Chapter 7

CHANGES IN GENE EXPRESSION AND SIGNAL TRANSDUCTION FOLLOWING IBOGAI NE TREATMENT

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Drug addiction may not be mediated by one neurochemical pathway and brain structure, but by a complex interaction of programs of gene expression with specific signal transduction pathways and environmental factors. The putative antiaddictive effect of ibogaine may result from the restoration of altered or disrupted programs of central and peripheral neuroadaptative processes involving programmed genes and their associated signaling mechanisms. As discussed in this chapter, because the pharmacotherapy of drug addiction has been largely disappointing, it is now more important than ever to consider new hypotheses. One new hypothesis being explored is that both the peripheral and central actions of abused substances contribute to drug addiction. It is suggested that an effective therapeutic agent for addiction may be obtained only when both peripheral and central actions of the processes contributing to addictions are considered. There is evidence from animal studies, and from anecdotal human studies, that the alkaloid ibogaine, and perhaps its metabolites, alter or regulate gene expression and signal transduction pathways and restore altered neuroadaptive processes arising from the loss of control due to drug addiction. We and others have observed that treatment with cocaine influences the regulation of certain genes in the brain, as indicated by the activation and inhibition of the expression sequence tags (ESTs) that have been isolated. The behavioral data presented here supports the notion that ibogaine restores the behavioral and neurogenetic alterations resulting from exposure to cocaine.

The recent advances in neurobiology have enabled the complex biological mechanisms underlying drug and alcohol addiction to be investigated at the cellular and molecular levels (1). Abused substances exert biological effects by interacting with cell membranes and receptors, and modify the function of proteins, which regulate signal transduction, intracellular pathways, and gene expression. Adaptation to the effects of abused substances is known to constitute a major determinant of the development of increased tolerance, withdrawal syndrome, and dependence. Thus, important targets for alcohol and other abused substance include second messengers, gene transcription factors, transmitter and voltage-regulated ion channels, GTP-binding proteins, and metabolizing enzymes (1). It is hoped that this research focus will identify important molecules for the development of drug and alcohol addiction, as this will certainly lead to identifying genes that are most critical in mediating addiction. A consideration of the contribution of environmental factors to addictions should not be underestimated. However, to develop novel pharmacologic therapies for treating or
preventing drug abuse, addiction, craving, or withdrawal symptoms when an individual is attempting to quit, we must first identify neural substrates and understand the mechanism by which abused drugs act at these target sites.

The involvement of coordinated programs of gene expression appears to be critical for many brain functions, including long-term memory and drug addiction (2). As shown in Figure 1, the cascade of intracellular signals mediated by receptors interacting with G proteins initiates the communication between extracellular signals and the nucleus to trigger specific patterns of gene expression (3). We have assumed that the initiation of compulsive drug use may

![Figure 1. The cAMP Signal Transduction Pathway](image)

**Figure 1. The cAMP Signal Transduction Pathway.** Schematic representation of the route whereby ligands at the cell surface interact with, and thereby activate, membrane receptors (R) and result in altered gene expression. Ligand binding activates coupled G-proteins (G), which, in turn, stimulate the activity of the membrane-associated adenylyl cyclase (AC). This converts ATP to cAMP, which causes the dissociation of the interactive tetrameric protein kinase A (PKA) complex into the active catalytic subunits (C) and the regulatory subunits (R). Catalytic subunits migrate into the nucleus where they phosphorylate (P) and thereby activate transcriptional activators such as Ca²⁺/cAMP response element binding protein (CREB), cAMP response element modulator (CREM), and activating transcription factor (ATF). These then interact with the cAMP response enhancer element (CRE) found in the promoters of cAMP-responsive genes to activated transcription.
involve the binding of the drug to a receptor resulting in activation or inhibition of the cAMP-dependent pathway. This will ultimately influence the transcriptional regulation of various genes through distinct promoter responsive sites (4). The cAMP-responsive element binding protein (CREB), the first CRE-binding factor to be characterized (5), is a transcription factor of general importance in both neuronal and other cells (3). CREB phosphorylation on Ser-133 promotes the activation of genes with an upstream CRE element (6). CREB phosphorylation and downstream gene expression can, in principle, be regulated by protein kinases under the control of cAMP (7), Ca²⁺ (8-11), or both (12). Alteration of CREB function specifically affects long-term synaptic changes and long-term memory, while sparing short-term changes (8, 13-16). Thus, the final transcriptional response to cAMP is the outcome of a complex interplay of nuclear targets activated by signal transduction events. In a stress model of anhedonia, our preliminary data (not shown) indicate a decrease in CREB phosphorylation. This is of significance and tremendous importance as anxiety and stress factors play a major role in the precipitation and maintenance of drug-seeking behavior (17). Our preliminary data (18) are supported by the growing consensus that genes influence behavior in both humans and animals, along with complex interactions with the environment. However, because any behavioral trait is likely to be affected by many genes acting in concert, the attempts to pin down which genes influence which behavior have proven difficult. Recent advances in genetic studies of human disease have linked some genes to some aspects of human biology, behavior, or disease. It is therefore timely that the tools are now available to discover the programs of gene expression that make the individual vulnerable to drug dependency.

