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Short communication

Repeated cocaine exposure attenuates the ability of 5-hydroxytryptamine to release striatal dopamine in vivo

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Abstract

Cocaine was administered repeatedly to rats and striatal microdialysis performed 1, 7, 14, or 21 days after the last cocaine injection. Local perfusion of 10 μM serotonin (5-hydroxytryptamine, 5-HT) increased basal dopamine levels approximately 7-fold (664 ± 79%, 813 ± 104% and 669 ± 62%, $n = 6$, $P < 0.0001$) in saline-treated controls, whereas in cocaine-treated animals the effect was significantly attenuated (432 ± 55%, 465 ± 61% and 497 ± 48% $n = 6$–10, $P < 0.03$) at 1, 7 and 14 days of withdrawal. The 5-HT effect on dopamine release returned to control levels 21 days after cocaine exposure and was not altered by acute cocaine treatment (30 mg/kg, i.p., 24 h prior). The results suggest that repeated cocaine administration attenuates the effectiveness of 5-HT on striatal dopaminergic activity.

Keywords: Microdialysis, brain; Cocaine; Dopamine; 5-HT (5-hydroxytryptamine, serotonin); Striatum

1. Introduction

Although the reinforcing effects of cocaine are primarily mediated by the activation of the mesolimbic dopamine system, little is known regarding the contribution of either the nigrostriatal dopamine system or the afferents which modulate this dopaminergic system, to the behavioral manifestations of cocaine withdrawal. Anatomical, electrophysiological and neurochemical (Benloucif et al., 1993) studies report that serotonin (5-hydroxytryptamine, 5-HT) neurons interact with the dopamine system both at cell body regions and terminal fields. The inhibition of transporter activity by acute cocaine treatment increases synaptic levels of both dopamine and 5-HT, subsequently leading to a decrease in both the electrical activity (White et al., 1992; Cunningham and Lakoski, 1988) and transmitter biosynthesis (Galloway, 1991) in these neuronal systems. Since the 5-HT neuronal system has a multifaceted influence on dopamine function, it is possible that cocaine's ability to alter dopamine function may be partly due to increases in synaptic 5-HT by cocaine.

As indicated above, (1) the dopamine system is essential for the reinforcing effects of cocaine, and (2) 5-HT influences dopamine function in several projection fields (i.e., striatum, nucleus accumbens and medial prefrontal cortex), thus it was of interest to determine the physiological significance of the 5-HT/dopamine interaction in an animal model of drug abuse. In this study, we tested the hypothesis that chronic exposure to cocaine alters 5-HT facilitation of dopamine release in the rat striatum. We also examined the 5-HT/dopamine interaction at several withdrawal timepoints since clinical observations indicate distinct withdrawal patterns after chronic cocaine exposure.

2. Materials and methods

Dialysis probes (4 mm exposed surface) of concentric design were implanted bilaterally in the anterior medial striatum (A, 0.7; L, 3.0; V, −7.0; with respect to bregma) of chloral hydrate anesthetized (400 mg/kg, i.p.) male Sprague-Dawley rats (275–350 g, Harlan, Indianapolis, IN, USA). The probes were perfused at a flow rate of 2 μl/min with artificial cerebrospinal fluid (aCSF) and samples were collected every 20 min (1 fraction) into 10 μl of high pressure liquid chromatography (HPLC) mobile phase and then immediately analyzed by HPLC with electrochemical detection (as previously described, Benloucif et

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al., 1993). For each subject, the baseline (3 stable fractions) was defined as control and drug treatment (local perfusion of 10 μM 5-HT for 1 fraction) was defined as percent change over control. The statistical significance of treatment-induced differences was calculated using Student's *t*-test (cocaine-treated group vs. appropriate saline-treated group). The cocaine treatment schedule followed that of Kalivas et al. (1992), a protocol that has been shown previously to elicit changes in dopamine neuronal activity. Rats were treated with either saline or cocaine (15 mg/kg, i.p. × 1 day followed by 30 mg/kg, i.p. × 5 days) and microdialysis performed either 1, 7, 14 or 21 days after the last treatment.

