Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 461 (2003) 19–25
www.elsevier.com/locate/ejphar

Targeted gene deletion of the 5-HT_{3A} receptor subunit produces an anxiolytic phenotype in mice

Stephen P. Kelley[1], Alison M. Bratt[2], Clyde W. Hodge*

Ernest Gallo Clinic and Research Center, Department of Neurology, University of California at San Francisco, Emeryville, CA 94608, USA

Received 4 November 2002; received in revised form 20 December 2002; accepted 24 December 2002

Abstract

Anxiety disorders are the most common psychiatric disorders. Typical medications used to treat patients are benzodiazepines or antidepressants that target serotonin (5-HT) activity. The ionotropic 5-HT_3 receptor has emerged as a potential therapeutic target because selective antagonist compounds reduce anxiety in rodents, primates, and humans. 5-HT binds to the extracellular N-terminus of the 5-HT_{3A} receptor subunit, but receptor activation is also enhanced by distinct allosteric sites. It is not known if specific molecular subunits of the 5-HT_3 receptor modulate anxiety. To address this issue, we characterized anxiety-like behavior of mice with a targeted deletion of the 5-HT_{3A} receptor subunit gene in the light/dark box, elevated plus maze, and novelty interaction animal models of anxiety. 5-HT_{3A} null mice exhibited an anxiolytic behavioral phenotype that was highly correlated across behavioral measures. This evidence indicates that the 5-HT_{3A} molecular subunit influences anxiety-like behavior. Pharmacotherapy that targets specifically the 5-HT_{3A} receptor subunit may provide a novel treatment for anxiety disorders.

Keywords: 5-HT, (5-hydroxytryptamine; serotonin); 5-HT_{3A} receptor; Anxiety; Animal model; (Mouse)

1. Introduction

Serotonin (5-HT) receptors are classified into seven groups (5-HT_{1-7}), comprising a total of at least 14 structurally and pharmacologically distinct mammalian receptor subtypes (Hoyer et al., 1994). At the molecular level, 5-HT receptors are mostly seven transmembrane-spanning, G-protein-coupled metabotropic receptors (Barnes and Sharp, 1999). The 5-HT_3 receptor is unique among this receptor family as the only member that is a ligand-gated ion channel (Derkach et al., 1989; Maricq et al., 1991). Cellular studies indicate that the 5-HT_3 receptor may form a cylindrical structure comprising five co-assembled subunits that surround a centrally gated channel (Boess et al., 1995). 5-HT binding occurs on the extracellular N-terminus of the 5-HT_{3A} subunit (Eisele et al., 1993), but receptor activation is also enhanced by pharmacologically distinct allosteric sites (Lovinger and Zhou, 1993). Electrophysiologically, 5-HT mediates rapid excitatory responses through ionotropic 5-HT_3 receptors (Derkach et al., 1989) and the activation of this channel results in rapid depolarization responses and subsequent desensitization (Lambert et al., 1989; Yakel and Jackson, 1988).

The 5-HT_{3A} receptor subtype was first cloned in 1991 (Maricq et al., 1991) and shown to be localized in the periphery and in limbic brain regions including the hippocampus, amygdala, and throughout the cortex (Tecott et al., 1993). Recently, a new class of 5-HT_3 receptor subunit (e.g., 5-HT_{3B}) was described and found to be co-expressed with the 5-HT_{3A} subunit in human amygdala, caudate, and hippocampus (Davies et al., 1999). Heteromeric assemblies of human 5-HT_{3A} and 5-HT_{3B} subunits show channel conductance, calcium permeability, and current–voltage properties that closely resemble neuronal 5-HT_3 channels (Davies et al., 1999). In rats, however, 5-

* Corresponding author. Present address: Department of Psychiatry, Bowles Center for Alcohol Studies, CB 7178, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA. Tel.: +1-919-843-4823; fax: +1-919-966-5679.
E-mail address: chodge@med.unc.edu (C.W. Hodge).
[1] Present address: Neurosciences Institute, Department of Pharmacology and Neuroscience, University of Dundee, Ninewells Hospital, Dundee DD1 9SY, Scotland, UK.
[2] Present address: Pharmacology Department, Organon Laboratories Ltd., Newhouse, Lanarkshire, ML1 5SH, Scotland, UK.

