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Nitric oxide-GAPDH transcriptional signaling mediates behavioral actions of cocaine

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Abstract

Psychotropic actions of cocaine are generally thought to involve its blockade of monoamine transporters leading to increased synaptic levels of monoamines, especially dopamine. Subsequent intracellular events have been less well characterized. We describe a signaling system wherein lower behavioral stimulant doses of cocaine, as well as higher neurotoxic doses, activate a cascade wherein nitric oxide nitrosylates glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to generate a complex with the ubiquitin-E3-ligase Siah1 which translocates to the nucleus. With lower cocaine doses, nuclear GAPDH augments CREB signaling, while at higher doses p53 signaling is enhanced. The drug CGP3466B very potently blocks GAPDH nitrosylation, hindering both signaling cascades and inhibits both behavioral activating and neurotoxic effects of cocaine. This system affords potentially novel approaches to the therapy of cocaine abuse.

Graphical abstract

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The authors declare none.

Keywords

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The role of cocaine as a behavioral stimulant was evident from its first introduction in the late nineteenth century. Scientists at the Merck Drug Company in Germany had isolated cocaine as the active ingredient in the extracts of the coca plant used to energize Peruvian mountain climbers. With a sample of cocaine provided by Merck, Sigmund Freud detected its stimulant actions (1). The sympathomimetic influences of cocaine were appreciated fairly early in the 20th century (2). Thus, when norepinephrine was established as the neurotransmitter of sympathetic neurons, it was natural to assume that it mediated effects of cocaine. Sympathomimetic amines act “directly” upon receptors or “indirectly” by facilitating the release of norepinephrine. Cocaine was established as an “indirect” sympathomimetic amine.

Definitive evidence as to how cocaine acts at a molecular level was provided in 1960 by Julius Axelrod, who employed the then recently developed [³H]norepinephrine. Axelrod showed that cocaine and other “indirect” sympathomimetic amines inhibited the accumulation of norepinephrine into sympathetic organs such as the heart, while “direct” amines lacked this activity. Thus, he established that indirectly acting sympathomimetic amines work by blocking the reuptake inactivation of norepinephrine (3,4).

Axelrod’s work focused upon norepinephrine, because it was the only accepted catecholamine neurotransmitter at that time. Dopamine was considered to be merely a

precursor to norepinephrine. About this time Arvid Carlsson reported levels of dopamine in the corpus striatum that were 10 times higher than those of norepinephrine, arguing that dopamine may be a transmitter in its own right (5). There ensued a body of research discriminating between dopamine and norepinephrine as mediators of cocaine effects, as well as the actions of amphetamine, which resemble those of cocaine. Relative potencies of amphetamine and cocaine derivatives as inhibitors of norepinephrine and dopamine uptake tended to favor dopamine as the mediator of cocaine's actions (6–10). Since cocaine also inhibits uptake of serotonin (11,12), there have been studies linking cocaine to serotonin as well as to dopamine (13,14). However, the current consensus opinion favors dopamine (15).

The nitric oxide/glyceraldehyde-3-phosphate dehydrogenase (GAPDH) signaling pathway

How do these actions of cocaine, occurring at cell membranes, impact intracellular events? A variety of workers have explored influences of cocaine upon nuclear signaling, such as chromatin remodeling (16–18). Moreover, transcription factors such as CREB and fosB have also been associated with cocaine (19–21). Transcriptional targets have been invoked, especially for BDNF, and immediate-early genes, such as c-fos and Arc, may also play important roles (21–23).

We were prompted to explore actions of cocaine in terms of messenger systems such as nitric oxide (NO) because of evidence that NO mediates a diversity of signaling systems both in the periphery and in the brain. Thus, NO was first identified as endothelial derived relaxing factor responding to actions of acetylcholine and bradykinin to relax smooth muscle of blood vessels (24–26). Cloning of the three principal forms of NO synthase (NOS) facilitated this effort. In particular, neuronal NO synthase (nNOS) appears responsible for the principal generation of NO in the brain (27–30).

How does NO influence overall cellular activity, especially nuclear events? The signaling cascade is initiated by various neurotransmitters, hormones and growth factors activating different forms of NOS. In the brain NMDA transmission provides the calcium that activates nNOS via its bound calmodulin (Fig. 1) (31,32). In blood vessels calcium similarly activates endothelial NOS (eNOS) (33). With inflammatory stimulation new protein synthesis of inducible NOS (iNOS) takes place to generate large quantities of NO (34). Signaling information reaches the nucleus via a pathway whereby NO elicits nitrosylation of glyceraldehyde-3-phosphate-dehydrogenase (GAPDH). Nitrosylated GAPDH acquires the capacity to bind to the ubiquitin E3 ligase Siah1, which possesses a nuclear localization signal and conveys nitrosylated GAPDH to the nucleus (35,36).

