Effects of a 5-HT_3 agonist and antagonist on inter-male aggression in *Mus musculus*

Matthew J. Crisp and Michael Kerchner
Department of Psychology, Washington College, Chestertown, MD 21620

Research has revealed an inverse relationship between serotonin (5-HT) levels in the brain and aggressive behavior. However, effects on aggression at the level of the receptor have yet to be elucidated for many 5-HT receptor subtypes. This study examined the effects of the 5-HT_3 receptor agonist *m*-chlorophenylbiguanide (*m*CPBG) and antagonist ondansetron on inter-male aggression in mice. Using a resident-intruder paradigm designed to assess both offensive and defensive aggression, male C57BL/6J mice received 1 mg/kg i.p. injections of either *m*CPBG, ondansetron, or an inactive vehicle and were subsequently exposed to male AKR/J mice for a period of 10 minutes. Attack latency and the proportion of time engaged in a range of defensive behaviors were recorded. Subject C57BL/6J mice were then immediately run in an open field test for an additional 10 minutes to examine any anxiolytic or sedative effects of the drugs. Results show no significant differences between drug groups in either offensive or defensive behavior, nor in measures of open field activity. However, an overall significant difference was observed between subjects of the offensive and defensive conditions in each drug group with regard to the open field test. In conclusion, this study did not reveal any significant effects of the 5-HT_3 agents on inter-male aggression, which may reflect a functional difference between the unique fast-acting 5-HT_3 receptor-channel and the remaining G-protein coupled 5-HT receptors. However, this conclusion is limited by the large variance in behavior combined with small sample sizes, or the possibility of a drug dose insufficient for behavioral effects.

Keywords: *m*CPBG, ondansetron, resident-intruder paradigm, offensive, defensive, C57BL/6J, AKR/J

Introduction

Serotonin, or 5-hydroxytryptamine (5-HT), is an important neurotransmitter in the human nervous system that plays an integral part in myriad neural functions, from nociception to mood and aggression. Serotonin's multifaceted roles in the nervous system are mediated by a family of 7 different receptor subtypes, many of which contain distinct subunits and differ in their mechanism of signal transduction. Such receptor heterogeneity allows serotonin to exert a number of different effects in the nervous system. Current research is focused on the molecular and behavioral characterization of these receptor subtypes to understand their individual roles in a variety of neurological, cellular, biochemical, and behavioral processes, in turn enabling investigators to specifically target particular receptor subtypes for pharmacological action. This study examines the pharmacological and behavioral effects of the specific serotonin subtype 3 (5-HT_3) agonist *m*-chlorophenylbiguanide (*m*CPBG) and antagonist ondansetron on the complex series of behaviors that constitute inter-male aggression.

Previous research has revealed an inverse relationship between CNS levels of 5-HT and aggressive behavior in both humans and rodents. Specifically, higher concentrations of 5-HT correspond to decreased aggression. A decrease in cortical 5-HT has been demonstrated to occur during and following an aggressive encounter in rats (van Erp & Miczek, 2000). In

humans, levels of the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) in the cerebral spinal fluid of non-suicidal patients with Axis I disorders – schizophrenia, depression, or bipolar disorder – display this inverse relationship with measures of aggressive behavior (Stanley et al., 2000). Suicide itself can be considered a form of self-directed aggression. Increased expression of 5-HT_{2A} receptors in the hippocampus and prefrontal cortex has been documented in adolescent suicide victims, further implicating altered serotonergic function in aggression (Pandey et al., 2002). Environmental factors, such as smoking, have also been associated with lower serotonergic function and increased aggression in patients with Axis I disorders (Malone et al., 2003).

