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Short communication

# Cocaine inhibits 5-$HT_3$ receptor function in neurons from transgenic mice overexpressing the receptor

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**Abstract**

Studies have shown that cocaine alters the function of recombinant 5-$HT_3$ receptors and that behavioral responses to cocaine are affected by 5-$HT_3$ receptor ligands. However, the actions of cocaine on brain 5-$HT_3$ receptors have not been characterized because these receptors are not abundantly expressed in most neuronal populations. We examined the effect of cocaine on 5-$HT_3$ receptor function in cultured hippocampal neurons from transgenic mice overexpressing the receptor. Cocaine competitively inhibited 5-$HT_3$ receptors with an $IC_{50}$ of $\sim$ 4 μM, indicating that brain 5-$HT_3$ receptors are important targets for the actions of this commonly abused substance.


*Keywords:* 5-HT (5-hydroxytryptamine, serotonin); Cocaine; Ligand-gated; Overexpression; Hippocampus; 5-$HT_3$ receptor

## 1. Introduction

5-Hydroxytryptamine type 3 (5-$HT_3$) receptors are ligand-gated ion channels that belong to the nicotinic acetylcholine receptor superfamily. Although these receptors are expressed in the brain at relatively low densities, it is thought that they may have important presynaptic functions that modulate neurotransmitter release. The function of 5-$HT_3$ receptors is regulated by substances of abuse, including ethanol and cocaine. Ethanol positively modulates both recombinant and native 5-$HT_3$ receptors in vitro (Lovinger, 1999; Lovinger and Zhou, 1998; Sung et al., 2000). Mice that overexpress this receptor show a decrease in ethanol consumption (Engel et al., 1998), which is probably due to enhanced ethanol sensitivity (Engel and Allan, 1999). Cocaine, on the other hand, has been shown to inhibit 5-$HT_3$ receptor function. To date, the mechanism of the interaction between cocaine and 5-$HT_3$ receptors has been studied mainly using non-neuronal cells, including *Xenopus* oocytes (Fan et al., 1995; Mair et al., 1998), human embryonic kidney cells (Brown et al., 1998) and neuroblastoma cells (Lambert et al., 1989). Moreover, only one study has examined the effects of cocaine on neuronal 5-$HT_3$ receptors. Specifically, Peters et al. (1993) found that 5-$HT_3$ receptors expressed in sensory neurons from the rabbit nodose ganglion are inhibited by cocaine. However, we are not aware of any study of the interaction of cocaine with this receptor in neurons of the central nervous system. Since behavioral studies of the effects of cocaine on transgenic mice overexpressing the 5-$HT_3$ receptor are currently underway, we decided to characterize the effects of this drug of abuse on currents mediated by these receptors in neurons from these animals.

## 2. Materials and methods

All chemicals were from Sigma or Fluka (St. Louis, MO). All animal procedures were approved by the University of New Mexico Health Sciences Center—Institutional Animal Care and Use Committee and were in accordance to National Institutes of Health guidelines. Electrophysiological studies were performed on hippocampal neuronal primary cultures that were prepared from postnatal days 3–4 homozygous or heterozygous B6SJL mice, as described previously (Costa et al., 2000). These mice were modified genetically to overexpress 5-$HT_{3A}$ subunits throughout the forebrain under the control of the calcium/calmodulin-dependent protein kinase α-II promoter (Engel et al., 1998).

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After 7–8 days in culture, whole-cell patch-clamp electrophysiological recordings were performed as described (Costa et al., 2000), with the exception that the internal solution contained in mM: 10 HEPES, 10 EGTA, 5 CsCl, 140 $CsCH_3SO_4$ (pH 7.4; 280 mosM). 5-HT, prepared fresh everyday, was applied for 3 s at 60-s intervals. A control experiment revealed that currents elicited by 10 μM 5-HT were reversibly inhibited by 94 ± 4% ($n = 3$) by the selective competitive 5-$HT_3$ receptor antagonist tropisetron (10 nM; data not shown). Cocaine was continuously applied in both the buffer- and agonist-containing solutions. Three agonist-triggered responses were recorded in the continuous presence of cocaine and then averaged; cocaine was pre-applied for 2 min prior to the first agonist application. The change in peak amplitude induced by cocaine was obtained with respect to the average of at least three control and three washout responses.

Data were initially analyzed by the Kolmogorov–Smirnov normality test (GraphPad Prizm; San Diego, CA). One-way analysis of variance (ANOVA) followed by Tukey's posthoc test and two-way ANOVA were used to subsequently analyze the data. Data are expressed as the mean ± standard error of the mean (S.E.M.).

