Short-Term Cocaine Treatment Causes Neuroadaptive Changes in $G_{\alpha_5}$ and $G_{\alpha_{11}}$ Proteins in Rats Undergoing Withdrawal

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ABSTRACT

One of the characteristics of drug dependence is that a drug has to be administered repeatedly before withdrawal effects can be observed. We have previously shown that withdrawal after 14 days of cocaine treatment produces a supersensitivity of hypothalamic 5-hydroxytryptamine (serotonin) 2A (5-HT$_{2A}$) receptors, which is accompanied by increases in the levels of $G_{\alpha_5}$ and $G_{\alpha_{11}}$ proteins. Unfortunately, the exact duration of cocaine treatment necessary to induce alterations in G protein levels during cocaine withdrawal is unknown. The present study investigated the minimum cocaine treatment period required to produce changes in protein levels of membrane- and cytosol-associated $G_{\alpha_5}$ and $G_{\alpha_{11}}$ proteins in the hypothalamic paraventricular nucleus, amygdala, and frontal cortex. Rats were injected with cocaine (15 mg/kg i.p., b.i.d.) for 0, 1, 3, 5, and 7 days and tested after 2 days of withdrawal. The levels of $G_{\alpha_5}$ and $G_{\alpha_{11}}$ proteins increased in the paraventricular nucleus and the amygdala, but not in the frontal cortex. Although 1 and 3 days of cocaine treatment were sufficient to maximally elevate the protein levels of $G_{\alpha_{11}}$ and $G_{\alpha_5}$ proteins in the amygdala, 5 days of treatment were required to maximally increase the levels of $G_{\alpha_{11}}$ and $G_{\alpha_5}$ proteins in the paraventricular nucleus. The data suggest that the amygdala shows a faster neuroadaptation to the effects of cocaine than the hypothalamic paraventricular nucleus. These findings provide insight into the relative importance of individual components of 5-HT$_{2A}$ receptor signal transduction system in regulating the overall sensitivity of this signaling in cocaine-treated rats.

Cocaine produces a variety of actions in neuronal function (Levy et al., 1994a). Cocaine binds with high affinity to neurotransmitter uptake sites on monoaminergic (serotonergic, dopaminergic, and noradrenergic) neurons in the brain and peripheral tissues, blocking the reuptake of serotonin, dopamine, and norepinephrine into the presynaptic neuron (Levy et al., 1994a).

The influence of cocaine on serotonergic neurotransmission has received increasing attention in recent years. The cocaine-induced reuptake blockade leads to an increased concentration of 5-HT in the synapse and thus to stimulation of postsynaptic 5-HT receptors (Hanson et al., 1987). Furthermore, cocaine reduces the activity of 5-HT neurons in the dorsal raphe (Cunningham and Lakoski, 1988), presumably as a consequence of increased stimulation of somatodendritic 5-HT$_{1A}$ autoreceptors in the dorsal raphe nucleus (Pan et al., 1989).