Examination of the effects of 5-HT on aggression and other behaviors at the level of 5-HT receptor subtypes using pharmacological and knockout approaches in rodents has produced a plethora of data, much of which focuses on the 5-HT_1 and 5-HT_2 receptors and their respective subtypes. Employing isolation-induced aggression and resident-intruder paradigms to examine inter-male aggression, these studies have reliably demonstrated that exposure of specific agonists for these receptor subtypes generally leads to a reduction in inter-male aggression. Conversely, inhibition of receptor function using specific antagonists generally promotes such aggression, although this relationship is not as sound. For example, the 5-HT_{1A} agonists eltoprazine, 8-OH-DPAT (8-hydroxy-2-(di-*n*-propyl-amino) tetralin), 5-Me-ODMT (5-methoxy-N,N-dimethyltryptamine), ipsapirone, and buspirone significantly reduce aggressive behaviors when administered prior to a hostile confrontation (Olivier et al., 1989; Bell & Hobson, 1994). Expansion of this relationship through additional studies between selective agents for 5-HT_{1A} receptors and aggression revealed that many of these agonists reliably reduce aggression without affecting motor performance (White et al., 1991; Sanchez et al., 1993; Mendoza et al., 1999). However, these and additional studies found that a number of 5-HT_{1A} antagonists also lead to a decrease in aggression (Mendoza et al., 1999). Such a discrepancy may be the result of these agents interacting with either pre- or post-synaptic 5-HT_{1A} receptors, other 5-HT receptor subtypes, or other neurotransmitter systems (Sanchez et al., 1993).

Regarding the 5-HT_{1B} receptor, knockout mice lacking this gene display heightened aggression compared to wild-types, implicating an important role for this receptor subtype in isolation-induced inter-male aggression (Saudou et al., 1994). Pharmacological studies support this role, in that 5-HT_{1B} agonists served to reduce aggression *in vivo* (Olivier et al., 1995). Studies examining the pharmacology of the 5-HT_2 receptor reveal that the selective 5-$HT_{2A/2C}$ agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane decreases aggression at high doses, but also elicits a characteristic side effect of 5-HT stimulation termed "wet dog shaking." (Sloviter et al., 1978) The relationship is not as clear with 5-HT_2 antagonists. While Sanchez et al. (1993) report no effects of 5-HT_2 antagonists on aggression, other studies by White et al. (1991) and Olivier et al. (1995) provide evidence that the specific 5-HT_2 antagonists DOI and ritanserin reduce aggression.

In addition to examining agonist and antagonist effects on the 5-HT_1 and 5-HT_2 receptors, the studies by White et al. (1991) and Sanchez et al. (1993) also examined the 5-HT_3 antagonists ondansetron and zacopride, but concluded that these compounds have no effect on mediating rodent inter-male aggression. However, the anxiolytic, or anxiety-reducing, and anti-emetic properties of these compounds have been well documented (Gandara et al., 1998). Effects of 5-HT_3 agonists on inter-male aggression have yet to be effectively studied.

In situ hybridization has shown that 5-HT_3 receptors exist in the peripheral and central nervous system (CNS). Within the CNS, 5-HT_3 mRNA is concentrated in the hippocampus, cerebral cortex, amygdaloid complex, hypothalamus, and cranial nerve nuclei in the brainstem (Tecott et al, 1993). Localization of 5-HT_3 receptors in these neural structures suggests that this fast-excitatory receptor may play important roles in emotional and behavioral responses, as well as other, less defined functions such as sensory perception, memory, and, possibly, motor control. The presence of 5-

HT_3 receptors in the amygdaloid complex and cerebral cortex suggests that these receptors have the potential to mediate aggression.

Paralleling research on 5-HT_{1A}, 5-HT_{1B}, and 5-HT_2 receptors, as well as investigations on the anxiolytic effects of 5-HT_3 antagonists, the results of this study were predicted to reflect an inverse relationship between 5-HT_3 activation and aggressive behavior, both offensive and defensive. Specifically, the 5-HT_3 agonist *m*CPBG was expected to attenuate inter-male aggression in both offensive and defensive conditions. Conversely, the 5-HT_3 antagonist ondansetron was hypothesized to enhance aggression. However, the anxiolytic effects of this drug could outweigh any positive effects on aggression.

Materials and Methods

Subjects

Seven week old C57BL/6J (30) male mice and AKR/J (6) male mice were obtained from The Jackson Laboratory (Bar Harbor, ME). The strains were chosen for their moderate aggression and ease of visual discrimination during antagonistic encounters. Immediately upon arrival, 15 C57BL/6J mice were housed in isolation, while the remaining 15 were socially housed in cohorts of three. The difference in housing defined the aggressive condition tested (described below). The C57BL/6J mice were then randomly and blindly assigned to one of three treatment groups. AKR/J mice were either socially housed in a group of three conspecifics or in isolation. Mice were housed in clear Plexiglas cages in a temperature controlled room with a 12-hr light/dark cycle. Food (Lab Diet 5015 Mouse Diet) and tap water were provided *ad libitum* and cages were cleaned once per week. Animal handling protocols were carefully reviewed and accepted by the Institutional Animal Care and Use Committee (IACUC) of Washington College.

