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The Role of Serotonin$_2$ Receptors in Mediating Cocaine-Induced Convulsions

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O'DELL, L. E., M. J. KREIFELDT, F. R. GEORGE AND M. C. RITZ. *The role of serotonin$_2$ receptors in mediating cocaine-induced convulsions.* PHARMACOL BIOCHEM BEHAV **65**(4) 677–681, 2000.—Previous research in our laboratory suggests that serotonin (5-HT) neurotransmission mediates the expression of cocaine-induced convulsions. The role of 5-HT in mediating this toxic effect of cocaine appears to be due to activation of 5-HT$_2$ receptors, because cocaine-induced convulsions are blocked by the 5-HT$_2$ antagonists cinanserin, ketanserin, and pirenperone. The present study utilized a number of compounds that display a high affinity for 5-HT$_2$ receptors to further examine the role of these sites in mediating this toxic effect of cocaine. Cocaine-induced convulsions were observed following pretreatment with various doses of the following 5-HT$_2$ antagonists: mianserin, metergoline, MDL 11939, and methiothepin. In addition, 1-(2-methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN 190) was tested to examine the influence of 5-HT$_1$ sites and the agonist compound 1-(3-triflurormethylphenyl)piperazine (TFMPP) was examined to further explore the role of 5-HT$_1$ and 5-HT$_2$ sites. Each 5-HT$_2$ antagonist attenuated cocaine-induced convulsions. Conversely, NAN 190 did not alter this toxic effect of cocaine. In addition, TFMPP significantly potentiated cocaine-induced convulsions. The results from this study support the hypothesis that 5-HT neurotransmission, acting primarily at 5-HT$_2$ receptors, plays an important role in mediating cocaine-induced convulsions. © 2000 Elsevier Science Inc.

Cocaine-induced toxicity Seizures Mianserin Metergoline MDL 11939 Methiothepin TFMPP NAN 190

COCAINE is a powerfully addicting drug of abuse that has been increasingly associated with toxic consequences, including convulsions and death. Research in our laboratory suggests that the convulsant effects of cocaine are mediated by serotonin (5-HT) neurotransmission. In previous studies utilizing multiple receptor site analyses we demonstrated that the potency of cocaine and related compounds to produce convulsions is highly correlated with binding of these compounds to the 5-HT transporter (16). In fact, the potency for binding at 5-HT transporters alone accounted for 78% of the variance in the potency of cocaine and related compounds for producing convulsions. Subsequent pharmacological studies in our laboratory supported the role of 5-HT in mediating this toxic effect of cocaine. For example, cocaine-induced convulsions are enhanced by pretreatment with 5-HT reuptake inhibitor (SSRI) compounds, such as fluoxetine, paroxetine, and citalopram (11). The role of 5-HT in mediating cocaine-induced convulsions is also supported by another laboratory demonstrating that this toxic effect of cocaine is enhanced by coadministration of a fenfleuramine racemer, which increases synaptic 5-HT levels (18).

Subsequent lines of research suggest that the role of 5-HT in mediating cocaine-induced convulsions appears to be mediated by 5-HT$_2$ receptors. Cocaine-induced convulsions are attenuated by the 5-HT$_2$ receptor antagonists cinanserin, ketanserin, and pirenperone (13,17,18). Pharmacogenetic studies in our laboratory also support the role of 5-HT$_2$ receptors in mediating cocaine-induced convulsions. Specifically, the density of these receptors appears to mediate sensitivity to the convulsant effects of cocaine across different strains of mice (13). Moreover, we found that cinanserin more potently attenuates cocaine-induced convulsions in mice exhibiting a lower density of 5-HT$_2$ receptors. The role of 5-HT$_2$ receptors in mediating cocaine-induced convulsions is also supported by the finding that this toxic effect of cocaine is enhanced by pretreatment with the preferential 5-HT$_{2C}$ agonists *m*-chlorophenylpiperazine (mCPP) and 6-chloro-2-(1-piperazinyl)pyrazine [MK212; (12)]. Collectively, these studies suggest that 5-HT

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neurotransmission, acting primarily at 5-HT_2 receptors, plays an important role in mediating cocaine-induced convulsions.