5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, which activate the dopamine mesoaccumbens pathway, play an important role in the behavioral effects of cocaine (McMahon et al., 2001). In addition, 5-HT$_{2A}$ receptors are expressed by neurons in the hypothalamic paraventricular nucleus (Zhang et al., 2002). Activation of 5-HT$_{2A}$ receptors in the hypothalamic paraventricular nucleus increases the secretion of ACTH, corticosterone, oxytocin, and prolactin (Van de Kar et al., 2001). Using neuroendocrine responses to the 5-HT$_{2A}$ receptor agonist DOI, we found increased sensitivity of 5-HT$_{2A}$ receptors that stimulate the secretion of ACTH, corticosterone, and prolactin after 42-h withdrawal from repeated cocaine treatment (15 mg/kg i.p., twice a day for 7 days) (Levy et al., 1992). The mechanism of this cocaine-induced supersensitivity of 5-HT$_{2A}$ receptors in the paraventricular nucleus might be an
altered expression of proteins associated with the 5-HT_{2A} receptor signaling pathway, including G_{q11} and G_{q111} proteins, and regulators of G protein signaling proteins, such as regulators of G protein signaling (RGS)4 and RGS7 proteins. Our previous study (Carrasco et al., 2003) showed that withdrawal (2 days) from chronic cocaine treatment (14 days, 15 mg/kg twice a day or using a binge protocol) produces a transient and region-specific increase in the levels of membrane-associated G_{q} and G_{q11} proteins in the hypothalamic paraventricular nucleus and the amygdala, but not in the frontal cortex (Carrasco et al., 2003). Exposure to chronic cocaine does not produce changes in the levels of membrane- or cytosol-associated 5-HT_{2A} receptors, RGS4 or RGS7 proteins in frontal cortex, amygdala, or paraventricular nucleus (Carrasco et al., 2003). These results support the conclusion that withdrawal from chronic cocaine treatment increases 5-HT_{2A} receptor function by altering postsynaptic signal transduction mechanisms, rather than increasing 5-HT_{2A} receptor density.

In this article, we focused on the minimum number of days of cocaine treatment required to change the levels of 5-HT_{2A} receptor signaling proteins in the hypothalamic paraventricular nucleus, amygdala, and frontal cortex. These regions were selected because of their prominent role in stress, anxiety, neuroendocrine function, and addiction (Carrasco and Van de Kar, 2003; Yun and Fields, 2003). The hypothalamic paraventricular nucleus plays a central role in mediating neuroendocrine responses to serotonergic activation (Bagdy, 1996). The hypothalamic paraventricular nucleus receives serotonergic projections from the raphe nuclei, which also send collaterals to other limbic structures, notably the amygdala (Liposits et al., 1987; Petrov et al., 1994). The amygdala is a limbic structure with interconnections to the cortex and the nucleus accumbens and plays a central role in the reinforcing effects of drugs of abuse (Yun and Fields, 2003).

Various drugs of abuse require repeated administration before withdrawal effects can be observed (Levy et al., 1994a). Because the immediate effect of cocaine is to reduce serotonergic firing rate (Cunningham and Lakoski, 1988), there is a good likelihood that the reduction in 5-HT release in the hypothalamus will gradually lead to supersensitive postsynaptic 5-HT_{2A} receptors. However, the minimum duration of cocaine exposure required to induce the adaptive mechanisms leading to an increase in the levels of G_{q} and G_{q11} proteins in the hypothalamic paraventricular nucleus and amygdala during withdrawal from cocaine has not been determined. In the present study, we evaluated the minimum number of days of cocaine treatment that will produce an increase in the levels of G_{q} and G_{q11} proteins in rats withdrawn from cocaine.

**Materials and Methods**

**Animal Treatment.** Male Sprague-Dawley rats (225–275 g) (Harlan, Indianapolis, IN) were housed two per cage in an environment controlled for lighting, temperature, and humidity. Food and water were available ad libitum. All procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory animals as approved by the Loyola University Institutional Animal Care and Use Committee. The rats were allowed to acclimate to their environment for at least 4 days before the start of the treatment period. Eight to 12 rats were randomly assigned to each group. Rats received injections of cocaine (15 mg/kg i.p., 8:30 AM and 3:30 PM) for 1, 3, 5, and 7 days. A control group of rats was injected with 0.9% saline (1 ml/kg i.p., 8:30 AM and 3:30 PM) for 7 days. All the rats were killed 2 days after the last injection. The brains were immediately removed, and the frontal cortex was dissected and frozen in liquid nitrogen. The remainder of the brain was frozen in dry ice and all tissues were stored at −80°C.

