Alterations in Fos-Related Antigen 2 and $\sigma_1$ Receptor Gene and Protein Expression Are Associated with the Development of Cocaine-Induced Behavioral Sensitization: Time Course and Regional Distribution Studies

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ABSTRACT

Repeated exposure to cocaine results in neuroadaptations that can alter the way the brain responds to subsequent stimuli. Earlier studies demonstrated that acute administration of cocaine up-regulates the immediate-early gene fos-related antigen 2 (fra-2) followed by a later up-regulation of $\sigma_1$ receptor gene and protein levels in brain regions involved in addiction and reward. To test whether such alterations could have long-term consequences on behavior, the present study was undertaken. Using a cocaine-induced behavioral sensitization model coupled with gene and protein expression studies in mice, the results show that cocaine induces the expression of fra-2, which leads to a progressive increase in $\sigma_1$ receptor gene and protein expression over a period of days. This progressive increase in $\sigma_1$ expression corresponds to the steady increase in the locomotor response to repeated cocaine administration in mice. The cocaine-induced changes in fra-2 and $\sigma_1$ receptor gene and protein expression occur in brain regions that subserve drug abuse, such as the cortex, striatum, and hippocampus, but not the cerebellum. Moreover, the prototypic $\sigma_1$ receptor antagonist 1-[2-(3,4-dichloropheny)ethyl]-4-methylpiperazine (BD1063) significantly attenuates both the molecular adaptations and behavioral sensitization induced by cocaine. These data suggest that repeated exposure to cocaine elicits alterations in fra-2 and $\sigma_1$ receptor-mediated mechanisms, which ultimately manifest as altered behavioral responses to cocaine.

Cocaine is a highly addictive substance. Acute cocaine administration can elicit stimulatory behaviors, convulsions, and even lethality, depending on its dosage (Matsumoto et al., 2003). Repeated, intermittent administration of cocaine may evoke a progressive enhancement of locomotor activity and stereotypic behaviors, termed as behavioral sensitization.

The neuronal circuits involved in the behavioral effects of cocaine consist of dopaminergic, glutamatergic, and GABAergic projections between the ventral tegmental area, nucleus accumbens, prefrontal cortex, hippocampus, and amygdala (Steketee, 2003; Kelley, 2004; Nestler, 2004; Wolf et al., 2004). In addition, some ion channels, including calcium, sodium, and potassium, contribute to the locomotor stimulatory and toxic effects of cocaine (Bauman and Diodomenico, 2002; Han et al., 2002; Morgan et al., 2003).

The involvement of $\sigma$ receptors in the actions of cocaine has also been actively investigated. Of the two established $\sigma$ receptor subtypes, $\sigma_1$ and $\sigma_2$, the $\sigma_1$ subtype seems to have the predominant role in modulating the actions of cocaine. At physiologically relevant concentrations, cocaine has preferential affinity for the $\sigma_1$ subtype (Matsumoto et al., 2002, 2003). $\sigma_1$ Receptors are found in the brain and peripheral tissues and are localized in cells on the plasma membrane and intracellular structures, such as the endoplasmic reticulum (McCann et al., 1994; Alonso et al., 2000; Hayashi and Su, 2007). Stimulated dopamine release can also be modulated through $\sigma_1$ receptors via signaling pathways involving protein kinase C (Nuwayhid and Werling, 2003). In addition, various pharmacological antagonists or antisense oligonucleotides targeting $\sigma_1$ receptors prevent cocaine-induced convul-
sions, lethality, locomotor activity, conditioned place preference, and behavioral sensitization in mice (Matsumoto et al., 2002, 2003; Romieu et al., 2004). Although these earlier studies provide compelling evidence for σ receptor antagonists as potential new medications for cocaine abuse, the mechanisms that underlie their ability to combat a wide array of behaviors are poorly understood.