The goal of the present study was to further examine the role of 5-HT_2 receptors in mediating cocaine-induced convulsions. Therefore, this toxic effect of cocaine was examined following pretreatment with a number of antagonists that exhibit a high affinity for 5-HT_2 receptors (i.e., mianserin, metergoline, MDL 11939, and methiothepin). Because some of these compounds also display an affinity for 5-HT_{1A} sites, cocaine-induced convulsions were also examined following pretreatment with NAN 190, an antagonist displaying relatively high selectivity for 5-HT_{1A} sites (19). To aid in further pharmacological comparisons, we also studied the effects of the 5-$HT_{2C/1B}$ agonist TFMPP on subsequent cocaine-induced convulsions. Taken together, this series of studies were designed to gain insight into the relative influence of specific 5-HT receptor subtypes in mediating cocaine-induced convulsions.

METHOD

Animals

Experimentally naive male C57BL/6J mice (60–100 days old) obtained from the Jackson Laboratories (Bar Harbor, ME) were used. Animals were housed in groups of same-sex littermates with ad lib access to Purina chow and tap water. All mice were maintained in a temperature controlled room (26°C) with a 12 L:12 D cycle (0700–1900 lights on). The experimental procedures, protocols, and housing conditions for this study were approved by the Institutional Animal Care and Use Committee at Amethyst Technologies, Inc. in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institute of Health.

Drugs

The following compounds were used: (−)cocaine HCl (National Institute on Drug Abuse), mianserin, metergoline, methiothepin, NAN 190, TFMPP (Research Biochemicals International), and MDL 11939 (Hoechst Marion Roussel, Inc). The doses are expressed as mg/kg base and all compounds were administered intraperitoneally in a volume of 10 ml/kg with saline vehicle. Metergoline, NAN 190, and MDL 11939 were administered in a suspension of 0.9% saline plus 1% polysorbate-80 vehicle, and all other compounds were administered in a 0.9% saline vehicle.

Behavioral Observations

Animals (n = 6–10 per group) were pretreated with mianserin (5.6, 10, or 17.8 mg/kg), metergoline (5.6, 10, 17.8, or 30 mg/kg), MDL 11939 (1.0, 3.2, 10, 17.8, or 30 mg/kg), methiothepin (0.1, 0.3, 1.0, 5.6, or 17.8 mg/kg), NAN 190 (5.6, 10, or 30 mg/kg), or TFMPP (10 or 30 mg/kg). The dose ranges were chosen to reflect a range of both effective and noneffective concentrations of the antagonists used. Animals (n = 44) pretreated with saline vehicle served as controls. The animals were then placed into 30 × 30 cm^2 Plexiglas chambers for 15 min and changes in the animals' behavior were monitored to allow for observation of any changes in behavior produced by the pretreatment compound alone. Previous studies have shown that 15 min was sufficient to allow for maximum behavioral and/or biochemical effects of the compounds tested (3,9,10). In addition, our behavioral observations indicated that 15 min was sufficient time to observe behavioral effects of these compounds.

Fifteen minutes following the first injection, mice receiving the antagonist compounds were injected with 100 mg/kg of cocaine, while mice receiving the agonist TFMPP were injected with 56 mg/kg of cocaine. Animals were then returned to the Plexiglas chambers where they were scored by a "blind" observer for the occurrence and latency of any of the following behavioral indices of overt convulsant activity typically produced by cocaine: wild running, clonus, tonus, and clonic–tonic [see (15)]. The occurrence of any one of the latter behaviors was scored as a convulsion, and an overall percentage for each group was obtained. Therefore, the data in Figs. 1 and 2 reflect the percentage of animals that exhibited a convulsion following pretreatment with the different serotonergic compounds as a function of control mice that received saline pretreatment. Animals were observed for 15 min, because previous studies in our laboratory have indicated that the percentage of convulsions occurring within 15 or 60 min postinjection does not differ, and this effect typically occurs within 2–4 min postinjection (11–13).