**Tissue Preparation.** Rat brains were placed in a cryostat at −10°C, and coronal sections were cut to obtain a 700-μm-thick section containing the paraventricular nucleus and rostral amygdala and a 1200-μm-thick section containing the caudal amygdala. The paraventricular nucleus and amygdala were microdissected from these frozen sections with the aid of a dissecting stereomicroscope. Plasma membranes of frontal cortex, amygdala, and paraventricular nucleus of the hypothalamus were prepared as described previously (Carrasco et al., 2003). All procedures were conducted at 4°C. Briefly, the frontal cortex, hypothalamic paraventricular nucleus, and amygdala were homogenized in 50 mM Tris buffer (pH 7.4) containing 150 mM NaCl, 10% sucrose, and 0.5 mM phenylmethylsulfonyl fluoride, and additional protease inhibitors purchased as a cocktail (containing 4-(2-aminoethyl)benzenesulfonyl fluoride, pepstatin A, trans-epoxyquinolin-1-4-4-4-4-guanidino)butane, leupeptin, and aprotinin) from Sigma-Aldrich (St. Louis, MO; 1.5 μl/30 mg of tissue). After centrifugation at 20,000g for 60 min, the supernatant was collected and stored at −80°C for further analyses of cytosol-associated protein levels. The pellets were collected and resuspended by sonication in a 20 mM Tris buffer (pH 8), containing 1 mM EDTA, 100 mM NaCl, 1% sodium cholate, and 1 mM dithiothreitol, plus the protease inhibitory cocktail (1.5 μl of cocktail/30 mg of tissue). The resuspended pellets were incubated while shaking for 1 h at 4°C and then centrifuged at 100,000g for 60 min. The supernatants were collected for the Western blot analyses of membrane-associated protein levels. Protein concentration was measured using a bicinchoninic acid protein assay kit (Pierce Chemical, Rockford, IL). The membrane proteins were stored at −80°C for Western blot analyses.

**Western Blot Analysis.** Samples containing 2 μg (hypothalamic paraventricular nucleus), 3 μg (amygdala), and 4 μg (frontal cortex) of protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis containing 0.1% SDS, 12.5% acrylamide/bisacrylamide (30:0.2), 4.6 M urea, and 275 mM Tris, pH 8.7. Gels were transferred electrophoretically to semi-dry blot to nitrocellulose membranes. After incubation with a blocking buffer (phosphate-buffered saline containing 0.2% casein and 0.1% Tween 20), the nitrocellulose membranes were probed overnight at 4°C with primary antibodies. Immunodetection was performed with the anti-G_{q1} (1:500; Santa Cruz Biotechnology, Inc., Santa Cruz, CA), anti-G_{q11} (1:500; Santa Cruz Biotechnology, Inc.), or anti-G_{q111} (1:6000; Santa Cruz Biotechnology, Inc.) antibodies. The overnight incubation with G_{q1}, G_{q11}, and G_{q111} antibodies was followed by incubation with peroxidase-labeled anti-rabbit antibody (1:2000; 1 h at room temperature; Santa Cruz Biotechnology, Inc.). Finally, the membranes were incubated with the enhanced chemiluminescence substrate solution (Amersham Biosciences Inc., Piscataway, NJ) and then exposed to X-ray film (Eastman Kodak, Rochester, NY). Protein loading for each lane was verified using an anti-actin antibody (1:20,000; Santa Cruz Biotechnology, Inc.). Negative controls included either omission of primary antibody or addition of preimmune rabbit immunoglobulins.

**Film Analysis.** Films were analyzed densitometrically using Scion Image software (Scion Corporation, Frederick, MD). The gray scale density readings were calibrated using a transmission step-wedge standard. The integrated optical density (IOD) of each band was calculated as the sum of the optical densities of all the pixels within the area of the band outlined. An adjacent area was used to calculate the background optical density of the film. The IOD for the film background was subtracted from the IOD for each band. Each sample was measured on three independent gels. All samples were standardized to controls and normalized to its respective actin level.