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(02)02960-6

HT_{3B} subunit transcripts are restricted to peripheral neurons(Morales and Wang, 2002), which suggests that rodent neural 5-HT_3 receptors might be 5-HT_{3A} homomeric receptors or heteromeric receptors containing 5-HT_{3A} subunits combined with subunits other than the 5-HT_{3B} subunit. Indeed, a comparison of electrophysiological properties of rodent neural 5-HT_3 receptors has shown the presence of receptors with both high and low conductances (Hussy et al., 1994; Jones and Surprenant, 1994; Yang et al., 1992), which suggests the presence of at least two different 5-HT_3 receptor channels (Fletcher and Barnes, 1998).

The antagonism of the 5-HT_3 receptor produces a range of anxiolytic effects in various animal models of anxiety (Costall and Naylor, 1991, 1992a,b; Costall et al., 1990). These effects have been demonstrated in both rodents (Barnes et al., 1992a) and in primates (Barnes et al., 1990). In rodent conflict models of anxiety such as the light/dark box (Costall et al., 1989a) and the elevated plus maze (Pellow and File, 1986; Pellow et al., 1985), selective 5-HT_3 antagonists such as tropisetron, ondansetron, and zacopride can produce anxiolytic behavioral profiles in both rats and mice (Barnes et al., 1992b; Cheng et al., 1984; Costall et al., 1989a; Jones et al., 1988) but some rodent studies have failed to find anxiolytic effects (Rodgers et al., 1997). Anxiolytic responses to 5-HT_3 antagonists have also been reported in other rodent ethological anxiety models such as social interaction tests (Cutler et al., 1997; Gao and Cutler, 1992a). Although the anxiolytic behavioral profile of 5-HT_3 antagonists is similar to diazepam in these behavioral models, there appears to be no corresponding increase in sedation or reduction of locomotor behavior (Jones et al., 1988). There have been few clinical studies utilizing 5-HT_3 antagonists to treat anxiety disorders; however, clinical trials have shown ondansetron to possibly be effective in treating generalized anxiety disorder (Freeman et al., 1997) and panic disorder (Schneier et al., 1996). In contrast to the pharmacological effects of 5-HT_3 antagonists demonstrated in rodent models of anxiety, the administration of 5-HT_3 agonists such as 2-methyl-5-HT has been demonstrated to produce anxiogenic effects when microinjected into either the dorsal raphe nucleus or the amygdala (Costall et al., 1989b).

Based on the evidence generated from the various 5-HT_3 antagonists across a range of anxiety models in both primates and rodents and the concomitant changes observed under 5-HT_3 agonists, it is reasonable to conclude that 5-HT_3 receptors are involved in the modulation of anxiety-related behavior. Since neural 5-HT_3 receptors might be homomeric or heteromeric receptors containing 5-HT_{3A} subunits, and 5-HT_3 antagonists show equal affinity for 5-HT_{3A} and 5-$HT3_{A/B}$ receptors (Brady et al., 2001), the functional significance of specific 5-HT_3 receptor subunits as they relate to anxiety has yet to be determined. To address this issue, we studied anxiety-related behavioral responses in mutant mice lacking the 5-HT_{3A} receptor subtype.

2. Methods

2.1. Mice

5-HT_{3A} receptor null mice were derived using homologous recombination as previously reported (Zeitz et al., 2002). F1 hybrid C57Bl/6J × 129 heterozygous progeny were backcrossed with C57Bl/6J mice to produce F9 generation congenics.

Heterozygotes from the F9 generation were bred to generate wild-type and 5-HT_{3A} null mutants used in the present study. Genotyping was conducted in Dr. David Julius' laboratory at the University of California at San Francisco as previously described (Zeitz et al., 2002). The experimenter conducting behavioral studies was blind to genotype. The mice were housed in plastic cages lined with Cell Sorb bedding and provided with food (Harlan, Indianapolis, IN) and water ad libitum. The vivarium was maintained on a 12-h light/dark cycle (lights on at 0600 h) at a temperature of 22 °C. All procedures were carried out in accordance with the *NIH Guide to Care and Use of Laboratory Animals* and institutional guidelines.