3. Results

Repeated exposure to cocaine attenuated the ability of locally perfused 5-HT to facilitate striatal dopamine release 1, 7 and 14 days after drug cessation. A single 20 min pulse of 5-HT (10 μM) increased extraneuronal dopamine levels 664 ± 79%, 813 ± 104%, 669 ± 61% over baseline (1.35 ± 0.17, 1.13 ± 0.35, 1.54 ± 0.35 fmol/μl, respectively, $n = 5–6$) in saline-treated controls, whereas the ability of 5-HT to increase striatal dopamine levels was significantly ($^{*}P < 0.03$) reduced to a 432 ± 55%, 465 ± 62%, 497 ± 48% increase over basal levels (1.63 ± 0.34, 1.57 ± 0.52, 1.45 ± 0.23 fmol/μl, respectively, $n = 6–10$) in cocaine-treated animals (Fig. 1). After 21 days of cocaine withdrawal, the ability of 5-HT to increase dopamine levels over baseline (0.98 ± 0.13 fmol/μl) did not differ from its effect in control animals (control baseline = 1.04 ± 0.28 fmol/μl, Fig. 1). Finally, to ensure that the observations made after 1 day of withdrawal were not due to residual effects of the last cocaine injection (delivered 24 h prior to dialysis), animals were treated acutely with one 30 mg/kg, i.p. dose of cocaine. Microdialysis performed 24 h later revealed that a single treatment with cocaine did not alter the ability of 5-HT (10 μM) to increase extracellular striatal dopamine levels (657 ± 113%, Fig. 2).

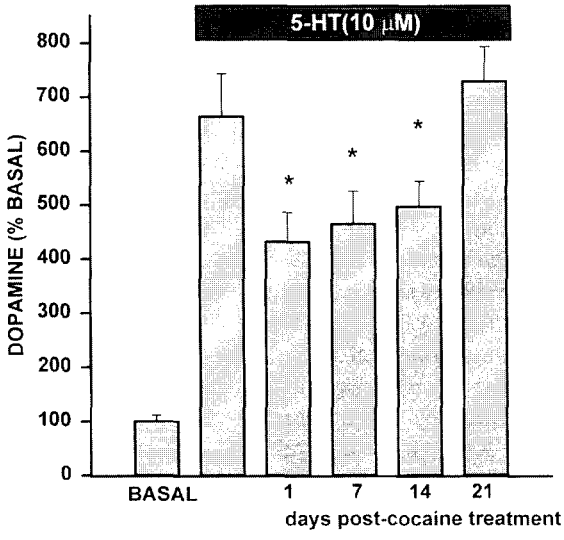

Fig. 1. Repeated cocaine exposure attenuated 5-HT facilitation of striatal dopamine release after 1, 7 and 14 days of withdrawal. 5-HT significantly enhanced extraneuronal dopamine levels nearly 7-fold (664 ± 79%, 813 ± 104%, 669 ± 61%, $n = 5–6$) in saline-treated controls, whereas the effect of 5-HT (10 μM) on dopamine was significantly attenuated (432 ± 55%, 465 ± 61%, 497 ± 48%, $n = 6–10$) in cocaine-treated animals at 1, 7 and 14 days of withdrawal, respectively ($^{*}P < 0.03$). Basal levels for saline-treated animals were 1.35 ± 0.17, 1.13 ± 0.35 and 1.54 ± 0.35 fmol/μl. Basal levels for cocaine-treated animals were 1.63 ± 0.34, 1.57 ± 0.52 and 1.45 ± 0.23 fmol/μl. After 21 days of cocaine withdrawal, the ability of 5-HT to increase dopamine levels (729 ± 65%, $n = 6$) over baseline (0.98 ± 0.13 fmol/μl) did not differ from the 5-HT effect on dopamine release (701 ± 97%, $n = 6$) over baseline (1.04 ± 0.28 fmol/μl) in control animals. Each bar represents the mean ± S.E.M.