**Statistics.** All data are expressed as the mean ± S.E.M., where n indicates the number of rats per group. A one-way analysis of vari-
Western Blot Analysis

**Gα11 Protein.** Gα11 protein was detected as a single band at approximately 40 kDa (Fig. 1A). The level of membrane-associated Gα11 protein in the hypothalamic paraventricular nucleus was elevated in rats treated with cocaine for 1 to 7 days and withdrawn for 2 days. The ANOVA revealed that cocaine treatment \( F(4,49) = 39.42315, \ p < 0.0001 \) had a significant main effect on the level of membrane-associated Gα11 protein. The increase in the levels of Gα11 protein ranged from 24.5% over the control levels (\( p < 0.01 \)) in rats that received cocaine treatment for 1 day to 95% over the control levels (\( p < 0.01 \)) in rats that received cocaine treatment for 5 days (Fig. 1A). Rats that received 7 days of cocaine treatment showed an increase of 69% over the control levels (\( p < 0.01 \)) (Fig. 1A). The levels of membrane-associated Gα11 protein were significantly higher in rats which received 5 days of cocaine treatment than those which received 1, 3, or 7 days of cocaine treatment (\( p < 0.01 \)) (Fig. 1A). Cocaine treatment did not have a significant effect on the levels of cytosol-associated Gα11 protein in the hypothalamic paraventricular nucleus \( F(4,49) = 0.25323, \ p > 0.9 \) (Fig. 1A).

Cocaine treatment had a significant main effect on the levels of membrane-associated Gα11 protein in the amygdala \( F(4,49) = 26.19591, \ p < 0.0001p \) (Fig. 1B). The peak in the levels of membrane-associated Gα11 protein in the amygdala was found after 1 day of cocaine treatment (107% over control levels; \( p < 0.01 \)). The levels of membrane-associated Gα11 protein in the amygdala stayed elevated during the first 5 days of cocaine treatment (\( p < 0.01 \)) (Fig. 1B). No significant differences were observed for the levels of membrane-associated Gα11 protein among 1 to 5 days of cocaine treatment (\( p > 0.05 \)). After 7 days of cocaine treatment, the levels of membrane-associated Gα11 protein (76% over control levels) were significantly lower than after 1, 3, or 5 days of cocaine treatment (\( p < 0.05 \)). No significant effect of cocaine was found on the levels of cytosol-associated Gα11 in the amygdala \( F(4,49) = 0.79477, \ p > 0.5 \) (Fig. 1B).

In the frontal cortex, cocaine treatment did not produce a significant change in the levels of membrane-associated Gα11 protein among 1 to 5 days of cocaine treatment \( F(4,49) = 0.55749, \ p > 0.69 \) or cytosol-associated \( F(4,49) = 0.3706, \ p > 0.8283 \) Gα11 protein (Fig. 1C) across the different treatment durations.

**Gαq Protein.** Gαq proteins were detected in the hypothalamic paraventricular nucleus, amygdala, and frontal cortex as a single band at approximately 42 kDa (Fig. 2). The levels of membrane-associated Gαq protein in the paraventricular nucleus were also affected by cocaine treatment \( F(4,49) = 23.63318, \ p < 0.0001 \). Cocaine treatment gradually increased the levels of membrane-associated Gαq (Fig. 2A): 60% over control levels after 1 day and 86% over control levels after 3 days of cocaine treatment (Fig. 2A). The peak levels of membrane-associated Gαq protein in the paraventricular nucleus were detected after 5 days of cocaine treatment (105% over control levels; \( p < 0.001 \)). The levels of membrane-associated Gαq protein were 60% over control levels (\( p < 0.01 \)) (Fig. 2A). After 7 days of cocaine treatment, the levels of membrane-associated Gαq protein were 60% over control levels (\( p < 0.01 \)) (Fig. 2A). The levels of cytosol-associated Gαq protein were also significantly altered by cocaine \( F(4,49) = 13.76312, \ p < 0.0001 \). However, whereas the levels of cytosol-associated Gαq protein were not significantly altered (\( p > 0.05 \)) during the first 5 days of cocaine treatment (Fig. 2A), a 30% reduction (\( p < 0.01 \)) was detected after 7 days of cocaine treatment.