Within the nucleus two different systems of signaling take place. With cytotoxic stresses, such as LPS-induced cell death for example, nuclear GAPDH activates the histone acetylating enzyme p300/CBP, which acetylates and activates p53 that in turn stimulates the cell death transcriptional targets, PUMA and Bax (Fig. 1) (37). By contrast, with less powerful, more physiologic stimuli, such as the neurotrophic factor BDNF (brain-derived neurotrophic factor) and NGF (nerve growth factor) a nuclear complex of nitrosylated GAPDH links Siah1 with the histone-methylating enzyme SUV39H1. This complex triggers

the degradation of SUV39H1 via the ubiquitin E3 ligase activity of Siah1 within the complex. Release from the methylation of histone lysines by SUV39H1 then facilitates acetylation of histone H3, which leads to CREB binding to DNA and increased expression of CREB-regulated genes such as c-fos and BDNF with associated augmentation of neuronal activity (Fig. 2) (38).

Cocaine in modest doses elicits behavioral stimulation, whereas at higher doses it is neurotoxic. We discovered that cocaine acts through the NO-GAPDH system with lower, stimulant doses activating the “neurotrophic” nuclear cascade, while higher, toxic doses act via the pathophysiologic PUMA/Bax system (39).

Behavioral and neurotoxic actions of cocaine both involve nitrosylated GAPDH

Our earlier studies discriminating “physiologic” and “pathologic” nuclear signaling for NO-GAPDH had employed markedly different stimuli for the two modes of nuclear actions (35–38,40,41). We wondered whether cocaine might provide a means of addressing both physiologic and pathologic actions with a single stimulus drug.

Dose-response relationships for cocaine are well established. In preliminary experiments we found that a dose range of 5 – 30 mg/kg cocaine provides behavioral stimulation without evidence of neurotoxicity, whereas 30 – 50 mg/kg doses are progressively neurotoxic. Both stimulant and neurotoxic treatment protocols of cocaine increased levels of nitrosylated GAPDH, with the neurotoxic regimen producing substantially greater effects. These effects were mediated via the neuronal NO system, as they were abolished in nNOS knockout mice (39).

We monitored nuclear events by measuring the binding of CREB to the c-fos promoter or the binding of p53 to the PUMA promoter. Single low doses of cocaine (5 – 30 mg/kg) increased CREB-c-fos promoter interactions, while multiple high doses (40 mg/kg) stimulated p53 binding to the PUMA promoter (39). We took advantage of our earlier evidence that NO-GAPDH signaling initiates a pathway leading to nuclear transcription of p53 and CREB (37). We showed that the behavioral stimulant regimen of cocaine would increase levels of the immediate early genes c-fos and Arc but not of PUMA, effects lost in nNOS knockouts. On the other hand, the neurotoxic regimen increased levels of PUMA and Bax but not c-fos (39).

We explored effects of stimulant and neurotoxic doses of cocaine on promoter interactions. Stimulant doses of cocaine markedly increased CREB/c-fos promoter binding, whereas neurotoxic cocaine doses increased p53/PUMA promoter binding (39).

All of the above effects were lost in nNOS knockouts. We established that NO was acting through GAPDH in subsequent experiments in mice. We made intrastriatal injections with lentiviral mRNA particles of wild type GAPDH or C150S mutants of GAPDH, which cannot be nitrosylated. We detected nitrosylation of wild type but not C150S mutant GAPDH following treatment with both behavioral and neurotoxic doses of cocaine. CREB

binding to the c-fos promoter and c-fos levels were both increased in mice overexpressing wild-type GAPDH but not GAPDH C150S. Similarly, at the neurotoxic cocaine doses p53 binding to the PUMA promoter as well as PUMA protein levels were increased in mice overexpressing wild-type GAPDH. Thus, GAPDH nitrosylation was evidently mediating both behavioral and neurotoxic actions of cocaine (39).

We sought a pharmacologic means of interrupting the NO-GAPDH system. Others had established that the monoamine oxidase inhibitor deprenyl is neuroprotective (42,43). These neuroprotective actions are unrelated to monoamine oxidase inhibition (44,45). Thus, deprenyl derivatives, such as CGP3466B (omigapil or TCH346) lack monoamine oxidase inhibitory activity but retain neuroprotective effects (Fig. 3) (46).

CGP3466B prevented GAPDH nitrosylation with remarkable potency, acting at concentrations lower than 1 nanomolar (47,48). Preventing GAPDH nitrosylation by treatment with CGP3466B was associated with inhibition of GAPDH binding to Siah1 as well as with nuclear translocation of GAPDH (48). As little as 0.15 mg/kg CGP3466B almost fully prevented the stimulation of GAPDH nitrosylation in the corpus striatum elicited by cocaine and prevented the cocaine-associated nuclear translocation of GAPDH (39).