In an earlier study combining behavioral pharmacological approaches with cDNA microarray analysis and reverse transcription-polymerase chain reaction (PCR) confirmations (Matsumoto et al., 2003; Liu et al., 2005), fos-related antigen 2 (fra-2), an immediate-early gene (IEG) and member of the fos family of transcription factors, was discovered to be up-regulated by cocaine and prevented by behavioral protective doses of BD1063, a σ1 receptor antagonist (Matsumoto et al., 2003; Liu et al., 2005). The ability of drugs to induce IEGs has been proposed to represent an early step in a chain of molecular events leading to synaptic reorganization, which underlie drug experience-dependent behavioral plasticity (Hyman and Malenka, 2001; McClung and Nestler, 2008). Because σ receptor ligands have also been reported to induce IEGs, particularly c-fos (Dahmen et al., 1996; Guitart and Farré, 1998), this raised the possibility that cocaine, through its interaction with σ receptors, could exert actions on IEGs.

After acute drug exposure, our earlier study demonstrated that cocaine-induced stimulation of fra-2 precedes a later up-regulation of σ1 receptor gene and protein expression in brain regions involved in addiction and reward (Liu et al., 2005). These findings led to the hypothesis that cocaine may exert effects on IEGs, such as fra-2, which in turn alter the regulation of target late genes, one of which may be the σ1 receptor. Because Fra-2 can form heterodimers with other transcription factors and bind to activator protein 1 (AP-1) sites (Herdegen and Leah, 1998; Smith et al., 2001), the presence of an AP-1 binding site on the σ1 receptor promoter (Seth et al., 1997) suggested a mechanism through which the gene could be transcriptionally regulated. Subsequent cocaine-induced increases in σ1 receptor expression may then lead to altered behavioral responses to later cocaine challenges, which can be expressed as sensitization.

Behavioral sensitization can be measured as an enhancement in locomotor hyperactivity upon repeated, intermittent administration with cocaine. This behavioral model has been used extensively to analyze neural adaptations associated with chronic cocaine exposure and withdrawal, and was used herein. In the present study, the σ1 receptor antagonist BD1063 (1-[2-(3,4-dichlorophenyl)ethyl]-4-methylpipеразин) was tested for its ability to modify the development of cocaine-induced behavioral sensitization. BD1063 has >400-fold better affinity for σ1 receptors compared with 5-hydroxytryptamine (5-HT2) receptors, and it has >1000-fold better affinity for σ1 receptors compared with the following binding sites: opioid receptors; N-methyl-D-aspartate receptors; dopamine D2 receptors; 5-HT1 receptors; α1, α2, and β-adrenergic receptors; muscarinic receptors; and dopamine, serotonin, and norepinephrine transporters (Matsumoto et al., 1995; Brammer et al., 2006). In addition, temporal and regional changes in fra-2 and σ1 receptor gene and protein expression were measured. Finally, the alterations in gene and protein expression were evaluated for associations with corresponding changes in behavior. Taken together, these studies were designed to test whether BD1063 can help prevent the occurrence of neural adaptations that result from repeated cocaine exposure and to determine the involvement of the σ1 receptor in the pathogenesis of cocaine-induced changes at the molecular and behavioral levels.

Materials and Methods

Subjects. Male, Swiss-Webster mice (24–28 g, n = 384) were acquired from Harlan (Indianapolis, IN) and housed in the University of Mississippi animal facility for at least 3 days before being used. Room temperature was maintained at 21°C. Lights were on from 6:00 AM to 6:00 PM. All procedures regarding the use and handling of animals were conducted as approved by the Institutional Animal Care and Use Committee serving the University of Mississippi.

Drug Application. Cocaine hydrochloride (Sigma-Aldrich, St. Louis, MO) was injected at a dosage of 10 mg/kg (1 mg/ml solution i.p.). Earlier studies revealed that this dose of cocaine produced peak locomotor stimulatory effects in our mice (McCracken et al., 1999). BD1063 (Tocris Bioscience, Ellisville, MO) was injected at a dosage of 30 mg/kg (3 mg/ml solution i.p.). In earlier studies, this dose of BD1063 produced robust attenuation of the locomotor stimulatory effects of the σ-active psychomotor stimulants cocaine (Matsumoto et al., 2001; Liu et al., 2005), methamphetamine (Nguyen et al., 2005), and 3,4-methylenedioxymethamphetamine (Brammer et al., 2006). It also attenuated cocaine-induced toxicities, such as convulsions and lethality (Matsumoto et al., 2001). With regard to the locomotor studies, BD1063 alone at the 30 mg/kg dose sometimes produced noticeable, although not statistically significant, decreases in locomotor activity; therefore, higher doses were not tested to avoid potential complications in data interpretation. Saline (10 ml/kg i.p.) was used as the control.