In the amygdala, the ANOVA indicated a significant main effect of cocaine treatment \( F(4,49) = 28.35248, \ p < 0.0001 \) on the levels of membrane-associated Gαq protein. In rats that received cocaine for 1 day, the levels of membrane-associated Gαq proteins were increased by approximately 74% over the saline control (\( p < 0.01 \)) (Fig. 2B). In rats that received cocaine for 3 days, the levels of membrane-associated Gαq proteins were significantly higher (74% over control levels; \( p < 0.01 \)). No significant differences were observed for the levels of membrane-associated Gαq proteins among 1 to 5 days of cocaine treatment (\( p > 0.05 \)). After 7 days of cocaine treatment, the levels of membrane-associated Gαq protein (76% over control levels) were significantly lower than after 1, 3, or 5 days of cocaine treatment (\( p < 0.05 \)). No significant effect of cocaine was found on the levels of cytosol-associated Gαq in the amygdala \( F(4,49) = 0.79477, \ p > 0.5 \) (Fig. 1B).
Fig. 2. Representative Western blots and densitometric analysis of the membrane and cytosol-associated Gαq in the hypothalamic paraventricular nucleus (A), amygdala (B), and frontal cortex (C) of rats injected twice a day with cocaine for 0, 1, 3, 5, and 7 days and then withdrawn for 2 days. Actin was used as a control of protein loading. The data represent the mean IOD ± S.E.M. as percentage of saline control (n = 6-8) measured in triplicate (++, p < 0.01, significant difference from rats injected with saline for 7 days; ⋆, p < 0.05, significant difference from rats injected with cocaine for 1, 3, and 7 days; ⋆⋆, p < 0.01, significant difference from rats injected with cocaine for 1, 3, and 5 days; ⋆⋆⋆, p < 0.05, significant difference from rats injected with cocaine for 1, 3, and 5 days; ⋆⋆⋆⋆, p < 0.01, significant difference from rats injected with saline for 7 days; #, p < 0.05, significant difference from rats injected with cocaine for 1, 5, and 7 days; ##, p < 0.01, significant difference from rats injected with saline for 7 days; &&, p < 0.05, significant difference from rats injected with cocaine for 1 day; &&&+, p < 0.05, significant difference from rats injected with cocaine for 1 day).

protein were even higher (103% over control levels; p < 0.01). The levels of membrane-associated Gαq protein in rats that received 3 days of cocaine treatment were higher than those which received 1, 5, or 7 days of cocaine treatment (p < 0.05) (Fig. 2B). After 5 and 7 days of cocaine treatment, the levels of membrane-associated Gαq protein were only elevated by 57% and 43% over control levels, respectively. Levels of cytosol-associated Gαq proteins in the amygdala were also affected by cocaine treatment [F(4,49) = 15.18487, p < 0.001]. The levels of cytosol-associated Gαq proteins were increased by 25% over the control levels in rats that received cocaine for 1 day (p < 0.005) and 55% in rats treated with cocaine for 3 days (p < 0.001) (Fig. 2B). The levels of cytosol-associated Gαq stayed elevated after 5 and 7 days of cocaine treatment by 50% and 45% higher over control levels (p < 0.01) (Fig. 2B). In rats treated with cocaine for 7 days, the levels of cytosol-associated Gαq proteins were still 43% higher (p < 0.01) than control levels (Fig. 2B).

In the frontal cortex, cocaine treatment did not alter the levels of membrane-associated Gαq protein [F(4,49) = 1.43207, p > 0.24] or the levels of cytosol-associated Gαq protein [F(4,49) = 0.55094, p > 0.69] (Fig. 2C).