Behavioral Testing

A resident-intruder paradigm was employed to assess both offensive and defensive aggression. In both conditions, an experimental C57BL/6J male was exposed to a conspecific AKR/J male; however, the role of the C57BL/6J males in the aggressive encounter, whether the resident or the intruder, was dependent on the condition and the housing of the subject. All encounters were recorded on video. For the offensive resident-intruder condition, isolated C57BL/6J mice served as the resident males and were exposed to socially housed AKR/J males. Two weeks after arrival, a 5-minute training session was conducted to expose the resident C57BL/6J males to the AKR/J intruders. Baseline behavioral measurements (described below) were recorded one week later over a three day period during 10-minute encounters between the resident C57BL/6J and an intruder AKR/J male. Immediately after the 10-minute encounter, the C57BL/6J mice were run in an open field test (PASF Software, San Diego Instruments) for 10 minutes to assess general activity and the subject's state of anxiety. Experimental measures were recorded one week after baseline. Isolated C57BL/6J males received one dose of either the inactive vehicle (10 mL/kg distilled water), the 5-HT_3 agonist *m*-chlorophenylbiguanide HCl (1 mg/kg), or the 5-HT_3 antagonist ondansetron HCl (1 mg/kg). Following a 15 minute acclimation period, an intruder AKR/J mouse was placed in the cage of the drugged resident and recorded for 10 minutes. Open field measurements were taken for the experimental resident immediately following the antagonistic encounter.

For the defensive resident-intruder condition, isolated AKR/J male mice served as the residents and were exposed to 15 socially housed C57BL/6J males. Two weeks after arrival, a 5-minute training session was conducted to ensure that the AKR/J males could defeat each C57BL/6J intruder. Defeat of the intruder was essential for the observation of a full repertoire of defensive behaviors. Both baseline and experimental measurements were taken following the same procedure and timeline as the offensive resident-intruder condition, with the exception that the socially housed experimental C57BL/6J males served as the intruders. The open field test was conducted immediately following an encounter.

Both resident-intruder conditions allowed the assessment of a range of behaviors.

Behaviors were analyzed directly from video footage and classified according to de Boer et al. (1999). For the offensive resident-intruder condition, aggressive behavior was assessed by measuring the latency to initial attack by the C57BL/6J resident. Assessment of defensive behavior was qualified by observing the proportion of time spent in submissive postures, freezing, flight, and retaliation. The open field test, a measure of general activity and animal anxiety, was scored by how often the animal tripped a grid of beams during the 10-minute trial.

Drugs

Ondansetron hydrochloride (1,2,3,9-tetrahydro-9-methyl-3-[(2-methyl-1H-imidazol-1-yl)methyl]-4H-carbazol-4-one hydrochloride; cat. no. O3639; lot no. 084K4714; molecular weight 293.37) was obtained from Sigma-Aldrich. *M*-chlorophenylbiguanide (cat. no. 0440; batch no. 4A/57696; molecular weight 248.11) was obtained from Tocris Bioscience. Both drugs were dissolved in distilled water (the inactive vehicle) to a concentration of 0.100 mg/mL and stored at 4°C. Drugs were used within 10 days. Injections were administered i.p. at a volume of 10 mL/kg.

Data Analysis

Data were analyzed using SPSS 13.0 statistical software. Attack latencies in the offensive resident-intruder condition between baseline and experimental groups were analyzed with respect to drug group using a one-way ANCOVA with baseline attack latencies as a covariate. Activity in the open field was divided into central, peripheral, and total activity. Activity among drug groups and resident-intruder conditions were compared using a two-way ANCOVA for each activity measure, with respective baseline activity measures used as covariates. Due to a significant difference between the offensive and defensive conditions, one-way ANCOVAs with baseline activities as covariates were employed on each activity measure in either aggressive condition to determine any significant effects of drug groups. Significance was judged at the $\alpha = .05$ level.

