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S-Glutathionylation and Redox Protein Signaling in Drug Addiction

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Abstract

Drug addiction is a chronic relapsing disorder that comes at a high cost to individuals and society. Therefore understanding the mechanisms by which drugs exert their effects is of prime importance. Drugs of abuse increase the production of reactive oxygen and nitrogen species resulting in oxidative stress. This change in redox homeostasis increases the conjugation of glutathione to protein cysteine residues; a process called S-glutathionylation. Although traditionally regarded as a protective mechanism against irreversible protein oxidation, accumulated evidence suggests a more nuanced role for S-glutathionylation, namely as a mediator in redox-sensitive protein signaling. The reversible modification of protein thiols leading to alteration in function under different physiologic/pathologic conditions provides a mechanism whereby change in redox status can be translated into a functional response. As such, S-glutathionylation represents an understudied means of post-translational protein modification that may be important in the mechanisms underlying drug addiction. This review will discuss the evidence for S-glutathionylation as a redox-sensing mechanism and how this may be involved in the response to drug-induced oxidative stress. The function of S-glutathionylated proteins involved in neurotransmission, dendritic spine structure, and drug-induced behavioral outputs will be reviewed with specific reference to alcohol, cocaine, and heroin.

1. INTRODUCTION

Substance use disorders are chronic, relapsing conditions, which exert deleterious consequences on individuals and society. Regrettably, despite extensive research and a continually evolving understanding of these conditions, current treatment options are limited and ineffective. Therefore, investigating common mechanisms that underlie addictive behavior in the search for novel therapies is of prime importance. In this review, we will present evidence that despite distinct neurochemical mechanisms, abuse of ethanol, cocaine, and heroin all produce oxidative stress, which in turn may induce S-glutathionylation of proteins. We contend that this redox-sensitive epigenetic modification alters protein signaling and may contribute to an addictive phenotype.

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2. OXIDATIVE STRESS

Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are products of aerobic metabolism generated during normal physiology and are important in modulating cellular physiologic processes including cell survival, proliferation, 1–4 differentiation, and apoptosis.[1–4] However, if local or systemic stress increases ROS or RNS production such that endogenous antioxidant defense mechanisms are overwhelmed, oxidative stress occurs.[1] This cytotoxic process has the potential to damage proteins, DNA, and lipids as well as to activate signaling pathways leading to apoptosis.[1,2,5,6] Therefore the balance between oxidants and antioxidants, or redox status, of a cell is critical to healthy cell survival and function. Redox status is highly compartmentalized within cells and it reflects different cellular activities, for example, the high metabolic activity in mitochondria requires a relatively more reducing environment than the cytosol.[7] Maintaining redox homeostasis is especially critical in the brain, an organ which consumes approximately 20% of the oxygen requirement of the body; has a high concentration of oxidation-prone polyunsaturated lipids and has lower levels of antioxidant enzymes.[8–11] Indeed, imbalance between ROS production and antioxidant capacity resulting in oxidative stress has been implicated in the etiology of neurologic disorders including those due to neuroinflammatory and neurodegenerative processes.[4,10,12]

3. S-GLUTATHIONYLATION OF PROTEINS OCCURS IN RESPONSE TO OXIDATIVE STRESS

Protection against oxidative and nitrosative stress is partially mediated by glutathione (GSH), a small, highly abundant hydrophilic γ-glutamyl-cysteine-glycine tripeptide. Oxidative-stress-induced reduction in GSH content in specific brain areas has previously been implicated in neurologic disorders including Parkinson's disease, schizophrenia, Alzheimer's, and epilepsy.[5,11,13] Due to the presence of the central cysteine, GSH exists in either a reduced form or, under oxidative conditions, as glutathione disulfide (GSSG).[14] Therefore the GSH:GSSG ratio provides an indication of redox metabolism within a particular cellular compartment.[14–16] Reduced GSH exists at high (2–3 mM) concentrations in brain tissues and contributes to the reducing status of the cell during normal homeostasis.[1,11,17] As a result of this reducing intracellular environment, many cytoplasmic proteins are rich in free cysteine thiols, which are available to undergo oxidative modification.[1,18] The conjugation of the cysteine residue in GSH with partially oxidized reactive protein thiols, including thiyls and sulfenic acid to form a mixed disulfide is known as S-glutathionylation.[3,11,19,20] ROS-mediated changes to protein thiols move sequentially via oxidation from cysteine sulfenic, sulfinic, and sulfonic acids, of which the latter two are irreversible reactions.[1] As the S-glutathionylation of a protein sulfhydryl group prevents its irreversible oxidation, S-glutathionylation is considered a protective mechanism in cells.[21] Oxidative stress results in an order of magnitude increase in protein S-glutathionylation clearly implicating S-glutathionylation as a redox-sensitive process.[22] In addition, S-glutathionylated proteins can be formed under conditions of nitrosative stress via the intermediate production of S-nitrosglutathione.[5,23] The capacity of a protein to be S-glutathionylated is determined by the accessibility of the cysteine residue in the three-

dimensional protein structure and the presence of surrounding basic amino acids, that is, a low pK_a value.[5,24] S-glutathionylation may occur either spontaneously or may be enzymatically catalyzed by glutaredoxin or members of the glutathione S-transferase (GST) family, which are widely expressed in the brain.[10,22,25–28] Deglutathionylation may occur spontaneously via reversal of the thiol-disulfide exchange reaction once excess oxidative stress has terminated and the GSH:GSSH ratio has increased, or via enzymatic action of thiol-disulfide oxireductases: glutaredoxin, sulfiredoxin, thioredoxin, and protein-disulfide isomerase.[1,18,23,25,29,30] When combined, the S-glutathionylation and deglutathionylation of proteins compose the S-glutathionylation cycle (Fig. 1).[16] The low molecular weight of GSH, which increases its capacity to interact with cysteine residues, combined with its abundance makes it a critical regulator of redox homeostasis.[1]

Although S-glutathionylation of proteins may be constitutive, that is, occurring under basal conditions, they are increased under conditions of increased ROS or RNS, which suggests that they are important in mediating the cellular response to oxidative and nitrosative stress.[1,21]

4. S-GLUTATHIONYLATION OF PROTEINS IS A REDOX-SENSITIVE SIGNALING MECHANISM IN CELLS

In addition to protecting proteins from irreversible oxidation, S-glutathionylation is increasingly being recognized as a means of redox-sensitive protein signaling.[31] The thiol groups of cysteines form intra- and intermolecular disulfide bridges and therefore play an important role in maintaining proper protein folding and structural stability.[14,18] Consequently, the binding of GSH to cysteine residues in proteins may alter the catalytic site or, even if not near the active site, may produce a conformational change such that it results in loss or gain of protein function.[21,32,33] In addition, S-glutathionylation imparts a negative charge to the protein and as a result may produce structural and functional changes.[16] Importantly, modification of cysteine residues via S-glutathionylation is a reversible process and any loss- or gain-of-function will be recovered following normalization of cellular redox status.[1] Therefore S-glutathionylation represents a mechanism by which changes in cellular redox status can be transduced into a functional response via post-translational protein modification, that is, S-glutathionylation is a means of cellular redox siganling.[1,20,34,35]