**Gαq Protein.** Gαq is a 40-kDa protein that has not been reported to associate with the 5-HT2A receptor signaling cascade. The determination of Gαq protein was used to verify the specificity of the effects of cocaine treatment on G proteins. Because Gαq proteins are not involved in 5-HT2A receptor signaling during withdrawal from chronic cocaine treatment, changes in the levels of membrane and cytosol-associated Gαq proteins were also evaluated as negative controls. The ANOVA for the levels of membrane and cytosol-associated Gαq protein in the paraventricular nucleus showed no significant main effect of cocaine treatment [F(4,49) = 0.60125, p > 0.66 and F(4,49) = 0.28564, p > 0.88, for membrane and cytosol, respectively; Fig. 3A]. In the amygdala, the statistical analysis for the levels of membrane and cytosol-associated Gαq proteins showed no significant main effects of cocaine treatment [F(4,49) = 0.55889, p > 0.69 and F(4,49) = 0.2732, p > 0.89, for membrane and cytosol, respectively; Fig. 3B]. Additionally, cocaine treatment did not affect the levels of membrane or cytosol-associated Gαq protein in the frontal cortex [F(4,49) = 1.21265, p > 0.31 and F(4,49) = 1.24992, p > 0.30, for membrane and cytosol, respectively; Fig. 3C].

**Discussion**

Chronic cocaine has substantial effects on 5-HT2A receptor function (Levy et al., 1994a). Cocaine administration (15 mg/kg i.p., twice a day for 7 days) enhances the head shake response elicited by a specific 5-HT2A receptor agonist (DOI) (Baumann et al., 1993). We have also previously reported that DOI-mediated increases in plasma levels of prolactin, corticosterone, and ACTH become supersensitive after an identical cocaine paradigm (Levy et al., 1992). Interestingly, in both the behavioral and the neuroendocrine studies, there was no increase in the maximal responses to DOI but instead a leftward shift in the dose-response curve (Levy et al., 1994a; Baumann and Rothman, 1998), suggesting alterations in the coupling state of post-receptor signal transduction mechanisms. Because no differences in the density of 5-HT2A receptors have been found in the frontal cortex, amygdala, nucleus accumbens, caudate putamen, and thalamus of cocaine-treated rats, when measured by quantitative receptor autoradiography and binding assays (Perret et al., 1998), the increased sensitivity must be due to changes in the efficiency of 5-HT2A receptor transduction mechanisms. 5-HT2A receptors are coupled via Gαq/11 proteins and Gβγ proteins to phospholipase C and A2 signaling cascades (Roth et al., 1998).

The present results indicate that the increase in the levels of Gαq and Gα11 proteins in the amygdala peaks during withdrawal from 1 to 3 days of cocaine treatment. In the hypothalamic paraventricular nucleus, the increase in the levels of Gαq and Gα11 proteins is more gradual and peaks after withdrawal from 5 days of cocaine treatment. Thus, cocaine induced a more rapid increase in the membrane-
**Go** Protein

**A. Paraventricular Nucleus**

![Protein Expression](image)

**B. Amygdala**

![Protein Expression](image)

**C. Frontal Cortex**

![Protein Expression](image)

Fig. 3. Representative Western blots and densitometric analysis of the membrane and cytosol-associated Go proteins in the hypothalamic paraventricular nucleus (A), amygdala (B), and frontal cortex (C) of rats injected twice a day with cocaine for 0, 1, 3, 5, and 7 days and then withdrawn for 2 days. Actin was used as a control of protein loading. The densitometric data represent the mean IOD ± S.E.M. as percentage of saline control (n = 6–8) measured in triplicate.

Associated levels of Go and Gα11 proteins in the amygdala than in the hypothalamic paraventricular nucleus, suggesting that the amygdala shows a faster neuroadaptation to the effects of cocaine. Although the neuroadaptations occurring in both of these brain regions may not be related, there are three lines of evidence supporting a role of the amygdala in the function of the hypothalamic paraventricular nucleus.

