Molecular Neurobiology of Drug Addiction

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The purpose of this review is to illustrate the ways in which molecular neurobiological investigations will contribute to an improved understanding of drug addiction and, ultimately, to the development of more effective treatments. Such molecular studies of drug addiction are needed to establish two general types of information: (1) mechanisms of pathophysiology, identification of the changes that drugs of abuse produce in the brain that lead to addiction; and (2) mechanisms of individual risk, identification of specific genetic and environmental factors that increase or decrease an individual's vulnerability for addiction. This information will one day lead to fundamentally new approaches to the treatment and prevention of addictive disorders. ***[Neuropsychopharmacology 11:77–87, 1994]***

KEY WORDS: *Synaptic transmission; Drug addiction; G proteins; Ventral tegmental area; Cyclic AMP; Nucleus accumbens*

EVOLVING MODELS OF SYNAPTIC TRANSMISSION

The most important area where molecular neurobiological investigations have contributed to drug addiction, and to biological psychiatry in general, is the increasingly complete and sophisticated picture of synaptic transmission these studies have provided. Figure 1 shows a working model of synaptic transmission around the time (1968–1976) that the first two volumes of the American College of Neuropsychopharmacology's *Generation of Progress* series were published. During this time, most studies in biological psychiatry focused on extracellular aspects of synaptic transmission, namely, on neurotransmitters and their interactions with reuptake sites, receptors, and ion channels located throughout the brain. More recent molecular studies have shed critical new light on this level of understanding of synaptic transmission. The cloning of numerous subtypes of reuptake proteins, receptors, and channels has refined our understanding of the mechanisms of action of psychotropic drugs. This information is also being used to develop drugs that interact with various protein subtypes with ever-increasing specificity. Such drugs will provide invaluable tools in basic studies of drug addiction and could also represent improved pharmacotherapeutic agents, although this latter possibility remains conjectural.

By the time the *Third Generation of Progress* was published in 1987 (Meltzer 1987), there was a much greater appreciation for the complexity of synaptic transmission (Figure 2). It was known that neurotransmitter-receptor regulation of ion channels represents only a small fraction of a neurotransmitter's effects on its target neurons. In addition to regulation of ion channels, it was becoming increasingly clear that neurotransmitters regulated virtually all processes that occurred within neurons, including gene expression. It was known further that many of the effects of neurotransmitters on ion channels, and all of the other effects of neurotransmitters on target neurons, are achieved through biochemical cascades of intracellular mes-

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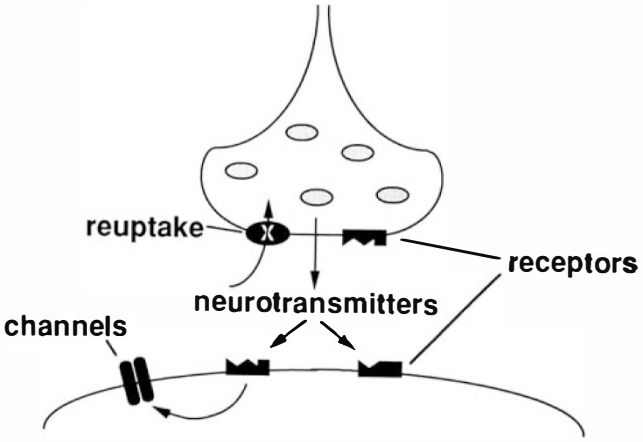

Figure 1. A working model of synaptic transmission, circa 1968–1976. During this period of time, synaptic transmission was conceived as the release of neurotransmitter from a nerve terminal, the binding of the neurotransmitter to specific receptor sites on target neurons, and the resulting alterations in the conductances of specific ion channels. The action of the neurotransmitter is then terminated by its reuptake into the nerve terminal.