The mammalian genome encodes 214,000 cysteine residues of which at least 10–20% are redox sensitive under biologic conditions.[36] Proteins susceptible to S-glutathionylation have multiple functions including signal transduction, calcium homeostasis, nitric oxide regulation, energy metabolism, cytoskeletal rearrangement, apoptosis, inflammation, and the expression, folding, trafficking, and degradation of proteins.[3,16,22,28,37–40] The diverse functions included in this list as well as the sheer number of S-glutathionylated proteins suggest both modification of individual protein function as well as modification of coordinated protein pathways in the cellular response to oxidative stress.[41,42] In addition, evidence suggests that S-glutathionylation impacts other important post-translational modifications including phosphorylation and S-nitrosylation.[2,16] Although the physiologic significance of these modifications are not yet fully known, as S-glutathionylation has the

capacity to increase, decrease, or leave unaltered protein function, it is clear that protein thiol redox state may act as a molecular switch by altering protein structure and/or function.[43]

S-glutathionylation fulfills the criteria of specificity, reversibility, and sensitivity under physiologic conditions necessary to be recognized as a regulatory mechanism.[1] S-glutathionylation occurs only on locally available and reactive thiols produce changes in protein function ensuring specificity in signaling.[36] Return to a reducing cellular environment results in a rapid and efficient deglutathionylation characteristic of a reversible system.[44] The degree of GSH conjugation to cysteine thiols is proportional to the aberration in redox status suggesting sensitivity of the system.[44] In addition, these criteria are fulfilled under a high GSH:GSSG ratio in intact cells suggesting that it is a physiologically relevant mechanism.[44] This suggests that cysteine residues in proteins serve as redox sensors capable of inducing changes in redox balance into integrated biologic outcomes.[36]

5. S-GLUTATHIONYLATION IN ADDICTION-RELATED PROTEIN SIGNALING

5.1 Drug Addiction, Dopamine, and Oxidative Stress

Drug addiction is a chronic relapsing disorder characterized by compulsive drug use, loss of control in drug intake despite potential negative consequences, and emergence of negative affect following drug withdrawal.[45] Acute impulsive drug use, defined as a tendency toward rapid unplanned actions and preference for immediate over delayed gratification, progresses to compulsive drug-seeking behavior, defined as the perseveration of maladaptive behavior.[45] This process produces cycles of preoccupation and anticipation of drug use, drug binge, and intoxication following drug consumption, and subsequent withdrawal and negative affect after drug-induced effects have diminished.[46]

Although drugs of abuse produce divergent effects in the brain, increased dopaminergic signaling primarily in the mesocortical and mesolimbic pathways, is a common underlying mechanism.[47–49] At high concentrations, auto-oxidation of the dopamine catechol ring produces quinone and semiquinone species, which reduces the GSH:GSSG ratio.[9,50–52] Alternatively, enzymatic oxidation of dopamine via monoamine oxidases results in the formation of hydrogen peroxide.[9] Due to the high oxidative potential of dopamine, it has been hypothesized that dopaminergic neuron terminals are more likely working closer to the limits of their antioxidant potential due to the need to store dopamine in a reduced state and to counteract ROS generated by dopamine metabolism.[53] Increased ROS production due to dopamine metabolism results in potentially neurotoxic effects especially at high dopamine concentrations such as those elicited by the use of drugs of abuse.[47] Evidence for the potentially neurotoxic role of endogenous dopamine in the striatum has been shown by measuring free and protein-bound cysteinyl-catechols, markers of oxidative damage, in response to intrastriatal injection of dopamine.[53] The resulting lesion and amount of cysteinyl-catechols produced were proportional to the concentration of dopamine injected, suggesting a causal and proportional relationship between striatal dopamine, modification of protein thiols, and neurotoxicity.[53] This relationship between dopamine toxicity and cellular redox status is at least partially attributable to GSH, as SK-N-SH neuroblastoma cells pretreated with a GSH-depleting compound showed elevated markers of apoptosis.[54] In

addition, incubation of astroglia-rich primary cultures in a dopamine-rich solution reduced extracellular GSH, indicating that GSH has a key role in combating the oxidative stress generated by elevated dopamine.[50] A similar effect was found in an experiment where incubation of astroglial cultures in dopamine showed an inverse dose-dependent relationship between dopamine concentration and GSH content.[50] Evidence for dopamine-induced oxidative stress specifically due to drugs of abuse has been shown in a binge methamphetamine paradigm in rats where methamphetamine-induced reductions in striatal GSH were recorded.[52] In addition to having potential neurotoxic effects, generation of ROS in response to drugs of abuse has been implicated in memory and learning processes, which underlie drug addiction behavior.[55]

Changes in protein S-glutathionylation can be expected under any conditions where GSH concentrations are decreased and therefore the oxidation of dopamine has also been implicated in protein S-glutathionylation.[1] In addition, GSTs have been implicated in the metabolism of dopamine.[9] Humans may possess one of four glutathione-S-transferase pi (GSTP) polymorphisms that arise from nucleotide transitions that change codon 105 from Ile to Val and codon 114 from Ala to Val.[22] These polymorphisms are associated with varying levels of functionality due to steric changes in the active site of the enzyme.[22] This implies that individual reactions to oxidative stress will be different and therefore by extension, this may modulate the varied effects of drugs of abuse on humans.[39] In support of this theory, evidence for GSTP polymorphisms and elevated risk of drug use has been found in both cocaine- and methamphetamine-using populations.[56,57] This suggests that dopamine-induced oxidative stress disturbs thiol homeostasis with potential downstream effects on protein function.[58]

The number of proteins that are recognized to undergo S-glutathionylation are increasing rapidly and many of these are implicated in drug addiction (Table 1). However, the physiologic relevance of whether these proteins exist in an oxidized or reduced state and the effect that this may have on addictive processes is not yet clearly understood. For this reason, the effect of S-glutathionylation on a limited number of proteins central to the effects relevant to psychostimulant, ethanol, and opioid addiction will be reviewed.

