DARPP-32 Mediates the Actions of Multiple Drugs of Abuse

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ABSTRACT

Drugs of abuse share the ability to enhance dopaminergic neurotransmission in the dorsal and ventral striatum. The action of dopamine is modulated by additional neurotransmitters, including glutamate, serotonin and adenosine. All these neurotransmitters regulate the phosphorylation state of Dopaminal serine/threonine protein phosphatase, PP-1. Phosphorylatine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32). Phosphorylation at Thr^{34} by protein kinase A converts DARPP-32 into a potent inhibitor of the multifunctioon at Thr^{75} by Cdk5 converts DARPP-32 into an inhibitor of protein kinase A. The state of phosphorylation of DARPP-32 at Thr^{34} also depends on the phosphorylation state of Ser^{97} and Ser^{130}, which are phosphorylated by CK2 and CK1, respectively. By virtue of regulation of these 4 phosphorylation sites, and through its ability to modulate the activity of PP-1 and protein kinase A, DARPP-32 plays a key role in integrating a variety of biochemical, electrophysiological, and behavioral responses controlled by dopamine and other neurotransmitters. Importantly, there is now a large body of evidence that supports a key role for DARPP-32-dependent signaling in mediating the actions of multiple drugs of abuse including cocaine, amphetamine, nicotine, caffeine, LSD, PCP, ethanol and morphine.

KEYWORDS: protein phosphorylation, psychostimulants, dopamine, striatum

DARPP-32, A MULTIFUNCTIONAL REGULATOR OF PROTEIN KINASES AND PROTEIN PHOSPHATASES

Dopamine- and cAMP-regulated phosphoprotein, Mr 32 kDa (DARPP-32), was initially discovered as a major target for dopamine-activated adenylyl cyclase and protein kinase A (PKA) in striatum.[1] Phosphorylation by PKA at Thr^{34} converts DARPP-32 into a potent high-affinity inhibitor of the multifunctional serine/threonine protein phosphatase, PP-1. The IC_{50} for inhibition of PP-1 is ~10^{-9} M.[2] Since DARPP-32 is expressed in very high concentration (~50 μM) in all medium spiny neurons,[3] including those in both the striatonigral and striatopallidal projection pathways (see below), and the concentration of all PP-1 isoforms is likely less than 20 μM,[4] a substantial proportion of PP-1 activity will be inhibited in response to even moderate increases in DARPP-32 phosphorylation.

Detailed structure-function studies have established that the first 40 amino acids at the NH_2-terminus of DARPP-32 are sufficient (when Thr^{34} is phosphorylated) for DARPP-32 to bind to and inhibit PP-1. Moreover, several different types of biochemical approaches have revealed that the remaining COOH-terminal portion of DARPP-32 serves an important role in the modulation of DARPP-32 function. In intact neurons, DARPP-32 is highly phosphorylated at Ser^{97} and Ser^{130} under basal conditions. Ser^{97} is phosphorylated by CK2 and Ser^{130} is phosphorylated by CK1. In vitro, phosphorylation of Ser^{97} of DARPP-32 increases the efficiency of phosphorylation of Thr^{34} by PKA.[5] In vitro, phosphorylation of Ser^{130} decreases the rate of dephosphorylation of Thr^{34} by PP-2B,[6] and in striatal slices, DARPP-32 phosphorylated at Ser^{130} is phosphorylated to a higher level at Thr^{34}.[7] The overall consequence of phosphorylation of DARPP-32 by CK1 or CK2 in intact cells is to increase the state of phosphorylation of Thr^{34}. The physiological role of these two phosphorylation events is to potentiate D1 dopaminergic signaling through the DARPP-32/PP-1 pathway.

