Role of Cdk5 in Neuronal Signaling, Plasticity, and Drug Abuse

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Key Words

Cdk5 · DARPP-32 · Drug abuse · Inhibitor-1 · Kinase · Phosphatase · Plasticity

Abstract

Functional and structural neuronal plasticity are mediated by a complex network of biochemical signal transduction pathways that control the strength of specific synapses and the formation of new synapses *de novo*. The neuronal protein kinase Cdk5 has been implicated as being involved in numerous aspects of both functional and structural plasticity through its regulation of signal transduction pathways. In this review the findings of a number of studies are summarized that have advanced our understanding of how Cdk5 may be involved in these processes. We focus on the modulation of protein phosphatase activity in both the hippocampus and basal ganglia, and review findings that indicate Cdk5 is likely to regulate neuronal plasticity in these brain regions. Studies showing involvement of Cdk5 in reward and motor-based plasticity, which are thought to underlie drug abuse, are discussed.

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Introduction

Intracellular signaling pathways regulate the physiological state of neurons, thereby controlling their excitability and responsiveness. During ‘slow’ neurotransmission, neurotransmitters released at the synapse activate receptors that are coupled to second messenger signal transduction pathways. An array of interconnected downstream signaling cascades alters various cellular processes that then affect ‘fast’ neurotransmission mediated by the ligand-gated ion channels associated with action potential generation. Protein phosphorylation and dephosphorylation, mediated by protein kinases and protein phosphatases, respectively, represent predominant biochemical mechanisms by which signals are promulgated. These intracellular regulatory mechanisms control two important features of the CNS, namely structural and functional plasticity. Functional plasticity refers to the regulation of the strength of existing synapses. Structural plasticity refers to the neurobiological processes by which new synaptic connections are made. These processes form the cellular basis for higher brain functions, including learning and memory. The principles of synaptic plasticity have been demonstrated to function in virtually every part of the brain where it has been assessed. The importance of

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neuronal plasticity to contextual-based learning and memory in the hippocampus, and in reward and motor-based learning in the dopamine circuitry of the basal ganglia has been well demonstrated. A number of signal transduction mechanisms regulating neuronal plasticity have been reported to be under the control of the neuronal kinase Cdk5. From these studies, a role for this kinase in balancing structural and functional plasticity may be hypothesized.

Functional Plasticity

Enzymes controlling protein phosphorylation at the synapse are of critical importance for the induction and maintenance of long-term changes in synaptic strength [1]. For example, control of protein dephosphorylation catalyzed by protein phosphatase-1 (PP-1) has been demonstrated to be a key regulatory step in the mediation of long-term potentiation (LTP) and long-term depression (LTD), the most commonly accepted models of synaptic plasticity associated with learning and memory [2–4]. PP-1 is a serine/threonine protein phosphatase that controls the phosphorylation state and activity of numerous downstream effector molecules known to govern synaptic strength, including NMDA receptors [5, 6] and AMPA receptors [7], Ca^{2+} /calmodulin (CaM)-dependent protein kinase II (CaMKII) [8], and cAMP response element-binding protein (CREB) [9]. PP-1 activity contributes to the induction of LTD [10] while inhibition of PP-1 has been shown to promote LTP [11, 12]. Protein phosphatase inhibitor-1 (inhibitor-1) is an endogenous regulator of PP-1 activity [13], and a physiological role for the regulation of PP-1 by inhibitor-1 has been suggested to mediate changes in synaptic strength.

In this model (fig. 1), biochemical cascades form pathways for modulating synaptic strength. Intense NMDA receptor stimulation results in increased intracellular Ca^{2+} concentrations, which causes activation of Ca^{2+} /CaM-dependent adenylyl cyclase. Increased cAMP levels result in activation of protein kinase A (PKA) and, in turn, phosphorylation of inhibitor-1 at amino acid residue Thr35. As a result, inhibitor-1 is converted into a potent inactivator of PP-1 ($\text{IC}_{50} = 1 \text{ nM}$). Inhibitor-1-mediated block of PP-1 allows CaMKII to achieve a Ca^{2+} -independent state of activity through autophosphorylation of residue Ser286. This, in turn, results in increased levels of phosphorylation of a number of CaMKII substrates that contribute to increased synaptic strength. Opposing this pathway, low frequency stimulation associated with LTD

results in selective activation of protein phosphatase-2B (calcineurin), which maintains inhibitor-1 in a dephosphorylated state. Recently this model has gained support from further studies demonstrating control of synaptic strength through regulation of postsynaptic PP-1 activity by inhibitor-1, as well as other PP-1 regulatory proteins [14]. Furthermore, inhibitor-1 knockout mice are deficient in LTP [15]. Moreover, expression of a constitutively active form of inhibitor-1 in transgenic mice has been reported to relieve the suppression of learning and memory under the control of PP-1 [16].

