A role for PKC in mediating stress-induced prefrontal cortical structural plasticity and cognitive function

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Impaired cognitive functioning plays an important role in several major psychiatric illnesses, including schizophrenia and bipolar disorder (BPD) (1, 2). Cognitive impairments can also appear before full-blown onset of illness (3) and affect key components of course and recovery, including illness progression, functional outcome, disability, and quality of life. Considerable interest therefore exists in elucidating the cellular mechanisms associated with these working memory deficits and developing improved therapeutics to treat them (4). Notably, in this issue of PNAS, Arnsten and colleagues (5) describe a novel role for protein kinase C (PKC) in mediating the effects of behavioral stress on both prefrontal cortical structural connectivity and prefrontal cortex (PFC)-dependent cognitive function. PKC has long been known to play a major role in regulating various forms of synaptic and neural plasticity; as we discuss below, the findings of Arnsten and colleagues, demonstrating an important role for PKC in mediating structural plasticity and prefrontal cortical function, have potentially important implications for developing novel therapeutics.

Previous research assessing the impact of stress focused predominantly on the hippocampus, but there is a growing appreciation that the PFC is also impacted by stress. For instance, chronic restraint stress causes progressive retraction and loss of dendritic spines in medial PFC regions (6, 7); these effects can last for weeks after stress ceases (8) and are associated with impaired performance on attentional tasks (7, 8). Arnsten and colleagues (5) observed similar changes in the medial PFC (5). They also found that stressed rats displayed progressively impaired performance on a task that assessed working memory, but not on a spatial discrimination task known to be independent of PFC functioning. Furthermore, working memory task performance was significantly correlated with dendritic spine density. These data are particularly important because they demonstrate that behavioral stress impairs working memory function, which relies on PFC neuronal connectivity. The study further found that PKC inhibition significantly altered the outcome of the stress-induced struc-

Fig. 1. PKC signaling hypothesis to explain stress-induced prefrontal cortical pyramidal cell structural modulation and PFC-dependent cognitive impairments. Stress causes noradrenergic hypersensitization and PKC activation through the $\alpha 1R/Gq/PLC$ pathway. Activated PKC phosphorylates MARCKS, and phospho-MARCKS induces dendritic spine loss or shrinkage. This is one plausible mechanism via which PKC inhibition can protect against stress-induced prefrontal cortical structural modification. Structural connectivity is the foundation for PFC-dependent cognitive function; thus, PKC blockade, which prevents stress-induced structural changes, rescues the stress-induced cognitive impairments. Dotted lines indicate that the pathway from PKC to cognitive impairment may occur independently from, although in a manner correlated with, structural modification of pyramidal cells.

tural modulation in the PFC and working memory.

This key finding builds on prior work suggesting that PKC is involved in prefrontal cortical response to stress. Stress exposure increases norepinephrine release in the PFC (9), which stimulates noradrenergic α -1 receptors ($\alpha 1Rs$) and activates the phosphatidylinositol (PI) signaling pathway and PKC (10). Consistent with this finding, PFC cognitive deficits are observed after exposure to pharmacologic stress, stimulation of $\alpha 1Rs$, or direct activation of PKC with phorbol esters in the PFC (10, 11). Conversely, PKC inhibition restores prefrontal cognitive functioning after all of these conditions (10, 11). Effects are also observed at the single-cell level,

where the firing of PFC neurons during cognitive tasks is reduced by activating PKC and restored by inhibiting it (10).

In toto, the data clearly demonstrate that PKC regulates cognitive functioning in the PFC and strongly suggest that stress may impair prefrontal cortical connectivity-dependent function via a PKC pathway. The study presented by Arnsten and colleagues (5) extends these findings and shows that pretreatment of rats with chelerythrine (a selective PKC inhibitor) before the daily restraint stress portion of the experiment diminished progressive impairment of working memory task performance. This reduction was more pronounced at the end of the chronic restraint stress when impairment was most severe. Perhaps somewhat surprisingly, pretreatment with a PKC inhibitor also attenuated the loss of dendritic spine density caused by the restraint stress. Thus, the data suggest that PKC inhibition not only protects against stress-induced *functional deficits*, but also protects against the effect of stress on *brain structure* in the PFC.