Experience in life, and encounters with pain or pleasure, may leave indelible impressions. The pleasurable experience from abused drugs may cause a change in the programs of gene expression. The nature and consequences of addictive disorders and other CNS disturbances may appear to be aberrant programs of gene expression, suggesting that the CNS of the drug-dependent individual may be “locked” into programs that no longer respond to appropriate external circumstances. The intensity of the pleasure or experience may be irreversible, and one cannot forget the pleasure because the rate of firing of neurons during the experience has been altered from the normal pattern of neuronal firing. The extent of dependency and the loss of control and the ultimate loss of plasticity—that is, incomplete loss or partial loss—may allow for reconditioning and regaining of control. Willpower to quit addiction may be difficult to mimic in the laboratory so as to study its mechanism and rate limiting facets. It appears that interference with the dopamine system may not restore the altered function and plasticity in drug dependence. The initial trigger or switch that leads to changes in programs of gene expression may be different for the different drugs of abuse.
Over the years, significant effort has been made to uncover valid genetic markers for the risk of drug and alcohol addiction. It is now recognized that drug and alcohol dependence is a chronic brain disease and a long-lasting form of neuronal plasticity. At the cellular and molecular level, there is a growing body of evidence that substances that cause addiction affect hormones and neurotransmitter-activated signal transduction leading to short-term changes in regulation of cellular functions and long-term changes in gene expression.

Complex, but defined, processes are emerging for the mechanisms leading to the development of drug tolerance and dependence arising from adaptations in post-receptor signaling pathways with the accompanying transcriptional regulators. This may initiate a cascade of altered programs of gene expression that underlie the long-term consequences of withdrawal and relapse that leads to drug seeking behavior. The number of post-receptor events shown in Tables I and II underscores the complexity of the processes leading to drug and alcohol dependence. Numerous studies have therefore demonstrated that chronic drug administration drives the production of adaptations in post-receptor signaling by

<table>
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<tr>
<th>TABLE I. SIGNAL TRANSDUCTION PATHWAYS ASSOCIATED WITH SUBSTANCE ABUSE AND PUTATIVE IBOGAINE EFFECTS</th>
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<tbody>
<tr>
<td><strong>Post-Receptor Mediated Signal Transduction</strong></td>
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<tr>
<td>Ca²⁺ signaling ions and other receptor and voltage operated channels</td>
</tr>
<tr>
<td>• K⁺, Na⁺, Ca²⁺ (P, T, N and L-types) ions</td>
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<tr>
<td>• (Na⁺K)-ATPase alpha-subunit</td>
</tr>
<tr>
<td>G protein-mediated signal transduction</td>
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<tr>
<td>• cAMP signal transduction</td>
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<tr>
<td>• Phosphorylation of adenyl cyclase</td>
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<tr>
<td>• cAMP-PKA-PKC systems</td>
</tr>
<tr>
<td>• CREB-dependent gene transcription</td>
</tr>
<tr>
<td>• API transcription factor gene expression (IEGs, c-fos, fos B, jun-B and c-jun, zif 268, krox-20.</td>
</tr>
<tr>
<td>Poly-phosphoinositide (PI) signaling pathway</td>
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<tr>
<td>Post-transcriptional palmitoylation of Gsα</td>
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<tr>
<td>Peripheral phospholipase D activity</td>
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<td>Lipid peroxidation</td>
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<tr>
<td>Microtubule associated protein</td>
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<td>Nuclear transcription factors</td>
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a tangled, but precise, web of signal transducers. The G protein-mediated signal transduction pathway may be crucial in the pathophysiology of drug dependency, so that actions involving G proteins may be candidate markers in the addiction process. The activity of the adenylate cyclase enzyme in the signal transduction pathway of many of the G protein coupled receptors appears to be involved in drug-seeking behavior. Furthermore, brain region-specific changes in multiple signaling pathways of activator protein (AP-1 transcription factor changes in gene expression in c-fos, fosB, jun-B, and c-jun) have also been linked to a long-lasting form of neuronal plasticity associated with drug and alcohol dependence. Receptor and voltage operated channels and G-protein-mediated phosphoinositol and protein kinases are among the other signal transduction pathways that may underlie the clinical manifestations of drug/alcohol dependence, tolerance, withdrawal, and addiction.