Evidence exists to suggest that some of these signaling pathways may be under the control of Cdk5, thereby implicating this kinase in the control of synaptic strength. For example, Cdk5 has been demonstrated to phosphorylate inhibitor-1 at Ser67 and reduce the efficiency of its phosphorylation by PKA [17]. Furthermore, the Cdk5 activators p35 and p39 have been shown to interact with CaMKII in a Ca^{2+} -dependent manner [18]. A number of other signaling pathways, such as the Ras/mitogen-activated protein kinase pathway, have been shown to be downstream of NMDA receptors. Furthermore, it is now recognized that a central component of the NMDA receptor signaling complex is the scaffold protein PSD-95 [19]. Moreover, neurotransmitter receptors such as metabotropic glutamate receptors (mGluRs) have been shown to be functionally and structurally associated with NMDA receptors and are involved in LTP [20, 21]. All of these pathways represent additional targets that may be either under the control of, or involved in, Cdk5-dependent modulation of synaptic strength. Indeed, some evidence has been presented to suggest that Cdk5 phosphorylates, and thereby regulates, the mitogen-activated protein kinase MEK [22] and PSD-95 [23]. It has also been suggested that Cdk5 modulates LTP induction by phosphorylating amino acid residue Ser1232 of the NR2A subunit of the NMDA receptor [24]. Studies also point to the possibility that Cdk5 activity is regulated via mGluR activation [25]. Finally, it has been suggested that Cdk5 can phosphorylate voltage-gated Ca^{2+} channels, thereby affecting neurotransmitter release [26]. Thus it would appear likely that Cdk5 is capable of modulating and mediating neuronal functional plasticity through a number of potential signaling pathways. It is through the regulation of these pathways that this protein kinase could contribute to higher brain functions such as learning and memory. Involvement of Cdk5 in plasticity is evidenced by its involvement in synaptic reorganization during kindling [27]. Furthermore, studies indicated that Cdk5 activity is required for associative learning [28]. However,

Fig. 1. Signal transduction model of synaptic plasticity. In this model, first proposed by John Lisman, low frequency stimulation of postsynaptic neurons results in a mild increase in intracellular Ca^{2+} levels, and activation of relatively low amounts of CaM, which bind Ca^{2+} with high affinity and activate calcineurin, maintaining inhibitor-1 in the dephosphorylated state (left). High frequency stimulation leads to activation of sufficient amounts of CaM to induce Ca^{2+} /CaM-dependent adenylate-cyclase activation and PKA-dependent phosphorylation of inhibitor-1 at Thr35 (right). This pathway, in combination with Ca^{2+} /CaM-dependent activation of CaMKII, causes autophosphorylation of CaMKII at Ser286, thereby allowing the kinase to become CaM-independent and contribute to strengthening of the synapse by a number of pathways. Cdk5 may influence a number of these pathways by, for example, interacting with CaM, phosphorylating NMDA receptors, inhibitor-1, and transcription factors (modified with permission from [95]).

more research is required to clearly elucidate which pathways are under the control of Cdk5. Ultimately, many of the same mechanisms by which Cdk5 functions in CNS development may be retained to facilitate neuronal plasticity in the adult brain.

Structural Plasticity

While axonal growth cone dynamics have been well characterized [29], it is only recently that dendritic spine motility has been documented [30]. Studies of the visual circuitry have demonstrated that the robust architectural dynamics of neurons in the developing brain are dimin-

ished in mature adult neurons [31, 32]. Furthermore, spine stability has also been shown to be affected by age [33]. However, it is widely accepted that spine formation and density are regulated by synaptic activity [34, 35], and a recent report demonstrates that spine structural plasticity is retained in the adult brain [36].

Evidence suggests that maintenance of spine morphology requires continual low-level activation of AMPA receptors by spontaneously released glutamate, whereas *de novo* spine formation is dependent upon NMDA receptor activation with subsequent stabilization by AMPA receptor activation [37, 38]. Coordination of actin cytoskeletal dynamics appears to be essential to structural plasticity. Signaling pathways that involve the Rho family of small GTPases are thought to be key regulators of actin polymerization [39]. Rho family members, including RhoA, Rac1, and Cdc42, have been shown to be involved in dendritic remodeling. Downstream of these proteins are other signaling molecules including PAK (p21 activated protein kinase), and LIM kinase that, in turn, regulate the ability of cofilin to organize actin [40]. Each step in this pathway serves as a potential site for regulation. In addition, many other molecules have been reported to alter spine motility and morphology, including steroid hormones [41], cadherins [42], ephrins [43], neuregulins [44, 45], neurotrophins [46], PSD proteins [47, 48], and cocaine and amphetamines [49–51]. It remains to be seen if all of these diverse pathways converge on a common mechanism such as Rho family GTPases.