A variety of recent studies have found that DARPP-32 is a physiological target for the proline-directed kinase, cdk5/p35, a cyclin-dependent kinase family member that is highly expressed in post-mitotic neurons where it is activated by the non-cyclin cofactor p35.[8] In vitro, phosphorylation of DARPP-32 at Thr^{75} does not alter the kinetics of phosphorylation by either CK1 or CK2. However, phosphorylation of Thr^{75} has a major inhibitory effect on the phosphorylation of Thr^{34} by PKA, and has a general inhibitory effect on phosphorylation of other PKA substrates. The resultant decrease in phosphorylation of Thr^{34} of DARPP-32 would act to inhibit D_1 dopamine signaling through the DARPP-32/PP-1 cascade.

DARPP-32 IS ENRICHED IN DOPAMINOCEPTIVE NEURONS

A prominent aspect of the distribution of DARPP-32 in the brain is its high enrichment in dopaminoceptive neurons in the striatum.[9] This distribution is very similar in all species studied, suggesting that it may be relevant to extrapolate functional data obtained in rodents to man. The highest

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levels of DARPP-32 are found in the striatum (caudate-putamen and nucleus accumbens), olfactory tubercle, bed nucleus of stria terminalis, and portions of the amygdaloid complex. These brain regions send projections to various target areas, including globus pallidus, ventral pallidum, the entopeduncular nucleus, and substantia nigra pars reticulata. Although concentrated in the striatum, it is important to note that moderate levels of DARPP-32 are found throughout the neocortex, with particular enrichment in layers II, III, and VI, in the dentate gyrus of the hippocampus and in the choroid plexus. There are also low levels of DARPP-32 in several other brain regions including hypothalamus and cerebellum. Within the striatum, DARPP-32 is found in medium-sized spiny neurons. Medium spiny neurons constitute the major cell type (95%) in the striatum, are inhibitory, and utilize GABA as their major neurotransmitter.[10] At the ultrastructural level, DARPP-32 has been found in most cytosolic subcellular compartments of medium spiny neurons, including dendrites, axons and axon terminals. Some nuclei also appear to contain DARPP-32 immunoreactivity.

Medium spiny neurons can be divided into two equally large subpopulations based on their peptide content and their projection areas.[11-13] One subpopulation contains substance P and dynorphin and projects directly to substantia nigra pars reticulata and the entopeduncular nucleus (the direct striatonigral pathway). The other subpopulation contains enkephalin and projects indirectly to these structures via relays in the globus pallidus and subthalamic nucleus (the indirect striatopallidal pathway). DARPP-32 is expressed at high levels in both striatonigral and striatopallidal neurons. Large-sized cholinergic interneurons and medium-sized GABAergic interneurons are devoid of DARPP-32 immunoreactivity.[14,15]

Regulation of the Phosphorylation of DARPP-32 by Dopamine, Glutamate, Serotonin and Adenosine

Within medium spiny neurons, a major role for dopamine is to modulate the actions of glutamate. Cross-talk between dopamine and glutamate plays important roles in the regulation of emotion, mood, reward, and cognition. Pertubations of these neurotransmitter systems are thought to contribute to the etiology of several common neuropsychiatric disorders, including schizophrenia, bipolar disorder and ADHD, and to play an important role in the actions of drugs of abuse. In addition to dopamine and glutamate, serotonin is also highly involved in the regulation of mood and reward and in the actions of several drugs of abuse. Indeed, the psychostimulants cocaine and amphetamine act on both dopamine and serotonin reuptake transporters and cause significant increases in the extracellular levels of these two monoamines in various regions of the brain, particularly in the striatum and nucleus accumbens.[16,17] We shall briefly review some of the studies of regulation of DARPP-32 by dopamine, glutamate and serotonin. As adenosine A_{2A} receptors are critical for the actions of caffeine and are important in the regulation of DARPP-32 phosphorylation in striatopallidal neurons, we will also briefly review the regulation of DARPP-32 by adenosine. For a more detailed discussion of these and other neurotransmitters, neuromodulators and neuropeptides see.[18]