The initial step in cocaine's behavioral effects is enhancement of dopamine actions upon D1 receptors, which are blocked by the D1 antagonist SCH23390 (49). We confirmed an association of D1 receptors with the NO-GAPDH cascade. Thus, the increase of GAPDH nitrosylation following both by stimulant and neurotoxic doses of cocaine was blocked by treatment with SCH23390. Accordingly, the NO-GAPDH pathway, like the major behavioral actions of cocaine, largely involves dopamine signaling through D1 receptors (39). These findings suggest that other direct or indirect D1 receptor agonists might stimulate the NO-GAPDH pathway.

Behavioral stimulant doses of cocaine increased nuclear degradation of SUV39H1 leading to associated diminished histone methylation and increased transcriptional activation of CREB along with increased expression of c-fos. CGP3466B blocked all of these actions (39).

Behavioral responses to cocaine paralleled these biochemical actions. Thus, CGP3466B treatment markedly reduced locomotor stimulation by cocaine without affecting basal locomotor activity. We also evaluated behavioral preferences for cocaine in the conditioned place preference model. In this system, preference for cocaine was reduced by treatment with CGP3466B. Neurotoxic effects of cocaine were also blocked by CGP3466B. We evaluated deprenyl, which would be predicted to elicit effects similar to CGP3466B based on its inhibition of GAPDH nitrosylation. Other monoamine oxidase inhibitors, such as rasagiline, lazabemide and pargyline, had no effect on GAPDH nitrosylation, indicating that deprenyl was not acting as a monoamine oxidase inhibitor. The prevention of GAPDH nitrosylation by deprenyl predicts that it ought to decrease behavioral effects of cocaine, which is what we observed. Whereas deprenyl substantially decreased cocaine's locomotor activation, rasagiline, pargyline and lasabemide, when combined with cocaine, elicited greater locomotor stimulation than cocaine alone. Deprenyl alone did not have an effect on

locomotor activity. This enabled us to conclude that deprenyl, like CGP3466B, reduced locomotor stimulant effects of cocaine by inhibiting GAPDH nitrosylation. Neurotoxic effects of cocaine were also blocked by deprenyl but not the other monoamine oxidase inhibitors (39).

Cytotoxic insults, acting via NO-GAPDH, lead to GAPDH binding to the protein acetylating enzyme p300, which in turn acetylates GAPDH leading to activation of p53's transcriptional activity and induction of PUMA (37). Neurotoxic doses of cocaine stimulated binding of p53-GAPDH as well as acetylation of both GAPDH and p53 in the striatum of intact mice. Activation of p53 led to increased p53-PUMA promoter binding as well as elevated PUMA levels. In contrast to the low doses of cocaine which activate c-fos but not PUMA, at high cocaine doses c-fos was undetectable, while PUMA expression was increased. CGP3466B blocked activation of p53 as well as induction of PUMA (39).

We evaluated cocaine neurotoxicity by examining apoptosis utilizing the TUNEL procedure. Neurotoxic doses of cocaine (30 – 50 mg/kg) substantially increased cellular apoptosis, which was prevented by CGP3466B. Cocaine (50 mg/kg) disrupted motor coordination in the rotarod test, effects reversed by CGP3466B. In contrast, the monoamine oxidase inhibitors rasagiline, pargyline and lazabemide failed to rescue cocaine-induced cell death and didn't improve rotarod performance. Under basal conditions in the absence of cocaine, these drugs also did not affect rotarod performance. The inability of deprenyl to elicit protective effects under these conditions might relate to its monoamine oxidase inhibition potentiating the disruptive influences of cocaine (39).

Conclusions

Our study indicates that the behavioral stimulant and neurotoxic actions of cocaine both involve nuclear translocation of NO-GAPDH which influences transcription. With the lower, behavioral stimulant doses cocaine signals largely through CREB and the c-fos promoter to increase transcription of genes such as BDNF and Arc (Fig. 4A). On the other hand, the high, neurotoxic doses appear to act largely through p53 mediated transcription of the pro-death genes PUMA and Bax (Fig. 4B) (39).

These findings fit with our other observations contrasting cytotoxic and neurotrophic signaling by the NO-GAPDH system. Thus, with cytotoxic insults nuclear GAPDH leads to enhanced acetylation by p300/CBP of p53 along with pro-death gene stimulation (37). In contrast, physiologic stimulation by neurotrophic agents BDNF and NGF is associated with assembly of GAPDH and Siah1 in a nuclear complex with the histone-methylating enzyme SUV39H1. In this complex the ubiquitin E3 ligase activity of Siah1 causes degradation of SUV39H1. The resultant diminished histone H3 methylation leads to enhanced acetylation of this histone and increased transcription by CREB with augmented neuronal outgrowth (38). Presumably the behavioral-stimulant and neurotoxic actions of cocaine reflect the signaling systems linked to CREB and p53 respectively (39).