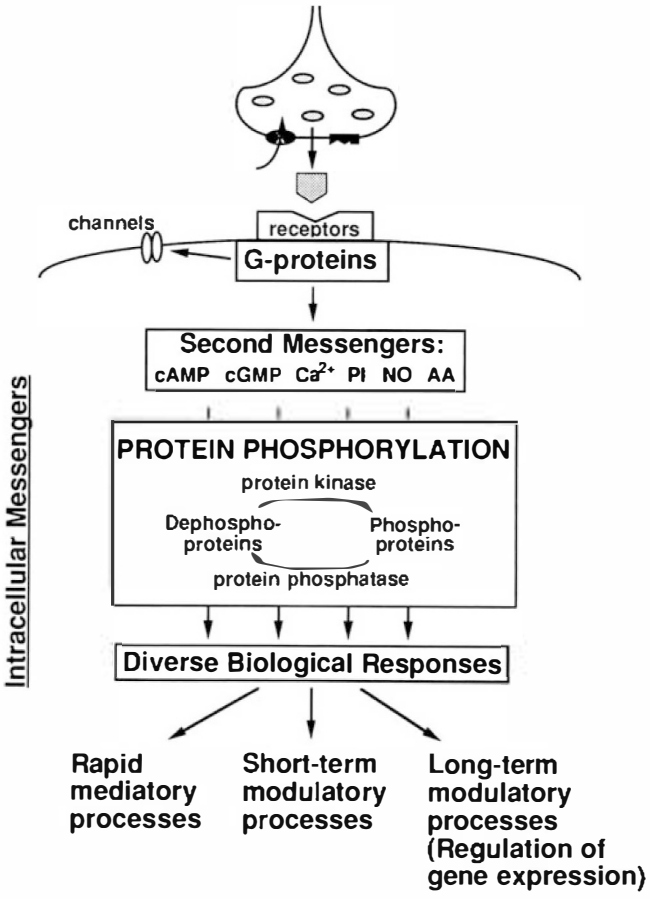

Figure 2. A working model of synaptic transmission, circa 1987. Studies in molecular neuroscience from 1976 to 1987 provided a much more complex view of synaptic transmission than that shown in Figure 1. These studies focused on the involvement of intracellular messenger systems, involving coupling factors (termed G proteins), second messengers [e.g., cAMP, cGMP, Ca^{2+}, nitric oxide (NO), and the metabolites of phosphatidylinositol (PI) and arachidonic acid (AA)], and protein phosphorylation (involving the phosphorylation of phosphoproteins by protein kinases and their dephosphorylation by protein phosphatases), in mediating multiple actions of neurotransmitters on their target neurons. Second messenger-dependent protein kinases (e.g., those activated by cAMP or Ca^{2+}) are classified as protein serine/threonine kinases, because they phosphorylate substrate proteins on serine or threonine residues. Each second messenger-dependent protein kinase phosphorylates a specific array of substrate proteins (that can be considered third messengers) and thereby leads to multiple biological responses of the neurotransmitter.

The multiple biological responses to a neurotransmitter can be divided into three main categories. In some cases, intracellular messengers mediate the actions of some neurotransmitters in opening or inhibiting particular ion channels. However, intracellular messengers mediate most of the many other actions of neurotransmitters on their target neurons. Some are relatively short-lived and involve modulation of the general metabolic state of the neurons, their ability to synthesize or release neurotransmitter, and the functional sensitivity of their various receptors and ion channels to various synaptic inputs. Others are relatively long-lived and are achieved through the regulation of gene expression in the target neurons. Thus, neurotransmitters, through the regulation of intracellular messenger pathways and alterations in gene transcription and protein synthesis, alter the numbers and types of receptors and ion channels in target neurons, the functional activity of the intracellular messenger systems in those neurons, and even the shape and numbers of synapses the neurons form. The figure is drawn to illustrate the amplification that intracellular messenger systems can give to neurotransmitter action: the single event of a neurotransmitter binding to its receptor (the first messenger level) can act through the second, third, fourth, etc. messenger levels to produce an increasingly wider array of physiological effects. Modified from Hyman and Nestler 1993.

sengers. These intracellular messengers consist of G proteins (GTP-binding membrane proteins), second messengers (such as cAMP and Ca^{2+}), and protein phosphorylation whereby the addition or removal of phosphate groups from all types of neural proteins alters their function and leads to the myriad of biological responses in question (see Nestler and Duman 1994; Nestler and Greengard 1994).

As the *Fourth Generation of Progress* (Bloom and Kupfer, 1994) approaches publication, we have gained an even greater appreciation of the complexity of synaptic transmission. As shown in Figure 3, we now know that neurotransmitter-receptor regulation of G proteins and second messenger-dependent protein phosphorylation pathways represents a small part of a neuron's intracellular regulatory machinery (see Duman and Nestler 1994). Neurons express high levels of trans-

membrane protein tyrosine kinases (such as the trk proteins) that serve as plasma membrane receptors for neurotrophins and many other growth factors. In addition to their role in development, these growth factors are now known to produce profound effects on fully differentiated, adult neurons. In fact, the traditional distinction between a neurotransmitter and a neurotrophin is becoming increasingly arbitrary. Neurons also express large numbers of cytoplasmic protein tyrosine kinases (such as src kinase) and protein serine/threonine kinases (such as the ERKs or MAP-kinases). Although these cytoplasmic enzymes are not regulated directly by extracellular signals, they are under the control of extracellular signals via second messenger-dependent and growth factor-dependent pathways.

The scheme shown in Figure 3 is still a simplified version of our current knowledge of synaptic transmission. The figure does not include the many types of protein phosphatases associated with each of these protein kinase systems that are also subject to regulation by extracellular and intracellular signals. The figure also does not adequately depict the numerous interactions that exist among the various intracellular messenger pathways. This high degree of interactions means that a primary perturbation in one particular pathway produces secondary, tertiary, etc., changes in other pathways, with regulation of these other pathways contributing eventually to the many biological responses of the original perturbation.

This information means that although most psychotropic drugs interact initially with proteins located at the extracellular aspect of the synapse (e.g., cocaine inhibits monoamine reuptake proteins and opiates activate opioid receptors), the numerous acute actions of these drugs are produced ultimately through the many intracellular messenger pathways that mediate these extracellular signals. Moreover, these intracellular messenger pathways play a central role in mediating the long-term effects that these drugs exert on brain function. This information, then, provides a conceptual framework within which highly specific and detailed hypotheses can be formulated concerning the precise mechanisms by which psychotropic drugs regulate brain function both acutely and chronically.