Statistical Analyses

The data in Figs. 1 and 2 reflect the number of animals exhibiting a cocaine-induced convulsion following pretreatment with various doses of the serotonergic compounds as a function of control animals that received saline pretreatment. The effects of each serotonergic compound on cocaine-induced convulsions were analyzed using the χ^2 distribution ($p < 0.05$).

RESULTS

Cocaine-induced convulsions were observed following pretreatment with compounds displaying a high affinity for 5-HT_2 receptors. The occurrence of convulsions is presented, because there were no differences between the frequency of convulsions and the latency to initiate this behavior. Overall, each of the 5-HT_2 antagonists significantly attenuated cocaine-induced convulsions, while the preferential 5-$HT_{2C/1B}$ agonist TFMPP potentiated this effect of cocaine. The effects of these compounds does not appear to be due to activation of 5-HT_{1A} sites, because NAN 190 did not significantly alter cocaine-induced convulsions.

Figure 1 illustrates the effects of various antagonists on convulsions produced by cocaine (100 mg/kg). Seventy-two percent of control mice receiving saline pretreatment exhibited convulsions, consistent with previous research in our laboratory using the same strain of mice (11–13,17). Cocaine-induced convulsions were significantly decreased in a dose-dependent manner by pretreatment with mianserin ($\chi^2 = 7.7$, $p < 0.03$), metergoline ($\chi^2 = 8.8$, $p < 0.05$), MDL 11939 ($\chi^2 = 14.5$, $p < 0.01$), and methiothepin ($\chi^2 = 14.2$, $p < 0.01$). Methiothepin was the most potent attenuator of cocaine-induced convulsions, because this effect was significantly attenuated by the 0.3 mg/kg dose of methiothepin relative to control animals ($\chi^2 = 4.4$, $p < 0.03$).

Some of the 5-HT_2 antagonists used in this study also exhibit an affinity for 5-HT_1 receptors. To examine whether the effects of these antagonists might be due in part to activation of 5-HT_{1A} sites, cocaine-induced convulsions were also examined following pretreatment with NAN 190, a preferential 5-HT_{1A} antagonist. Figure 2 (left panel) illustrates the effects of NAN 190 on convulsions produced by cocaine (100 mg/kg). NAN 190 did not significantly alter cocaine-induced convul-