5.2 Altered Neurotransmission in Response to Ethanol, Cocaine, and Opioids Share Common Signaling Mechanisms

Drug addiction involves a sensitization in the neural processes that control drug reward such that the motivation to consume drug is increased as addiction progresses.[90] Although the mechanisms underlying drug of abuse signaling are complex and not yet fully elucidated, several common substrates exist (Fig. 2). The rewarding effects of drugs are mediated by the mesolimbic dopamine system where increased dopamine release results in binding to postsynaptic dopamine receptors, which are broadly classified into type 1 (D1R) and type 2 (D2R) receptors.[80,90] D1R stimulation activates the cyclic AMP (cAMP)-dependent protein kinase pathway leading to transcription factor induction and phosphorylation of downstream proteins.[91] Briefly, binding to D1Rs increases cAMP with subsequent activation of cAMP-dependent protein kinase or protein kinase A (PKA).[80] Activation of PKA induces phosphorylation of dopamine and cAMP-regulated phosphoprotein 32 kDa (DARPP-32) at

threonine 34, which subsequently inhibits protein phosphatase-1, a regulator of gene transcription.[79] D1R-mediated signaling also increases the translocation of PKA to the nucleus to evoke a nuclear cascade including activation of the transcription factor ΔFosB, activation of which increases cyclin-dependent protein kinase 5 (Cdk5).[79] Cdk5 then phosphorylates DARPP-32 at threonine 75 (at the expense of threonine 34 phosphorylation) to dampen PKA signaling and disinhibit protein phosphatase-1 in a negative feedback mechanism.[66,79,92] Conversely, D2Rs are negatively coupled to adenylyl cyclase with a resultant decrease in cAMP and instead signal via a cAMP-independent process of Akt/GSK3 activation.[79,90] Binding to D2Rs activates phospholipase C, which hydrolyzes phosphatidylinositol-4,5-bisphosphate to form diacylglycerol and inositol triphosphate after which diacylglycerol binds to most of the isoenzymes of PKC to activate them.[83] PKC then acts to phosphorylate serine/threonine residues of the target protein.[83]

D1Rs and D2Rs are colocalized with glutamate receptors in medium spiny neurons of the nucleus accumbens (NAc) and as such the two interact to produce the long-term changes in synaptic plasticity and structure that are associated with drug addiction.[80] Dopaminergic neurotransmission in response to drugs produces reward while glutamatergic signaling codes the context of this reward to result in drug-related learning and memory.[79,80] Glutamatergic neurons from the prefrontal cortex and other limbic areas innervate the ventral tegmental area and NAc to drive dopaminergic neuronal activity or to modulate the activity of dopaminoceptive neurons.[79] Increased dopamine-dependent activity of PKA affects glutamatergic receptors and neurotransmission. PKA-mediated phosphorylation of serine 897 of the GluN1 subunit of glutamatergic *N*-methyl-D-aspartate receptors (NMDAR) controls their trafficking to the membrane surface.[80] In addition, phosphorylation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPAR) at Ser845 by PKA increases binding of AMPARs to anchor proteins required for insertion into the cell membrane.[93,94] Phosphorylation at Ser845 of AMPARs also primes extrasynaptic receptors for insertion into the synaptic space.[95] PKA-induced increase in Cdk5 affects glutamatergic signaling via phosphorylation of postsynaptic density 95 (PSD95), a postsynaptic scaffolding protein that links NMDARs to the cytoskeleton.[96] Cdk5 activation results in smaller PSD95 clusters and therefore reduced NMDAR levels in the synapse.[96] The net result is an increase in the AMPAR:NMDAR ratio, which underlies many forms of plasticity and is required for the induction of long-term potentiation learning.[49,97] Recordings from midbrain slices show that ethanol, morphine, and cocaine produce a significant increase in AMPAR:NMDAR ratio reflecting an increase in basal excitatory strength.[49,97]

The rewarding properties of opiates are mediated primarily by μ-opioid receptors found in the ventral tegmental area and NAc.[49] Binding to μ-opioid receptors of GABAergic interneurons in the VTA disinhibits dopaminergic projection neurons to increase dopamine release in the NAc.[49] Heroin, an opioid drug, exerts effects on the brain by binding to postsynaptic $G_{i/o}$ opioid receptors located postsynaptically on NAc medium spiny neurons with the result that $G_{i/o}$ dissociates into α and βγ subunits.[98] $G\alpha_i$ inhibits adenylyl cyclase activity and thereby cAMP production while $G\alpha_5$ and Gβγ dimers activate adenylyl cyclase, to increase cAMP and PKA.[98] Increased Gβγ activates phospholipase C leading to the activation of PKC and increasing downstream target signaling in an extracellular signal-related kinase (ERK) dependent process.[99] PKC is capable of acutely desensitizing

dopamine receptors.[100] PKA and PKC are implicated in the long-term changes to opioid abuse with PKC mediating the phosphorylation and subsequent internalization of opioid receptors.[101] In addition, the chronic opioid administration upregulation of PKC correlates with the development of tolerance to opioids.[101] This effect is possibly due to binding to opioid receptors, increasing diacylglycerol, and thereby PKC activity.[101] Moreover activation of PKA following chronic opioid use phosphorylates μ-opioid receptors disrupting coupling of these receptors to G_i proteins.[101]

6. S-GLUTATHIONYLATION OF PKA, PKC, Cdk5, AND ACTIN MAY INFLUENCE DRUG BEHAVIOR

In keeping with their role in drug-induced neurotransmission, PKA, PKC, and Cdk5 mediate behavioral responses to drugs of abuse, while alterations in the cytoskeletal protein actin may underlie the effects of drugs of abuse on synaptic strength.

PKA signaling in the NAc is upregulated in response to repeated cocaine use.[102] A study examining the effect of activation or inhibition of the PKA system in the rat NAc found that inhibition of PKA signaling reduced self-administration and induced relapse of cocaine-seeking behavior; a finding explained by a heightened sensitivity to cocaine.[102] In contrast, a PKA activator resulted in increased cocaine self-administration and produced nonspecific motor activity on both drug paired and inactive levers, that is, increased nonreinforced locomotor responses.[102] The effect of PKA inhibition on drug self-administration was repeated in a study where bilateral infusion of PKA inhibitors into the NAc in rat reduced cocaine self-administration and induced relapse after withdrawal suggesting that the effects to cocaine were sensitized.[103] In a further study, administration of a PKA activator to rat NAc increased the motivation for drug consumption measured by an increase in the progressive ratio responding (the effort that the rat was willing to expend in order to obtain a cocaine reward) while the opposite was true following administration of a PKA inhibitor.[81] When combined these results suggest that cocaine-induced alterations in PKA activity have direct relevance to drug-seeking behavior.[81] Of relevance to this review, the C subunit of PKA can be inactivated by the S-glutathionylation of cysteine 199 located in the activation loop, which inactivates the kinase in a redoxsensitive manner and which may allow dephosphorylation of threonine197, which is responsible for coordinating the active conformation and optimal enzymatic activity.[82] This suggests that PKA activity is a potential target of S-glutathionylation-mediated redox signaling control.[104]