C. Ibogaine: Beyond Dopamine in the Nucleus Accumbens

The problem of drug addiction continues to affect modern society with severe consequences. Despite extensive research efforts, the neurobiological mechanisms responsible for compulsive and uncontrollable drug use remain poorly understood (21). Therefore the long-term use of ingestion of psychostimulants, like cocaine and amphetamines, narcotics like heroin, benzodiazepines like diazepam, and the recreational use of alcohol, nicotine in smoking, and cannabinoids in marijuana may cause addiction with craving and withdrawal syndrome acting as a deterrent from cessation from drug and/or alcohol use. For
many years, the central dopamine hypothesis of reward and reinforcement in drug addiction has been associated with elevated dopamine levels in the nucleus accumbens (Acb), which has therefore been suggested as the central and final common neuroanatomical target for abused drugs in the brain (22-26). As discussed recently (27), if the dopamine hypothesis of reward and the Acb brain structure associated with reinforcement were all but proven, then manipulation of the dopamine system should provide medications for drug and alcohol addicts in the clinic. But since pharmacological treatment of drug and alcohol addiction has largely been disappointing, new therapeutic approaches and hypothesis are needed. Although, it has been previously recognized that the reward centers in the brain consist of multiple systems and neuroanatomical sites, emerging data have started to challenge the dopamine hypothesis of reward involving the Acb circuitry (28). The studies in normal and cocaine addicts using positron emission tomography (PET) are associated with metabolic abnormalities in the orbitofrontal cortex and the striato-thalamic-orbitofrontal circuit, which has now been postulated as a common mechanism underlying drug and alcohol addiction (28,29). Furthermore, there are other dopamine independent mechanisms involving other neurotransmitters like glutamate (30,31), γ-aminobutyric acid (GABA), dynorphin, serotonin (5-HT), and cholecystokinin (CCK), in the Acb and in other brain regions like the frontal cortex, hippocampus, locus coeruleus, lateral hypothalamus, or the periaqueductal gray, that are potential neural substrates for the rewarding properties of psychostimulants, benzodiazepines, barbiturates, opiates, and phencyclidine hydrochloride (PCP) (32). Since the usefulness in treating any addiction with dopaminergic agents has been limited (33,34), one emerging potential, yet controversial, therapeutic agent is ibogaine, an indole alkaloid isolated from the bark of the African shrub, Tabernanthe iboga.