Results

Offensive and defensive resident-intruder conditions

Mean ± *S.E.* values for both offensive and defensive conditions with respect to drug group are graphically depicted in Figure 1. In the offensive condition, the mean (± *S.E.*) attack latency for resident C57BL/6J males exposed to the inactive vehicle, *m*CPBG, and ondansetron were 279.0 (±108.1), 315.0 (± 117.6), and 337.2

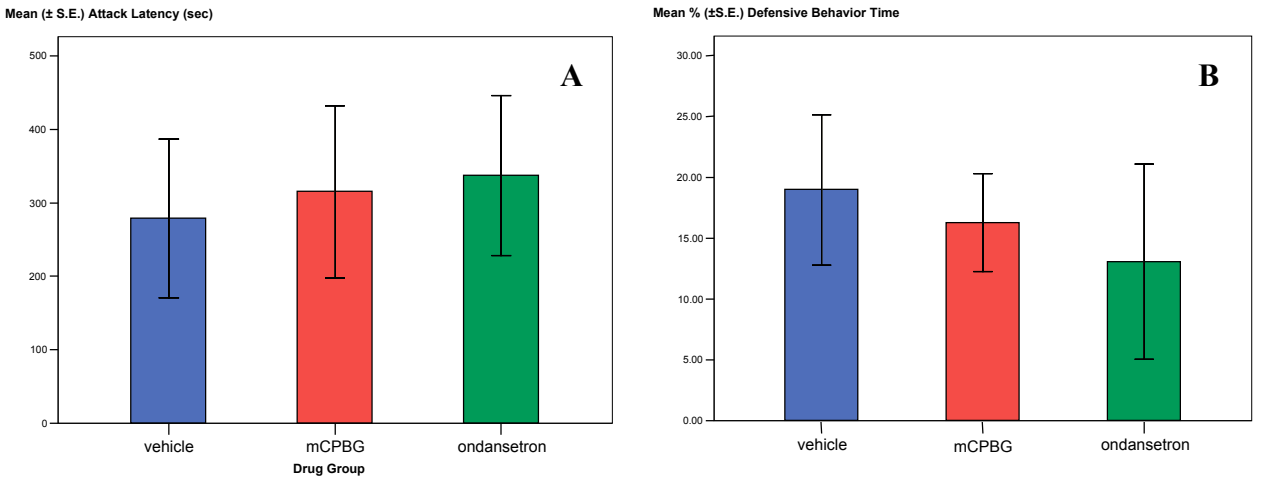

Figure 1. In the offensive condition (A), significant differences were not observed between the vehicle ($n = 5$), *m*CPBG ($n = 5$), and ondansetron ($n = 5$) with respect to attack latency. Similarly, no significant differences were seen between the vehicle ($n = 5$), *m*CPBG ($n = 5$), and ondansetron ($n = 4$) regarding the proportion of time spent in defensive behavior in the defensive condition (B).

(± 109.1) seconds, respectively. A one-way ANCOVA, controlling for initial group differences, revealed no significant differences between mean attack latencies of experimental animals with respect to drug group, $F < 1$. Intruder C57BL/6J males in the defensive condition exposed to the inactive vehicle, *m*CPBG, and ondansetron spent an average of 18.97%, 16.27%, and 13.06% in defensive behaviors. No significant differences were observed between drug groups using a one-way ANCOVA, with differences in resident AKR/J aggression as covariates, $F < 1$.

Open field activity

Activity in the open field was divided into central, peripheral, and total activity. A one-way ANCOVA with baseline activity measures as a covariate revealed that the offensive condition possessed significantly greater activity than the defensive condition for all activity measures: central $F(1,26) = 4.642$, $p < .05$; peripheral $F(1, 26) = 9.144$, $p < .05$; total $F(1, 26) = 10.533$, $p < .05$. However, when one-way ANCOVAs controlling for baseline activity were performed for each condition, no significant differences between drug groups were found ($F < 1$ for all drug groups in each condition, except offensive central activity $F[2, 12] = 1.247$, $p > .05$). Mean ± *S.E.* activity values for each open field measure, clustered by resident-intruder condition, are shown in Figure 2.