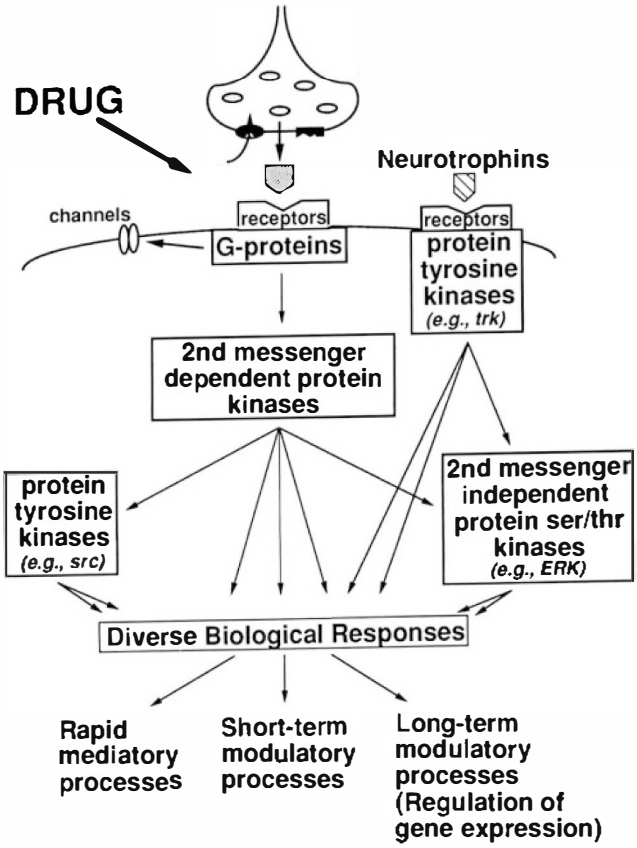

Figure 3. A working model of synaptic transmission, circa 1994. It is now apparent that the brain contains many important intracellular regulatory pathways in addition to those regulated directly by G proteins and second messengers. Brain contains numerous protein serine/threonine kinases that are not regulated directly by second messengers (e.g, the ERKs or MAP kinases). In addition, the brain contains numerous types of protein tyrosine kinases (that phosphorylate substrate proteins on tyrosine residues), some of which reside in the receptors for neurotrophins and most other growth factors (e.g., the trk proteins), and others that are not associated with growth factor receptors (e.g., src kinase). Each of these various protein kinases are highly regulated by extracellular stimuli. The second messenger-dependent protein kinases are regulated by receptor-G protein-second messenger pathways as shown in Figure 2. The receptor-associated protein tyrosine kinases are activated upon growth factor binding to the receptor. The second messenger-independent protein serine/threonine kinases and the protein tyrosine kinases that are not receptor associated seem to be regulated indirectly via the second messenger-dependent and growth factor-dependent pathways as depicted in the figure. The brain also contains numerous types of protein serine/threonine and protein tyrosine phosphatases, not shown in the figure, which are also subject to regulation by extracellular and intracellular stimuli. Thus, the binding of neurotransmitter to its receptor extracellularly results in numerous short- and long-term biological responses through the complex regulation of multiple intracellular regulatory pathways and the phosphorylation or dephosphorylation of numerous substrate proteins. Modified from Duman and Nestler 1994.

HYPOTHETICAL ROLE OF G PROTEINS AND THE cAMP PATHWAY IN DRUG REINFORCEMENT AND CRAVING

A large behavioral and pharmacological literature has revealed considerable information concerning the extracellular actions of drugs of abuse (see Wise 1990; Kuhar et al. 1991; Koob 1992). For example, as shown in Figure 4, cocaine, via increasing synaptic levels of dopamine, would activate all known subtypes of dopamine

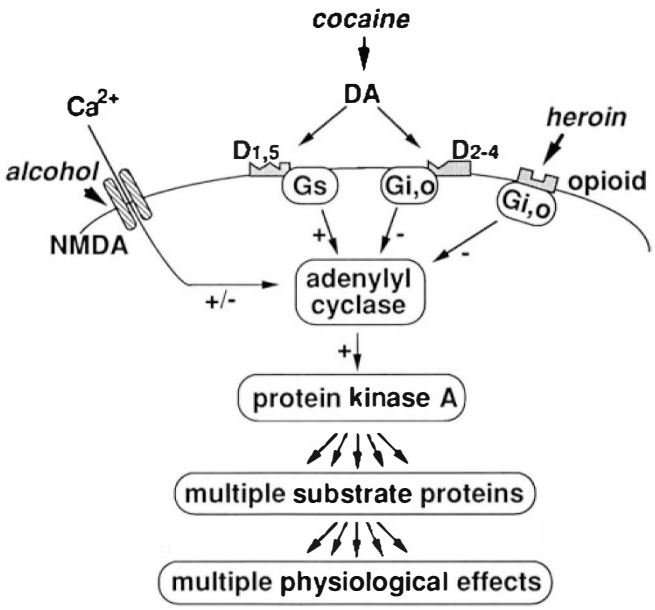

Figure 4. Hypothetical role of G proteins and the cAMP pathway in mediating some of the acute effects of heroin, cocaine, and alcohol on mesolimbic dopamine function. This model is based on the known extracellular mechanisms of action of these drugs and the known receptor and post-receptor mechanisms that would be expected to follow. The figure focuses on G proteins and the cAMP pathway and therefore represents only a small portion of the many receptor and postreceptor mechanisms that likely mediate acute drug effects on this and other neural pathways.

receptors throughout the brain. Heroin would activate the growing number of opioid receptors now known to be expressed in the brain. Recent work has even provided insight into some of the proteins with which alcohol interacts initially, such as the NMDA glutamate receptor (as depicted in the figure), as well as the $GABA_A$ receptor and L-type calcium channels (not shown) (Wafford et al. 1991; Morrisett and Swartzwelder 1993). Clearly, cocaine, heroin, and alcohol produce very different effects in different brain regions and peripheral tissues, but growing evidence has shown that the actions of these drugs converge on the mesolimbic dopamine system (See Wise 1990; Kuhar et al. 1991; Koob 1992). This neural pathway consists of dopaminergic neurons in the ventral tegmental area (VTA) and their numerous projections, particularly those to the nucleus accumbens (NAc) and prefrontal cortex. The mesolimbic dopamine system appears to play a critical role in mediating the acute reinforcing actions of these and other drugs of abuse. In addition, the mesolimbic dopamine system may be one of the sites where drugs of abuse produce chronic adaptations that underlie the long-term changes in drug reinforcement mechanisms (e.g., craving) that are the core feature of addiction clinically (Nestler 1992; Nestler et al. 1993).