Dopamine: Five different G protein-coupled dopamine receptors have been identified. D_1 receptor subtypes (D_1, D_5) stimulate adenylyl cyclase, whereas D_2 receptor subtypes (D_{2S}, D_{2L}, D_3, D_4) inhibit adenylyl cyclase.[19] In addition, both D_1-type and D_2-type receptors have been shown to regulate intracellular Ca^{2+} levels. D_1-type receptors can interact with calcyon and influence Ca^{2+}-dependent signaling via G_q-coupled release of Ca^{2+} from intracellular stores[20] D_2-type receptors are coupled directly to phospholipase C and the production of inositol triphosphate. As a result, activation of D_2-type receptors leads to activation of the Ca^{2+}/calmodulin-dependent protein phosphatase, calcineurin (also known as PP-2B).[21] D_2 receptors are found on dopaminergic nerve terminals, where they are thought to play an autoinhibitory role, and postsynaptically on medium spiny neurons. D_1 and D_3 receptors are predominantly expressed postsynaptically on medium spiny neurons. Anatomical studies have shown that striatonigral neurons contain high levels of D_1 receptors, whereas striatopallidal neurons predominantly express D_2 receptors.[22] However, biochemical and physiological evidence supports the idea that there is also a population of medium spiny neurons that express both D_1 and D_2 types of receptor.[23,24]

As a consequence of the actions of the different types of receptors, dopamine regulates the state of phosphorylation of DARPP-32 in a bidirectional manner. In striatal slices or whole animals, activation of D_1 receptors, via stimulation of PKA, results in phosphorylation of DARPP-32 at Thr^{34}.[21,25] This effect is counteracted by the activation of D_2 receptors via inhibition of adenylyl cyclase and via stimulation of Ca^{2+}-dependent activation of calcineurin.[21;26] Activation of D_1 receptors also decreases the phosphorylation state of DARPP-32 at Thr^{75} by a process that appears to involve the PKA-dependent activation of PP-2A.[27] Thus, enhanced dopaminergic transmission via D_1 receptors leads to a decreased phosphorylation of Thr^{75}-DARPP-32, which reduces inhibition of PKA and thereby facilitates signaling via the PKA/Thr^{34}-DARPP-32/PP-1 cascade.

Glutamate: The regulation of DARPP-32 phosphorylation by glutamate is complex and involves the actions of both ionotropic (NMDA and AMPA) and metabotropic (mGlu) glutamate receptors. Glutamate, released at glutamatergic nerve terminals, activates NMDA- and AMPA-type receptors, leading to a decrease in DARPP-32 Thr^{34}

phosphorylation through the Ca^{2+}-dependent activation of calcineurin.[28,29] Activation of NMDA and AMPA receptors also results in a decrease in Thr^{75} phosphorylation.[29] Surprisingly, the regulation of Thr^{75} phosphorylation is not mediated through Ca^{2+}-dependent activation of calcineurin, but rather via Ca^{2+}-dependent activation of PP-2A, and involves a mechanism that is not yet fully understood. The primary action of glutamate through NMDA and AMPA receptors is to reduce phosphorylation of Thr^{34} and as a result to activate PP-1. However, under conditions where Thr^{34} phosphorylation is low, the dephosphorylation of Thr^{75} and the resulting disinhibition of PKA caused by glutamate may act to potentiate dopamine/D_1 receptor/PKA/phospho-Thr^{34} DARPP-32 signaling.

Metabotropic glutamate receptors are subdivided into three groups: group I (mGlu1 and mGlu5 receptors), group II (mGlu2 and mGlu3 receptors), and group III (mGlu4, mGlu6, mGlu7, and mGlu8 receptors).[30] Group I mGlu receptors are expressed in both direct and indirect pathway neostriatal neurons,[31] and group II and III mGlu receptors are expressed on the terminals of corticostriatal afferents.[32] Activation of mGlu5 receptors in striatal slices stimulates DARPP-32 Thr^{34} phosphorylation, an effect that is dependent on activation of adenosine A_{2A} receptors by endogenous adenosine.[33] This study has further suggested that there is a synergistic interaction of mGlu5 receptors and adenosine A_{2A} receptors at the level of production of cAMP, and that this effect of mGlu5 receptors requires MAP kinase signaling.