Body weight, feeding, and drinking were characterized in male adolescent (24–36 days of age) 5-HT_{3A} null mice ($n = 17$) and wild-type controls ($n = 17$). Adult (90–120 days of age) experimentally naïve male mice were used in locomotor and novel object experiments. The same mice were tested in the light/dark box and elevated plus maze in randomized counterbalanced order. This allowed direct within-subject correlation of performance on the two standard tests of anxiety-like behavior.

2.2. Locomotor activity and habituation

A photobeam-based tracking system (MED Associates, St. Albans, VT) was used to track the movement of the mice and to calculate distance traveled in an open field enclosed with clear acrylic sidewalls (43.2 × 43.2 × 30.5 cm). Prior to each session, the open field was wiped clean with 2.5% glacial acetic acid to avoid any confounding odors. The mice were then transported from an adjacent room, placed in the corner of the open field, and left to behave freely for 60 min. This procedure was repeated for three consecutive days.

2.3. Light/dark box

A plastic test box (27 × 16 × 12.7 cm) was positioned in an open field arena enclosed by a sound-attenuating chamber. A partition in the center of the test box separated the covered black-walled side from the open white-walled side. The white side of the test chamber was illuminated by a 28-

V incandescent bulb located 12.7 cm above the floor of the test box. There was no other light source present within the sound-attenuating chamber. A photobeam-based tracking system (MED Associates) was used to track the movement of the mice within the test box and to calculate time spent in the black or white areas of the box. The test box was wiped clean with 2.5% glacial acetic acid before each test session. Prior to the beginning of the test session, the mice where transported from an adjacent room in their home cages to a dark testing room and placed within an unlit, empty, sound-attenuating chamber and left to habituate for 60 min. Following the habituation period, the mice were taken from their home cages and placed into the white area of the test box enclosed by a sound-attenuating chamber. The mice were then left to explore the test box for 5 min. The dependent variables recorded were the time spent in the white area and the latency to cross from the white area to the black area.

2.4. Elevated plus maze

The mice were transferred from the housing unit to the test room and left to habituate to the novel environment for at least 4 days prior to testing. The test apparatus was an elevated plus maze consisting of two open arms and two closed arms, placed on a table and elevated 60 cm from the surface of the table. The plus maze was constructed of wood and painted white. The arms of the plus maze were 9 cm in width and 30 cm in length. The middle area of the plus maze was 85 × 85 cm. For the closed arms, a wall 12.2 cm in height was affixed to all the sides of the arm excluding the entrance from the middle area. Prior to each test session, the plus maze was wiped clean with 2.5% glacial acetic acid. At the beginning of a test session, each mouse was placed in the middle zone and left to explore the plus maze for 10 min. A video tracking system (Columbus Instruments, Columbus, OH) was used to record the behavior of the mice in the plus maze. The dependent variables recorded were the time spent on the open arms of the plus maze and the total number of entries into the open arms.

2.5. Novel object interaction

The same photobeam-based tracking system (MED Associates) used in the locomotor habituation experiment was also used in this experiment. The novel object consisted of a centrifuge tube cap (32 × 12 cm) placed in the center of the open field. Prior to each session, the open field and the novel object were wiped clean with 2.5% glacial acetic acid to avoid any confounding odors. The mice were then transported from an adjacent room placed in the corner of the open field and left to behave freely for 60 min. The dependent variables were total time ambulatory and total ambulatory counts in the object zone during the initial 10 min of the test. The object zone was defined as the area (12.7 × 12.7 cm) around the novel object. All mice were habituated to the open field environment during 3 × 60 min locomotor habituation trials conducted on three consecutive days prior to the novel object interaction test.