Locomotor Activity. Locomotor activity was measured using an automated activity monitoring system (San Diego Instruments, San Diego, CA). Plexiglas testing chambers were surrounded by 16 × 16 photobeam arrays, and locomotor activity was quantified as the number of beam breaks made by the mice as they moved around the apparatus. Mice (n = 12 for each data point) were individually adapted to testing chambers for 15 min, after which they received two injections separated by a 15-min interval of one of the following combinations of treatments: saline + cocaine, BD1063 + cocaine, saline + BD1063, or saline + saline for 5 consecutive days (days 1–5). After a 10-day drug-free period, the mice were challenged with cocaine (day 15). Locomotor activity recording started immediately after the second injection and continued for 30 min on days 1, 2, 3, 4, 5, and the challenge day (day 15). The behavioral data were evaluated using analysis of variance followed by post hoc pairwise comparisons using Bonferroni’s tests, to determine whether the differences between the groups were statistically significant.

Brain Tissue Sampling. On days 1, 3, 5, and 15, immediately after the locomotor recordings, brain tissues (n = 12) were collected and dissected into different regions (left half brain, right cortex, right cerebellum, right hippocampus, and right striatum) for the gene (n = 6 for each data point) and protein (n = 6 for each data point) expression studies.

Real-Time PCR. Total RNA was prepared from each brain sample using TRIzol reagents (Invitrogen, Carlsbad, CA) following the standard protocol for RNA extraction. First-strand cDNA synthesis was performed using SuperScript II RNase H- Reverse Transcriptase (Invitrogen) and random decamers (Ambion, Austin, TX).

PCR primers were designed using Primer Express software version 2.0 (Applied Biosystems, Foster City, CA) (Table 1) and were synthesized by Integrated DNA Technologies, Inc. (Corvalle, IA) using a standard desalting purification method. A stock solution was prepared by diluting the oligos to 100 pmol/μl in sterile water and then stored at −70°C.

For the PCR reactions, the following were combined: 2 μl of cDNA template, 12.5 μl of master mix, 0.125 μl for each primer, and 10.25 μl of master mix, 0.125 μl for each primer, and 10.25 μl.
μl of sterile water for a total volume of 25 μl. The mixture was then put into an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Thermal cycling was initiated at 50°C for 2 min, followed by a first denaturing step at 95°C for 10 min, and then 40 cycles at 95°C for 15 s and at 60°C for 1 min. Threshold cycle (Ct) was determined by the analysis software (SDS; Applied Biosystems).

Relative gene expression levels were evaluated by the ΔΔCt method. 28S rRNA was used as a reference. The data from the assays were subject to analysis of variance and post hoc tests (Bonferroni’s pairwise comparisons) to determine whether the differences between the experimental groups were statistically significant.

**Western Blots.** Pulverized tissue samples were homogenized in T-PER Tissue Protein Extraction Reagent (Pierce Biotechnology, Rockford, IL) using a ratio of ~1 g of tissue to 20 ml of T-PER Reagent. The samples were centrifuged at 10,000 rpm for 5 min, and the supernatants were collected. For small brain regions, like the striatum and hippocampus, tissues from two mice were pooled for protein extraction. Protein concentration was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA), and each sample was normalized to its total protein concentration.