Group I mGlu receptors also regulate the phosphorylation of DARPP-32 at Thr^{75} and Ser^{130}. Treatment of striatal slices with a group I mGlu receptor agonist stimulates the activity of CK1, leading to an increase in the phosphorylation of DARPP-32 at Thr^{75} and Ser^{130}.[34,35] Detailed analysis of the mechanism of CK1 activation revealed that group I mGlu receptors, coupled to G_q, activate phospholipase C and stimulate the generation of inositol triphosphate, leading to an increase in intracellular Ca^{2+}. The increased intracellular Ca^{2+} activates calcineurin, causing dephosphorylation of inhibitory autophosphorylation sites in CK1. Activation of CK1 causes phosphorylation of Ser^{130} and results in activation of Cdk5 and phosphorylation of Thr^{75} by an unknown mechanism.

The relative temporal contributions of ionotropic and metabotropic glutamate receptors in mediating effects of glutamate on phosphorylation of DARPP-32 at Thr^{34} and Thr^{75} have been recently studied.[36] Treatment with glutamate caused a complex change in DARPP-32 Thr^{34} phosphorylation. An initial rapid increase in Thr^{34} phosphorylation was NMDA/AMPA/mGlu5 receptor-dependent and was mediated through activation of a neuronal nitric oxide synthase/nitric oxide/cGMP/cGMP-dependent kinase signaling cascade. A subsequent decrease in phosphorylation was attributable to activation of an NMDA/AMPA receptor/Ca^{2+}/calcineurin signaling cascade. This decrease was followed by rephosphorylation via a pathway involving a mGlu5 receptor/phospholipase C and MAP kinase signaling cascade. Treatment with glutamate initially decreased Thr^{75} phosphorylation through activation of NMDA/AMPA receptor/Ca^{2+}/PP-2A signaling. Thereafter, glutamate slowly increased Thr^{75} phosphorylation through activation of mGlu1 receptor/phospholipase C signaling.

Serotonin: Various serotonin receptors, including 5-HT_{1B}, 5-HT_{1F}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4, and 5-HT_6, are expressed in medium spiny neurons in the striatum.[37] These receptors act primarily via the following second messenger systems: 5-$HT_{1B/E}$ receptors decrease cAMP formation, 5-$HT_{2A/C}$ receptors increase inositol triphosphate and diacylglycerol production, 5-HT_3 receptors increase Na^+ and Ca^{2+} influx, and 5-HT_4 and 5-HT_6 receptors increase cAMP formation. Studies in striatal slices and whole animals have shown that serotonin stimulates phosphorylation of DARPP-32 at Thr^{34} and decreases phosphorylation at Thr^{75} primarily via activation of 5-HT_4 and 5-HT_6 receptors.[38] Serotonin stimulates phosphorylation of Ser^{130}–DARPP-32 primarily via 5-HT_2 receptors. As a consequence of their actions on DARPP-32 phosphorylation, these three serotonin pathways act synergistically to inhibit PP-1.