Ibogaine is used by some African societies in high doses during initiation ceremonies and rituals, and in low doses to combat hunger and fatigue. In Western cultures, new claims indicate that a single dose of ibogaine eliminates withdrawal symptoms and reduces drug cravings for extended periods of time (35). The mechanism of action associated with the ability of ibogaine to block drug-seeking behavior is currently incompletely understood, and a number of studies suggest that ibogaine has a broad spectrum of action on multiple systems (36,37). It is speculated that this broad spectrum of activity on opiate, serotonin, dopamine, choline, glutamate, N-methyl-D-aspartate (NMDA), sigma, noradrenergic, monoamine transporters, neurotensin, kappa-opioid, and other hormonal systems may, in part, contribute to the putative antiaddictive properties of ibogaine. As it is now doubtful that the mesolimbic dopamine acts by directly producing feelings of pleasure or euphoria (38), we have to move beyond the nucleus accumbens and dopamine hypothesis of reward. In place of the dopamine hypothesis, our working hypothesis is that the molecular events that underlie the development of compulsive drug-seeking behavior involve multiple brain sites.
and systems in drug reinforcement. These molecular switches lead to neuroplastic alterations in specific signal transduction systems that turn on/off subsets of genes that precipitate the behavioral manifestations of loss of control and compulsive drug or alcohol use. Alcohol and abused drugs turn on the switch, and withdrawal, craving, or relapse turns off the switch that creates the neuroadaptive addiction cycle. It is therefore possible that ibogaine, through its multiple actions, can restore the hedonic homeostatic dysregulation caused by drug and alcohol abuse (39). In these continuing studies we are using in vivo and in vitro systems to study the effects of cocaine and other abused substances and to determine whether these effects can be blocked by ibogaine.

II. In Vitro Action of Cocaine on Ca^{2+} and Protein Kinase C Signaling

As discussed below, ibogaine was shown to block some of the actions of cocaine in vivo. While an intact organism is desirable for studying in vivo effects, the in vitro system is also valuable in exploring the mechanism of action in isolated preparations. Thus, a number of investigators have used PC-12 cells to study the effects of alcohol, but not that of cocaine on cell function (40-42). It was demonstrated that chronic alcohol exposure increases protein kinase C (PKC) activity and regulation of Ca^{2+} channels in PC12 cell lines. The PC 12 clonal cell line of neural crest origin possesses the ability to secrete dopamine and other neurotransmitters that are known to be affected by cocaine. These cells are coupled to the second messenger systems necessary for signal transduction in response to a variety of stimuli. PKC consists of a family of closely related isoforms, which differ in their localization and pharmacological properties. It is a major mediator of transducing signals to the interior of the cell, and it is activated in vivo by Ca^{2+} and diacylglycerol. The activity and translocation of PKC has been implicated in a number of cellular and neuronal functions. Previous studies have therefore suggested a role of PKC in the modulation of ethanol effects on receptor function in cells of central nervous origin. The aim of the present study was to determine the activity and expression of PKC isoforms, along with changes in Ca^{2+} levels following incubation of PC 12 cells with cocaine. While alcohol-induced increases in PKC levels have been associated with the up-regulation of Ca^{2+}, we demonstrate the ability of cocaine to disrupt signal transduction of PC 12 cells.

The treatment of PC 12 cells with cocaine (0.01-3.0 mM) modified the activity and expression of the PKC isoforms and increased the intracellular levels of Ca^{2+} in the cells. SDS-PAGE and Western blotting analysis of the PC 12 cell
Figure 2. Total activity of Protein Kinase C (PKC) (top panel) and the Differential Expression of PKC Isoforms. For the PKC activity, the PC 12 cells were treated with or without cocaine for 6 days, and the total activity in the homogenates was analyzed as described. The immunoblotts derived from Western analysis were scanned and quantified, and the expression of the PKC isoforms presented in arbitrary units is shown in the lower panel.
homogenates with antibodies against PKC α, β, γ, δ, ε, and ζ after incubation with doses of cocaine are shown in Figure 2. The spectrum of the effects of increasing doses of cocaine varied according to the isoforms. At doses up to 7.0 mM, cocaine was lethal to the PC 12 cells and inhibited the expression of all the isoforms examined (data not shown). There was inhibition of the expression of PKCα at low dose of 0.01 mM and increased expression at higher doses (0.1-3.0 mM) of cocaine. Immunoblotting with the anti-PKCβ antibody detected an 80-kDa protein, whose expression increased as the dose of cocaine increased. While the expression of PKCδ also increased with increasing doses of cocaine, the expression of PKCγ and PKCε decreased with increasing doses of cocaine, with the expression of PKCζ remaining significantly unchanged. The incubation in the presence or absence of the antigenic peptides allowed identification of the PKC isoforms by the occurrence of immunolabeled bands, which were not seen when antigenic control peptide was present. Interestingly, the total activity of PKC increased with increasing concentrations of cocaine and declined with concentrations above 3.0 mM when compared to PC 12 cells that were not exposed to cocaine, as shown in Figure 2. The levels of Ca²⁺ in the PC 12 cell homogenates with or without incubation with cocaine were measured using fura-2 and analyzed with the SPEX AR-CM fluorometer. The Ca²⁺ levels significantly increased with increasing concentrations of cocaine in the PC 12 cells compared to the controls, as shown in Table III. These data, therefore, confirm that the antibodies used can detect PKCs α, β, γ, δ, ε, and ζ in the PC 12 cells, and show that cocaine differentially affects the expression of the subtypes of protein kinase C.

