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Influence of the dopaminergic system, CREB, and transcription factor-κB on cocaine neurotoxicity

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Abstract

Cocaine is a widely used drug and its abuse is associated with physical, psychiatric and social problems. Abnormalities in newborns have been demonstrated to be due to the toxic effects of cocaine during fetal development. The mechanism by which cocaine causes neurological damage is complex and involves interactions of the drug with several neurotransmitter systems, such as the increase of extracellular levels of dopamine and free radicals, and modulation of transcription factors. The aim of this review was to evaluate the importance of the dopaminergic system and the participation of inflammatory signaling in cocaine neurotoxicity. Our study showed that cocaine activates the transcription factors NF-κB and CREB, which regulate genes involved in cellular death. GBR 12909 (an inhibitor of dopamine reuptake), lidocaine (a local anesthetic), and dopamine did not activate NF-κB in the same way as cocaine. However, the attenuation of NF-κB activity after the pretreatment of the cells with SCH 23390, a D1 receptor antagonist, suggests that the activation of NF-κB by cocaine is, at least partially, due to activation of D1 receptors. NF-κB seems to have a protective role in these cells because its inhibition increased cellular death caused by cocaine. The increase in BDNF (brain-derived neurotrophic factor) mRNA can also be related to the protective role of both CREB and NF-κB transcription factors. An understanding of the mechanisms by which cocaine induces cell death in the brain will contribute to the development of new therapies for drug abusers, which can help to slow down the progress of degenerative processes.

Key words: Cocaine; Apoptosis; NF-κB; CREB; BDNF; Neurotoxicity

Introduction

Drug abuse and addiction constitute a public health problem of great importance: both affect many people and cause a wide variety of consequences to society. Cocaine is an abused drug with a high prevalence worldwide. According to the United Nations Office on Drugs and Crime (UNODC, 2011 <http://www.unodc.org/documents/data-and-analysis/WDR2011/World_Drug_Report_2011_ebook.pdf>), although cocaine use has declined, it is still one of the most abused drugs in the USA. Cocaine inhibits the dopamine transporter (DAT) in neuron terminals, causing an increase in extracellular dopamine levels. Activation of dopamine transmission in the mesocortico-limbic system is a common characteristic of all addictive drugs. This system originates in the ventral tegmental area (VTA) and projects mainly to the nucleus accumbens (NAc) and prefrontal cortex (PFC). Repeated exposure to cocaine leads to neuroadaptations in the mesocorticolimbic system that are associated with the development of addiction (1,2). Addiction is a chronic relapsing disease (3) and its treatment is the most expensive of the neuropsychiatric disorders (4), mainly owing to the costs of health care, productivity loss, and crime (UNODC, 2011).

Cocaine Toxicity

Cocaine users seek the effects of euphoria (feeling of well-being), self-confidence, and increased alertness. However, cocaine abuse can also lead to adverse effects such as anxiety, paranoia, self-centered behavior, dysphoria, and delusions (5). Moreover, many studies have demonstrated a variety of toxic effects of cocaine in humans (5-8) and animals (9,10). A study of 332 cocaine users in São Paulo found that one-fifth had severe

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seizures and death resulting from its chronic use (11). It has also been demonstrated that the use of high doses of cocaine is associated with violent behavior, including murder and suicide (12). The stimulant effects of cocaine can lead to a rapid increase in its intensity due to a sensitization process related to drug craving and increased intake, leading to the use of increasing concentrations of the drug by chronic users (13). There are reports that cocaine causes cardiovascular, neuromuscular, and central nervous system toxicity, and complications such as infections, kidney and lung injury, liver toxicity, and reproductive disorders (14). The occurrence of epilepsy and psychiatric and neurological deficits is also related to the use of this drug (15). Cocaine can affect cellular morphology or function, including: inhibition of neurite extension (extensions of the cell bodies of neurons) (16), changes in the function and morphology of mitochondria (17), reduced dilation of the endoplasmic reticulum (18), and abnormal lysosomal proteolysis (19). Cognitive disorders such as learning and memory deficits are reported in most chronic users of cocaine (20-22) and in children of dependent mothers (23-25). Prenatal exposure to cocaine (26,27) may affect fetal development because cocaine is able to cross the placenta (28) and accumulate in the fetus (29,30). The consequences - for developing neurons - of *in utero* exposure to psychostimulants are regarded as a major area of interest. It is estimated that approximately 30,000-160,000 newborns are exposed to cocaine *in utero* per year (Mathias, 1995 <http://archives.drugabuse.gov/NIDA_Notes/NNVol10N1/NIDASurvey.html>). However, the consequences of prenatal cocaine use and the mechanism of action by which this drug exerts its effects have not been widely investigated. It has been reported that newborns exposed to cocaine *in utero* tend to have low birth weight, decreased head circumference, systemic hypertension, tachycardia (31,32), and deficits in cognitive development (33,34). The cognitive abnormalities detected in the first year of life appear to contribute to learning and attention disabilities at school age (35). The changes associated with intrauterine exposure to cocaine may be related to molecular adaptations or to anatomical changes in specific brain regions, such as the anterior cingulate cortex, prefrontal cortex, and middle frontal areas that regulate cognitive and emotional development (25).