Adenosine: Adenosine is found intra- and extracellularly in all organs of the body. Most adenosine is formed via breakdown of ATP intracellularly and transported to the extracellular space via equilibrative transporters. Extracellular adenosine acts via G-protein-coupled receptors of which two, A_1 and A_{2A} receptors, are abundantly expressed in the brain. Adenosine A_1 receptors inhibit, and A_{2A} receptors stimulate, adenylyl cyclase. A_1 receptors have a widespread distribution in the brain, with the highest levels in hippocampus and cerebellum, whereas A_{2A} receptors are almost exclusively found in striatum where they are restricted to striatopallidal neurons.[39] In striatal slices, the A_{2A} receptor agonist CGS21680 was found to increase the level of DARPP-32 phosphorylated at Thr^{34} in a concentration-dependent manner.[40] When treatment with CGS 21680 was combined with treatment with SKF81297, a selective D_1 agonist, an additive response was observed both on cAMP levels and Thr^{34}-DARPP-32 phosphorylation. In whole animals, in vivo, antagonists at A_{2A} and D_1 receptors had an additive effect in reducing DARPP-32 phosphorylation.[25] A_{2A} receptors are co-localized with D_2 receptors. Since A_{2A} receptors increase and D_2 receptors decrease cAMP levels, adenosine-dopamine interactions are in most instances antagonistic. Indeed, the A_{2A} antagonist, SCH 58261, significantly counteracted the increase in Thr^{34}-DARPP-32 phosphorylation that was observed following treatment with selective D_2 receptor antagonists.[25] Likewise, the ability of D_2 antagonists to increase Thr^{34}-DARPP-32

phosphorylation was dramatically reduced in A_{2A} receptor KO mice. These data provided further support for the notion that adenosine acting on A_{2A} receptors provides a basal tonic activity of the cAMP/PKA/Thr^{34}-DARPP-32 pathway, which is necessary to mediate many of the effects of dopamine acting via D_2 receptors. It has also been shown that A_{2A} agonism, via cAMP-dependent mechanisms, decreases the phosphorylation at Thr^{75}-DARPP-32.[41]

Role of DARPP-32 Phosphorylation in the Actions of Drugs of Abuse

It is well established that the dopaminergic system plays an important role in reward-related behaviors, and drugs with reinforcing properties share the ability to increase dopaminergic transmission.[42,43] By virtue of its regulation by dopamine, as well as by other neurotransmitters linked to the actions of drugs of abuse, DARPP-32 is positioned to play an important role in either mediating or modulating the short, and perhaps long, term actions of drugs of abuse. The generation of DARPP-32 knockout (KO) mice,[44] and the more recent generation of mice in which individual phosphorylation sites have been mutated,[45] have provided powerful animal models for use in studies of the role of DARPP-32 in the actions of drugs of abuse. A wide variety of approaches using these mouse models have demonstrated that DARPP-32 mediates many biochemical, electrophysiological, gene transcriptional, and behavioral effects of dopamine. A general feature of the results obtained is that DARPP-32 is required for the actions of physiological concentrations of dopamine, while the consequences of the lack of DARPP-32 are less pronounced at higher, supraphysiological, concentrations of dopamine. We will briefly summarize these recent studies of DARPP-32 mutant mice as well as summarize studies of the regulation of DARPP-32 phosphorylation by a variety of drugs of abuse.[18,46,47]

Cocaine and amphetamine: Cocaine inhibits reuptake of dopamine, whereas amphetamine promotes release of dopamine from nerve terminals through a weak-base-mediated reverse transport mechanism.[42,43] Acute treatment of mice with cocaine or amphetamine increases the phosphorylation of Thr^{34}-DARPP-32 and Ser^{130}-DARPP-32, but decreases the phosphorylation at Thr^{75}.[43,45] A recent immunohistochemical study[48] has demonstrated that the psychostimulant-induced phosphorylation of Thr^{34}-DARPP-32 occurs primarily in striatonigral neurons. Chronic treatment with cocaine upregulates the expression of both Cdk5 and p35 in striatum, and this leads to increased phosphorylation of Thr^{75} of DARPP-32 and consequently to decreased phosphorylation of Thr^{34}.[49] A well-known behavioral effect of repeated cocaine administration is the development of sensitization. Notably, intraaccumbal application of Cdk5 inhibitors was found to potentiate cocaine-induced behavioral sensitization,[49] indicating that Cdk5 is likely to be involved in counteracting this sensitization phenomenon.