These results demonstrate the ability of cocaine to affect the activity and expression of PKC isoenzymes in PC 12 cells. The effect of cocaine was dose dependent and specific for the different isoforms of PKC. The differential expression of PKC isoforms was accompanied by increased total PKC enzyme activity and Ca²⁺ levels. These effects of cocaine in the expression and activity of PKC in the PC 12 cells share some similarities and differences with the results previously obtained with ethanol in PC 12 cells (42). It was demonstrated that

<table>
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<tr>
<th>Treatment</th>
<th>Δ[Ca²⁺]i nM</th>
<th>% Control</th>
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<tr>
<td>Control</td>
<td>43.70 ± 5</td>
<td>100</td>
</tr>
<tr>
<td>0.01 mM cocaine</td>
<td>349.30 ± 25</td>
<td>799</td>
</tr>
<tr>
<td>0.10 mM cocaine</td>
<td>270.40 ± 19</td>
<td>619</td>
</tr>
<tr>
<td>1.00 mM cocaine</td>
<td>19273.00 ± 2001</td>
<td>44103</td>
</tr>
<tr>
<td>3.00 mM cocaine</td>
<td>21137.00 ± 1998</td>
<td>48368</td>
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chronic ethanol exposure increased the levels of PKCδ and ε and PKC-mediated phosphorylation in cultured neural cells (40,42). The results obtained indicated that, like alcohol, cocaine increased the levels of PKCδ, but, unlike alcohol, increasing doses of cocaine decreased the expression of PKCε. Furthermore, a number of studies have suggested a role of PKC in the modulation of alcohol effects on receptor function in cells of CNS origin, and the basis of some of these pharmacological effects may be related to the PKC-derived transduction mechanisms (41,45,46). PKC activity has also been linked with tolerance to the effects of alcohol (42) and morphine (47). In the CNS, alcohol and cocaine are known to disrupt a number of hormonal and neurotransmitter systems, including dopaminergic mechanisms that may be associated with compulsive alcohol and drug use and relapse. Since not all the physiological actions of the multiple dopamine systems can be explained by the modifications of the cAMP-dependent pathway, some studies have suggested an involvement of the inositol phosphate-generating system (48). There is increasing experimental evidence for the existence of cross-talk or interaction between multiple signal transduction systems (40,48) in the action of drugs that modulate the dopamine system, including psychostimulants like amphetamine. The data obtained in this study suggest some role of PKC in the effects of cocaine and lend further support to the probable existence of cross-talk between multiple signal transduction systems.