A substantial body of research points to a central role for Cdk5 in coordinating cytoskeletal organization and vesicle trafficking associated with changes in neuronal cell morphology and structural plasticity [52–55]. Immunocytochemical studies show that p35 localizes at the cell periphery in lamellipodial and filopodial structures and is important for neurite outgrowth [56, 57]. Overexpression of p35 and p39 induces actin reorganization [57]. Cdk5 has been shown to induce PAK1 hyperphosphorylation in a Rac-dependent manner, resulting in reduction of PAK1 kinase activity, thereby effecting downstream actin cytoskeletal dynamics. Cdk5 also associates with the actin cross-linking molecule, α -actinin-1, in a Ca^{2+} -dependent manner [18]. In addition, Cdk5 has been suggested to mediate semaphorin-dependent regulation of actin organization that is essential to dendrite orientation [58]. Furthermore, Cdk5 may affect neurite extension via phosphorylation of MAP1B [59, 60]. Cdk5 phosphorylates β -catenin and, in this manner, may function in cadherin-mediated adhesion as part of a Cdk5/p35/N-cadherin/ β -catenin complex [61, 62]. Moreover, neuregulins have

been demonstrated to rely upon Cdk5 to mediate ErbB-dependent neuromuscular junction formation [63]. Perhaps Cdk5/ErbB interactions are also important during the formation of central synapses as well.

Neostriatal Signaling

The principles of neural plasticity, which have been defined in the hippocampus, would appear to apply to the dopamine neurotransmission circuitry of the basal ganglia, which includes the dorsal striatum and nucleus accumbens [64]. It has been well demonstrated that dopamine modulates synaptic plasticity in medium spiny neurons of the striatum [65]. Furthermore, repeated exposure to drugs of abuse that target dopamine neurotransmission has been shown to affect striatal synaptic plasticity [66–70].

The striatal specific protein DARPP-32 is an important integrator of signal transduction pathways. DARPP-32, a homologue of inhibitor-1, is converted into a potent inhibitor of PP-1 when phosphorylated by PKA at Thr34 [71]. This pathway is invoked by activation of D1 dopamine receptors [72] and is opposed, as with inhibitor-1 in the hippocampus, by activation of calcineurin that dephosphorylates the PKA site [73]. DARPP-32 is also phosphorylated by Cdk5 at Thr75, which prevents it from being phosphorylated by PKA and causes it to function as an inhibitor of PKA [74]. Thus, the activity of Cdk5 in the striatum would appear to oppose the effects of the dopamine signaling cascade and possibly enhance pathways such as cortical glutamate input, causing increased intracellular Ca^{2+} levels. Studies using knockout mice have suggested a role for DARPP-32 in regulating striatal LTP and LTD [75]. Furthermore, disruption of corticostriatal plasticity has been associated with increased PKA-dependent phosphorylation of DARPP-32 levels [76]. It is quite likely that, through regulation of the dopamine/PKA/DARPP-32 pathway, Cdk5 is able to influence plasticity in the striatum.

Drug Addiction

The transition to the drug-addicted state is increasingly viewed as a consequence of experience-dependent neural and behavioral plasticity [69, 70]. Addictive drugs act on the brain's natural reward systems, which have evolved to provide advantages to organisms under natural selective pressures [77]. The overwhelming rewarding and rein-

forcing stimuli of repeated administration of drugs of abuse, such as psychomotor stimulants, result in changes in the neural circuitry of the mesocorticolimbic (and perhaps the nigrostriatal) pathways of the dopaminergic system [49–51]. These changes result in continued drug-seeking and drug administration, despite a variety of deleterious effects.

The mechanisms by which drugs (e.g. nicotine, ethanol, cocaine, amphetamine and opiates) are able to induce addiction vary considerably; nonetheless, similarities exist. For example, all drugs of abuse achieve their action by altering the intracellular signal transduction pathways associated with normal synaptic neurotransmission [78]. Indeed, cocaine causes an increase in synaptic dopamine levels by blocking dopamine reuptake in the basal ganglia, in turn stimulating the dopamine signaling cascade. Repeated exposure to cocaine leads to changes in several components of dopamine signaling, changes in gene expression, and changes in the neuronal circuitry of dopaminergic neurons [72, 79–85].

Stimulation by addictive drugs results in a transient burst in immediate early gene expression in the striatum [69, 86–88] (fig. 2). This effect may be mediated partly by CREB, which is also activated in these regions by drugs of abuse. While the activation of CREB and c-Fos may be short-lived, one highly stable Fos family protein, Δ FosB, has been found to accumulate and persist in striatal neurons in response to chronic exposure to drugs of abuse. The effect of Δ FosB as a target of drugs of abuse has been characterized by studies using genetically altered mouse models, in which the gene encoding Δ FosB has been ablated or placed under the control of an inducible striatal specific promoter [86, 89, 90]. DNA microarray analysis of inducible transgenic mice that overexpress Δ FosB revealed that Cdk5 is a downstream target of Δ FosB in the striatum, including the nucleus accumbens, which is especially implicated in drug addiction [69].