Boiled protein samples (40–60 μg) and molecular weight (mol. wt.) standards [Precision Plus Protein Standards (5 μl); Bio-Rad] were resolved on 12 or 15% SDS-polyacrylamide gels, electrophoresed, and transferred onto nitrocellulose membranes. The membranes were then blocked with bovine serum albumin for Fra-2 or nonfat dry milk for σ1 receptors. The membranes were incubated with the following primary antibodies: Fra-2 (Q20, sc-604; 1:1000; Santa Cruz Biotechnology, Inc., Santa Cruz, CA); σ1 receptors (1:1000; Aves Labs, Tigard, OR); and β-actin (I19, sc-1616; 1:5000; Santa Cruz Biotechnology, Inc.). Next, the following horseradish peroxidase-conjugated secondary antibodies were incubated: anti-rabbit IgG for Fra-2, anti-goat IgG for β-actin (Santa Cruz Biotechnology, Inc.), and anti-chicken IgY for σ1 receptors (Aves Labs). NIH/3T3 nuclear extracts (Santa Cruz Biotechnology, Inc.) were used as a positive control for the Fra-2 antibody, and depleted antibodies against σ1 receptor antigens (Aves Labs) were used as a negative control for the σ1 receptor antibody. Immunoreactivity was visualized using enhanced chemiluminescence. Western blots for each protein was repeated at least three times.

The optical density reading of each band was quantified using Quantity One software (Bio-Rad) with background subtraction. The data from each experimental band was normalized to β-actin and then subject to analysis of variance. If there was an overall significant effect, Bonferroni’s pairwise comparisons were used for post hoc evaluations.

**Results**

**Behavioral Sensitization.** The results confirm that cocaine can induce behavioral sensitization. The σ1 receptor antagonist BD1063 can block the development of cocaine-induced locomotor sensitization. Figure 1 illustrates the time course of locomotor activity changes during the development of behavioral sensitization. Mice exhibited a gradual increase in locomotor activity during 5 consecutive days of repeated cocaine administration (p < 0.001). On the challenge day (day 15), all of the animals received cocaine, and locomotor activity increased in all treatment groups. However, the cocaine treatment group (Sal + Coc, days 1–5) displayed much higher locomotor activity than the other treatment groups (Fig. 1; p < 0.001). The BD1063 pretreatment group (BD + Coc, days 1–5) also showed some increase in locomotor activity, with statistically significant differences compared with the saline control group (Sal + Sal) on days 3, 4, 5, and 15 (p < 0.05). The level of locomotor activity in the BD1063 pretreatment group (BD + Coc) was significantly lower than the cocaine treatment group (Sal + Coc) on days 2, 3, and 4 (p < 0.05). No behavioral sensitization developed in the saline and BD1063 alone treatment groups (Sal + Sal and BD + Sal).

**Gene Expression.** Figure 2 shows the time course and regional distribution of fra-2 gene expression in the different

![Fig. 1. Effects of cocaine (10 mg/kg i.p.) and BD1063 (30 mg/kg i.p.) in mice on the development of behavioral sensitization. On days 1 through 5, mice received one of the following combinations of treatments: saline + cocaine (Sal + Coc), BD1063 + cocaine (BD + Coc), saline + BD1063 (Sal + BD), or saline + saline (Sal + Sal). After a 10-day drug-free period, on day 15 all of the mice were challenged with cocaine (10 mg/kg i.p.). Locomotor activity recording started immediately after the drug treatments and continued for 30 min. The cocaine treatment group showed increasing locomotor activity in 5 consecutive administration days and the challenge day, reflecting the development of cocaine-induced behavioral sensitization. The BD1063 pretreatment group also showed increasing locomotor activity in 5 consecutive administration days and the challenge day, but the locomotor activity was lower than the cocaine treatment group. No behavioral sensitization developed in the saline and BD1063 alone treatment groups. *** p < 0.001.](image)
treatment groups. Cocaine up-regulated fra-2 gene expression in the half brain, hippocampus, striatum, and cortex (Fig. 2, A–D) on days 1, 3, and 5, but not in the cerebellum (Fig. 2E). Similar to earlier studies involving acute drug exposures, pretreatment with BD1063 diminished the cocaine effect (Liu et al., 2005; Matsumoto et al., 2001). On day 15 when all of the animals received cocaine, up-regulation of fra-2 gene expression was exhibited in all treatment groups, suggesting that fra-2 gene up-regulation is associated with cocaine administration. This result confirms earlier data showing that acute administration of cocaine stimulates fra-2 gene expression (Liu et al., 2005).