Protein kinase C (PKC) isozymes phosphorylate and regulate the activity of some G-protein-coupled receptors and altered levels of PKC have been associated with opiate drugs, ethanol, and cocaine.[83,105,106] While many functions of PKC are protective and required for normal cellular functioning, excessive or prolonged increase in PKC activity may be deleterious.[88,107] Cocaine reinstatement (a model of relapse) following withdrawal is at least partially due to D2R-dependent increases in PKC signaling in the NAc shell with increased D1R or direct pathway signaling to promote relapse.[108] Indeed, a study of cocaine-primed reinstatement of cocaine-seeking behavior showed an increase in phosphorylated PKCγ.[109] Injection of PKC inhibitors into the NAc core inhibits cocaine-seeking in rats while injection

of inhibitors into the ventral tegmental area reduces dopamine release in the NAc as well as cocaine-induced hyperlocomotion. ZIP-mediated inhibition of PKC reduced cocaine sensitization, suggesting that the λ and ξ isozymes specifically play an important role in nonassociative cocaine memory and cocaine addiction.[84] A role for PKC in drug-associated memory was also found in a study where administration of a PKC inhibitor after cocaine administration prevented the development of cocaine preference measured in a conditioned place-preference paradigm.[83] A role for PKC in the rewarding effects of ethanol has also been found with PKCε knockout mice consuming less ethanol than their wild-type counterparts.[107] S-glutathionylation of PKC has been shown to negatively regulate its function.[85]

Chronic cocaine upregulates Cdk5, a serine/threonine kinase, as well as its activator p35.[65] This Cdk5/p35 complex has been implicated in neuronal development and survival, trafficking, transport, and dopaminergic neurotransmission.[65] Cdk5 activation following drug use follows the action of ΔFosB transcription factor, which is upregulated in response to drugs of abuse such as psychostimulants.[65] The relationship between ΔFosB and Cdk5 in the context of cocaine addiction was investigated in mice with inducible and targeted striatal expression of ΔFosB.[66] Adult rats injected with cocaine increased Cdk5 expression in the caudate putamen and NAc[66] while intra-accumbal injection of a Cdk5 inhibitor increased cocaine-induced locomotor sensitization.[66] In a separate study, infusion of a Cdk5 inhibitor into the NAc increased behavioral sensitization to cocaine and cocaine self-administration with increased lever presses for cocaine administration.[110] Increased GSTP decreases Cdk5 function suggesting that S-glutathionylation of Cdk5 may be inhibitory.[67]

Excitatory synapses modify the strength of synapses due to previous experience in a neuroadaptive process known as synaptic plasticity, which allows for the integration of experience-dependent learning.[94,111,112] However, this response is hijacked by drugs of abuse resulting in aberrant plasticity where drugs can produce long-lasting changes in motivation and reward behavior via changes in synaptic plasticity, structure, and function.[45,79,113] The persistence of addiction even following prolonged withdrawal suggests that drugs induce long-term changes in neuronal structure and function that extend beyond the period of intoxication producing a long-lasting drug-induced plasticity that might underlie addictive behavior.[91,114] As the majority of excitatory synapses are formed with dendritic spines, change in the shape or numbers of dendritic spines reflects changes in experiential learning.[111] Rodents chronically treated with cocaine display increased density of immature spines on medium spiny neurons and these structural changes in dendritic spine morphology may persist for several months following the onset of withdrawal.[115] A chronic intermittent ethanol exposure model of alcohol use disorder found that alcohol increased dendritic spine density.[116] Changes in the actin cytoskeleton mediate changes in spine morphogenesis. S-glutathionylation of G-actin on cysteine 374 inhibits its polymerization into F-actin, the form of the protein responsible for increased length and spine head diameter required for the induction of LTP.[21,59,61] In addition, Cdk5 is critically involved in neuronal activity–-mediated spine morphogenesis due to phosphorylation of S6K, a signaling protein which increases protein synthesis.[117]

7. S-GLUTATHIONYLATION IN ALCOHOL ADDICTION

Alcohol is a widely abused drug with 17.7 million people in the United States meeting the criteria for alcohol dependence or abuse in 2012.[118] The effects of alcohol on the brain are complex and varied with ethanol consumption affecting norepinephrine, serotonin, dopamine, opioid, and GABA signaling.[119,120] The primary effects of ethanol are exerted via GABAA and NMDARs to enhance GABA and inhibit glutamate signaling, which when combined produces an overall depressive effect.[45,121] However, with increasing use, NMDAR function is increased and this is further exacerbated in withdrawal to produce cognitive effects such as delirium.[121] In addition, chronic alcohol use and repeated withdrawal downregulates the sensitivity of $GABA_A$ receptor signaling to reduce the locomotor and cognitive effects of ethanol while enhancing the action of $GABA_B$ receptors, which are responsible for decreasing neuronal excitability.[121] Ethanol results in excitation of ventral tegmental area dopamine neurons, increasing dopamine release in NAc projections, which when combined with activation of the endogenous opioid reward system to increase endorphin content in the NAc, is responsible for the rewarding properties of alcohol.[121] Furthermore, ethanol also increases serotonin levels in the NAc.[121]

7.1 Ethanol Metabolism Increases ROS Production

Strong evidence exists for ethanol-induced increases in ROS production with both chronic exposure to ethanol and subsequent withdrawal resulting in oxidatively modified lipids and proteins that may damage glia and neurons.[122] Animal studies have found increased brain lipid peroxidation products in ethanol-exposed rats while stimulation of hippocampal astrocytes with ethanol has been shown to increase ROS generation in the mitochondria.[123,124] Studies of alcoholic patients have found a strong correlation between increased levels of plasma malondialdehyde, a marker of lipid peroxidation, and blood alcohol content at the time of admission.[125] In addition, alcoholic patients have increased blood hydroxyl ion levels and increased levels of the antioxidant enzyme Cu, Zn-superoxide dismutase, and Mn-superoxide dismutase, indicative of oxidative stress.[126,127] Evidence for oxidative-stress-induced damage, as measured by TUNEL-staining, has been found in postmortem superior frontal cortex and hippocampus samples of alcoholic patients.[128] Evidence for the importance of oxidative stress in the metabolism of alcohol has also been found in models of fetal alcohol spectrum disorder where reduced antioxidant defenses during embryonic and fetal stages may explain the sensitivity of the developing brain to the toxic effects of ethanol.[129] When combined this suggests that ethanol increases free radical generation and that the deleterious effects of chronic alcoholism may be associated with oxidative-stress-induced damage. Given the relationship between ethanol and ROS production, it is perhaps unsurprising that acute and chronic ethanol consumption has been associated with decreased GSH levels.[130–132] Postmortem samples from patients with a history of chronic alcohol use found an overall reduction in brain GSH content compared to controls, which may be explained by increased conjugation of GSH to the oxidative metabolites of ethanol.[133] This ethanol-induced reduction in GSH may produce a compensatory increase in serum gamma glutamyltransferase, which occurs following a history of prolonged ethanol consumption and has been used as a biomarker of alcoholic behavior.[134,135]