From studies of DARPP-32 KO and phospho-site knockin mice, DARPP-32 is likely to be involved in changes in gene transcription that are important for maintaining altered synaptic function and for initiating adaptive morphological changes of the type believed to underlie the effects of various drugs of abuse. Treatment of animals with cocaine increases the phosphorylation state of CREB, ELK, and multiple immediate early genes.[42,43] CREB, c-Fos, Fras, and many other immediate early genes regulate gene transcription and may coordinate alterations in gene expression, leading to long-term changes in neuronal function. Dopamine, via activation of D_1 receptors and PKA, stimulates phosphorylation of CREB at Ser^{133} in striatum,[50] and the dephosphorylation of CREB at Ser^{133} is under the control of PP-1.[51] Other transcription factors, such as ELK, are regulated by the MAP kinase signaling cascade.[42] Notably, dopamine receptor-mediated activation of MAP kinase, CREB, c-Fos, and ΔFosB is strongly attenuated in DARPP-32 KO mice.[13,52,53]

A detailed study of the regulation of the MAP kinase signaling cascade by DARPP-32 showed a prominent role of Thr^{34}-DARPP-32/PP-1 signaling.[48] Inhibition of PP-1 appears to be important at several levels for activating the ERK cascade. On the one hand, it prevents extracellular regulated kinase (ERK) dephosphorylation by striatal enriched phosphatase (STEP), by maintaining this tyrosine phosphatase in a phosphorylated, inactive, state. On the other hand, PP-1 is also critical upstream of ERK, because MEK phosphorylation in response to psychostimulants was dramatically reduced in DARPP-32 KO mice.

Several alterations in the behavioral responses to acute and chronic treatment with cocaine are exhibited in DARPP-32 KO mice. An attenuated locomotor responsiveness to a single injection of cocaine is found in DARPP-32 KO mice compared with wild-type mice.[44] In contrast, following chronic treatment with cocaine, DARPP-32 KO mice show increased locomotor sensitization as compared with wild-type mice.[54] The different involvement of DARPP-32 in acute and chronic responses to cocaine may be related to the differences in the relative levels of Thr^{75}-DARPP-32 and Thr^{34}-DARPP-32, which have been found following acute vs chronic treatment with cocaine, and the antagonistic relationship between these two sites.[27,49] The acquisition of place-preference is also significantly attenuated in DARPP-32 KO mice.[55,56]

The effects of D-amphetamine on two other behavioral parameters—sensorimotor gating and repetitive movements—were also greatly diminished in DARPP-32 KO, Thr^{34}Ala-DARPP-32 and Ser^{130}Ala-DARPP-32 mice.[45]

Opioids: Opiates regulate striatal function via an indirect action on mesencephalic dopamine neurons and a direct action on opioid receptors located within striatum.[43] There is a relatively abundant expression of all three opioid receptor sub-types (μ, δ, and κ) in striatum.[57,58] κ opioid receptors are expressed primarily on dopaminergic nerve terminals, whereas μ- and δ-receptors are expressed in medium spiny neurons. μ-receptors appear to be enriched in striatonigral neurons where they are colocalized with D_1 receptors. δ-receptors are highly expressed in cholinergic interneurons, but there is also some expression in striatopallidal neurons. Both μ- and δ-receptors are negatively coupled to adenylyl cyclase. In striatal slices, activation of opioid receptors was found to modulate the effects of dopamine and adenosine on DARPP-32 phosphorylation at Thr^{34}.[59] Thus, consistent with the cellular localization of its receptor, the μ-opioid receptor agonist, DAMGO, inhibits the increase in DARPP-32 phosphorylation induced by SKF 81,297, a D_1 receptor agonist, but not by CGS 21,680, an A_{2A} receptor agonist. Conversely, the δ-opioid receptor agonist, DPDPE, inhibits DARPP-32 phosphorylation induced by activation of A_{2A} receptors, but not by activation of D_1 receptors.