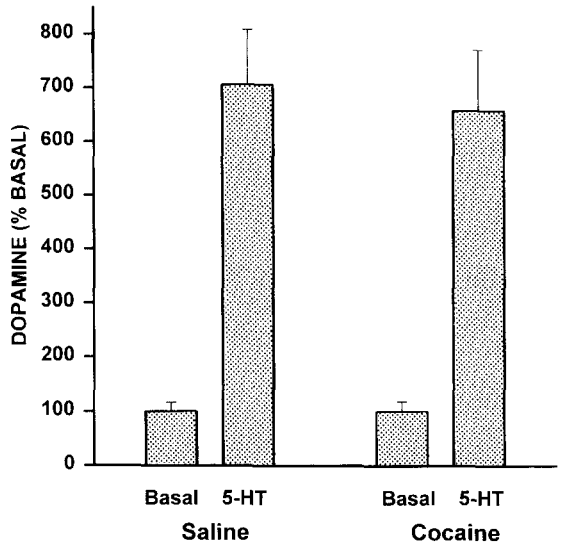

Fig. 2. The ability of 5-HT (10 μM) to facilitate dopamine release was not affected by acute (24 h prior) treatment with cocaine (30 mg/kg, i.p.) suggesting that the effects seen after 1 day of withdrawal from repeated cocaine treatment are not due to residual cocaine. Each bar represents the mean ± S.E.M. ($n = 6$).

4. Discussion

This study examined the ability of 5-HT to influence striatal dopamine release in rats withdrawn from chronic cocaine treatment. The results suggest that repeated cocaine treatment (15 mg/kg, i.p. × 1 day followed by 30 mg/kg, i.p. × 5 days) significantly attenuated the effect of 5-HT (10 μM) on dopamine release at 1, 7, and 14 days of withdrawal. This effect was reversible in that the effect of 5-HT on dopamine release returned to control values after 21 days of withdrawal. These observations suggest that repeated cocaine treatment produces a functional alteration in the interaction between 5-HT and dopamine which seems to be dependent upon the length of withdrawal from the last cocaine injection.

Recently, the role of 5-HT in cocaine reinforcement and withdrawal has received much attention. For example, the activity of the serotonergic system was inversely correlated with cocaine self-administration in rats such that pretreatment with selective serotonin reuptake inhibitors suppressed cocaine self-administration (Carroll et al., 1990) and dorsal raphe lesions increased cocaine self-administration (Loh and Roberts, 1990). Moreover, the activation of both the 5-HT and dopamine systems was essential to emulate cocaine's influence on striatal transcription factor gene expression (Bhat and Baraban, 1993) and to elicit cocaine-like electrophysiological effects on nucleus accumben neurons (White et al., 1992). These studies illustrate the synchronous interactions between 5-HT and dopamine systems with respect to the behavioral and cellular effects of chronic cocaine.

Repeated cocaine administration differentially alters the sensitivity of 5-HT_1 and 5-HT_2 receptor functions in vivo. For example, 5-HT_{1A} autoreceptors, which are known to regulate the firing rate of dorsal raphe neurons, become supersensitive after chronic cocaine administration (Cunningham, 1995). On the other hand, 5-HT_2 receptors, which control the neuroendocrine release of oxytocin, are rendered subsensitive after repeat cocaine administration (Levy et al., 1992). Clearly, cocaine can exert a diverse but profound influence on the sensitivity of 5-HT receptors. Thus it can be speculated that the observations made in the present study may be due to an alteration in the sensitivity of the 5-HT receptor(s) regulating dopamine terminal excitability.