It is known that PKC is a soluble enzyme in its active state, and translocation to the plasma membrane is required for its activation by Ca²⁺ and phospholipids (49). However, not all PKC isoenzymes are calcium dependent. It was not surprising that the levels of Ca²⁺ were increased by the treatment with cocaine, because any one or a combination of the following mechanisms can be speculated to be involved: (1) by increasing Ca²⁺ channels as reported for ethanol (42); (2) by inhibition of the plasma membrane Ca²⁺ATPase pump, endoplasmic reticulum Ca²⁺ pump, mitochondrial Ca²⁺ uptake; and/or (3) due to the stimulation of the release of Ca²⁺ from internal storage by opening intracellular Ca²⁺ channels. Cocaine may also activate phospholipase C, which hydrolyzes phosphoinositol biphosphate (PIP2) yielding inositol triphosphate (IP3) and diacylglycerol (DAG), which, in turn, promotes translocation of PKC to the membrane, enhancing activation. The IP3 can release Ca²⁺ from internal storage sites and thus increase intracellular Ca²⁺([Ca²⁺])ₜ). The high levels of Ca²⁺ may also stimulate Ca²⁺ dependent proteases to degrade membranes and inhibit translation and transcription. Some or all of these proposed mechanism may be implicated in cocaine-induced apoptosis, observed at higher doses of cocaine. Although the mechanisms by which cocaine increases Ca²⁺ levels remain to be established, it is attractive to speculate that just like alcohol, cocaine may also increase the number and function of Ca²⁺ channels in the neural PC 12 cell line (42). Of course, cocaine may also be acting by mechanisms independent of the voltage-dependent Ca influx. In summary, this part of the study showed that cocaine
differentially altered the expression of PKC isoforms accompanied by increased levels of Ca\textsuperscript{2+} and total PKC activity. It is suggested that the differential expression of PKC isozymes may demonstrate distinct roles of PKC isoforms in the actions of cocaine. Thus, the PC 12 cell model may be exploited to further understand the neurobiology of cocaine’s action in neural systems. We are currently looking at the effects of ibogaine on a number of signaling pathways. Some experimental evidence appears to suggest that ibogaine’s action on signal transduction is more robust when that signal has been altered by abused substances. For example, ibogaine was reported to potentiate the inhibition of adenylyl cyclase by serotonin (\textsuperscript{50}). The mechanism by which ibogaine and noribogaine elicited a concentration-dependent increase in receptor-mediated inhibition of adenylyl cyclase activity is unclear. Since ibogaine and noribogaine did alter adenylyl cyclase activity, the enhanced inhibition of enzyme activity appears to represent functional antagonism (\textsuperscript{50}).

III. Effects of Ibogaine on the Action of Cocaine \textit{In Vivo}

For many years, the powerful reinforcing effects of psychostimulants, including cocaine and other abused drugs, have been linked to the mesocorticolimbic dopamine system and its connections (\textsuperscript{22-26}). Although dopamine is still thought to play a critical role in motivation and reward, it is doubtful that the mesocorticolimbic dopamine acts directly as the brain reward center (\textsuperscript{38}). However, it is now conceptualized that rather than signaling pleasure as previously thought, the neurotransmitter dopamine may be released by neurons to highlight significant stimuli (\textsuperscript{38}). The neurobehavioral effects of cocaine may be linked to a number of factors, including the route of administration, the dose of cocaine, the environmental cues, and the co-administration of other substances, including alcohol. It is unlikely that the overall neurobehavioral effects of cocaine are due to a single neurotransmitter action in one pathway in the central and peripheral nervous system. It is more likely that they are the result of the effects on multiple systems. The broad spectrum of action of ibogaine, therefore, makes it attractive to investigate whether it will functionally block an \textit{in vivo} action of cocaine that is linked to emotionality/stress, which may be a factor in drug dependency.

The acute and subacute effects during treatment and withdrawal from ibogaine on the performance of ICR mice in the elevated plus-maze test did not show any clear dose-response profile of action following the acute administration of ibogaine, as shown in Figure 3. The data obtained following treatment with ibogaine, or the combination with cocaine, were compared to the effect of
Figure 3 The Acute and Subacute Effects During Treatment and Withdrawal (W/D) from Ibogaine (top panel), and the Influence of Ibogaine on Cocaine Withdrawal in the Elevated Plus-Maze Test System. The time spent in the open arms (sec) of the plus-maze following the 5-minute test session is shown. Following withdrawal from cocaine (1.0 mg/kg), ibogaine (2.5 mg/kg) was administered daily for 4 days accompanied by daily testing. Significant differences from vehicle-treated animals are indicated as *p<0.05 or +p<0.05 (one-way ANOVA followed by Dunnet’s test).
vehicle-treated control mice. While the decrease in time spent in the enclosed arms at the lowest dose of 1.0 mg/kg may indicate an antiaversive action, the 2.5 mg/kg dose induced an aversive action in the open arms characterized by a decrease in the time spent in these arms. Ibogaine at the doses used had no significant effect on the time spent in the central platform. The number of entries into the open and closed arms was reduced only at the 2.5 mg/kg dose acutely (data not shown). At the highest dose of ibogaine used, the time spent and number of entries into the open and enclosed arms and the central platform were not different from controls. The repeated treatment of mice with ibogaine induced aversive and antiaversive behavior to the open arms on day 4 and day 13, respectively. An antiaversive behavior of mice injected with a 5-mg/kg dose was also recorded on day 10, as shown by the reduced time spent in the enclosed arms. This subacute treatment with ibogaine did not affect the time the animals spent in the central platform. Following withdrawal from the 14-day treatment with ibogaine, there were no differences in the time spent and number of entries into the open arms, enclosed arms, and central platform in comparison to control animals, as shown in Figure 3.