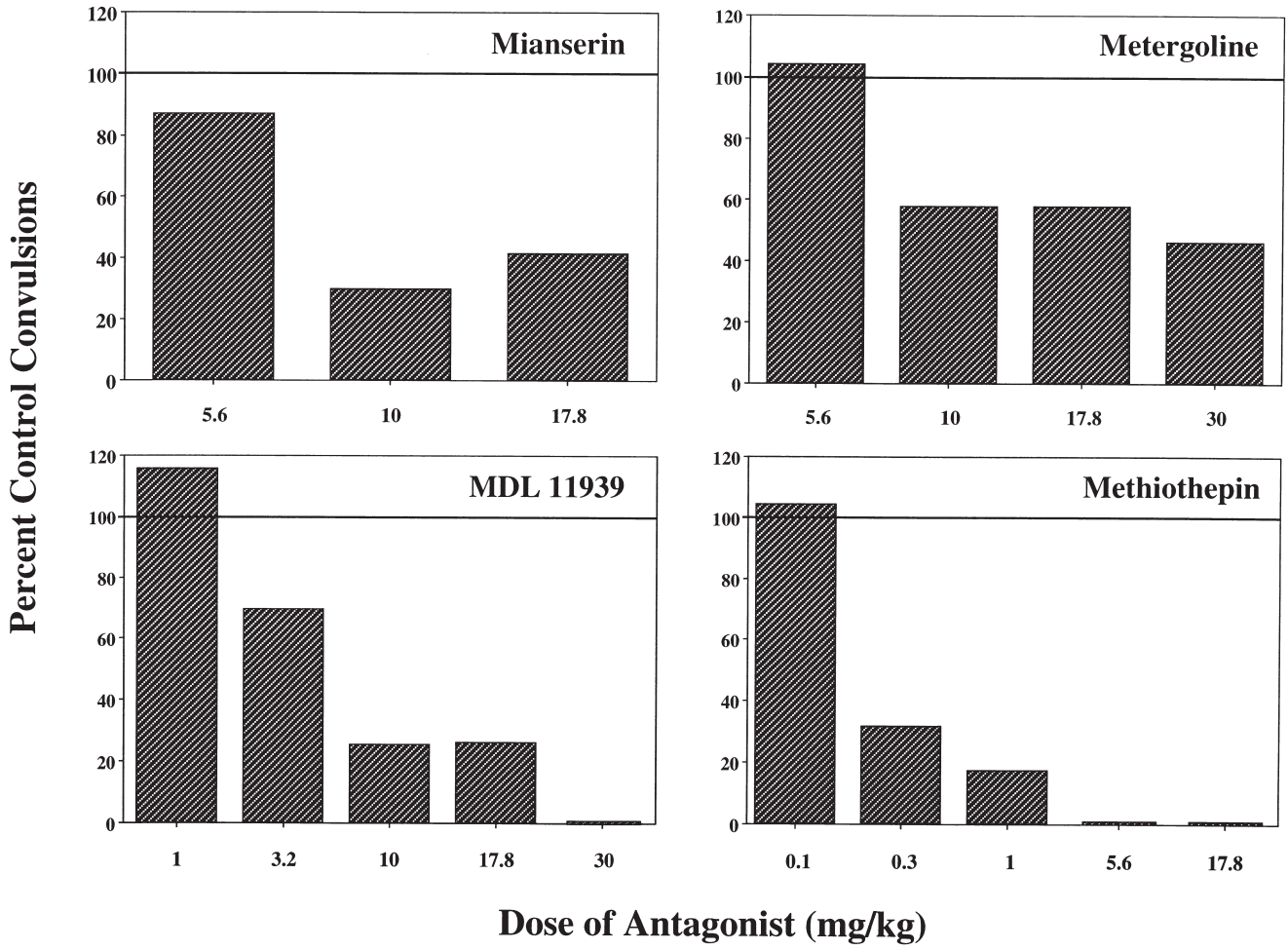

FIG. 1. Percentage of control convulsions produced by cocaine (100 mg/kg) following pretreatment with mianserin (top left), metergoline (top right), MDL 11939 (bottom left), or methiothepin (bottom right). Seventy-two percent of animals pretreated with saline vehicle exhibited convulsions, such that values below 100% reflect a decrease in convulsions relative to control animals. Each of the 5-HT_2 antagonists significantly attenuated cocaine-induced convulsions relative to control mice receiving saline pretreatment ($p < 0.05$).

sions at any dose tested, suggesting that the effects of the other antagonists were not likely due to activation of 5-HT_{1A} receptors.

Figure 2 (right panel) illustrates the effects of the 5-HT agonist TFMPP on convulsions produced by cocaine. Animals in this study received a lower dose of cocaine (56 mg/kg) that produced convulsions in 25% of saline-pretreated mice, consistent with our previous findings (12,13). TFMPP pretreatment produced an increase in cocaine-induced convulsions $\chi^2 = 6.0, p < 0.01$. Pretreatment with this agonist doubled (10 mg/kg) and tripled (30 mg/kg) the incidence of cocaine-induced convulsions relative to saline controls.