Another possibility that may account for the observed changes in 5-HT/dopamine interactions include modifications to either transporter density or reuptake activity for these two monoamine systems. However, this explanation is not supported by other studies in that a majority of published reports suggest that repeated cocaine treatment does not influence either radioligand binding or [^{3}H]dopamine uptake in vitro (for review see Zahniser et al., 1995). Although intermittent cocaine alters 5-HT transporter binding in cortical brain regions, it fails to modify striatal 5-HT transporter binding (Cunningham, 1995).

It could be argued that cocaine-induced alterations in the intrinsic regulatory parameters controlling dopamine function may account for the diminished effect of 5-HT on dopamine release. For example, studies have shown that repeated cocaine treatment reduced dopamine synthesis in vivo (Brock et al., 1990). Nonetheless, decreased measures of dopamine neurochemistry have not been consistent in that repeated injections of cocaine produced no significant changes in the levels of dopamine, 5-HT, their precursors or their metabolites in post mortem tissue assays from various rat brain regions including the striatum (Baumann et al., 1993). It has been suggested that the craving which accompanies cocaine abstinence may be related to dopamine depletion; however, the evidence does not support an actual depletion of dopamine but rather points to a diminution of dopamine neurotransmission. Indeed, diminished effectiveness of excitatory afferent inputs, such as the effects reported herein, may contribute to an attenuation in dopamine transmission.

Even though dopamine tissue content may remain unchanged after chronic cocaine administration, other studies have shown that cocaine significantly influences extracellular basal dopamine levels. For example, intermittent cocaine schedules cause an initial rise in extracellular dopamine levels at early withdrawal timepoints, followed by a decrease in basal dopamine levels at later withdrawal timepoints in the rat nucleus accumbens (as measured by no net flux, for review see Weiss et al., 1995). Alterations in these levels may be due to transient desensitization of autoregulatory mechanisms (White et al., 1992). Although a correlation exists between basal dopamine levels and autoreceptor sensitivity, the relationship between basal dopamine levels and withdrawal symptoms is not as straightforward (Weiss et al., 1995).

Animal models of cocaine withdrawal using intracranial electrical self-stimulation suggest that the withdrawal process commences immediately following the removal of cocaine access (Markou and Koob, 1991). The withdrawal symptom, craving, is attributed to decreased dopamine neurotransmission and, as mentioned above, dopamine hypoactivity has been documented in the rat nucleus accumbens during late withdrawal periods from chronic cocaine. It is interesting to note, however, that dopamine activity in the nigrostriatal pathway may be reduced at early withdrawal periods. For example, 7 days postcocaine treatment, A9 somatodendritic autoreceptors exhibit supersensitivity to the dopamine agonist apomorphine (Zhang et al., 1992). The results from the present study provide additional evidence for a reduction in A9 dopamine neurotransmission at early withdrawal timepoints, most likely due to an alteration in 5-HT input.

It is important to stress that the phenomenon of cocaine withdrawal is described clinically as a syndrome consisting of multiple symptoms. Early withdrawal from repeated cocaine use is characterized by depression, craving, dysphoria, anhergia, anxiety and panic disorder, symptoms often associated with not only the mesoaccumbal dopamine pathway, but also with other dopamine and serotonergic systems (Gawin, 1991). In fact, many of these symptoms are indicative of a depressed 5-HT system, which may account for the results of recent clinical studies which report that selective 5-HT reuptake inhibitors, such as fluoxetine, relieve a portion of craving experienced during cocaine withdrawal (Walsh et al., 1994). Conversely, lowering 5-HT levels by tryptophan depletion also reduces the level of euphoria experienced during acute cocaine exposure in humans (Aronson et al., 1995). After chronic cocaine use, self-administration of psychomotor stimulants to promote dopamine release may represent a compensation for the decreased effectiveness of 5-HT on dopamine neurotransmission noted herein. Moreover, the attenuated

effectiveness of 5-HT on dopamine release at early withdrawal periods may explain the ability of 5-HT enhancing strategies to diminish cocaine self-administration in rats and craving in humans.

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