7.2 Increased ROS Production May Contribute to the Behavioral Effects of Ethanol

Ethanol is metabolized primarily by astrocytic catalase and cytochrome P450 IIE1 in the brain to produce acetaldehyde in a process that leads to increased generation of ROS including hydroxyl and hydroxyethyl radicals.[124,125,136–138] In addition to being potentially cytotoxic, oxidative stress might underlie some of the behavioral features associated with ethanol consumption.[139] Acetaldehyde reacts with 5-hydroxytryptamine in serotonergic neurons to produce 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline.[120] The oxidation of this product by hydroxyl radicals produces greater flux in ROS with the potential to cause oxidative damage.[120] The resultant damage to serotonergic neurons and concomitant decrease in 5-hydroxytryptamine is believed to contribute to increased alcohol preference and ultimately addiction.[120] In addition, the GSH conjugates of 1-methyl-6-hydroxy-1,2,3,4-tetrahydro-β-carboline are oxidized to form 8-S-glutathionyl-1-methyl-1,2,3,4-tetrahydro-β-carboline-5,6-dione, which binds to $GABA_B$ receptors, stimulation of which results in increased ethanol-addictive-type behaviors such as locomotor stimulation and increased risk of relapse.[120] Mice administered alcohol and ebselen, a hydrogen peroxide scavenger, showed reduced locomotor activity when allowed to explore an open-field apparatus suggesting that there is a proportional relationship between brain catalase-H_2O_2 activity and ethanol-induced locomotor stimulatory effects.[139] A further study found that administration of nitropropionic acid, an inhibitor of mitochondrial complex II which increases free radical production, increased locomotor activity in ethanol-treated mice.[140] In addition, mice exposed to hyperoxic conditions to increase brain hydrogen peroxide content, showed increased locomotor activity.[141] These results suggest that ethanol-induced redox signaling may contribute to the behavioral and molecular effects of alcohol in alcohol addiction.[120]

7.3 Protein S-Glutathionylation in Response to Ethanol

While direct evidence for the role of protein S-glutathionylation in the response to ethanol-induced oxidative stress is scarce, a number of studies point to the possible involvement of S-glutathionylating enzymes in alcohol addiction. Ethanol administered in drinking water to rats over 4 weeks increased GST levels in the cerebellum suggesting that increased oxidative stress in response to ethanol consumption increased protein S-glutathionylation.[142] Alpha-lipoic acid, a redox-modulating agent and thiol replenisher, scavenges free radicals and was used to mitigate the effects of ethanol-induced oxidative damage produced in a mouse hippocampal cell line.[143] Rodent studies have also indicated that the transcription factor, Nrf2, is activated by ethanol administration and in turn activates several antioxidative cytoprotective gene clusters including the enzymes involved in GSH homeostasis: γ-glutamyl transpeptidase, GSH synthase, and γ-glutamyl cysteine ligase.[144] In human studies, proteomic analysis of the corpus callosum of healthy and alcoholic postmortem corpus callosum found an alcohol-induced increase in GSTP in the corpus callosum genu.[145] In addition, elevated levels of GST mu were found in the corpus callosum body of alcoholic patients.[146] Reduced levels of GST have been indicated as a potential contributor to the sensitivity of the developing fetal brain to the toxic effects of ethanol and may be a contributor to the enhanced sensitivity of the fetus to oxidative stress.[147] When combined this provides preliminary evidence for the involvement of S-glutathionylation in the response to ethanol-induced oxidative stress.

8. S-GLUTATHIONYLATION IN COCAINE ADDICTION

In 2012, 1.1 million Americans met the criteria for dependence/abuse of the psychostimulant cocaine.[118] Cocaine activates dopaminergic systems in the NAc and amygdala via inhibition of the dopamine transporter.[45] In addition, cocaine blocks serotonin and noradrenaline transporters to glutamate and GABA in the NAc.[45,148]

8.1 Cocaine Metabolism Increases ROS Production

Cocaine is metabolized by either spontaneous or enzymatic hydrolysis or alternatively via oxidation by P450 with the sequential production of N-oxidative metabolites: norcocaine, *N*-hydroxynorcocaine, and norcocaine nitroxide.[149] ROS including hydrogen peroxide and superoxide anion radicals are produced during the oxidative metabolism of cocaine and are believed to induce cellular redox cycling with the end product of increased oxidative stress.[150,151] The positive charge of cocaine at physiologic pH may result in accumulation of cocaine in the mitochondria and its capacity to dissipate mitochondrial membrane potential.[152,153] Cocaine appears to directly inhibit mitochondrial complex I activity to increase ROS accumulation and potentially generate oxidative stress.[153,154] In addition, cocaine has been found to downregulate complex I subunit expression as determined by differential display of genes induced by cocaine when measured in the cingulate cortex.[155] Cocaine administration has also been suggested to increase metabolic activity in the brain, evidenced by a significant increase in temperature, that might contribute to the generation of ROS, as they are a product of physiologic respiratory processes.[156] Evidence for cocaine-induced oxidative stress is clear. Increased ROS levels are found in the striatum and prefrontal cortex of cocaine-treated rats while administration of cocaine to mice has been studied primarily as a means of determining cocaine-induced toxicity.[156] Cocaine administration to mice also produces elevated levels of lipid peroxidation products in the striatum and cortex.[157] Single or repeated cocaine administration in rats increases the formation of hydrogen peroxide in prefrontal cortex mitochondria and increased lipid peroxide production in the striatum and prefrontal cortex.[154] A concomitant increase in the antioxidant superoxide dismutase and GSH peroxidase that co-occurred with the increase in ROS was also found.[154] Therefore acute or chronic cocaine administration was shown to increase ROS production in the dopaminergic brain areas of the striatum and prefrontal cortex and produce a compensatory increase in antioxidant activity.[154] The resulting increase in ROS generation in the brain after cocaine administration, affects the antioxidant status as evidence by decreased GSH concentration and reduced catalase activity.[158,159] Human studies have also found that increased ROS production may produce harmful effects with a study examining cocaine-induced changes in the prefrontal cortex of human cocaine users and in rats administered cocaine finding increased degradation of nuclear PARP-1, a sensor of DNA damage and marker of apoptosis, which was considered potentially indicative of oxidative-stress-induced apoptotic signaling.[113]