In vitro studies investigating the effects of cocaine in cell culture neuroglioblastomas (36), PC12 cells (37), cortical neurons of fetal mice (38), and neuronal precursor cells (39) have reported changes in the growth and differentiation of neurons and in the activation of cell death pathways. In addition, it has been reported in animal studies that prenatal exposure to cocaine causes morphological brain abnormalities and cognitive deficits after birth (34,40). Taken together, these data suggest that cocaine can cause a variety of adverse effects on neuronal development.

Cocaine effects on CREB and NF-κB

The induction by cocaine of the immediate expression of genes involved in apoptotic cascades has been reported by several researchers and it is believed that this effect is mediated primarily by the stimulation of D1 receptors (41,42). Thus, it is suggested that cocaine causes changes in gene transcription that can be associated with some long-lasting functional changes (43). CREB is a transcription factor that can be phosphorylated by several protein kinases. CREB proteins comprise a family that binds to a particular sequence of DNA, called the cAMP response element (CRE) (44). Activation of CREB involves several steps; the phosphorylation of serine 133 (45) and the recruitment of CREB-binding protein (CBP) are crucial (46,47). CREB plays an important role in mediating the effects of cAMP and neurotransmitters that act on gene expression via the cAMP pathway. Some of the genes that contain CRE sites express Fos, proencephalin, somatostatin, tyrosine hydroxylase, α1-Na, K-ATPase, and vasoactive intestinal peptide (48,49). The activation of the cAMP pathway is regulated by the dopaminergic system, so the transcription factor CREB seems to be involved in the effects of chronic administration of psychostimulants (50).

NF-κB plays an important role in regulating the inflammatory response and cell death (51). NF-κB is a transcription factor found in a variety of cell types, including neurons and microglia (52). NF-κB can be activated by pro-inflammatory stimuli such as: pathogen-derived lipopolysaccharide (LPS); cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-1 β (IL-1β); and reactive oxygen species (53-55).

NF-κB proteins comprise members of the Rel/NF-κB family, forming homo- and heterodimers through a combination of the p65 (or RelA), p50, p52, c-Rel, or RelB subunits. It is constitutively expressed in the cytoplasm where it is bound to IκB, a protein that masks the nuclear localization signal of NF-κB, thereby retaining it in the cytoplasm (56). Inducers of NF-κB act through intracellular signaling cascades that activate the IκB kinases (IKKs), which phosphorylate two specific N-terminal serines of IκBα, resulting in IκBα polyubiquitination and degradation in the 26S proteasome (57).