Because of its great potency and selectivity, CGP3466B is a valuable tool for discriminating diverse actions of cocaine and other stimuli. It prevents GAPDH nitrosylation at

subnanomolar concentrations (47,48). Low nanomolar levels of deprenyl also inhibit GAPDH nitrosylation (48). Both drugs prevent transcriptional signaling by cocaine and diminish behavioral and neurotoxic actions of cocaine (39).

Might there be therapeutic implications of these findings? CGP3466B and related drugs exert neuroprotective actions in animal models of motor neuron disease (50), muscular dystrophy (51,52), amyotrophic lateral sclerosis (46) and Parkinson's Disease (53,54), though limited clinical trials in Parkinson's Disease have not been promising (55). It is worth noting that CGP3466B post-treatment had neurorescuing effects in multiple neurotoxicity models (46). Our findings suggest that CGP3466B and related drugs may be useful in treating cocaine overdoses as well as its behavioral actions (39). Actions of other stimulant drugs and a wide range of psychopharmacologic agents may be influenced by manipulation of the NO-GAPDH signaling cascade.

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List of Abbreviations

Arc	Activity-regulated cytoskeleton-associated protein
Bax	B-cell lymphoma-associated X protein
BDNF	Brain-derived neurotrophic factor
CBP	CREB-binding protein
c-fos	Cellular fos. Fos is an oncogene, the name is derived from FBJ (Finkel–Biskis–Jenkins) murine osteogenic sarcoma virus
C150S	Cysteine 150 to serine mutation
CREB	cAMP response element-binding protein
fosB	Delta FBJ murine osteosarcoma viral oncogene homolog B
eNOS	Endothelial nitric oxide synthase
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
LPS	Lipopolysaccharide
NGF	Nerve growth factor
nNOS	Neuronal nitric oxide synthase
NO	Nitric oxide
NOS	Nitric oxide synthase
p300	A histone acetyltransferase
p53	Tumor suppressor protein that protects from DNA damage
PUMA	p53 upregulated modulator of apoptosis

SUV39H1	Suppressor Of variegation 3–9 homolog 1 (Histone methyltransferase that specifically trimethylates 'Lys-9' of histone H3)
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling

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Figure 1.

The NO-GAPDH-Siah1 cell death cascade in neurons. Severe stress in the brain induces the NO-GAPDH-Siah1 cell death pathway. Glutamate excitotoxicity is illustrated as an example of such severe stress. Stimulation of the NMDA receptor with toxic levels of glutamate leads to an influx of calcium that activates nNOS through calmodulin. Activated nNOS induces the nitrosylation of GAPDH. SNO-GAPDH binds Siah1, which in turn escorts GAPDH to the nucleus. Once in the nucleus, GAPDH binds to p300/CBP and activates it via

acetylation. P300/CBP induces cell death by activating p53-mediated transcription of cell death genes.

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Figure 2.

The NO-GAPDH-Siah1 cascade regulates neuronal differentiation. Physiologic stimuli such as BDNF stimulate nNOS mediated nitrosylation of GAPDH, its binding to Siah1 and the translocation of both proteins to the nucleus. In the nucleus the GAPDH-Siah1 complex binds to the histone methyltransferase suppressor of variegation 3–9 homolog 1 (SUV39H1). Siah1 ubiquitinates SUV39H1 leading to its degradation. This leads to decreased methylation of histone H3 at lysine 9 and relief of gene expression silencing. Degradation of

SUV39H1 leads to increased acetylation of histone H3 on lysine 9 and in turn CREB mediated gene expression leading to increased neurite outgrowth.

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Omigapil
(TCH346 or CGP3466B)

Selegiline
(L-deprenyl)

Figure 3.
The structures of CGP3466B and l-deprenyl.

Figure 4.

GAPDH nitrosylation pathway mediates cocaine effects. **(A)** A recreational dose of cocaine induces dopamine-1 receptor (D1R) dependent nNOS-mediated nitrosylation of GAPDH. SNO-GAPDH binds Siah1, and both proteins translocate to the nucleus. Degradation of SUV39H1 by Siah1 leads to increased CREB dependent gene expression and mediates the behavioral effects of cocaine. **(B)** A toxic dose of cocaine, on the other hand, stimulates the NO-GAPDH-Siah1 cell death cascade inducing cell death via activation of p300/CBP and p53 mediated gene expression.