## 3. Results

As shown in Fig. 1, cocaine reversibly inhibits, in a concentration-dependent manner, the current elicited by 5-HT. The cocaine $IC_{50}$ concentration was 4.2 μM (95% confidence interval 3.1–5.6 μM) with a Hill slope of

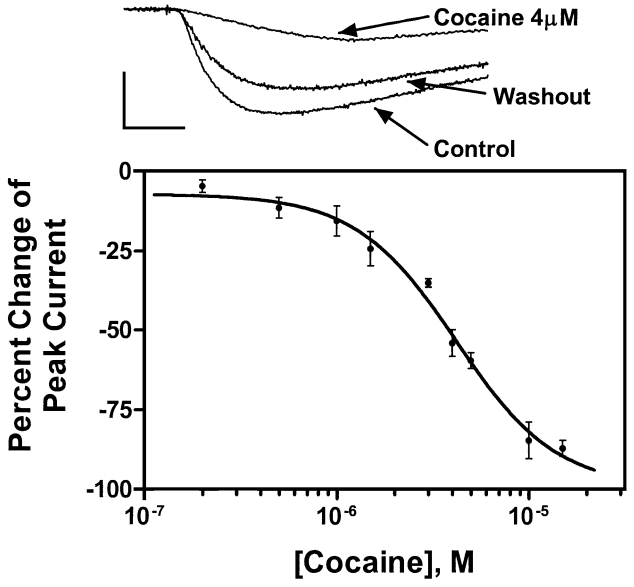


Fig. 1. Inhibitory effect of cocaine on 5-$HT_3$ receptor-mediated currents. Top panel: Sample trace showing whole-cell currents evoked by 5-HT (3 μM) in the absence and presence of cocaine (4 μM). Scale bar represents 100 pA and 1 s. Bottom panel: Dose–response curve for the cocaine-induced inhibition of 5-$HT_3$ receptor-mediated currents. Each symbol represents the mean ± S.E.M. of 7–10 neurons. See text for results of statistical analyses.