First, injection of ketanserin, a 5-HT2 receptor antagonist, into the amygdala inhibited the effect of photic stress on the release of ACTH and corticosterone (Feldman et al., 1998). Second, as mentioned earlier, activation of 5-HT2A receptors expressed by neurons in the hypothalamic paraventricular nucleus increases the secretion of ACTH, corticosterone, oxytocin, and prolactin (Van de Kar et al., 2001). We recently reported (Zhang et al., 2002) that when MDL 100,907, a 5-HT2A receptor antagonist, is injected directly into the hypothalamic paraventricular nucleus, it completely blocks the prolactin and oxytocin responses to DOI, whereas the same dose of MDL 100,907 produces an incomplete inhibition of the ACTH response to DOI, suggesting a role of extrahypothalamic structures in the regulation of ACTH release. Last, 5-HT2A receptors are found in oxytocin and corticotrophin-releasing factor-immunoreactive cells in the hypothalamic paraventricular nucleus, whereas in the amygdala, 5-HT2A receptors are found in corticotrophin-releasing factor-immunoreactive cells (Zhang et al., 2001; Gray et al., 2003). 5-HT2A receptors are not found on enkephalin neurons, which are part of an inhibitory local circuit in the amygdala, further emphasizing their excitatory role in this brain area (Gray et al., 2003). Interestingly, a number of studies suggest that amygdaloid corticotrophin-releasing factor plays an important role in the expression of cocaine-induced behavior (Yun and Fields, 2003).

We further speculate that serotonin release in the amygdala activates 5-HT2A receptors (located on corticotrophin-releasing factor-expressing neurons) and induces corticotrophin-releasing factor secretion from terminals in the brainstem that mediate anxiety and stress-like responses. Thus, 5-HT2A receptors could activate corticotrophin-releasing factor neurons in the amygdala, which excite dorsal raphe neurons to induce ACTH release via their projection to hypothalamic neurons (Commons et al., 2003). Also, direct projections from the central amygdaloid nucleus to the hypothalamic paraventricular nucleus could mediate this effect (Gray et al., 1989). In this way, the activation of 5-HT2A receptors in the amygdala could be one mechanism by which neuroendocrine (hypothalamic corticotrophin-releasing factor) and autonomic/behavioral (amygdaloid corticotrophin-releasing factor) responses are integrated during responses to stressful or aversive stimuli (Gray et al., 2003). Thus, it is possible that in rats withdrawn from cocaine, an exacerbation of 5-HT2A receptor signaling in the amygdala, produced by an increase in the membrane-associated levels of Go and Gα11, could lead to a supersensitivity of the 5-HT2A receptor signaling in the paraventricular nucleus.

The increase in Go and Gα11 protein levels during cocaine withdrawal may be due to three different phenomena: 1) an increased expression of the Go proteins specifically at the cell membrane, 2) a general increase in Go protein levels in both membrane and cytosolic cellular compartments, and 3) a translocation of the G proteins from the cytoplasmic to the membrane portion. Because no reductions in the levels of cytosol-associated Go and Gα11 proteins were found, it is not likely that the supersensitivity of 5-HT2A receptor signaling in cocaine-treated rats involves a redistribution or translocation of Go and Gα11 proteins from the cytosol to the plasma membrane in the amygdaloid-hypothalamic neuronal circuits. The significant increase in the cytosol-associated levels of Go and Gα11 proteins in the amygdala could involve increase in the mRNA levels encoding Go and Gα11 proteins or decreased rate of degradation of these proteins in this brain region. Although Go and Gα11 proteins are targets for N-myristoylation but not ADP-ribosylation (Chen and Manning, 2001), there is no evidence that treatment with cocaine leads to changes in the post-translational modifications of Go or Gα11 proteins.