Based on our knowledge of intracellular messenger pathways (see Duman and Nestler 1994), specific hypotheses can be formulated concerning the post-receptor mechanisms underlying the acute and chronic actions of drugs of abuse on the mesolimbic dopamine system. A small portion of such hypotheses, focused on regulation of the cAMP pathway, is shown in Figure 4. Cocaine activation of D_1 and D_5 dopamine receptors would be expected to activate the cAMP pathway via the stimulation of Gs, the activation of adenylyl cyclase, the generation of cAMP, and the activation of cAMP-dependent protein kinase. The protein kinase would then phosphorylate numerous substrate proteins, which would mediate many of the acute actions of cocaine on mesolimbic dopamine function. Cocaine would also activate D_2, D_3, and D_4 dopamine receptors, which may be located on a partially different subset of neuronal elements in the mesolimbic dopamine system, although this remains to be established with certainty. Activation of these receptors would exert the opposite effect on the cAMP pathway, through the recruitment of Gi and Go and the inhibition of adenylyl cyclase. Heroin activation of most types of opioid receptors, which are also coupled to Gi and Go, would produce similar effects. Even alcohol, through direct actions on NMDA glutamate receptors and indirect regulation of the state of deplorarization of neurons, would be expected to regulate the cAMP pathway. This is based on the knowledge that cellular levels of Ca^{2+} can exert powerful stimulatory or inhibitory effects on adenylyl cyclase, depending on the type of adenylyl cyclase expressed in neurons of the mesolimbic dopamine system (see Nestler and Duman 1994). It should be emphasized that each of these various actions of cocaine opiates, and alcohol could potentially occur both in presynaptic and postsynaptic elements in these brain regions. Clearly, the information provided in Figure 4 represents only a small portion of the expected consequences of activation of the various receptors and G proteins indicated; the figure fails, for example, to show expected regulation of the Ca^{2+} and phosphatidylinositol pathways (see Agranoff and Fisher 1994; Nestler and Greengard 1994). As information is obtained concerning the role of the cAMP pathway in drug addiction, the possible involvement of these other intracellular messenger pathways must also be investigated.

DIRECT EVIDENCE FOR A ROLE OF G PROTEINS AND THE cAMP PATHWAY IN DRUG REINFORCEMENT AND CRAVING

The tools of molecular neurobiology coupled with more traditional neuropharmacological disciplines can now directly test the validity of the hypotheses shown in Figure 4. For example, growing evidence supports a role for Gi and Go in the acute and chronic actions of drugs

Table 1. Evidence Supporting a Role for Gi and Go in the Acute and Chronic Actions of Drugs of Abuse on the Mesolimbic Dopamine System

1. Direct inactivation of Gi/Go in the NAc regulates cocaine and heroin self-administration behavior.
2. Chronic administration of cocaine or opiates decreases levels of Gi/Go in the NAc.
3. Chronic administration of cocaine decreases levels of Go in the VTA, an effect that parallels the development of locomotor sensitization, and direct inactivation of Gi/Go in the VTA mimics locomotor sensitization.

Based on data from Nestler et al. 1990; Steketee et al. 1991; Terwilliger et al. 1991; Striplin and Kalivas 1992; Self et al. 1994.

Table 2. Evidence Supporting a Role for the cAMP Pathway in the Acute and Chronic Actions of Drugs of Abuse on the Mesolimbic Dopamine System

1. Direct activation or inactivation of the cAMP pathway in the NAc exerts opposite effects on cocaine and heroin self-administration behavior.
2. Chronic administration of cocaine, opiates, or alcohol upregulates the cAMP pathway in the NAc.
3. Direct activation of the cAMP pathway in the NAc facilitates cocaine-induced locomotor activity and locomotor sensitization.

Based on data from Terwilliger et al. 1991; Cunningham and Kelley 1993; Self et al. 1993; Miserendino and Nestler 1994; Nestler et al. 1994.

of abuse on the mesolimbic dopamine system (Table 1). First, direct manipulation of these G proteins in the NAc has been shown to regulate cocaine and heroin self-administration behavior (Self et al. 1994). In these experiments, pertussis toxin, which inactivates Gi and Go, was administered bilaterally into the NAc. It was found that this treatment produces a rightward shift in the dose-response functions for both cocaine and heroin self-administration (Figure 5). It was further found that the time course by which pertussis toxin administration produces these changes in drug self-administration closely parallels the time course by which the toxin inactivates Gi and Go in this brain region as determined by biochemical measures (Self et al., 1994). In addition, in separate studies, chronic administration of opiates or cocaine has been shown to reduce levels of Gi and Go selectively in the NAc (Nestler et al. 1990; Terwilliger et al. 1991). Together, these studies provide increasing evidence for the hypothesis that these G proteins mediate some of the acute reinforcing actions of drugs of abuse, and that chronic adaptations in the G proteins may represent part of the pathophysiological mechanisms underlying drug addiction.

Chronic cocaine is also known to decrease levels of Go in the VTA (Nestler et al. 1990), changes that correlate temporally with the induction of locomotor sensitization (Striplin and Kalivas 1992) (see Table 1). Moreover, local administration of pertussis toxin into the VTA has been shown to mimic aspects of locomotor sensitization behavior (Steketee et al. 1991). These data indicate that Go may play a role in the acute and chronic effects of cocaine on locomotor activity, another behavior known to be regulated by the mesolimbic dopamine system (see Kalivas and Stewart 1991).

