Cocaine and the AP-1 Transcription Factor Complex

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ABSTRACT: Cocaine addiction in humans develops gradually with repeated administrations and persists long after cocaine has cleared the body. The mechanisms underlying this persistent form of neuroplasticity are not understood and can involve both structural and biochemical mechanisms. The long time course for cocaine addiction in humans and for development of cocaine self-administration in animal models suggests the involvement of alterations in gene expression leading to altered signaling in the brain. In the striatum (Str) and nucleus accumbens (NAc) of rats, pretreatment with repeated cocaine administrations downregulates the induction of various immediate early genes (IEGs) by a subsequent acute challenge with cocaine. Some of these downregulated IEGs encode Fos-related components of the activator protein-1 (AP-1) complex, which is likely to regulate a number of genes important for neuronal function. Interestingly, repeated cocaine administration induces novel delta FosB-related proteins (called chronic Fos-related antigens (Fras)) in the NAc and Str that replace the downregulated isoforms of Fos. Unlike the acutely induced, short-lasting isoforms of Fos and FosB, the chronic Fras persist long after the last cocaine administration. The known form of delta FosB per se lacks the domain required to activate transcription. If the chronic Fras are similar in structure to delta FosB, then the induction of chronic Fras likely leads to a blockade of AP-1-dependent transcription resulting in altered gene expression. We are presently purifying the chronic Fras to obtain amino acid sequence in order to directly examine our hypothesis about the effects of repeated cocaine administration on AP-1-dependent transcription and gene expression in the brain.

INTRODUCTION

Addiction to cocaine depends on the gradual development of persistently altered behavioral responses to repeated administration of drug or drug-related cues (for reviews, see Refs. 1–4). These altered responses include increased craving for cocaine when it is absent and tolerance to higher drug doses when it is administered. They also include locomotor sensitization and conditioned place preference in rats, as well as 'punding' in humans. There is a great deal of evidence that the critical anatomical substrate of the acute rewarding effects of drugs of abuse is the mesoaccumbens pathway extending from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). In recent years, these same 'reward regions' have been implicated in the chronic changes underlying addiction, including the altered responses to cocaine (for reviews, see Refs. 1–3, 5). Investigations of neurotransmitter level changes or alterations in receptor number or affinity in the VTA and NAc following repeated administration of cocaine have been contradictory or inconsistent, suggesting that post-receptor alterations may underly the altered behavioral responses. Work from the laboratory of Eric Nestler and many other laboratories has shown a variety of drug-induced alterations in the levels

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of specific mRNA messages and proteins within post-receptor signal transduction pathways in the VTA and NAc (for reviews, see Refs. 2, 3, 5). These alterations are likely to have direct effects on neuronal signaling and thus on behaviors with and without the drug in the system. But how do these alterations in protein levels come about?

REGULATION OF GENE EXPRESSION

The process of regulating cellular protein levels, from DNA to proteins, is called gene expression. Gene expression can be regulated by alterations in protein translation or degradation, as well as mRNA transcription or degradation. Most recent work has focused on transcription regulation (for reviews on transcription regulation, see Refs. 6, 7). mRNA is transcribed from DNA by RNA polymerase II. A large complex of proteins called general transcription factors are critically involved in the binding of RNA polymerase II at the transcription start site and initiating polymerase activity. The promoter region, usually found upstream of the coding sequence of the gene, contains unique DNA sequences called enhancers that bind to proteins called specific transcription factors. These specific transcription factors interact with the general transcription factors, directly and indirectly, to regulate the formation of the general transcription initiation complex and thus regulate the transcription rate of specific genes. Many different specific transcription factors have been identified. Some are present at basal levels in the cell and are regulated by post-translational modifications (*e.g.*, phosphorylation of Ser-133 on cyclic adenosine monophosphate (cAMP)-responsive binding protein (CREB)). Others are rapidly induced and degraded and require de novo protein synthesis (*e.g.*, cFos). The latter are encoded by immediate early genes (IEGs) and are thought to induce further alterations in gene expression.

THE AP-1 TRANSCRIPTION FACTOR

The activator protein-1 (AP-1) complex is a well studied specific transcription factor complex that is composed of IEG proteins (for review, see Ref. 8). It is a heterodimer formed by the cFos protein, or its related isoforms, dimerizing with the Jun protein, or its related isoforms. The isoforms of Fos are often called Fras (Fos-related antigens); they include Fra-1, Fra-2, FosB, and the truncated form of FosB, called delta FosB. The isoforms of cJun include JunB and JunD. The AP-1 complex binds to a specific DNA sequence, called the AP-1 site, in the promoter of a number of genes important for neuronal function to either activate or repress transcription.