Cdk5 is a particularly interesting neuromodulatory target of drugs of abuse because, as discussed, it has been shown to regulate dopamine neurotransmission through phosphorylation of DARPP-32 [74]. In agreement with enhanced Cdk5 expression in inducible Δ FosB transgenic mice, Cdk5 expression was found to be upregulated in rat striatum in response to chronic exposure to cocaine [69]. Similarly, mRNA and protein levels of the Cdk5 activator, p35, were also upregulated by induced overexpression of Δ FosB and by chronic exposure to cocaine. Once upregulated, Cdk5/p35 appears to exert an initial desensitizing effect by reducing the efficacy of D1 dopamine receptor-mediated signaling, as assessed by PKA phosphorylation

of specific substrates in striatal slices from rats given chronic cocaine. The overall desensitizing effects of Cdk5 upregulation were also manifested as attenuation in cocaine-induced locomotor behavior. Animals given intra-accumbens infusions of the Cdk5 inhibitors roscovitine or olomucine exhibited marked elevation in cocaine-induced increases in locomotor behavior in comparison to animals in which vehicle alone or inactive congener was infused. These effects became apparent after 3–4 consecutive days of treatment with cocaine, in agreement with gene expression-dependent adaptations. Thus, Cdk5 activation serves as a negative feedback homeostatic mechanism invoked in response to chronic exposure to cocaine [69, 91] (fig. 2). These findings also raised the possibility that Cdk5 plays a role in the long-term adaptations to cocaine and other drugs of abuse that result in addiction.

The ability of drugs of abuse to cause persistent neural adaptations via altered gene expression may be related to the alterations in the neural circuitry associated with addiction. Structural changes in the neuronal architecture in response to chronic exposure to cocaine and other psychomotor stimulants have been well demonstrated. Repeated stimulant exposure increases the number of dendritic branch points and spines both of medium spiny neurons in the nucleus accumbens and pyramidal neurons in the medial prefrontal cortex [49, 50]. Interestingly, chronic exposure to opiates has the opposite effect on spine density [92], supporting the idea that different drugs of abuse may have opposite effects on common mechanisms of neuronal structural plasticity. Based on this literature, it could be hypothesized that drug-induced changes in Cdk5 gene expression directly mediate the effect of cocaine (and, possibly, other drugs of abuse) on dendritic morphology in the nucleus accumbens. Indeed, the effect of chronic cocaine on spine density in this region was shown to be completely blocked by intra-accumbens infusion of roscovitine [51]. In keeping with the idea that changes in structural plasticity induced by drugs of abuse are dependent on Cdk5 activity, it has been suggested that opiates, which reduce spine density, cause a reduction in Cdk5 expression in human addicts as well as rats [93]. Finally, it is interesting to consider that the effects of another, arguably addictive, psychomotor stimulant, caffeine, may be mediated via Cdk5-dependent regulation of dopamine signaling [94].

Fig. 2. Schematic of cocaine effects on the dopamine D1 receptor signaling cascade, gene expression, and Cdk5-mediated effects. Cocaine enhances dopamine signaling by blocking reuptake of dopamine at the synapse (top). Chronic stimulation of the D1 dopamine receptor/cAMP/PKA pathway leads to PKA-dependent changes in gene expression which target Cdk5 for upregulation via Δ FosB. Increased Cdk5-dependent phosphorylation of DARPP-32 at Thr75 results in attenuation of dopamine signaling (functional plasticity) but may commit striatal neurons to adaptive changes in connectivity or spine formation structural plasticity that result in addiction.

Conclusions

An integrated and complex network of signaling pathways forms the basis of synaptic plasticity both in the hippocampus and the basal ganglia. Normal, as well as aberrant, neurotransmission modulates neuronal circuitry through these biochemical signaling pathways. Cdk5 activity has now been implicated to affect many of these pathways. In particular, Cdk5 may modulate synaptic strength through its ability to regulate protein phosphatase activity. Therefore, Cdk5 may directly alter both functional and structural plasticity and influence learning and memory. As an example, chronic exposure to cocaine causes overwhelming dopamine D1 receptor stimulation that elicits a Cdk5-dependent homeostatic response (al-

tered functional plasticity). However, this response irreversibly dedicates the affected neurons to a process of morphological change and dendritic spine formation (altered structural plasticity), which is also dependent on Cdk5 activity. In this manner, Cdk5-dependent changes in neuroplasticity, which are associated with learning and memory, may result in the formation of the addictive state.

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