Similar types of information have been obtained to support a role of the cAMP pathway in drug reinforcement mechanisms (Table 2). These studies have made use of cholera toxin, which activates Gs, and analogs of cAMP that regulate cAMP-dependent protein kinase (Self et al. 1993). For example, as shown in Figure 6, direct administration of activators or inhibitors of the

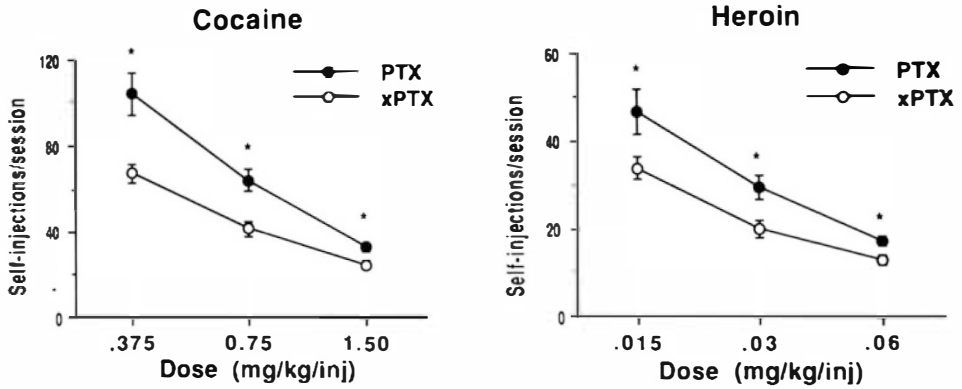

Figure 5. Effects of bilateral pertussis toxin (PTX) injections into the NAc on the dose-response relationship of intravenous cocaine or heroin self-administration. The data are expressed as the mean ± SEM of self-administration totals as a function of injection dose for daily 3-hour sessions conducted at the end of maintenance testing. Control rats received similar injections of inactive pertussis toxin (xPTX). Asterisks indicate that values differ from xPTX-treated controls; * $p < .01$, student's *t*-test. From Self et al. 1994.

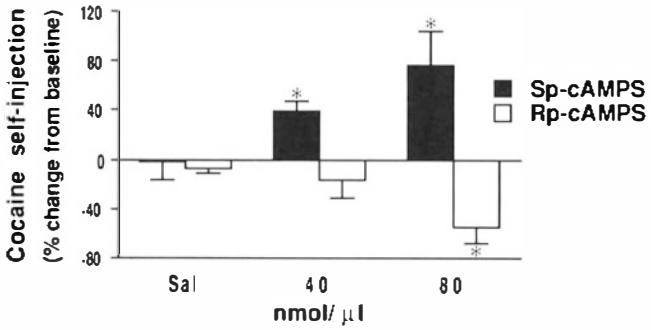

Figure 6. Effects of bilateral cAMP analog injections into the NAc on the maintenance of preestablished intravenous self-administration of cocaine (0.5 mg/kg/injection). The cAMP analogs used included: Sp-cAMPS (an activator of cAMP-dependent protein kinase) and Rp-cAMPS (an inhibitor of cAMP-dependent protein kinase). The data are expressed as the mean ± SEM of self-administration totals from daily 3-hour test sessions following the injection of the cAMP analog or vehicle. Asterisks indicate that values differ from vehicle-treated control; * $p < .05$ by student's *t*-test. Data from Self et al. 1993.

protein kinase into the NAc produces opposite effects on cocaine self-administration (Figure 6), and more preliminary data indicate a similar action on heroin self-administration. In addition, chronic treatment of rats with opiates, cocaine, or alcohol has been shown to up-regulate the cAMP pathway in the NAc, with drug-induced increases seen in levels of adenylyl cyclase and cAMP-dependent protein kinase (Terwilliger et al. 1991; Nestler et al. 1994). Together, these findings provide the first direct evidence for the hypothesis (Nestler 1992; Nestler et al. 1993) that the cAMP protein phosphorylation pathway does indeed regulate the acute reinforcing properties of drugs of abuse, and that chronic adaptations in this intracellular pathway may represent part of the pathophysiological mechanisms involved in drug addiction. Similar types of studies support a role of the cAMP pathway in the NAc in cocaine-induced locomotor activity and sensitization (Cunningham and Kelley 1993; Miserendino and Nestler 1994).

Ultimately, acute and chronic drug regulation of G proteins and the cAMP pathway will result in changes in mesolimbic dopamine function through the phosphorylation, by cAMP-dependent protein kinase, of numerous types of substrate proteins. A major focus of current research, then, is on identifying the specific substrates whose phosphorylation mediates drug reinforcement mechanisms in the VTA–NAc pathway acutely and chronically.

A ROLE FOR ALTERATIONS IN GENE EXPRESSION IN DRUG ADDICTION

Among the many possible substrate proteins involved in drug addiction mechanisms are those that regulate gene expression. There are two general types of evidence to suggest that alterations in gene expression may contribute importantly to drug addiction. First, drug regulation of many of the target proteins identified to date in the mesolimbic dopamine system (e.g., G proteins and the cAMP pathway) occurs at the messenger RNA level (see Nestler et al. 1993; Vrana et al. 1993). Chronic cocaine treatment has also been reported recently to regulate mRNA expression of the dopamine transporter in the VTA-NAc pathway (Cerruti et al. 1994). Second, it has been known for many years that many prominent features of drug addiction in people and laboratory animals can persist for a long time, perhaps a lifetime, after discontinuation of drug exposure. These considerations have stimulated many investigators to examine directly the regulation of gene expression by drugs of abuse.

These studies have focused on classes of nuclear regulatory proteins, termed transcription factors, that bind to specific sequences of DNA located in the promoter regions of certain genes and thereby increase or decrease the rate at which those genes are transcribed (Hyman and Nestler 1993). Prominent examples of transcription factors in the brain include CREB (cAMP response element binding protein), Fos, and related proteins. Figure 7 illustrates a general scheme by which drug regulation of gene expression may contribute to addiction. Drug-induced alterations in intracellular messenger pathways would result in alterations in transcription factor function, either through the direct phosphorylation of transcription factors or through regulation of their expression. Such regulation of transcription factors would then alter the expression of specific target proteins, which would result in the adaptive changes in brain function that are responsible for addiction. Although there is a plethora of reports that acute drug treatment or other acute stimuli can result in regulation of transcription factors in the brain, what would appear to be particularly relevant to addiction per se are long-term changes in nuclear regulatory mechanisms, which have been more difficult to identify.