PSYCHOSTIMULANTS REGULATE THE AP-1 TRANSCRIPTION FACTOR

The psychostimulants cocaine and amphetamine have been shown to rapidly induce the IEGs, including cFos and the Fras at the mRNA and protein levels in the NAc and striatum (Str).[9–12] The mRNAs encoding these proteins obtain peak levels 45–60 minutes after drug administration and return to control levels by about 2 hours. The proteins obtain peak levels at about 2–3 hours and return to control levels by 4–8 hours. Interestingly, one day following chronic treatment with cocaine (twice daily for 14 days), an acute challenge of cocaine no longer induces the mRNA encoding cFos, the Fras, JunB, and zif268.[11,12] There is a downregulation of the IEG response induced by cocaine. In contrast, the levels of most all the proteins in the pathway leading to induction of cfos are increased following chronic psychostimulant administration. These

include adenylyl cyclase, protein kinase A, cAMP regulated element binding protein (CREB), as well as CREB phosphorylation at the Ser-133 site, which is necessary for activation of cfos transcription.[2,13] Thus, downregulation of IEGs must involve either downregulation of an activating pathway, such as via the serum response element (SRE) or sis-inducible element (SIE) sites in the promoter of cfos, or upregulation of a repressing pathway, such as the observed increase in dynorphin levels leading to increased kappa-receptor-mediated decreases in adenylyl cyclase activation.[13–16]

Downregulation of acute induction of the mRNAs encoding proteins of the AP-1 complex suggests that induction of the AP-1 complex and its DNA binding activity should also be reduced following chronic cocaine administration. Gel shift assays were used to measure the amount of binding by the AP-1 protein complex to the specific DNA sequence of the AP-1 consensus site. As expected AP-1-dependent binding increased rapidly after acute cocaine administration and returned to control levels by 8 hours.[11,17] Surprisingly, AP-1 binding was still highly elevated one day following the last chronic cocaine administration and returned to control levels with a half-life of about 7 days. This was an approximately 50-fold increase over the half-life of the acutely induced AP-1 complex. On the other hand, further induction of AP-1 binding by acute cocaine challenge was significantly downregulated as expected.[11] The chronic AP-1 complex also had slightly different affinities for AP-1-like DNA sequences.[18] The different binding abilities to these sequences suggest that the chronic AP-1 complex may bind to different AP-1-like sites in different promoters with different affinities. This would have qualitative, in addition to quantitative differences in effects on AP-1-dependent transcription in the chronically administered animal.

THE AP-1 COMPLEX CONTAINS NOVEL PROTEINS CALLED CHRONIC FRAS

Supershift assays with an antibody that recognizes an epitope common to cFos and all the other Fras disrupts DNA binding by both the acutely and chronically induced AP-1 complexes.[11,17,18] This indicates that both AP-1 complexes contain cFos or a Fos-related protein. Immunoblots with this same Fra antibody show the expected induction of a number of acutely induced Fras, including cFos, following acute cocaine administration. The various Fras have different time courses, but all return to control levels within 8 hours. In contrast, one day following the last chronic cocaine administration, there is a doublet of Fra-immunoreactive bands with molecular weights of 35–37 kDa. Similar to the chronic AP-1 complex, these proteins persist with a half-life of about 7 days. The time courses, supershift assay, and immunoblots all suggest that these 35–37-kDa Fras are responsible for the long-lasting chronic AP-1 complex. We call these long-lasting proteins the 'chronic Fras,' because they are induced significantly only by repeated administrations and not by single acute administrations.

The chronic Fras and chronic AP-1 complex were even more strongly induced when the twice-daily administrations were reduced to once daily for the same number of total administrations.[19] When the rate of administration was reduced again to once every three days, chronic Fra levels were similar to that for twice-daily administrations. Altering the frequency of repeated cocaine administrations had a different effect on the ability of subsequent acute cocaine challenges to induce the acute Fras. Acute induction of the Fras was significantly downregulated following twice-daily administrations of chronic cocaine.[19] When the interval between administrations was increased to one day, downregulation was less evident and altogether absent with three-day intervals. Thus, the chronic Fras and the chronic AP-1 complex are unlikely to play a direct role in the downregulation of the acutely induced Fras.