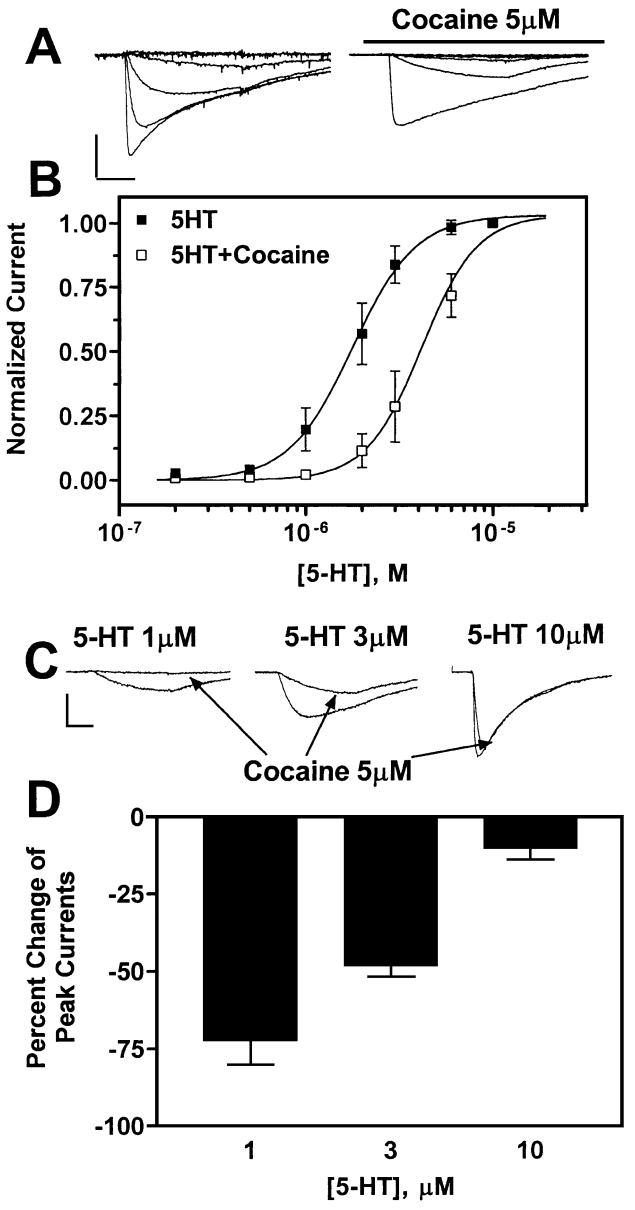


Fig. 2. Cocaine is a competitive inhibitor of native 5-$HT_3$ receptors. (A) Sample traces showing whole-cell currents evoked by increasing concentration of 5-HT in the absence (left traces) and in the presence (right traces) of cocaine 5 μM. Scale bar represents 100 pA and 1 s. (B) Summary of the effect of cocaine. Data were normalized with respect to the maximum current elicited by 5-HT (10 μM). Each point represents the mean ± S.E.M of four neurons ($P < 0.001$ by two-way ANOVA). (C) Sample traces showing whole-cell currents evoked by increasing concentrations of 5-HT in the absence and in the presence of cocaine 5 μM. Scale bar represents 100 pA and 1 s. (D) Summary of the effects of cocaine. Each bar represents the mean ± S.E.M. of five different recorded neurons ($P < 0.05$ by one-way ANOVA followed by Turkey's posthoc test). Data were normalized with respect to the current elicited by 5-HT alone.

−1.6 (95% confidence interval −2.4 to −0.9). We then tested the effect of cocaine on the 5-$HT_3$ receptor dose–response curve (Fig. 2A–B). We found that cocaine induces a parallel shift of the dose–response curve to the right; the $EC_{50}$ concentrations were 1.8 μM (95% confidence interval

1.5–2.0 μM) and 4.2 μM (95% confidence interval 3.6–4.9 μM) for 5-HT alone and 5-HT in the presence of 5 μM cocaine, respectively. The Hill slopes were 2.6 (95% confidence interval 1.7–3.4) and 2.9 (95% confidence interval 1.97–3.88) for 5-HT alone and 5-HT in the presence of 5 μM cocaine, respectively. We also tested if the inhibition of 5-$HT_3$ receptor-mediated currents by cocaine was dependent on the 5-HT concentration. As shown in Fig. 2C–D, cocaine was more effective at lower 5-HT concentrations.

## 4. Discussion

The purpose of this study was to assess the effect of cocaine on native 5-$HT_3$ receptors expressed in central nervous system neurons. In agreement with other reports, we found that cocaine inhibits 5-$HT_3$ receptor-mediated responses in a dose-dependent manner (Brown et al., 1998; Fan et al., 1995; Lambert et al., 1989; Mair et al., 1998; Peters et al., 1993). Our finding that cocaine inhibits 5-$HT_3$ receptor-mediated responses with an $IC_{50}$ of 4.2 μM is consistent with the results of a number of studies with recombinant 5-$HT_3$ receptors expressed in *Xenopus* oocytes. These studies reported $IC_{50}$ values between 5.5 and 6.1 μM (Belelli et al., 1995; Mair et al., 1998). Moreover, Breitinger et al. (2001) recently reported that cocaine competes for 5-HT binding to 5-$HT_3$ receptors expressed in neuroblastoma cells with a dissociation constant of 7 μM. However, other studies have reported more potent actions of cocaine. For instance, Fan et al. (1995) found that cocaine inhibits recombinant 5-$HT_3$ receptors expressed in *Xenopus* oocytes with an $IC_{50}$ of 0.7 μM. Moreover, Brown et al. (1998) reported an $IC_{50}$ of 0.4 μM for receptors expressed in human embryonic kidney-293 cells and Peters et al. (1993) an $IC_{50}$ of 83 nM for native receptors expressed in cultured nodose ganglion neurons. Thus, it appears as if the actions of cocaine on 5-$HT_3$ receptors are dependent on the expression system and animal species. Variability in $IC_{50}$ values could be due to differences in subunit composition or post-translational modifications. It is possible that the presence of the 5-$HT_{3B}$ subunit in these receptors alters pharmacological responses to cocaine as it does for tubocurarine (Davies et al., 1999). Alternatively, $IC_{50}$ variability could be simply due to differences in agonist occupancy of the 5-$HT_3$ receptor. It can be concluded, however, that the presence of cocaine decreases 5-$HT_3$ receptor occupancy, which is supported by our finding that it induces a right shift of the agonist dose–response curve and also by the fact that it inhibits to a greater extent currents activated by lower concentrations of 5-HT. Thus, cocaine behaves as a typical competitive antagonist of the 5-$HT_3$ receptor in neurons from the transgenic mice.

In conclusion, we found that cocaine inhibits the function of 5-$HT_3$ receptors in cultured hippocampal neurons from mice overexpressing the receptor. Plasma concentrations of cocaine in patients addicted to this drug usually are in the low micromolar range (0.73–1.02 μM) (Javaid et al., 1978). Thus, present results and those of the studies discussed above strongly suggest that 5-$HT_3$ receptors are important targets of cocaine at pharmacologically relevant concentrations. Since 5-$HT_3$ receptors are thought to regulate neurotransmitter release, it would be interesting to determine if cocaine also alters the presynaptic actions of this receptor.

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