3. Results

3.1. Locomotor activity

To determine if any overt neurobiological changes exist due to the targeted mutation, we first tested 5-HT_{3A} null mice and wild-type littermates in a novel open field environment for three consecutive days. The null mutation produced no effect on spontaneous locomotor activity (Fig. 1A). Moreover, locomotor activity by both genotypes decreased as a function of days in the test environment [$F(2,255) = 12.211$, $P < 0.001$] and time during each test session [$F(5,255) = 36.827$, $P < 0.001$], which indicates normal habituation to the environment (Fig. 1A). This finding is consistent with pharmacological evidence, which indicates that 5-HT_3 receptor antagonists have no effects on locomotor behavior (Jones et al., 1988). To further examine the general health of the mice, we measured body weight (Fig. 1B), food intake (Fig. 1C), and water intake (Fig. 1D) during a 24-h period. The absence of differences in these measures indicates that the targeted deletion of the 5-HT_{3A} receptor subunit did not alter homeostatic systems that regulate the growth and survival of the organism.

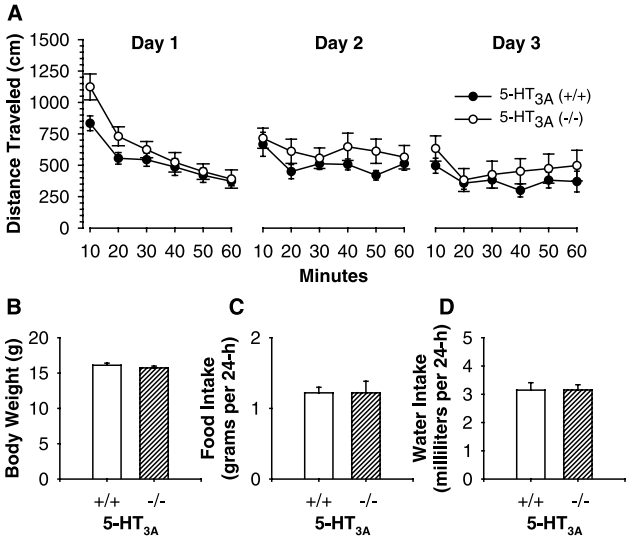

Fig. 1. Deletion of the 5-HT_{3A} receptor subunit did not produce major changes in motor or consummatory behavior. Spontaneous locomotor activity and habituation to an open field environment measured during three daily 60-min test sessions (A). Mean distance traveled (± S.E.M.) averaged for each 10-min time bin in wild-type littermate controls (●, $n = 7$) and 5-HT_{3A} null mice (○, $n = 8$). A genotype × day × 10-min time bin ANOVA revealed no significant difference in genotype. Body weight (B), food intake [g/day] (C), and water intake [ml/day] (D) were unaffected by deletion of the 5-HT_{3A} receptor subunit ($n = 17$ per genotype).

3.2. Light/dark box

The light/dark box is a validated mouse model of anxiety (Costall et al., 1989a). 5-HT_3 antagonists consistently increase time spent in the aversive, illuminated compartment of the box, thus indicating an anxiolytic effect (Bill et al., 1992). 5-HT_{3A} null mice exhibited a twofold increase in percentage of time spent in the illuminated white area of the test box as compared to wild-type littermate controls [$F(1,14) = 5.243$, $P < 0.05$] (Fig. 2A). Although null mutant mice on the average displayed higher latencies to enter the dark zone than wild types, this difference was more variable [$F(1,14) = 1.588$, $P > 0.05$].

3.3. Elevated plus maze

The elevated plus maze is another widely used, validated, rodent model of anxiety (Pellow et al., 1985). 5-HT_3 antagonists increase the dwell time or number of entries into the aversive open arms of the plus maze (Artaiz et al., 1995), which indicates an anxiolytic-like profile. 5-HT_{3A} null mice visited the open arms of the plus maze twice as many times as wild-type controls [$F(1,13) = 14.430$, $P < 0.01$] (Fig. 2B), which corresponds with other anxiolytic-like performance on the open arms of the maze (Table 1). In order to determine if this anxiolytic-like effect was a function of nonspecific exploratory behavior of the maze, a statistical analysis of covariance (ANCOVA) was conducted with the number of visits to the closed arms as a covariate. Closed arm entries did not predict performance on the open arms [$F(1,12) = 8.983$, $P > 0.05$]. Additionally, a linear regression analysis revealed that the percentage of total time spent in the illuminated zone of the light/dark box was a significant predictor of the total number of visits in the open arms of the elevated plus maze ($R^2 = 0.41$, $P < 0.05$) (Fig. 2C). Thus, 5-HT_{3A} null mice exhibit highly correlated performance on behavioral tests that rely on motor suppression (light/dark box) or activation (plus maze), which suggests that the anxiolytic-like phenotype is not related to motor ability or general exploration.