Figure 3 illustrates the time course and regional distribution of frac1 receptor gene expression in the different treatment groups. No frac1 receptor gene expression changes were observed on day 1. However, cocaine-induced up-regulation of the frac1 receptor gene was observed in the half brain, hippocampus, striatum, and cortex after 3 days of treatment (Fig. 3, A–D), but not in the cerebellum (Fig. 3E). Up-regulation of frac1 receptor gene expression was also seen on the challenge day (day 15), but this change was statistically significant only in the striatum ($p < 0.05$). Similar to our earlier study involving acute drug exposures (Liu et al., 2005), pretreatment with BD1063 diminished the cocaine effects to levels comparable with those measured in the saline and BD1063 alone treatment groups.

**Protein Expression.** Figure 4 illustrates the time course and regional distribution of Fra-2 protein expression in the different treatment groups. No significant changes were shown between the treatment groups in all brain regions studied. However, although Fra-2 protein up-regulation did not reach statistical significance, a small increase in Fra-2 expression was still observed in half brain, striatum, hippocampus, and cortex (Fig. 4, A–D).
Figure 5 illustrates the time course and regional distribution of $\sigma_1$ receptor protein expression in the different treatment groups. Significant up-regulation of $\sigma_1$ receptor protein expression was observed in the half brain, striatum, and cortex ($p < 0.05$; Fig. 5, A, C, and D) on days 3, 5, and 15. Up-regulation of $\sigma_1$ receptor protein expression was also observed in the hippocampus, although the change did not reach statistical significance (Fig. 5B). No up-regulation was observed in the cerebellum (Fig. 5E). Similar to our earlier study involving acute exposures (Liu et al., 2005), pretreatment with BD1063 diminished the cocaine effects and showed a similar protein expression level as treatment with saline or BD1063.

**Association between Molecular and Behavioral Changes.** Figure 6 illustrates the concomitant and progressive increase in $\sigma_1$ receptor gene and protein expression with the increase in cocaine-induced locomotor activity (development of sensitization) over time. In contrast, the groups that were pretreated with BD1063 did not exhibit the increases in $\sigma_1$ receptor gene and protein expression (Figs. 3 and 5) and also showed a significant reduction in the sensitizing effects of cocaine (Fig. 1; correlation not shown). The relationship between $\sigma_1$ receptor expression and cocaine-induced behavior was evident in the hippocampus, striatum, and cortex (Fig. 6, A–C), but not the cerebellum (data not shown), suggesting its relevance for understanding neuroadaptations that result in altered responses to repeated exposure to cocaine. A similar association was not observed with fra-2 gene and protein expression (correlation not shown).

**Discussion**

The ability of $\sigma$ receptor antagonists to block the development of behavioral sensitization induced by cocaine has been...
reported earlier (Witkin et al., 1993; Ujike et al., 1996). The present study confirmed this result, with the prototypic \( \alpha_1 \) receptor antagonist BD1063 significantly attenuating the development of cocaine-induced behavioral sensitization. Whereas the BD1063 pretreatment group showed lower locomotor activity than the cocaine sensitized treatment group, it should be noted that some sensitization characteristics remained. This pattern of results suggests that pathways in addition to \( \alpha_1 \) receptors are also involved in the development of behavioral sensitization. However, \( \alpha_1 \) receptors are important enough to the overall development mechanisms that antagonizing them alone significantly attenuates behavioral sensitization.