Importantly, the chronic Fras and the chronic AP-1 complex are also induced by a number of chronic treatments that are very different from cocaine administration, including chronic electroconvulsive seizures (ECS), chronic tranylcypromine, kainic acid, and chronic apomorphine following 6-hydroxydopamine (6-OHDA) lesions.[17,18,20] Chronic Fras were found only in those regions where the same acute treatment induces cFos and the acutely induced Fras. This allows one to hypothesize that repeated perturbations of a neuron gradually induce the chronic Fras as an adaptive mechanism for inducing further alterations in gene expression that are more appropriate for responding to long-term, versus short-term, changes in the neuron's environment.

WHAT ARE THE IDENTITIES OF THE CHRONIC FRAS?

Immunoblotting with an antibody against the N-terminus of FosB and delta FosB labeled a 35-kDa band with the same migration rate and time course as the chronic Fra bands.[18,21,22] In contrast, the chronic Fras were not labeled by an antibody for the C-terminus of FosB, which is absent from delta FosB. An independently derived antibody against FosB and delta FosB disrupted DNA binding by the chronic AP-1 complex,[21] confirming the presence of a FosB-like protein in the complex. Importantly, the chronic Fras could not be induced in the brains of FosB knockout mice,[23,24] which strongly suggested that the chronic Fras are a product of the fosB gene. As a whole, the data suggest that the chronic Fras are identical to delta FosB, which also has a molecular weight of approximately 35 kDa. However, unlike the chronic Fras, the acutely induced delta FosB protein is rapidly induced, returns to control levels after 8 hours, and migrates slightly faster on one- and two-dimensional electrophoretic gels.[18,21] Additionally, there are two sets of chronic Fras: one with an isoelectric point (pI) similar to that of delta FosB, while the other has a very different pI not explained by phosphorylation states.[18,21] Attempts to dephosphorylate the chronic Fras have failed to make them migrate similarly to the acutely induced delta FosB (unpublished observations). Thus the exact identity of the chronic Fras remains undetermined.

The similarity of the chronic Fras to delta FosB allows one to hypothesize about the effects of the chronic Fras on neuronal transcription. Two studies using the F9 teratocarcinoma cell line, have shown that the C-terminus, which is absent from delta FosB, is necessary for transcriptional activation by the FosB protein.[25,26] This suggests that the chronic Fras may act as a dominant negative for AP-1-dependent transcription. However, two other studies using the NIH 3T3 cell line have shown significant transcriptional activation by delta FosB, similar to that of cFos.[27,28] Therefore, the function of chronic Fras is clearly undetermined. The transcriptional activating ability of delta FosB and thus of the chronic Fras can be different depending on the cells in which it is induced. Therefore, a determination of the *in vivo* effects of chronic Fras on AP-1-dependent transcription must await studies performed with neurons *in vivo* as opposed to cultured cells. However, relevant information under conditions validated by *in vivo* studies could be obtained from studies with cultured cells.

THE CHRONIC FRAS HETERODIMERIZE WITH JUND

Supershift experiments indicate that the chronic Fras heterodimerize primarily with JunD.[21] It has been suggested that JunD is not as strong an activator as cJun, which is found in the acutely induced AP-1 complex. Thus again, the chronic AP-1 complex could act as a dominant negative for AP-1-dependent transcription. However, although JunD homodimers have been shown not to activate AP-1-dependent transcription as

well as cJun homodimers, JunD is just as active as cJun when heterodimerized with cFos.[29] So again, it is difficult to predict from cell culture studies what the effects of the chronic AP-1 complex will be *in vivo.*

FUTURE DIRECTIONS

Purification of the chronic Fras is the next step in the investigation of chronic Fras and the chronic AP1 complex. Purification is necessary for 1) a determination of what allows them to be induced significantly only by chronic and not acute treatments; 2) what modifications allow them to persist for a half-life 50-fold longer than the acute Fras; 3) detailed studies of the effects of these modifications on AP-1-dependent transcription both in cell culture and *in vivo. In vivo* experiments are also necessary to determine which AP-1-dependent genes are regulated *in vivo,* as opposed to cultured cells, by the chronic, as well as acute Fras. This will allow one to examine their significance in the molecular mechanisms underlying drug addiction. With this information, one may be able to specifically regulate the long-term effects of repeated drug abuse without altering other effects of the drug. This may help both treatment compliance and specifically target the long-term craving that underlies the truly damaging effects of drugs of abuse.

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