Recent work has made progress in this area by delineating long-term effects of cocaine on Fos-like transcription factors. Several laboratories have reported that acute administration of cocaine or other stimulants results in the induction of Fos and several related immediate early gene products (e.g., FosB, Fra2, Jun, and JunB) in the NAc and caudate/putamen (Graybiel et al. 1990; Young et al. 1991; Hope et al. 1992; Cole et al. 1992; Nguyen et al. 1992). Induction of these proteins is associated, as would be expected, with an increase in AP-1 DNA binding activity (Young et al. 1991; Hope et al. 1992). AP-1 refers to the sequence of DNA to which Fos and related proteins bind.

In contrast, chronic cocaine administration produces very different actions on this transcription factor fam-

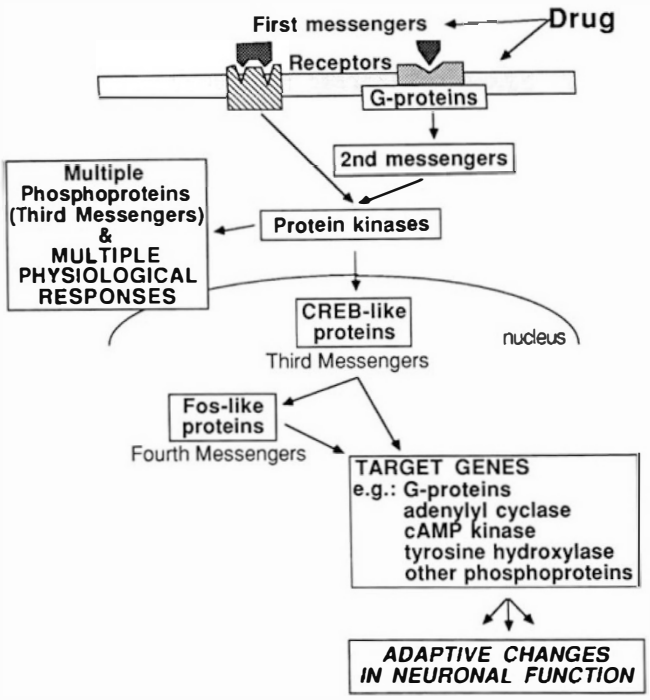

Figure 7. Schematic illustration of the hypothetical role played by gene expression in drug addiction. According to this scheme, an initial extracellular effect of a drug of abuse would trigger changes in multiple intracellular messenger pathways in target neurons. Changes in the intracellular messengers would result in numerous physiological responses to the drug (as shown in Figure 3), including alterations in gene expression. The latter types of alterations would occur through the regulation of many classes of nuclear, DNA-binding proteins, termed transcription factors, such as CREB and Fos. CREB-like transcription factors refer to those that are regulated by extracellular agents primarily through changes in their degree of phosphorylation. Fos-like transcription factors refer to those that are expressed at very low levels under basal conditions and regulated by extracellular agents primarily through induction of their expression (presumably via CREB-like proteins). Both types of transcription factors would then result in altered levels of expression of specific target proteins that underlie the adaptive changes in brain function associated with addiction. Modified from Nestler 1992.

ily (Hope et al. 1992). A course of chronic cocaine dramatically reduces the ability of a subsequent acute exposure to the drug to induce Fos and the other immediate early gene products. However, chronic cocaine treatment is paradoxically associated with a robust induction of AP-1 DNA binding activity. These findings suggest that chronic cocaine treatment may induce different types of Fos-like proteins compared to the acute situation. Direct support for this possibility has been obtained recently (Hope et al., 1994) through the analysis of Fos-like proteins by one- and two-dimensional blot immunolabeling procedures utilizing an antibody that recognizes a moiety in the Fos molecule that is shared by numerous Fos-like proteins. These blot immunolabeling studies have shown that acute and chronic cocaine treatments are associated with the induction of *different* Fos-like proteins, and that some of the Fos-like proteins induced under chronic cocaine-treated conditions do not correspond to any known proteins. These novel proteins, designated chronic Fra's (*F*os-*r*elated *a*ntigens), show different functional properties compared to c-Fos and the other acute Fra's. First, the chronic Fra's exhibit a relatively long half-life: whereas the acute Fra's (and the associated acute
to control levels within 12 to 24 hours following acute cocaine administration, the chronic Fra's (and the associated chronic AP-1 binding complex) remain at maximal levels 24 hours following the last chronic dose of cocaine and are at half-maximal levels one week later (Hope et al. 1994). The chronic and acute AP-1 complex also show different binding affinity toward certain AP-1 sites, consistent with the possibility that the chronic and acute Fra's may differ in their interactions with certain target genes.

Figure 8 shows a working hypothesis concerning the physiological significance of these observations (Nestler et al. 1993). According to this scheme, acute cocaine exposure leads to the induction of a transient AP-1 complex composed of Fos and related immediate early gene products. This transient complex presumably mediates some of the short-term effects of cocaine on gene expression. In contrast, chronic cocaine exposure leads to the induction of a persistent AP-1 complex composed of different Fos-like proteins. An AP-1 complex of different half-life and composition would be expected to produce some different effects on gene expression, including some unique to the chronic-treated state. The induction of such altered AP-1 complexes highlights the fact that in addition to the traditional *quantitative* characterizations of chronic drug effects on the brain as representing either up- or down-regulation (i.e., sensitization or tolerance), chronic drug treatments also result in *qualitative* changes in brain function, that is, that the chronic-treated state is simply different from the control or acute-treated state in important respects.