One possible avenue underlying the protective effects of PKC inhibition is via the blockade of myristoylated alanine-rich PKC C substrate (MARCKS) phosphorylation (Fig. 1). MARCKS is an actin filament cross-linking protein. In cultured hippocampal neurons, PKC activation causes loss and shrinkage of spines, a process abolished in neurons that overexpress nonphosphorylatable MARCKS (12). Consistent with this finding, overexpression of pseudophosphorylated MARCKS also causes loss and shrinkage of spines. It is thus possible that overactivation of PKC results in MARCKS dysfunction and spine loss. This hypothetical mechanism observed in hippocampal neurons could potentially exist in cortical neurons; however, PKC phosphorylates a number of distinct substrates and, therefore, other pathways may also play a role.

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The findings of Arnsten and colleagues (5) may also be of considerable clinical importance, given the well-known impairments in working memory observed in schizophrenia and BPD (1, 2). Indirect evidence exists that PKC signaling plays a role in the pathophysiology and treatment of BPD (13). Studies have reported that the PKC ratio of membrane to cytosolic fractions of platelets from manic patients was higher than in controls, as was the serotonin-induced translocation of PKC from cytosolic to membrane fraction. PKC activity and the translocation of PKC to the membrane were also elevated in post-mortem cerebral cortical tissues from individuals with BPD. Human genetic findings have also implicated PKC signaling in BPD and schizophrenia. For instance, two completely independent genomewide association studies identified diacylglycerol kinase ϵ (DGK ϵ), an enzyme involved in the phosphorylation/inactivation of diacylglycerol (DAG), an endogenous activator of PKC (Fig. 1)] as a risk gene for BPD (14, 15). Genetic association studies further suggest that variants of regulator of G protein signaling 4 (RGS4) confer risk for schizophrenia (16) and, perhaps, BPD (17) (Fig. 1). However, the influence of the DGK ϵ and RGS4 risk variants on protein function is largely unknown, as is the question of whether the protein functional alterations are causally linked to behavioral abnormalities associated with the illnesses.

In view of the potential role of elevated PKC in BPD, it is noteworthy that two mainstays in the treatment of BPD (lithium and valproate) attenuate PKC isozyme levels when administered in

therapeutically relevant paradigms (18). Furthermore, these effects are only observed after chronic administration and in a time frame consistent with clinical antimanic effects. Three small, independent, clinical studies found that tamoxifen (an antiestrogenic PKC inhibitor

The data clearly demonstrate that PKC regulates cognitive functioning in the PFC.

that readily crosses the blood–brain barrier) exerted antimanic effects in patients with bipolar mania (13); these effects were observed relatively rapidly and in a time frame consistent with PKC inhibition in the central nervous system (CNS).

The findings reported by Arnsten and colleagues (5) suggest that PKC inhibition may also play a contributory role in preventing/reversing the gray matter reductions observed in patients with BPD. Although BPD is not a classic neurodegenerative disorder, structural neuroimaging studies have demonstrated regional volumetric reductions, including reduced gray matter volumes in areas of the orbital and medial PFC, in individuals with BPD (18). Postmortem neuropathological studies similarly show reduced cortex volume and region- and layer-specific reductions in number, density, and/or size of neurons and glial cells in the subgenual PFC, orbital cortex, and dorsal anterolateral PFC (18).

In addition, longitudinal studies of individuals with BPD found that lithium increased gray matter volume (19), and several independent, cross-sectional studies demonstrated that lithium-treated patients with BPD had increased gray matter volumes compared with untreated patients (reviewed in ref. 18). These findings are also consistent with preclinical studies noting that chronic lithium treatment increased dendritic length and spine density in the CA3 hippocampal subregion and prevented chronic restraint stress-induced loss of length and spine density in this region (20). It should also be noted that, in addition to its effects on PKC, lithium may exert neurotrophic effects via a variety of mechanisms (18).

In conclusion, the findings of Arnsten and colleagues (5) suggest that PKC signaling may be key to mediating stress-induced prefrontal cortical structural plasticity and cognitive function. Furthermore, these data suggest that brain-penetrant selective PKC inhibitors may be useful in treating disorders associated with such impairments. Although developing such treatments will undoubtedly be challenging, the success of kinase inhibitors such as Gleevec, Iressa, and Herceptin in cancer treatment demonstrate the feasibility of using these agents safely and effectively. The important work highlighted in their article suggests several key and much-needed future research avenues for elucidating the links between psychiatric disorders, stress, and cognitive impairments and devising novel therapeutics to treat these deficits and improve quality of life for the millions of patients who suffer from these devastating disorders.

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