8.2 Increased ROS Production May Contribute to the Behavioral Effects of Cocaine

Several studies have found evidence for an association between oxidative stress and behavioral outcomes following cocaine administration. To determine the effects of ROS on cocaine-induced signaling in reinforcement processes, a nonspecific ROS scavenger, PBN,

was administered to rats trained to self-administer cocaine and produced a significant reduction in the number of active lever presses compared to saline-treated controls.[156] This effect was found to be mediated by superoxide anions, as administration of the superoxide dismutase mimetic TEMPOL, a compound shown to reduce superoxide radical generation, produced a similar dose-dependent reduction in cocaine self-administration.[156,160] Both TEMPOL and PBN were also shown to alleviate ROS-induced damage by reducing 8-hydroxyguanosine staining in NAc neurons.[156] Peripheral administration of TEMPOL also reduced cocaine-enhanced dopamine release measured by fast scan cyclic voltammetry.[156] When combined this suggests that the rewarding and reinforcing properties of drugs are at least in part mediated via the effects of ROS.[156]

TEMPOL was also investigated in a study examining the link between the attenuation of oxidative stress and change in behavioral response to cocaine.[161] Cocaine increased lipid peroxidation and nitric oxide radical content in the prefrontal cortex and NAc in both *in vitro* and *in vivo* experiments that were accompanied by a decrease in the total antioxidant capacity *in vitro*. These oxidative-stress-induced changes were attenuated by administration of TEMPOL.[161] The behavioral response to cocaine was measured by locomotor sensitization where cocaine produced a persistent increase in locomotor activity.[161] This was attenuated by the administration of TEMPOL prior to the administration of cocaine while TEMPOL alone had no effect on locomotor activity.[161] These results suggest that the attenuation of cocaine-induced oxidative stress reduces the induction and expression of behavioral sensitization to cocaine providing a link between oxidative stress and behavioral measures.[161]

The association between cocaine-induced oxidative stress in the brain and the oxidative sensor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), which is involved in learning and memory, was investigated in an experimental model of cocaine administration in rats.[162] Changes in synaptic plasticity are required for the memory and learning processes that underlie drug addiction and NF-κB has been implicated in experience-dependent synaptic plasticity as well as the response to oxidative stress.[162] Administration of cocaine to rats with subsequent spatial memory training in the Morris water maze found reduced GSH and GSH-peroxidase levels in the hippocampus although there were no changes in caspase-3 or NF-κB activity.[162] NF-κB activity was decreased by cocaine in the frontal cortex, and was negatively correlated with memory retrieval experiments whereas training in the Morris water maze occurred prior to the administration of cocaine.[162] When combined these results suggest an additional role for NF-κB in cocaine-related learning and memory, separate to that involved in the response to toxic levels of cocaine as suggested by the lack of increase in caspase-3 and the involvement of the prefrontal cortex, an area implicated in addiction-related learning and memory.[162]

A study investigating the effects of cocaine self-administration on markers of oxidative stress differentiated between the motivational aspects of cocaine and the purely pharmacologic responses to cocaine by using a yoked triad scheme whereby contingent responding to cocaine by one rat resulted in a similar administration of cocaine to another rat and saline to a third rat.[163] In this paradigm, active cocaine administration produced an increase in superoxide dismutase activity in the hippocampus, frontal cortex, and dorsal

striatum and a corresponding decrease in malondialdehyde formation with no change in superoxide dismutase activity in those rats that passively received cocaine at exactly the same dose.[163] Withdrawal from cocaine produced elevations in malondialdehyde levels in the hippocampus and prefrontal cortex in rats both actively and passively receiving cocaine suggesting that oxidative stress may be involved in the process of craving.[163] Therefore, oxidative stress occurs during both drug taking and withdrawal with oxidative-stress-induced changes occurring in brain regions associated with motivational use of cocaine and cocaine-induced learning and memory.[163] These results may be partially explained by the motivation aspect of active cocaine administration, which results in greater dopamine release than those rats that receive cocaine passively.[163] This study concludes that oxidative stress is involved in the motivational aspects of voluntary cocaine use possibly via an effect on learning and memory processes.[163]

8.3 Protein S-Glutathionylation in Response to Cocaine

The potential role of GSTP in cocaine-mediated neuroplasticity and related addictive behavior has been previously suggested and was investigated in a study examining protein S-glutathionylation in response to chronic and acute cocaine.[39,164] Chronic cocaine treatment as well as an additional acute challenge increased S-glutathionylation of proteins as measured by a decrease in free sulfhydryl groups, an indirect measure of the amount of GSH conjugated to cysteine residues.[164] In contrast, GSTP levels were reduced by daily cocaine administration but were increased after acute cocaine administration in previously chronic cocaine-treated animals.[164] GSTP knockout mice showed enhanced behavioral sensitization to cocaine and greater preference for cocaine measured in the conditioned place-preference paradigm, an effect replicated by the pharmacologic inhibition of GSTP by ketoprofen injection. Therefore it was concluded that reductions in GSTP may lead to enhanced synaptic plasticity in the response to cocaine.[164]

9. S-GLUTATHIONYLATION IN HEROIN ADDICTION

Heroin is one of the most dangerous of the abused drugs producing a wide range of deleterious effects on the brain including gray matter loss, neuronal apoptosis, mitochondrial dysfunction, synaptic defects, and reduced adult neurogenesis.[165] Although heroin is not widely abused (approximately 467,000 Americans were classified as heroin dependent in 2012), it has a high abuse potential.[118] Heroin, a 3,6-diacetyl ester of morphine, is an opioid analgesic, which is metabolized to morphine in the body.[165] Heroin activates opioid receptors in the ventral tegmental nucleus, nucleus, and amygdala through direct or indirect actions via interneurons.[45] Although the μ-opiate receptor is the primary target of heroin in the brain, heroin also affects dopaminergic, GABAergic, glutamatergic, and serotonergic signaling.[165] Heroin has been shown to upregulate neuronal nitric oxide synthase, which increases brain nitric oxide levels, in the right temporal cortex and left hypothalamic paraventricular nucleus, an area of the classical reward system, of individuals who died from heroin abuse.[165]

9.1 Heroin Metabolism Increases ROS Production

Heroin metabolism occurs via deactylation in the active metabolites morphine and 6-monoacetylmorphine and it is believed that the effects of heroin on opioid receptors occurs primarily through its metabolites via 6-monoacetylmorphine.[166] Long-term heroin abuse induces oxidative stress and reduces total antioxidant capacity, possibly due to elevated dopamine and autoxidation of catecholamines.[167] In addition, incubation of primary cell culture rat cortical neurons in a heroin-containing medium indicated that heroin decreases the mitochondrial membrane potential, which may increase ROS generation.[166] Evidence for increased heroin-induced ROS production has also been found in human studies with heroin users having a greater pro-oxidative than antioxidative balance measured in blood.[168,169] In addition, malondialdehyde levels are increased and total antioxidant capacity is decreased in the cortex of heroin users in comparison to controls with a proportional relationship between malondialdehyde and blood opioid concentration.[170]