DISCUSSION

This study represents one of several converging lines of evidence suggesting that the initiation of cocaine-induced convulsions is mediated by 5-HT_2 receptors. The current results indicate that this toxic effect of cocaine is attenuated by a series of structurally distinct antagonists displaying a high affinity for 5-HT_2 receptors (7,8,19). This is consistent with previous findings that cocaine-induced convulsions are inhibited by the 5-HT_2 antagonists cinanserin, ketanserin, and pirenperone (13,17,18).

The results reported here also indicate that this toxic effect of cocaine is facilitated by TFMPP, an agonist displaying a high affinity for 5-HT_{2C} receptors (19). This finding is consistent with research indicating that cocaine-induced convulsions are potentiated by the preferential 5-HT_{2C} agonists mCPP and MK212 (12). Although the agonist findings suggest that 5-HT_{2C} receptors play a role in mediating cocaine-induced convulsions, the antagonists used in this study display similar affinities for 5-HT_{2A} and 5-HT_{2C} receptors (2,19). Thus, it is possible that 5-HT_{2A} receptors play a role in the observed effects of these compounds in decreasing cocaine-induced convulsions. However, each of the 5-HT_{2C} agonists that we have studied exhibits a preferential selectivity for 5-HT_{2C} receptors relative to 5-HT_{2A} sites. For example, TFMPP is approximately 60-fold more selective for 5-HT_{2C} receptors relative to 5-HT_{2A} sites (19), though recent research

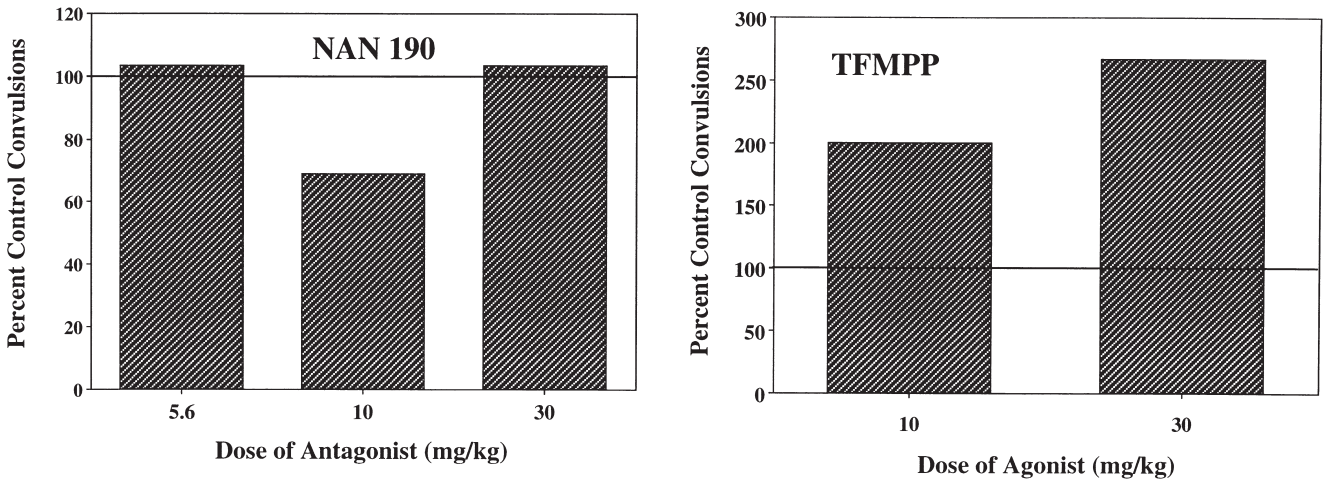

FIG. 2. The left panel reflects the percentage of control convulsions produced by cocaine (100 mg/kg) following pretreatment with NAN 190. Seventy-two percent of animals pretreated with saline vehicle exhibited convulsions, such that values below 100% reflect a decrease in convulsions relative to this control value. The right panel reflects the percentage of control convulsions produced by cocaine (56 mg/kg) following pretreatment with TFMPP. Twenty-five percent of animals pretreated with saline vehicle exhibited convulsions, such that values above 100% reflect an increase in convulsions relative to this control value. TFMPP significantly potentiated cocaine-induced convulsions relative to control mice receiving saline pretreatment ($p < 0.01$).