Previous studies suggest that the ability of drugs of abuse, such as cocaine, to induce immediate-early genes represents an early step in a chain of molecular events leading to synaptic reorganization and forms of drug experience-dependent behavioral plasticity, such as sensitization (Hyman and Malenka, 2001; McClung and Nestler, 2008). In the present study, we focus on fra-2, which belongs to the fos family of IEGs, because earlier studies indicate that this transcription factor is particularly sensitive to the actions of cocaine that involve \( \alpha_1 \) receptors (Liu et al., 2005). In the current study, the up-regulation of fra-2 gene expression after each cocaine administration suggests that this gene plays an important role in the development of behavioral sensitization. Further-

Fig. 4. Time course of Fra-2 protein levels in different brain regions, showing slightly higher expression in the cocaine treatment group in half brain, hippocampus, striatum, and cortex. The difference was not statistically significant. Mice received one of the following combinations of treatments on days 1–5: saline + cocaine (Sal + Coc), BD106 + cocaine (BD + Coc), BD106 + saline (BD + Sal), or saline + saline (Sal + Sal). Cocaine was administered at a dose of 10 mg/kg i.p., and the BD1063 dose was 30 mg/kg i.p. After a 10-day drug-free period, on day 15 all of the mice were subsequently challenged with cocaine (10 mg/kg i.p.). Brains were removed 30 min after drug administration, immediately after behavioral testing. A, half brain; B, hippocampus; C, striatum; D, cortex; and E, cerebellum.
more, the up-regulation of fra-2 only occurred in brain regions related to addiction and reward (striatum, hippocampus, cortex), further implicating the alterations of fra-2 expression in drug-related neuroadaptations, rather than nonspecific activation of nervous system functions.

Although no significant changes in Fra-2 protein expression were observed in the different treatment groups, Fra-2 protein displayed modest increases in the striatum, hippocampus, and cortex of cocaine-treated animals. This result is consistent with the results of our earlier acute study in which Fra-2 protein expression was up-regulated after acute cocaine exposure, but it required at least 1 h before achieving statistical significance (Liu et al., 2005). The measurements in the current study were taken 30 min after drug administration, immediately after behavioral testing. A, half brain; B, hippocampus; C, striatum; D, cortex; and E, cerebellum. *; p < 0.05 and **, p < 0.01.

In contrast to the changes in fra-2, which probably represent an early, immediate response to cocaine, the alterations in $\sigma_1$ receptor gene and protein expression accumulated and persisted over time. The up-regulation of $\sigma_1$ receptor gene expression was observed after 3 days of cocaine exposure in the half brain, striatum, hippocampus, and cortex, but not in the cerebellum. This pattern of temporal and regional expression is consistent with our hypothesis that $\sigma_1$ receptors serve as a target for fra-2, and the change represents one of the neuroadaptations resulting from repeated cocaine exposure. Such a progressive increase in $\sigma_1$ receptor gene expression.

Fig. 5. Time course of $\sigma_1$ receptor protein level in different brain regions, showing cocaine-induced up-regulation in half brain, hippocampus, striatum, and cortex. Mice received one of the following combinations of treatments on days 1–5: saline + cocaine (Sal + Coc), BD106 + cocaine (BD + Coc), BD106 + saline (BD + Sal), or saline + saline (Sal + Sal). Cocaine was administered at a dose of 10 mg/kg i.p., and the BD1063 dose was 30 mg/kg i.p. After a 10-day drug-free period, on day 15 all of the mice were subsequently challenged with cocaine (10 mg/kg i.p.). Brains were removed 30 min after drug administration, immediately after behavioral testing. A, half brain; B, hippocampus; C, striatum; D, cortex; and E, cerebellum. *; p < 0.05 and **, p < 0.01.
The gene and protein expression patterns in the hippocampus were also significant in the current sensitization study. This pattern of gene and protein expression differs from our earlier study involving a single cocaine administration, in which no significant changes in \(\sigma_1\) receptor gene and protein expression changes were seen in the hippocampus (Liu et al., 2005). This result implies that changes in the expression of the \(\sigma_1\) receptor gene and protein may occur later in the hippocampus than in other regions, such as the striatum and cortex. This would be consistent with the established role of the hippocampus in learning and memory, which could serve as a neural substrate for some of the adaptive changes that occur in the brain after repeated cocaine exposures. Many investigators hypothesize that learning and memory and drug addiction are modulated by the same neurotrophic factors, share certain intracellular signaling cascades, and depend on activation of transcription factors such as cAMP response-element binding protein (CREB) (Kelley, 2004; McClung and Nestler, 2008). The importance of the hippocampus to learned aspects of drug addiction is also supported by reinstatement of drug taking after stimulation of glutamatergic fibers in this brain region (Vorel et al., 2001). Therefore, changes in gene and protein expression in the hippocampus after repeated cocaine exposures may underlie important persistent changes characteristic of the addicted state, whereas fewer changes in the hippocampus may be expected upon single acute administration of cocaine.