9.2 Increased ROS Production May Contribute to the Behavioral Effects of Heroin

Several studies have found evidence that heroin-induced ROS production may be involved in the behavioral effects of the drug. A study using a mouse model of heroin examined the effect of antioxidant treatment combined with heroin on markers of oxidative stress and withdrawal behavior.[171] Mice were administered heroin daily with dosages increasing over the course of 40 days, and were divided into three groups: those that received only heroin, those that received heroin coadministered with antioxidants, or those that received antioxidant supplementation for 10 days following the end of heroin administration.[171] Heroin administration increased oxidative stress as measured by increased percentage of cells with DNA damage and increased carbonyl and malondialdehyde content in brain cells.[171] A corresponding decrease in superoxide dismutase, catalase, and GSH peroxidase activity, and total antioxidant capacity with increasing heroin dose was also found.[171] Administration of antioxidants either concurrently or after heroin administration, reduced markers of oxidative damage.[171] Naloxone-precipitated withdrawal behavior in these mice were measured and suggested that the administration of heroin had produced a dependent phenotype.[171] Interestingly, antioxidant administration resulted in a significant reduction in withdrawal behaviors suggesting that oxidative stress mediated the withdrawal behavioral phenotype.[171] In a separate study, naloxone-precipitated withdrawal increased malondialdehyde and decreased GSH levels measured in whole blood suggesting that oxidative stress is also involved in withdrawal.[172] These changes in malondialdehyde and GSH were reduced by administration of the antioxidants vitamin E or melatonin.[172]

Evidence of a link between oxidative stress and the reinforcing properties of morphine, the primary metabolite of heroin, has also been found. Administration of TEMPOL and the subsequent reduction in oxidative stress in the NAc shell prevented the expression of morphine-conditioned place preference.[173] A study examining the relationship between morphine-induced oxidative stress and withdrawal behavior in rats found that morphine increased malondialdehyde levels, which was further increased by naloxone-induced withdrawal.[174] Treatment with alpha-lipoic acid lowered malondialdehyde levels and symptoms of withdrawal further suggesting that oxidative stress is involved in the morphine addiction and withdrawal phenotype.[174] Administration of the antioxidant hydrogen sulfide

combined with naloxone-precipitated heroin withdrawal has also been shown to significantly decrease behavioral withdrawal symptoms as well as malondialdehyde levels while increasing GSH content and superoxide dismutase, GSH peroxidase, and catalase activity.[175]

9.3 Protein S-Glutathionylation in Response to Heroin

A study examining the effect of antioxidants on heroin-induced oxidative stress and withdrawal behavior may provide evidence for the role of protein S-glutathionylation in mediating the response to heroin. In the study, mice administered heroin at increasing dosages twice daily over the course of 15 days showed reduced total antioxidant capacity and increased ROS production measured in blood and increased thiobarbituric acid and carbonyl content measured in brain.[176] In addition, heroin-treated mice showed significantly more naloxone-precipitated heroin withdrawal behavioral signs than mice administered saline.[176] Treatment with the antioxidant, rosmarinic acid, reduced the levels of ROS in blood and thiobarbituric and carbonyl levels in brain while increasing total antioxidant capacity.[176] Moreover, antioxidant treatment, including treatment with rosmarinic acid, reduced exploring, shaking, and jumping behavior, markers of heroin withdrawal, suggesting that oxidative stress mediates heroin withdrawal-induced behavior.[176] Although this study did not directly measure protein S-glutathionylation, rosmarinic acid has been shown to increase GSTP.[177,178] Therefore, it is possible that S-glutathionylation may be involved in mediating the effects of rosmarinic acid on heroin withdrawal.

10. FUTURE RESEARCH

Further research into drug-induced S-glutathionylation may reveal targets for future treatment. Indeed, the antioxidant and *N*-acetyl prodrug of cysteine, has shown great promise as a treatment in animal models of alcohol and cocaine abuse, reducing both drug-seeking behavior and symptoms of withdrawal.[179–181] NAC is taken up into glia by the cystine/glutamate exchanger (X_c^-), which is responsible for providing the cysteine necessary for the synthesis of GSH and therefore the supply of GSH to neurons by astrocytes.[182–184] Interestingly, X_c^- is itself an unconfirmed target of S-glutathionylation, due to its low pK_a cysteine residues (cysteines 197 and 414). Oxidative-stress-induced reduction in intracellular GSH has been found to increase X_c^- activity, possibly as a mechanism of normalizing redox homeostasis.[185] In keeping with this, NAC was found to reverse GSH depletion in rat astroglial cells following cocaine-induced oxidative stress.[186,187] Regrettably, clinical trials of NAC efficacy as a treatment for substance use disorders have received mixed results.[188] However, these results do suggest that drugs affecting glutathionylation are valid areas of research in addiction pharmacotherapy. In line with this, development of drugs that increase GSTP activity or reversibly inhibit glutaredoxin might represent a novel and highly effective means of modulating drug-induced S-glutathionylation and addictive behavior. However, as the effects of S-glutathionylation on protein function are varied, modification of S-glutathionylation status of specific proteins will be a further challenge.

11. CONCLUSIONS

S-glutathionylation is an understudied post-translational protein modification that allows for sensitive and reversible signaling in response to altered redox status. Increased generation of

ROS with subsequent oxidative stress is a mechanism shared by all drugs of abuse despite their acting on diverse molecular targets. As a result, drug-induced protein S-glutathionylation may translate transient increases in ROS into enduring modifications in biochemistry, neural responses, and ultimately behavior. Indeed, evidence exists for S-glutathionylation of key proteins involved in drug-induced neurotransmission such as PKA, PKC, and Cdk5, which regulate AMPAR and NMDAR insertion into the postsynaptic membrane to influence drug-related learning and memory. Furthermore, S-glutathionylation also affects actin cycling to influence dendritic spine morphology and synaptic strength. As this field of study progresses, the number of proteins confirmed as targets of S-glutathionylation will likely grow and it is possible that many of these may be involved in addictive processes. For this reason, further research in targeting S-glutathionylation as a potential therapy for drug addiction is warranted.

Although a wide body of evidence suggests an important role for S-glutathionylation in drug addiction, this area of research is still relatively unexplored. The role of S-glutathionylation in drug addiction is complicated by its modification of multiple proteins, the function of each of which may be increased, decreased, or unaltered by this post-translational modification. Furthermore, research to date has not examined differences in drug-induced oxidative stress between different cellular compartments, an important line of research given that redox status compartmentalization allows for subcellular specificity in redox signaling.[7] However, previous research has linked neurodegeneration in Parkinson's disease with upregulated GSTP in neuronal synaptosomal fractions.[189] This suggests that GSTP-mediated S-glutathionylation can be modified in the postsynapse, an area where drugs of abuse exert their initial effects. Similarly, glutaredoxin has been found in neuronal cytoplasmic fractions.[190] However, the effect of drug-induced oxidative stress on GSTP and glutaredoxin activity within specific cellular compartments is unknown.