suggests that TFMPP also produces an endogenous release of 5-HT which may augment its agonist properties (1). Furthermore, mCPP is 10-fold more selective and MK212 25-fold more selective for 5-HT_{2C} receptors relative to 5-HT_{2A} sites (5,7,8). Thus, while 5-HT_{2A} effects cannot be ruled out, the effects of the compounds used in this study on cocaine-induced convulsions are likely due to actions of these drugs at the 5-HT_{2C} receptor subtype.

The finding that methiothepin inhibited cocaine-induced convulsions more potently than the other antagonists studied suggests that neurotransmitter receptors other than 5-HT_2 sites may be involved in mediating this toxic effect of cocaine. Methiothepin displays nanomolar affinity at 5-HT_6 and 5-HT_7 sites, and it is possible that these receptors also play a role in mediating this toxic effect of cocaine (8). It is of potential interest that the potencies of the compounds used in this study to block convulsions are consistent with the previously reported rank order of affinities of these compounds for 5-HT_7 receptors (6). In addition, it is possible that the potency of methiothepin is related to additional effects at muscarinic sites, which appear to play an inhibitory role in mediating cocaine-induced convulsions. It has been shown that methiothepin, but not cinanserin, facilitated K^+-evoked release of acetylcholine from striatal slices (4). Thus, the attenuation of cocaine-induced convulsions by methiothepin may be due in part to an increase in cholinergic neurotransmission. This possibility is consistent with our previous finding that the potency of cocaine-like compounds to produce convulsions is inversely related to the affinity of these compounds at muscarinic M_1 and M_2 sites (16,17). This suggests that cholinergic neurotransmission at these sites may play an inhibitory role in mediating cocaine-induced convulsions. Therefore, the potent effects of methiothepin may be due to additive effects of this compound at muscarinic and/or other 5-HT receptors.

The results of this study do not provide evidence that 5-HT_1 receptors significantly influence cocaine-induced convulsions, although some of the drugs used in this study exhibit a relatively high affinity for 5-HT_1 sites (14,19). For example, methiothepin has an affinity for 5-HT_{1B} sites that is similar to that of 5-HT_2 sites and TFMPP is only fivefold more selective for 5-HT_2 sites relative to 5-HT_{1B} sites (8,19). Despite this, it is unlikely that the effects of these compounds were due to activation of 5-HT_{1B} sites, because mianserin displays approximately 800-fold selectivity for 5-HT_2 sites relative to 5-HT_{1B} receptors, and this compound attenuated cocaine-induced convulsions (7). The present study also suggests that 5-HT_{1A} sites do not play a role in mediating cocaine-induced convulsions. This is based on the finding that cocaine-induced convulsions were not significantly altered by NAN 190, a compound displaying 200–600 fold higher selectivity for 5-HT_{1A} sites relative to other 5-HT_1 subtypes (19).

Converging lines of evidence from our laboratory support the hypothesis that 5-HT_2 receptors play a role in mediating cocaine-induced convulsions. First, pharmacogenetic studies support this hypothesis, because the density of 5-HT_2 receptors appears to mediate genetic sensitivity to cocaine-induced convulsions, and cinanserin more potently attenuated this effect of cocaine in mice exhibiting a lower density of 5-HT_2 receptors (13). In addition, the results of this study support previous pharmacological findings that cocaine-induced convulsions are attenuated by antagonists and facilitated by agonists that preferentially bind to 5-HT_2 receptors (12,13,17,18). Taken together, these studies suggest that 5-HT_2 receptors play an important role in mediating cocaine-induced convulsions.

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