Although the precise mechanisms through which these neuroadaptations occur have yet to be fully elucidated, the literature suggests the involvement of a number of potential pathways. In our working hypothesis, activation of \(\sigma_1\) receptors by cocaine is expected to increase intracellular calcium levels, which is typical of \(\sigma_1\) receptor agonists (Hayashi et al., 2000; Hayashi and Su, 2007). In earlier studies, calcium has been shown to be capable of inducing Fra-2 (Herdegen and Leah, 1998). Similar to other members of the fos family, Fra-2 is known to form heterodimeric complexes with other proteins, such as Jun and CREB family members (Herdegen and Leah, 1998). Fra-2 is known to be capable of inducing Fra-2 (Herdegen and Leah, 1998), providing an alternate pathway through which Fra-2 could theoretically stimulate transcription. Moreover, exposure of cells or animals to cocaine is known to increase \(\sigma_1\) receptor mRNA and protein expression. Therefore, BD1063 could attenuate the ability of cocaine to interact with \(\sigma_1\) receptors, thereby preventing this cascade.

Concomitant to the stimulation of \(\sigma_1\) receptors, cocaine also indirectly activates dopaminergic systems, where one consequence is an increase in cAMP levels (see Zhang et al., 2005). cAMP is also known to activate Fra-2 (Herdegen and Leah, 1998), providing an alternate pathway through which \(\sigma_1\) receptors could be transcriptionally regulated and expression levels altered. cAMP can also activate extracellular signal-regulated kinase, leading to the stimulation of other transcription factors such as CREB and subsequent induction of immediate-early genes such as c-fos (Lu et al., 2006). In separate studies, cAMP-induced activation of extracellular signal-regulated kinase has been linked to increased expression of \(\sigma_1\) receptors (Cormaci et al., 2007). Although the
mechanisms for this latter effect is unknown, c-fos could serve as a bridge to the increase in σ1 receptors because our earlier microarray studies showed that in addition to fra-2, cocaine stimulated the expression of c-fos, and this could be attenuated by pretreatment with BD1063 (Liu et al., 2005).

In summary, pretreatment with BD1063 before each cocaine exposure significantly attenuated the development of cocaine-induced behavioral sensitization. Moreover, BD1063 also prevented cocaine-induced changes in fra-2 and σ1 receptor gene and protein expression. Taken together, the results suggest that the portion of cocaine’s effects that are mediated through σ1 receptors are highly relevant to understanding the neurobiology of drug abuse. The data suggest important elements of an intracellular cascade that, when activated by repeated cocaine exposure, elicit molecular neuroadaptations that parallel altered functional behavioral responses. Further studies of changes in fra-2 and σ1 receptor gene and protein expression in brain regions related to drug abuse may provide insights into the complex array of psychological and physiological alterations that are characteristic of addiction.

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Bauman JL and DiDomenico RJ (2002) Cocaine-induced channelopathies: emerging results suggest that the portion of cocaine’s effects that are mediated through σ1 receptors are highly relevant to understanding the neurobiology of drug abuse. The data suggest important elements of an intracellular cascade that, when activated by repeated cocaine exposure, elicit molecular neuroadaptations that parallel altered functional behavioral responses. Further studies of changes in fra-2 and σ1 receptor gene and protein expression in brain regions related to drug abuse may provide insights into the complex array of psychological and physiological alterations that are characteristic of addiction.


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