Discussion

This study failed to show that the 5-HT_3 agents *m*CPBG and ondansetron have a reliable effect on either offensive or defensive murine inter-male aggression. Similarly, no significant differences between drug groups with respect to open field activity were present. These results may be due to a number of factors. First, the 5-HT_3 receptor may not play a significant role in mediating inter-male aggression, which may reflect its radical structural differences compared to other serotonin receptors. Pharmacologically, these drugs may act as poor 5-HT_3 agents *in vivo*, failing to reach and bind to the receptors in the brain, or may have been ineffective at the tested concentration. However, the large within-subject variance, combined with a small sample size (4-5 subjects per drug group and condition), make discovering any relatively small effects of these drugs on aggression difficult.

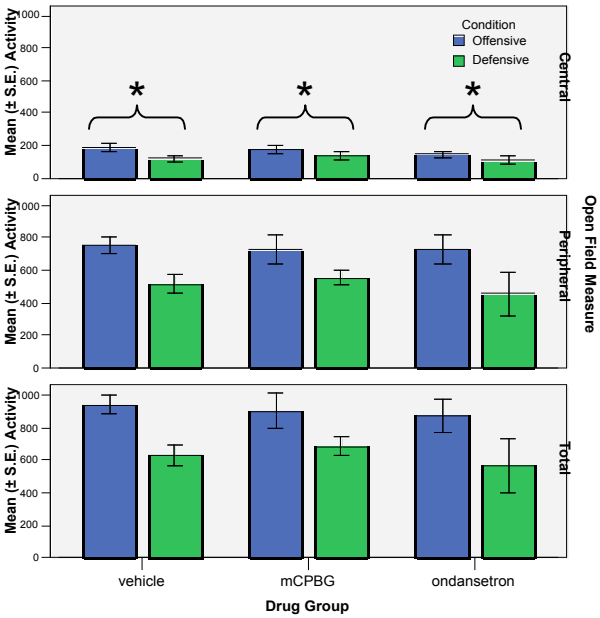

Figure 2. Analysis of open field activity revealed that the offensive group ($n = 15$) possessed significantly greater activity than the defensive group ($n = 14$) in each activity measure. However, no significant differences were observed between drug groups in any activity measure. (*denotes significance at the .05 level)

The single significant difference within the open field activity of the subjects in both offensive and defensive conditions may be explained by differences in housing or by the effects of the hostile encounter. Considering the former, housing mice in isolation could account for anxiogenic effects in an open field test, which would explain the larger difference in peripheral activity versus central activity. However, the effects of the latter cannot be ruled out. Research has documented altered serotonergic function both during and following an antagonistic encounter in rats (van Erp & Miczek, 2000). Perhaps this effect is dependent on the role the rodent plays in an encounter, whether establishing dominance or suffering defeat. Such an effect may partially explain the

observed differences in open field activity between aggressive conditions. Future trials comparing open field activity in isolated and socially housed mice to victorious or defeated mice could provide the data to further study this observed effect.

Suggestions for future research include increasing the sample size for each drug group, implementing video analysis software, and testing a range of doses for each 5-HT_3 agent. Expanding the sample size for each drug group and resident-intruder condition would decrease the variance observed in behavior and activity. If the drugs did have a subtle effect on behavior, this may only be observed with a large group. A larger subject pool would also enable a better selection of only moderately aggressive resident mice of both strains, thus decreasing initial group differences between drugs and conditions. Behavioral assessment using video analysis software, where behaviors are fed into a computer at specified time intervals, would serve to increase the accuracy and range of observed behaviors. This would prove a valuable tool since many behaviors occur rapidly and intermittently and cannot be accurately measured by the human hand. Finally, developing a dose-response curve for each drug by testing a wider dose range would be ideal to determine the dose-dependent relationship between the tested 5-HT_3 agents and behavior. One of the reasons that this study did not support the original hypothesis may have been due to a drug dose too low for any behavioral effects to be noted. In that case, analyzing the effects of the drugs over a range of doses may reveal subtle effects in behavior.

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Corresponding Author

Matthew J. Crisp
Washington College Department of Psychology
mcrisp2@washcoll.edu
300 Washington Avenue
Chestertown, MD 21620