The influence of ibogaine (2.5 mg/kg) on cocaine withdrawal in the plus-maze test showed that on withdrawal from 1.0 mg/kg cocaine, an intense aversion into the open arms was blocked by ibogaine (see Figure 3). The data obtained add to the growing evidence that ibogaine, its congeners, and perhaps its metabolites, may have value in the treatment of drug and alcohol dependency. This conclusion is supported by other animal and human anecdotal and clinical evidence (37). Although there are some negative data, a number of animal studies indicate that ibogaine reduces some of the behavioral manifestations associated with cocaine administration and withdrawal (37). For example, in a study with mice, ibogaine reduced cocaine consumption in a drinking preference model (51). In another mouse study, ibogaine did not reduce the withdrawal manifestations following naloxone-precipitated withdrawal in morphine-dependent mice (52). In rats, ibogaine has been shown to decrease morphine self-administration (53,54), reduce the frequency of withdrawal induced by naloxone (53,55), and decrease intravenous cocaine self-administration (56). The effects of ibogaine on alcohol consumption was also investigated in animals, and it was demonstrated that ibogaine and one of its metabolites, noribogaine, reduces alcohol consumption in a number of alcohol-preferring rat lines (57-59). There are suggestions that the use of ibogaine in the treatment of drug and alcohol abuse be viewed with some degree of caution (60) because of its hallucinogenic properties and perhaps toxicity, but it is difficult to ignore the balance of evidence now emerging from animal and human data. In a preliminary study of seven individuals addicted to opiates, three who had at least 1.0 gm ibogaine, had remained drug free for 14 weeks (61). Therefore, there is some merit in the further investigation of the value of ibogaine in drug and alcohol dependence, which may form a template for the
development of novel compounds for substance abuse pharmacotherapy.

While the neuronal and molecular basis for the putative antiaddictive properties of ibogaine, its congeners, and its metabolites are incompletely understood, it has been postulated that these ibogaine-like compounds may be countering the multiple actions of abused substances (59). Most abused substances are known to have effects and interactions with one or multiple systems, including activation of gene expression and signal transduction, serotonin, dopamine, GABA, glutamate, noradrenergic, opiates, and hormones, particularly stress hormones (59). Because of the promiscuity of action of ibogaine, it is not surprising that it has shown promise preclinically and in the clinic. Thus, several approaches including pharmacological, histochemical, biochemical, behavioral, radioligand binding, toxicological, spectrometry, synthesis, and more recently molecular biology and genetics have been used to probe the action of this alkaloid. Overall, consensus data support the multiple effects of ibogaine. There is increasing interest in the genetic and signaling molecules that are important in the multiple actions of ibogaine. It appears that ibogaine may be restoring the intricate interactions within and between signaling pathways that are disrupted by abused substances. Intriguingly, because of the tangled web of cellular signaling mechanisms, there is no doubt that the more we know about signal transduction, the more we realize that more has yet to be discovered (62). Therefore, as hypothesized above, ibogaine may be switching off a subset of genes that have been turned on by alcohol and abused drugs. One transduction cascade that has been associated with the chronic administration of opiates or psychostimulants is the cAMP signal transduction pathway, which leads to CREB phosphorylation and downstream gene expression that, in principle, can be regulated by protein kinases (63,64).