When IκBα is degraded, NF-κB migrates to the nucleus and modulates the transcription of target genes involved in cell death. Evidence obtained in our laboratory showed, by electrophoretic mobility shift assay, that 1.0 mM cocaine induced activation of NF-κB in PC12 cells after 6 h of incubation (58). The activation of the p50/p65 subunit of NF-κB by cocaine is linked to the activation of the D1 dopamine receptor (58). Cocaine concentrations used in our study were similar to those previously used by others in different cell types (59,60). Cocaine-induced NF-κB activation was also observed in macrophages (61), human brain endothelial cells (62), and PC12 cells (63). In addition, *in vivo* studies in mice showed that chronic administration of cocaine induced NF-κB activation in NAc (64). It is important to note that high constitutive NF-κB activity mediates resistance to oxidative stress in neuronal cells (65) and in agents that inhibit NF-κB activation-induced apoptosis in response to several neurotoxins (66,67). In fact, in our study, the inhibition of NF-κB significantly increased cell death accelerated by cocaine treatment, suggesting that this transcription factor plays a protective role in cocaine-treated PC12 cells (58). In addition, Lee et al. (62) showed an anti-apoptotic effect of NF-κB in PC12 cell death induced by auto-oxidized dopamine. Taken together, the results showed that concentrations of cocaine comparable to the concentration that has been reported in plasma levels (0.3 μM to 1 mM) of subjects who use this drug (60,68) can induce changes in transcription factors that are important to the inflammatory response and innate immune response (69) and to cell death and the cell protection response.

Cell Death and Cocaine

Cell death can occur by two distinct mechanisms: necrosis and apoptosis. Necrosis, also called pathological or accidental cell death, occurs when cells are exposed to an extreme variation of their physiological conditions (such as hyperthermia and hypoxia) with consequent damage of the membrane, leading to cell death. Apoptosis, unlike necrosis, is a selective and regulated process important for embryogenesis, development, and the depletion of infected cells. However, a change in the process of apoptosis can lead to the development of some neurodegenerative diseases such as stroke, Alzheimer's disease, and Parkinson's disease (70).

Given the diversity of situations that can cause neuronal death by apoptosis, it is not surprising that several components of signal transduction have been described that participate in this process. Among them is the loss of growth factors with neurotrophic activity such as neuronal growth factor (71), which can be caused by an increased release of cytokines such as TNF-α (72), the excitotoxicity caused by an excessive increase in the concentration of excitatory amino acids such as glutamate (73), or by the increased oxidative stress and modulation of transcription factors such as NF-κB (74).

In nervous tissue, apoptosis and necrosis may coexist or occur sequentially, with the mode of cell death being influenced by the intensity and duration of harmful stimuli and also by the energy state of the cell (75). One way to induce apoptosis is by the release of mitochondrial cytochrome c and the subsequent activation of caspases 3, 6, and 7 (76).

In addition to the caspase-dependent apoptotic process, caspase-independent events may also occur (77). One of the most studied proteins involved in apoptosis is caspase-independent apoptosis-inducing factor, which, when released from mitochondria, translocates to the nucleus, where it induces DNA fragmentation independent of caspases. The proteins that form part of the Bcl-2 family (Bax, Bak, Bcl-XL, Bcl-2, and others) regulate programmed cell death, the integrity of the mitochondria, and cytochrome c release (78). By acting on mitochondria, these proteins have an important role in determining death and cell survival (79). The Bax protein is considered pro-apoptotic and the Bcl-2 protein anti-apoptotic (60). The expression of Bcl-2 is increased in neurons that survived ischemic strokes, and a reduction in this protein exacerbates neuronal death (80). The expression of Bcl-2 can be induced by several promoters that bind to its regulatory region, including CREB (81). Thus, the Bcl-2/Bax balance is crucial to the regulation of cell death.

Brain-derived neurotrophic factor (BDNF) is a neurotrophin that plays an important role in neuronal protection. The intracellular signaling of BDNF occurs by the binding of neurotrophin to its receptor TrκB and by the activation of protein tyrosine kinases in the cytoplasm (82). BDNF is regulated by many factors, including transcription factors such as NF-κB and CREB. These, too, are indirectly regulated by the expression of the receptor TrκB, which requires the presence of secondary events such as increased cAMP or Ca^{2+} to be efficiently inserted in the plasma membrane, thereby being able to transduce the signal triggered by the binding of BDNF (83).

Several reports suggest that cell death by apoptosis plays an important role in the induction of neuronal loss caused by cocaine and other psychostimulants (84,85). It has been shown that cocaine activates the mitochondrial apoptotic pathway, decreasing the levels of mitochondrial cytochrome c and activating caspases 3 and 9 in cultured neuronal cortex (59). In myocardial cells, cocaine inhibits a complex of the respiratory chain of mitochondria (68) and decreases the mitochondrial membrane potential and ATP levels in cardiomyocytes (86). Finally, the results of microarray experiments (87) suggest that mitochondrial function and energy metabolism are affected in the brains of cocaine human abusers.

