe and effective small molecule modulators of PKD would have the potential to be first-in-class therapies for neurological disorders.
♦ Small molecules have the advantage of the potential for high receptor activity, target enzyme selectivity, cell permeability, blood-brain barrier permeability, not eliciting immune responses, oral bioavailability, enhanced metabolic stability and the capacity for relatively cost effective large-scale manufacturing.

BACKGROUND OF INVENTION

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Synaptic plasticity depends on the dendritic trafficking of AMPA-type glutamate receptors (AMPARs) (Isaac et al., 2007; Kennedy and Ehlers, 2006). The localization of AMPARs can be dynamically regulated by both NMDA-type glutamate receptors (NMDARs) and group I metabotropic-type glutamate receptors (mGluRs) (Shepherd and Huganir, 2007). Calcium influx through NMDARs triggers signaling cascades that regulate both removal and insertion of AMPARs from the synapse (Kennedy and Ehlers, 2006). mGluRs are Gαq-coupled, seven transmembrane receptors that control synaptic strength both by initiating internalization of AMPARs (Shepherd and Huganir, 2007) and by promoting specific subunit expression at synaptic sites (Mameli et al., 2007). While much work has been done to understand the mechanisms and molecules by which NMDARs and mGluRs regulate the localization of AMPARs, they remain incompletely understood.

The novel kinase protein kinase D1 (PKD1) is likely to be involved in this process. It plays an important role in protein trafficking and has diverse cellular functions in multiple subcellular compartments including the trans-Golgi network (TGN), the nucleus, and the mitochondria (Wang, 2006a). Since PKD1 has numerous targets within cells, its localization to subcellular domains plays a key role in its regulation. PKD1 has several structural domains that target it to specific sites (Baron and Malhotra, 2002; Jamora et al., 1999; Sanchez-Ruiloba et al., 2006), and dynamic translocation of PKD1 has been demonstrated in response to stimulation (Rozengurt et al., 2005). Activation of PKD1 usually depends on phosphorylation by PKC isoforms downstream of Gαq signaling (Rey et al., 2004). While PKD1 is highly expressed in brain, little is known about its localization or regulation in neurons.

The function of PKD1 in protein trafficking has been well established. In several cell types, including neurons (Sanchez-Ruiloba et al., 2006), dominant-interfering versions of PKD1 prevent trafficking of proteins from the TGN to the plasma membrane. While in some cases it appears that the regulation of this trafficking is dependent on specific interactions between PKD1 and the cargo protein (Sanchez-Ruiloba et al., 2006; Yeaman et al., 2004), PKD1 also regulates the lipid content of the TGN which can directly influence the budding of these intracellular membranes (Hausser et al., 2005). In addition to its trafficking roles at the TGN, PKD1 promotes the recycling of β-integrins in fibroblasts (Woods et al., 2004).

PKD also controls epigenetic mechanisms of gene regulation. PKD can phosphorylate at least three members of the class II family of histone deacetylases (HDACs)(Wang, 2006b). Phosphorylation of HDACs 4, 5, or 7 by PKD keeps them from shuttling to the nucleus where they are known to acetylate histones and suppress gene expression(Vega et al., 2004).
STAGE OF DEVELOPMENT
The program has successfully elucidated critical roles for PKD in the regulation of neuronal function and has discovered evidence linking aberrant PKD activation to neurological disease.

We found that PKD1 is a point of convergence for different glutamatergic signaling pathways that independently control its activity and its subcellular localization. PKD is phosphorylated and activated by stimulation of mGluRs. By contrast, PKD responds to calcium influx through NMDARs by undergoing a complex 2-step translocation. First, PKD translocates from the cytoplasm to the plasma membrane and then from the plasma membrane to dendritic structures that co-localize with several markers of early recycling endosomes. With mutagenesis, we have discovered the residues of PKD that are required for each step.

These PKD-associated structures are also highly co-localized with the AMPAR subunit GluR2. We have discovered that disruption of PKD function with a dominant-interfering version of PKD1 or knock-down of endogenous PKD1 using siRNA disrupts the localization of GluR2. With versions of GluR2 fused to pH-sensitive forms of GFP, we have measured the kinetics of GluR2 internalization and re-insertion and have found that PKD regulates the process of insertion of GluR2-containing AMPARs. Together, these findings indicate that PKD is required for normal AMPA receptor trafficking and regulates the kinetics of AMPAR reinsertion. The dual regulation of PKD by mGluR and NMDARs and the localization of PKD to early endosomes suggests it may be positioned to respond to synaptic activity and govern the fate of internalized AMPARs (e.g., degradation vs. reinsertion).

To test this hypothesis directly, we generated CNS-specific PKD1 deficient mice by crossing a line of transgenic mice carrying a floxed PKD1 allele with a line of transgenic mice that express CRE-recombinase under the control of a neural-specific nestin promoter. PKD1 knockout mice are viable, breed normally, and exhibit no gross CNS developmental defects. We evaluated hippocampal slices from these animals electrophysiologically and found that synaptic transmission at the Schaffer collateral-CA1 synapse was normal. Long-term potentiation at those synapses could also be induced normally with high frequency stimulation. However, long-term depression, induced by NMDAR-dependent low frequency stimulation or mGluR-dependent stimulation was absent. Interestingly, LTD in the VTA is thought to be required for addiction and AD model mice show deficits in LTD.

We have also evaluated PKD1 deficient mice for behavioral deficits. PKD1 mice exhibit the most profound deficit in hippocampal spatial learning and memory that we have ever observed. They also have deficits in novel object recognition, which is generally believed to be more a measure of cortical learning and memory. They have a small but statistically significant deficit in open field exploration but are otherwise normal in tests of anxiety, depression, and overall motor function.

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We have also found that PKD regulates HDAC5 localization in neurons. Brief stimulation of primary neurons with agonists of the metabotropic glutamate receptor agonist, ACPD, is sufficient to trigger the rapid extrusion of HDAC5 from the nucleus. Interfering with PKD1 with siRNA knockdown blocks the ability of mGluR stimulation to stimulate HDAC5 translocation indicating that PKD is required for the process. We have also discovered that the induction of the activity-regulated cytoskeletal (Arc) gene by synaptic activity requires PKD function. Arc induction is necessary for memory consolidation and is dysregulated in AD.

Finally, we have examined PKD phosphorylation and activation in mouse models of Alzheimer’s disease (J20) and Huntington’s disease (HD). Compared with littermate controls, we observed a significant increase in PKD phosphorylation in brain extracts prepared from these mice. Considering the well-established abnormalities in synaptic function that are early features of these models, we speculate that aberrant PKD activation may play a role in disease phenotypes.

During the course of these studies, a variety of proprietary and non-proprietary tools have been developed to facilitate these investigations. We have developed (1) proprietary PKD isoform specific antibodies, (2) expression constructs for wild-type and mutated versions of PKD, (3) effective siRNA against PKD and a lentiviral delivery system and, (4) small molecule inhibitors of PKD that are effective in neurons, developed by Dr. Kevan Shokat (UCSF) and tested first by us. We have generated neuron-specific PKD knockout animals.

**PROPOSED R&D**
Additional preclinical studies are required to establish the importance of PKD to AD and other neurological diseases.

**LICENSING POTENTIAL**
Gladstone seeks to develop and commercialize by an exclusive or non-exclusive license agreement and/or sponsored research with a company active in the area.

**PATENT STATUS**
♦ Patent application filed.


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