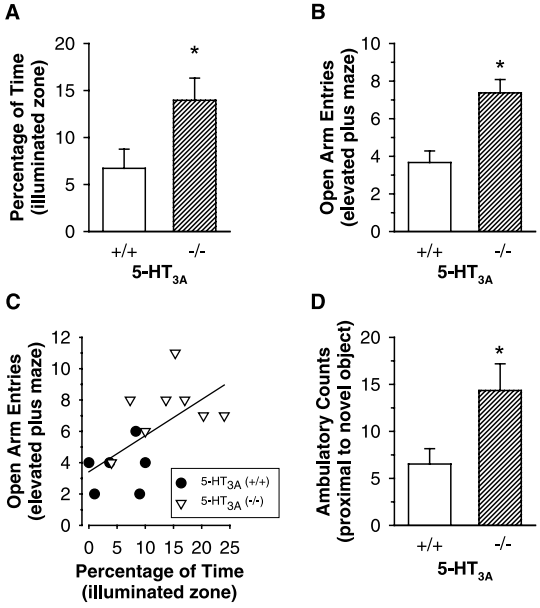

Fig. 2. Anxiolytic behavioral profile of mice lacking the 5-HT_{3A} receptor gene. (A) In the light/dark box test, the percentage of time spent in the illuminated zone of the light/dark box was significantly greater in 5-HT_{3A} receptor knockouts ($n = 8$) as compared to wild-type littermate controls ($n = 7$) [$F(1,14) = 5.243$, $P < 0.05$]. (B) Effects of 5-HT_{3A} receptor subunit deletion on behavior in the elevated plus maze. The number of entries in the open arms of the elevated plus maze was significantly greater in the 5-HT_{3A} receptor knockouts ($n = 8$) as compared to wild-type littermate controls. (C) Linear regression comparing elevated plus maze behavior and light/dark box performance. The percent of time spent in the illuminated zone was found to be a significant predictor of the number of entries into the open arms of the elevated plus maze for wild-type littermate controls (●, $n = 6$) and 5-HT_{3A} knockouts (▽, $n = 8$) ($R^2 = 0.41$, $P < 0.05$). (D) Ambulatory counts (photobeam breaks) proximal to the novel object in the center of an open field. 5-HT_{3A} receptor null mice ($n = 17$) displayed significantly higher ambulatory counts than wild-type controls ($n = 20$), in the object zone of the open field, evidencing higher exploratory behavior and reduced anxiety in response to a novel object. All data are expressed as mean (± S.E.M.). * Indicates significant difference from wild-type control by ANOVA.

Table 1
Behavioral performance on the open arms of the elevated plus maze indicates anxiolytic-like phenotype of 5-HT_{3A} null mice (−/−) as compared to wild-type controls (+/+)

Behavioral measure	5-HT_{3A} (+/+)	5-HT_{3A} (−/−)	Significance value[a]
Distance traveled (cm)	88.9 (25.1)	153.2 (16.4)	$P < 0.05$
Ambulatory time (s)	12.2 (3.4)	22.4 (2.5)	$P < 0.05$
Resting time (s)	15.2 (9.7)	35.4 (16.6)	NS
Other behaviors[b] (s)	12.5 (4.4)	28.0 (4.9)	$P < 0.05$
Total visit time (s)	39.8 (16.6)	85.7 (14.3)	NS

Values are mean (± S.E.M.).
NS = nonsignificant.
[a] Significance value is based on ANOVA.
[b] Other behaviors include grooming, stretch–attend postures, and stereotypic nonambulatory movements.