The isolation and cloning of the chronic Fos-like proteins will make it possible to study their role in altering the expression of various putative target proteins (e.g., G proteins and the cAMP pathway) identified in the mesolimbic dopamine system. Of course, one attractive feature of the chronic Fos-like proteins, and the long-term changes in gene expression they suggest, is that they could account for the long-lasting nature of the biochemical adaptations that have been observed in the mesolimbic dopamine system following chronic drug exposure and for the long-lasting changes in brain function associated with addiction.

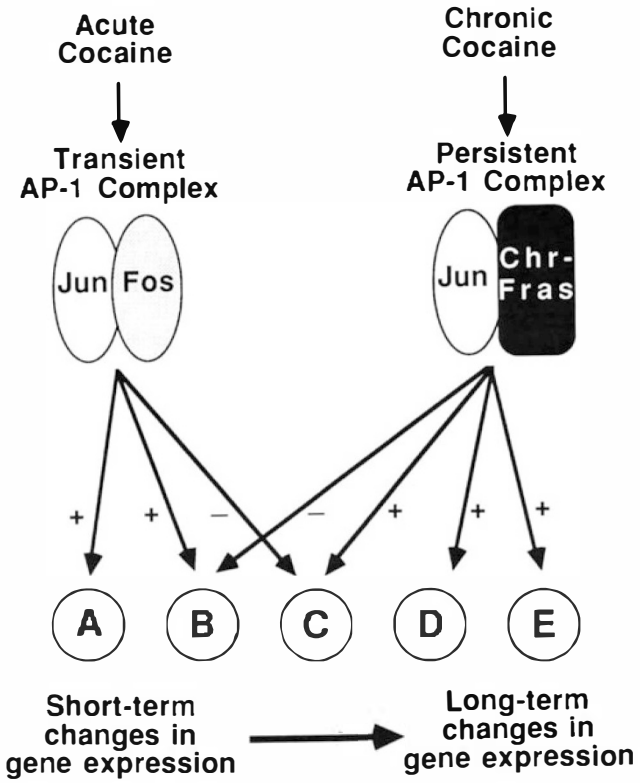

Figure 8. Scheme illustrating the regulation of AP-1 binding proteins by acute and chronic cocaine. Acute cocaine administration transiently induces several AP-1 binding proteins in the NAc and caudate/putamen, including Fos, FosB, Fra2, c-Jun, and JunB. Induction of these transcription factors could mediate some of the rapid stimulatory (+) and inhibitory (−) effects of cocaine on the expression of neural genes (A to E) that contain AP-1 binding sites. In contrast, chronic cocaine administration results in the persistent induction of AP-1 binding activity that is accounted for by different Fos-like (and possibly Jun-like) proteins, including recently identified proteins designated chronic Fra's (*F*os-*r*elated *a*ntigens). Such chronic, persistent AP-1 complexes of altered composition would be expected to exert different transcriptional effects compared to the acute AP-1 complexes and could mediate some of the long-term effects of cocaine on gene expression. From Nestler et al. 1993.

CONTRIBUTION OF INTRACELLULAR MESSENGER PROTEINS TO INDIVIDUAL RISK FOR DRUG ADDICTION

As more is learned of the pathophysiology of drug addiction through the investigation of intracellular messenger pathways, it will be possible to identify specific genetic and environmental factors that determine an individual's predilection for drug addiction. Such factors presumably determine drug addiction vulnerability by influencing an individual's responsiveness to the multiple positively and negatively reinforcing effects of a drug of abuse after acute or chronic exposure.

The importance of genetic factors in establishing individual responsiveness to drugs of abuse is indicated by differences in drug-related behaviors among different inbred animal strains (e.g., Lewis and Fischer rats). The Lewis rat self-administers opiates, cocaine, and alcohol to a greater extent than the Fischer rat (George and Goldberg 1989), and develops a greater degree of conditioned place preference to cocaine and morphine (Guitart et al. 1992; Kosten et al. 1994). The Lewis rat also exhibits a greater facilitation in brain self-stimulation thresholds in response to cannabinoids than the Fischer rat (Gardner and Lowinson 1991). These findings raise the possibility that the Lewis rat is inherently more "drug-preferring" than the Fischer rat. However, the observation that Lewis and Fischer rats also show different locomotor responses to cocaine after acute and chronic administration (George et al. 1991; Kosten et al. 1994), raises the alternative possibility that, rather than a difference in drug preference per se, the two rat strains may differ in their inherent sensitivity to certain of the effects of drugs of abuse.

Interestingly, Lewis and Fischer rats show marked inherent differences in several biochemical parameters in the mesolimbic dopamine system (see Table 3 for summary). The NAc of the drug-naive Lewis rat contains lower levels of Gi and higher levels of adenylyl cyclase and cAMP-dependent protein kinase compared to the NAc of the drug-naive Fischer rat (Guitart et al. 1993). In this manner, the Lewis rat (as compared to the Fischer rat) resembles outbred Sprague-Dawley rats treated chronically with opiates, cocaine, or alcohol. Related studies, also summarized in Table 3, have similarly shown that the VTA of the drug-naive Lewis rat (as compared to the VTA of the drug-naive Fischer rat) shows different levels of a number of phosphoproteins known to be altered in Sprague-Dawley rats in the chronic drug-treated state (see Nestler 1992; Nestler et al. 1993). These studies support the possibility that the various intracellular messenger proteins identified could not only contribute to the pathophysiology of drug addiction but also represent part of the biochemical mechanisms that determine inherent responsiveness to drugs of abuse, including drug preference.