We confirmed that cocaine treatment induced PC12 cell death by apoptosis and that necrosis was associated with mitochondrial dysfunction, increased LDH release, activation of caspase 3, decreased Bcl-2 expression, and increased α-spectrin cleavage. In our experiments, we found an increase in BDNF mRNA levels 6 h after treatment with cocaine, indicating a transitory rise in this neurotrophin. BDNF regulates the differentiation and apoptosis of neurons and glial cells (88), and the increase in BDNF may be considered as a line of defense against the apoptosis process caused - in our model - by cocaine. In fact, the increase in BDNF mRNA levels could be linked to the activation of NF-κB and CREB (89). The protective role of NF-κB in cocaine treatment of PC12 cells may be associated with the expression of anti-apoptotic genes, such as BDNF. However, the compensatory mechanisms for cell death induced by cocaine are ineffective at

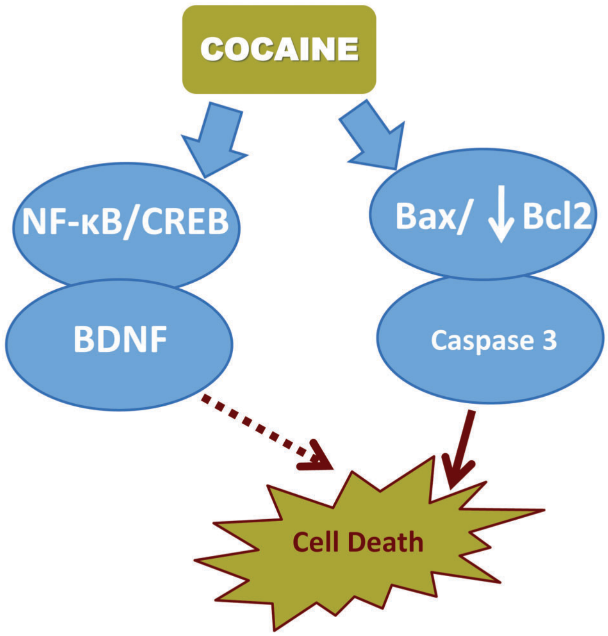

Figure 1. Schematic representation of the neurotoxic action of cocaine in PC12 cells. The treatment of PC12 cells with cocaine can alter the Bax/Bcl-2 ratio, reducing the Bcl2 levels, which could lead to activation of caspase 3 and the triggering of the cell death process seen after 24 h of treatment. On the other hand, to protect the cell from cocaine toxicity, both NF-κB and CREB are activated at 6 h. The activation of these transcription factors could lead to transcription of anti-apoptotic genes, such as brain-derived neurotrophic factor (BDNF), that act to reverse the process of cell death.

terminating the apoptosis process later (Figure 1). It is interesting to note that methamphetamine and 3,4-methylenedioxymethamphetamine can also induce apoptosis in the same way as cocaine, but cocaine seems to be less toxic (84). This may be due to the induction of protective systems (e.g., NF-κB and BDNF) by cocaine. Therefore, the activation of both transcription factors may represent a compensatory mechanism to limit cell death associated with cocaine drug abuse.

Although we have considered the induction of apoptosis related to mitochondrial dysfunction as the prime pathway involved in cocaine neurotoxicity, it is important to consider other alternative pathways that can also play an important role in this process, such as the NADPH oxidase pathway. In fact, cocaine is associated with severe oxidative stress in cardiomyocytes involving the production of reactive oxygen species, leading to MAPK activation and an apoptosis process that is mediated by NOX2 (90).

An understanding of the mechanisms by which cocaine induces cell death in the brain will contribute to the development of new therapies designed to slow the progress of neurodegenerative processes in drug abusers.

Cocaine can cause damage to the newborn children of pregnant women who use cocaine during the pregnancy. As the migration behavior of neurons ultimately determines their connectivity, synaptic potential, and success of neurotransmission, cocaine may produce behavioral and anatomical alterations as a result of maternal cocaine use during pregnancy by acting on neuronal guidance. An understanding of the mechanisms by which cocaine leads to motivational alterations in offspring may ultimately pave the way for the development of strategies for educational intervention programs and/or potential pharmacological treatments.

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