Nicotine: Nicotine has been shown to modulate dopaminergic neurotransmission mainly by enhancing dopamine release in nigrostriatal and mesolimbic dopaminergic systems.[60,61] Five types of α subunits (α2-α6) and three types of β subunits (β2-β4) constitute α and β type heteromeric nicotinic acetylcholine receptors (nAChRs), whereas α7 subunits constitute homomeric nAChRs.[62] Four major populations of nAChRs are expressed at dopaminergic terminals in the striatum, with the major subtype containing α4β2 subunits.[63] The other predominant subtype, α7, is expressed at glutamatergic terminals in the striatum.[64]

Acute application of nicotine in mouse neostriatal slices produces a dose-dependent response, with a low concentration (1 μM) causing a sustained decrease in DARPP-32 Thr^{34} phosphorylation, and a high concentration (100 μM) causing a transient increase in Thr^{34} phosphorylation.[65] Using a variety of pharmacological reagents, nicotine at a low concentration (1 μM) has been found to stimulate α4β2-containing nAChRs at dopaminergic terminals. Activation of α4β2 nAChRs results in the release of dopamine, and the released dopamine selectively activates dopamine D_2 receptor signaling, leading to a reduction in phosphorylation at Thr^{34}. Conversely, nicotine at a high concentration (100 μM) was found to stimulate both α4β2 nAChRs at dopaminergic terminals and α7 nAChRs at glutamatergic terminals. Activation of α7 nAChRs results in the release of glutamate. Co-activation of α4β2 nAChRs by nicotine, and of NMDA/AMPA receptors by glutamate, synergistically induces robust dopamine release, leading to the activation of dopamine D_1 receptors and phosphorylation of Thr^{34}. Thus, depending on the amount of dopamine released locally in response to low and high concentrations of nicotine, either dopamine D_2 receptor signaling in striatopallidal neurons or dopamine D_1 receptor signaling in striatonigral neurons is predominantly activated.

Lysergic acid diethylamide (LSD) and Phencyclidine (PCP): LSD and other related hallucinogens act via multiple serotonin receptors, including 5-HT_2, 5-HT_5 and 5-HT_6 receptors.[66,67] Behavioral studies using discrimination paradigms have suggested a major role for 5-HT_2 receptors in mediating the discriminative stimulus effects of hallucinogens.[68] Stimulation of 5-HT2 receptors increases Ser^{130}-DARPP-32 in slices and LSD also significantly increases phosphorylation of Ser^{130}-DARPP-32 in mice.[45] LSD had similar effects on Ser^{130}-DARPP-32 in slices prepared from striatum and in striata from whole animals. LSD also increased phosphorylation at Thr^{34}-DARPP-32. In slices, but not in striata from whole animals, LSD decreased Thr^{75}-DARPP-32.

PCP acts as a non-competitive antagonist at NMDA receptors and causes a complex behavioral response. In general, NMDA receptor antagonists can produce psychotic symptoms in humans that are indistinguishable from acute episodes of schizophrenia. As a result, glutamatergic dysfunction has been proposed as an underlying cause of the pathophysiology of schizophrenia.[69] Hence, NMDA receptor antagonists, such as PCP, have been used extensively to pharmacologically model psychosis in animals. However, PCP also potently regulates the reward system. Rats can be trained to self-administer PCP.[70] Interestingly, this effect appears to be largely dopamine-independent. Furthermore, PCP-induced locomotor stimulation and potentiation of cocaine reinforcement, can occur without a concomitant increase in dopamine release.[71,72] PCP as well as MK801, another NMDA antagonist, increases Thr^{34}-DARPP-32 phosphorylation.[21,45] PCP also increases Ser^{130}-DARPP-32, but has no effect on Thr^{75}-DARPP-32.[45]

The pattern of DARPP-32 phosphorylation induced by D-amphetamine, LSD, and PCP would be predicted to result in synergistic inhibition of PP-1. In accordance with these biochemical observations, the effects of LSD and PCP, as well as of D-amphetamine, on sensorimotor gating and repetitive movements were reduced in DARPP-32 KO mice and in mice with point mutations at Thr^{34} or Ser^{130} of DARPP-32.[45]