The importance of environmental factors in drug addiction vulnerability is demonstrated by the well-established ability of many exogenous treatments to alter drug-related behaviors. An animal's responsiveness to a drug of abuse can be altered by prior exposure to that drug itself, to other drugs, or to environmental stimuli. For example, glucocorticoid treatment and environmental stress have been reported to augment the reinforcing and locomotor-activating properties of opiates, cocaine, and amphetamine, and prior exposure to these drugs of abuse can exert the same effects (e.g., see Cole et al. 1990; Kalivas and Stewart 1991; Piazza et al. 1991).

Table 3. Summary of Lewis-Fischer Strain Differences in Biochemical Parameters in the Mesolimbic Dopamine System

	Drug Naive Conditions Lewis vs Fischer	Chronic Morphine-Treated Conditions Δ in S-D†
NAc		
cAMP kinase	L > F	↑
Adenylate cyclase	L > F	↑
Giα	L < F	↓
Tyrosine hydroxylase	L < F	↓*
VTA		
Tyrosine hydroxylase	L > F	↑
Neurofilaments	L < F	↓
Glial fibrillary acidic protein	L > F	↑

The symbols used in this table (<, >, ↑, ↓) refer to differences in levels of enzyme activity (for adenylyl cyclase and cAMP-dependent protein kinase) or levels of immunoreactivity (for Giα, tyrosine hydroxylase, neurofilaments, and glial fibrillary acidic protein).

* In this case, the downward arrow refers to a decrease in phosphorylation state that would be expected to have a similar functional effect as a decrease in total amount.

† S-D, Sprague-Dawley rats.

For references, see Nestler 1992; Nestler et al. 1993.

Genetic and environmental factors cannot be viewed as separate, independent variables; rather, it is the interaction between the two that determines an individual's predilection for drug addiction. An illustration of such interactions are the findings that chronic exposure to drugs of abuse or to glucocorticoids produces different behavioral effects, and different biochemical adaptations in the mesolimbic dopamine system, in Lewis and Fischer rats (Guitart et al. 1992, 1993; Ortiz and Nestler 1993). In this way, Lewis and Fischer rats provide a novel experimental system to investigate the specific genetic and environmental factors that combine to determine the biochemical phenotype in the mesolimbic dopamine system and the various drug-related behaviors regulated via this neural pathway. The fact that Lewis and Fischer rats are genetically divergent, such that strain differences probably exist for a large number of genes, does not limit their usefulness in the study of drug addiction. This situation is, in fact, clinically relevant in terms of the known differences in susceptibility to the various effects of drugs of abuse observed among people, who also differ greatly in their individual genetic makeup. Lewis and Fischer rats could be used to identify specific genes related to drug addiction by cross-breeding the two strains through several generations to identify biochemical and behavioral traits that cosegregate, followed by genetic linkage analysis. Various drug-regulated proteins (e.g., those shown in Figure 7) could serve as candidate genes in these studies. These investigations in rats could be followed by genetic studies in clinical populations to determine whether the same or related genes may contribute to drug addiction vulnerability in people. Identification of genes that predispose individuals for addiction would provide leads in the development of new therapeutic agents, revolutionize diagnostic capabilities, and enable the targeting of preventive measures to particularly vulnerable individuals.

CONTRIBUTIONS OF INTRACELLULAR MESSENGER PROTEINS TO DRUG DEVELOPMENT EFFORTS

As an increasing number of intracellular messenger proteins are identified as contributing to the pathophysiology or individual risk of drug addiction, these proteins could be used to develop novel pharmacotherapeutic agents for the treatment and prevention of drug addiction. At the very least, measures of the intracellular messenger proteins in rats can be used as a novel procedure by which to screen test compounds preclinically for their potential antiaddiction properties. For example, does drug X prevent or reverse cocaine's ability to produce adaptations in G proteins, the cAMP pathway, etc., in the mesolimbic dopamine system? In contrast to all current drug development efforts in the area of drug addiction, which are focused on test agents with a specific pharmacological property (e.g., selective antagonism of a dopamine receptor subtype), this new screen would enable the rational testing of novel drugs with diverse pharmacological actions.

It is even conceivable that the intracellular messenger proteins themselves could be targeted by new therapeutic agents. After all, some of the most effective and widely used drugs are directed at intracellular messenger pathways. Aspirin and all other nonsteroidal antiinflammatory agents inhibit cyclo-oxygenase,

the first enzyme in the generation of prostaglandin, leukotriene, and endoperoxide intracellular messengers (Piomelli and Greengard 1990). FK-506 and related immunosuppressive agents bind to a newly discovered class of protein, the immunophilins (also present at high levels in brain), that regulate the activity of specific protein phosphatases (see Steiner et al. 1992). Lithium, one of the most effective psychotherapeutic agents available, directly regulates the activity of adenylyl cyclase, G proteins, and certain inositol phosphatases, actions that presumably result in the drug's antimanic and antidepressant effects (see Hyman and Nestler 1993). The targeting of intracellular messenger proteins would represent a dramatic departure for drug development efforts in psychiatry and would clearly be of high risk. On the other hand, such efforts offer the potential of tremendous breakthroughs; drug development efforts in psychiatry over four generations have failed to result in fundamentally more efficacious agents, with fundamentally new mechanisms of action, in the treatment of drug addiction or other neuropsychiatric disorders.

CONCLUSIONS

The fact that many features of drug addiction in people can be reproduced accurately in laboratory animals means that studies of drug addiction offer a unique advantage in the investigation of the neurobiological mechanisms underlying a complex and clinically important behavioral abnormality. Molecular adaptations that occur in the brain in association with drug addiction in animal models can be related to behavioral aspects of addiction in the animals, and eventually to the clinical situation. This information will lead to an improved understanding of drug addiction and to the development of more effective treatments and preventive measures. It is also anticipated that advances made in the field of drug addiction will have important implications for how we approach the pathophysiology and treatment of other major neuropsychiatric disorders.

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