**IV. Expression of Genes Regulated by Ibogaine Using cDNA Microarray Analysis**

Microarray technology has been described as a minirevolution in science and medicine and holds tremendous potential in unraveling programs of expression in normal and disease states (19,20). Because of its relative simplicity and power, it has been referred to as the new frontier in gene discovery and expression analysis and can be used to study programs of gene expression and profiling gene expression patterns of many genes in a single experiment. Microarray analysis has already been used in a number of laboratories to answer different kinds of research questions relating to gene expression (19,20). This study utilizes commercially available Atlas mouse cDNA expression arrays on which 588
mouse cDNAs have been immobilized. Two Atlas arrays are used, along with the reagents needed to make the cDNA probes. These cDNA probes are prepared from RNA isolated from the brains of mice that have been treated with cocaine, or ibogaine, or vehicle control groups. The expression levels of these known genes following the treatment in the three groups can then be compared and analyzed. It must be recognized that this technology has its limitations, and this study did not involve the detailed preparation of the DNA arrays, but only uses the commercially available arrays. Examples of differential gene expression patterns in two groups using cDNA expression arrays are presented in Figure 4, while Tables I and II list the putative genes and signaling molecules regulated by abused drugs/alcohol and ibogaine. Further research is required to characterize the most important genes regulated by ibogaine. Two different technologies, both with tremendous potential application in ibogaine research, human disease, biology, and behavior, are the use of gene-targeting approaches and differential display polymerase chain reaction (DDPCR). We have used the DDPCR procedure and obtained some preliminary data. Our preliminary DDPCR data

Figure 4. Differential Gene Expression patterns in Two Groups Using cDNA Expression Arrays. Example of differential gene expression patterns with subtle changes that can be detected during data analysis. Putative changes observed following administration of a number of abused substances may be normalized by treatment with ibogaine.
may suggest that abused substances are involved in the regulation of certain genes in the brain. If ibogaine reverses the action of cocaine on gene expression in the brain, as it does on cocaine-induced behaviors, characterization of the ESTs we have obtained may lead to the isolation and identification of genes induced or inactivated by ibogaine. Targeted gene disruption and gene manipulation technologies have been applied to neuroscience research. A number of novel genes have recently been identified and cloned, but the regulation of their expression is unknown. Homologous recombination enables the study of the physiological consequences of the absence of a specific gene. Recently, the function of a number of genes was studied by invalidating their genes. Once the complete genes activated or inactivated by ibogaine are known, then the functions of the genes can be analyzed by homologous recombination.

V. Conclusions

It is currently recognized that addiction is a chronic relapsing brain disease (65), for which behavioral and effective treatment is urgently needed. Unfortunately, effective drug-abuse treatment continues to be elusive, and the efficacies of new treatments for drug and alcohol addiction have been largely disappointing. The discovery that ibogaine, an emerging, controversial, potential treatment for alcohol and drug addiction, along with the recognition that the mesocorticolimbic dopamine may not, after all, underlie the reward pathway as previously hypothesized, may facilitate and aid rapid progress in substance-abuse research beyond the nucleus accumbens and dopamine hypothesis of reward. As reported recently, since ibogaine’s excitatory effect on ventral tegmental area neurons was not long lasting, nor does it persistently alter cocaine- or morphine-induced changes in dopamine neuron impulse (66), it was concluded that other mechanisms must be explored to account for the proposed antiaddictive properties of ibogaine. For the in vitro studies, it was reported that cocaine disrupts signal transduction in PC 12 cells by altering the expression and activity of PKC isoforms and Ca^{2+} levels. Since cocaine differentially altered the expression of PKC isoforms accompanied by increased levels of Ca^{2+} and total PKC activity, it remains to be determined if ibogaine will block the effects of cocaine on the expression of PKC isozymes and activity. For the in vivo studies, it was demonstrated acutely that ibogaine induced an aversive behavior in the ICR mice in the plus-maze test. Ibogaine did not by itself precipitate withdrawal anxiogenesis in the mouse model, but it reversed the withdrawal aversions caused by cessation from cocaine administration. Therefore, it was concluded that if anxiety or stress is a factor in drug dependency, then the antiaddictive property of
Ibogaine in vivo may be associated with modifying the CNS neurotransmission that may be involved in anxiety. The ability of ibogaine to alter drug-seeking behavior may thus be due to the combined actions of the parent drug and metabolite at key pharmacological targets that modulate the activity of drug-reward circuits. Thus, further studies are required to establish the efficacy of ibogaine and the design of ibogaine-like compounds for substance treatment that lack the toxicity and hallucinogenic profile of ibogaine. Finally, the mapping of the human genome will enable us to identify all the potential gene products that could be involved in addictions and the action of ibogaine.

References

7. CHANGES IN GENE EXPRESSION AND SIGNAL TRANSDUCTION