Caffeine: Caffeine, the most commonly used psychostimulant, acts as an antagonist at A_1 as well as A_{2A} adenosine receptors.[73] A role for A_1 adenosine receptors in the stimulatory actions of caffeine was initially suggested. However, it is now recognized that blockade of A_{2A} receptors is critical for the actions of caffeine.[73,74] Recent studies have provided strong evidence for an involvement of DARPP-32 in the stimulatory actions of caffeine. Systemic administration of caffeine, or of SCH58261, a selective A_{2A} receptor

antagonist, causes an increase of Thr^{75}-DARPP-32 in wild-type mice.[41] The stimulatory effects of caffeine and SCH 58,261 on locomotor activity, seen in wild-type mice, were greatly reduced in DARPP-32 KO mice. The blockade of a basally active A_{2A}/PKA/PP-2A/Thr^{75}-DARPP-32 pathway may, therefore, play a critical role for the stimulatory actions of caffeine.

Ethanol: DARPP-32 appears to be involved in both acute and long-term responses to ethanol. Conditioned place preference studies of wild-type and DARPP-32 KO mice indicate a requirement for DARPP-32 in mediating ethanol reward.[75] DARPP-32 KO mice also exhibit a significant decrement in ethanol self-administration. However, DARPP-32 KO mice show a greater sensitivity to the motor stimulant effect produced by a single injection of ethanol. Another recent study has shown that DARPP-32 appears to play a role in ethanol reinforcement by acting to regulate the ability of ethanol to inhibit NMDA receptor function.[76] In the presence of ethanol, NMDA synaptic currents are generally reduced. In brain regions containing DARPP-32, dopamine, via D_1 receptors, stimulates the PKA-mediated phosphorylation of the NR1 subunit of the NMDA receptor at Ser^{897}. In DARPP-32 KO mice this regulation of NMDA receptors is absent, and activation of D_1 receptors does not prevent the ability of ethanol to inhibit NMDA receptors. Interestingly, moderate levels of ethanol increase phosphorylation of Thr^{34} in striatal slices, although the mechanism involved has not been determined.

SUMMARY

A large body of work over the last two decades has found that DARPP-32, when phosphorylated at Thr^{34}, acts as an amplifier of PKA-mediated signaling through its ability to potently inhibit PP-1. This amplifying property of DARPP-32 is critical for dopaminergic signaling, but it is also utilized by multiple other neurotransmitters, including glutamate, serotonin and adenosine, in the striatum and other brain regions. In addition to its role as a PP-1 inhibitor, DARPP-32 when phosphorylated at Thr^{75} inhibits PKA. Upon dopaminergic neurotransmission, the phosphorylation state at Thr^{75} is reduced allowing disinhibition of PKA and further increasing phosphorylation at Thr^{34}. This complex positive feedback loop potentiates dopaminergic signaling.

An important component of our studies of DARPP-32 function, particularly those related to the actions of drugs of abuse, has been the use of DARPP-32 KO mice. More recently these studies have been complemented by generation of mice in which each of the 4 phosphorylation sites have been individually mutated. In future studies, these "phosphomutant" mice will be used to further dissect the precise roles played by Thr^{34}, Thr^{75}, Ser^{97} (the mouse equivalent of Ser^{102}) and Ser^{130} in the stimulatory and rewarding effects of drugs of abuse.

Other recent studies have indicated that the actions of dopamine and adenosine differ between sub-populations of striatal neurons. For example, a recent immunohistochemical study,[48] demonstrated that amphetamine and cocaine increase levels of Thr^{34}-DARPP-32 phosphorylation mainly in striatonigral neurons. It will be important in the future to analyze DARPP-32 phosphorylation in either striatonigral or striatopallidal neurons in response to the actions of different drugs of abuse. We hope that these and other novel types of approach will enable us to identify protein kinases, protein phosphatases and phosphoprotein substrates that mediate the actions of drugs of abuse and that could be novel targets for development of treatments for drug dependence.

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