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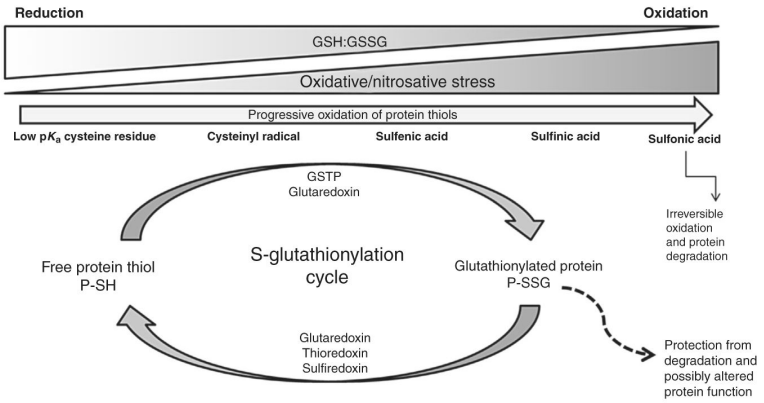

Figure 1.

S-glutathionylation is a reversible oxidative modification of cysteine residues. In response to increased oxidative stress, GSH is conjugated to low pK_a cysteine residues (P-SH) by GSTP or glutaredoxin to form a glutathionylated protein (P-SSG). This process is referred to as S-glutathionylation. Upon return to a more reducing environment, the reverse process is catalyzed by glutaredoxin, thioredoxin, or sulfiredoxin. S-glutathionylation protects against irreversible oxidation of protein thiols and may alter protein function.

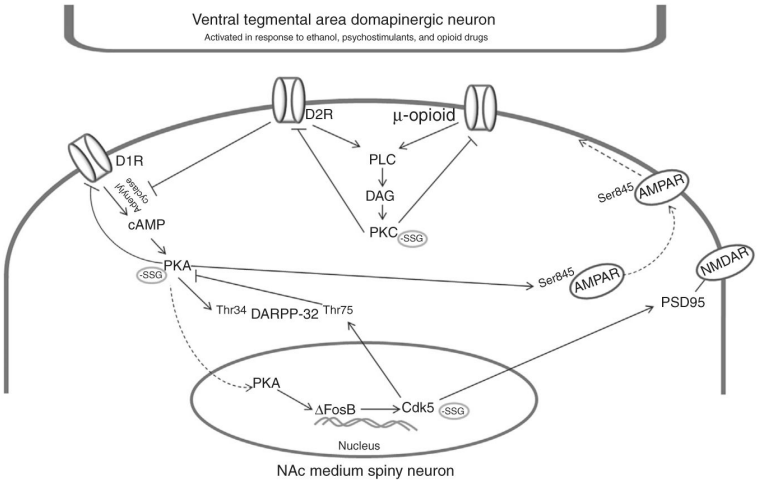

Figure 2.

Drugs of abuse share common signaling pathways. Ethanol, cocaine, and heroin increase dopamine release from ventral tegmental area dopaminergic neurons. Stimulation of D1Rs increases cAMP levels and activates PKA, which phosphorylates threonine34 of DARPP-32. Translocation of PKA to the nucleus increases ΔFosB-mediated transcription, which in turn elevates Cdk5. Cdk5 phosphorylates threonine75 of DARPP-32, which subsequently inhibits PKA activity. Cdk5 also phosphorylates PSD95, a cytoskeletal protein responsible for stabilizing NMDARs in the membrane. Phosphorylation decreases the size of PSD95 aggregates and thereby reduces synaptic NMDAR levels. PKA increases phosphorylation of serine845 of AMPARs, which increases their insertion into the neuronal membrane and their lateral diffusion into the synaptic field. Binding to D2 and μ-opioid receptors increases PLC activity and thereby DAG levels, which activates PKC. PKC-mediated phosphorylation of D2 and μ-opioid receptors results in desensitization. PKA, PKC, and Cdk5 are targets for S-glutathionylation, which decreases their activity.

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Table 1

Proteins Susceptible to S-Glutathionylation are Involved in Drug Addiction

Protein	Function	Relevance to Addiction	Effect of Glutathionylation
Actin	Structural cytoskeletal protein[59]	Actin polymerization is required for dendritic spine remodeling in response to drug use[59,60]	Polymerization is inhibited[61]
Alcohol dehydrogenase	Catalyzes alcohol metabolism	Reduced alcohol dehydrogenase activity results in increased alcohol levels in brain tissue[62]	Glutathionylation has been confirmed but effect is unknown[63]
Caspase 3	Proapoptotic factor[47]	May induce apoptosis in response to drug-induced oxidative stress[47]	Reduced[64]
Cdk5	Neuronal development, survival, trafficking and transport[65]	Phosphorylates DARPP-32 to reduce dopaminergic signaling via D1Rs[66]	Reduced[67]
Cofilin	Depolymerization of filamentous actin[68]	Important for actin cycling, which is required for dendritic spine remodeling in response to drug use[68,69]	Glutathionylation has been confirmed but effect is unknown[70]
COMT	Catecholamine catabolism	COMT is responsible for the degradation of dopamine, the neurotransmitter responsible for drug reward. Increased COMT activity is positively associated with drug abuse[71,72]	Reduced[73]
GSTP	Phase II detoxification enzyme[74]	Conjugates GSH to target proteins in response to oxidative stress to modulate target protein function[28]	Reduced[28]
Myosin	Cytoskeletal structural protein	Involved in dendritic spine morphogenesis and controls the dendritic spine actin cytoskeleton.[75] Dendritic spine remodeling occurs in response to drug use[69]	Reduced[76]
NF-κB	Redox-sensitive transcription factor[4]	Required for dendritic spine remodeling in response to drug use and plays a role in the induction of long-term potentiation[77]	Reduced[78]
PKA	Kinase responsible for phosphorylation of multiple downstream targets including DARPP-32[79]	Involved in AMPAR:NMDAR trafficking and localization.[80] Increased PKA activity correlates with increased motivation to consume drugs[81]	Reduced[82]
PKC	Kinase responsible for phosphorylation of multiple targets including G-protein-coupled receptors[83]	Increased PKC activity correlates with increased drug-related memory, drug seeking, and reinstatement behavior[83,84]	Reduced[85]
Profilin	Polymerization of globular actin[86]	Important for actin cycling, which is required for dendritic spine remodeling in response to drug use[69]	S-glutathionylation has been confirmed but effect is unknown[70]

Protein	Function	Relevance to Addiction	Effect of Glutathionylation
Tyrosine hydroxylase	Rate-limiting enzyme in dopamine synthesis[87]	Dopamine is responsible for the acute rewarding effects of drugs[88]	Reduced[89]

A small subset of the proteins that have been identified as susceptible to S-glutathionylation are included in the table and their relevance to drug addiction is also included.