Additionally, it is possible that other proteins involved in the function of the 5-HT2A receptor and Go and Gα11 proteins could be modified by cocaine treatment. These include two families of G protein regulators important to the specificity and kinetics of the G protein signaling: the RGS pro-
tions and the activator of G protein signaling (AGS) family. In a previous report (Carrasco et al., 2003), we did not find changes in the levels of membrane and cytosol-associated levels of RGS4 and RGS7 proteins, two RGS proteins associated with the Gq and G11 protein signaling (Hollinger and Hepler, 2002), in the hypothalamic paraventricular nucleus, amygdala, or frontal cortex. However, other RGS proteins are up-regulated by cocaine (Bishop et al., 2002; Rahman et al., 2003). RGS4 shows a biphasic response in the locus coeruleus, with decreased RGS4 mRNA levels after acute administration and increased levels of RGS4 mRNA after chronic administration (Bishop et al., 2002). Also, the protein content of RGS9 is increased by chronic cocaine in the nucleus accumbens (Rahman et al., 2003). In human cocaine overdose abusers, up-regulation of RGS3 and down-regulation of RGS12 mRNAs in the ventral tegmental area have been reported (Tang et al., 2003). On the other hand, AGS3 one member of the AGS proteins is up-regulated in the prefrontal cortex during withdrawal from cocaine (Bowers et al., 2004). AGS3 binds to Gq proteins and inhibits GDP dissociation (Bowers et al., 2004). To the best of our knowledge, there is no current evidence of AGS proteins associated to the Gq and G11 protein signaling.

The activity of the hypothalamic-pituitary-adrenal axis before, at the time of, and subsequent to cocaine exposure seems to be an important determinant of whether individuals will engage in cocaine-seeking behavior (Mantsch et al., 2003). Whereas acute administration of cocaine stimulates the hypothalamic-pituitary-adrenal axis in rats (Rivier and Vale, 1987; Levy et al., 1991), chronic cocaine administration is associated with neuroadaptive changes that lead to attenuated responses to cocaine in rats and humans (Levy et al., 1993; Zhou et al., 2003). Rats chronically treated with cocaine showed a deficit in serotonergic nerve terminal function as seen from a reduced ACTH response to serotonin-releasing drugs p-clopaomphatine and fenfluramine (Levy et al., 1994b; Van de Kar et al., 1995). On the other hand, withdrawal from cocaine is associated with activation of the hypothalamic-pituitary-adrenal axis in rats (Peltier et al., 2001; Zhou et al., 2003). This enhanced response of the hypothalamic-pituitary-adrenal axis during withdrawal from chronic cocaine has been reported 24 to 48 h after the last cocaine injection (Peltier et al., 2001; Zhou et al., 2003). Similar results have been reported in human cocaine addicts (Vesovi et al., 1992; Baumann et al., 1995). These observations suggest that after development of adaptation or tolerance to chronic cocaine that produces deficits in the serotonergic activity, and during the withdrawal period, there is an increase in the activity of the hypothalamic-pituitary-adrenal axis. This increased activity would be associated to the supersensitivity of the 5-HT2A receptors and can be measured as increased 5-HT2A receptor-mediated release of ACTH, corticosterone, and prolactin. This supersensitivity of postsynaptic 5-HT2A receptors in the hypothalamic-pituitary-adrenal axis may be mediated through increased levels of membrane-associated of Gq or G11 proteins in the hypothalamic paraventricular and/or amygdala. However, at this point in time we cannot conclusively show whether the changes observed in Gq or G11 proteins are due to cocaine treatment or the withdrawal period.

In summary, our results reveal unique neuroadaptive mechanisms in the hypothalamic paraventricular nucleus and the amygdala but not frontal cortex of rats withdrawn from cocaine. The mechanisms by which cocaine mediates its region-specific effects are incompletely understood, although some behavioral and neuroendocrine effects associated with 5-HT2A receptor regulation seem to involve increased levels of membrane-associated Gq and G11 proteins. These findings provide insight into the relative importance of individual components of the 5-HT2A receptor signal transduction system in regulating the overall sensitivity of this signaling transduction pathway.

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