3.4. Novel object interaction

Neophobia, as measured by ambulatory responses to a novel object, is also indicative of anxiety state in rodents (van Gaalen and Steckler, 2000). With anxiolytic-like behavioral profiles evidenced in the previous experiments, we predicted that mice lacking the 5-HT_{3A} receptor subunit would interact more than wild-type controls with a novel object placed in the aversive center area of an open field. Accordingly, 5-HT_{3A} knockout mice engaged in more ambulatory behavior proximal to the novel object [$F(1,36) = 5.219$, $P < 0.05$; Fig. 2D] and spent more time in close contact with the novel object [$F(1,36) = 4.423$, $P < 0.05$] than wild-type controls. This indicates that mice lacking the 5-HT_{3A} subunit displayed a higher propensity to explore the novel object, a strong indicator of reduced anxiety.

4. Discussion

The primary finding of this study is that targeted gene deletion of the 5-HT_{3A} receptor subunit produced an anxiolytic-like behavioral phenotype in mice. Initial screens of 5-HT_{3A} null mice and wild-type littermate controls found no significant differences in motor ability, open field exploration, habituation to a novel environment, food or water intake, or body weight. This suggests that the null mutation did not alter the function of major homeostatic systems. However, the behavioral profile exhibited by the 5-HT_{3A} null mice was consistent with reduced anxiety as characterized by three validated animal models.

Reduced anxiety-like behavior exhibited by the 5-HT_{3A} null mice in the light/dark box corresponds with a number of reports showing the potential anxiolytic activity of 5-HT_3 receptor antagonists (Barnes et al., 1990, 1992a; Bill et al., 1992; Costall et al., 1993; Gao and Cutler, 1992b; Jones et al., 1988; Sanchez, 1995). In fact, of the numerous methods employed to examine the anxiolytic action of 5-HT_3 antagonists, the light/dark box has yielded the most consistent results across drugs, laboratories, and species (Olivier et al., 2000). Accordingly, in the present experiment, the 5-HT_{3A} receptor null mice spent significantly more time in the illuminated and fear-provoking section of the box than wild-type littermate controls, which suggests reduced anxiety.

An anxiolytic-like effect of 5-HT_{3A} deletion was also evident in the elevated plus maze. This behavioral paradigm is essentially a conflict model of anxiety, whereby the positive drive to explore a novel environment is opposed by the negative drive to avoid a fear-provoking environment—in this case, the open arm of the plus maze (Rodgers and Dalvi, 1997). Although 5-HT_3 antagonists have been demonstrated to produce anxiolytic behavioral profiles in some studies (Artaiz et al., 1995; Costall et al., 1993), other investigations find no effect (File and Johnston, 1989; Rodgers et al., 1997). In this study, 5-HT_{3A} knockout mice made significantly more entries into the anxiety-provoking open arms of the plus maze. The results of the ANCOVA demonstrate that the anxiolytic action of the 5-HT_{3A} deletion was specific to the open arms of the maze and not due to generalized locomotor activity. Finally, the linear regression of the percentage of time spent in the illuminated zone (light/dark box) on the number of entries into the open arms (elevated plus maze) revealed a significant correlation between the two measures of anxiety from two different behavioral models. Given that the measurements obtained from the light/dark box are temporal rather than locomotor-based, as in the plus maze, the significant correlation between the two behavioral paradigms strongly supports the validity of a conclusion for reduced anxiety in 5-HT_{3A} knockout mice.

Additional evidence provided by the novel object interaction test further supports the conclusion that mice lacking the 5-HT_{3A} receptor subunit exhibit an anxiolytic-like behavioral profile. Enhancements of locomotor activity in the vicinity of a novel object and novel object-directed exploration have previously been used as indices of anxiolysis in both the rat (Klebaur and Bardo, 1999) and the mouse (Malleret et al., 1999). 5-HT_{3A} null mice displayed an increased activity profile in the region close to the novel object, further suggesting a state of reduced anxiety in these mice.

In contrast to the apparent anxiolytic-like effect of 5-HT_{3A} receptor deletion observed in the present study, a growing number of studies have failed to find anxiolytic effects of 5-HT_3 antagonists (File and Johnston, 1989, also see Olivier et al., 2000, 50, for a review; Kshama et al., 1990; Rodgers et al., 1997). Similarly, a comprehensive study showed that ondansetron was predictive of anxiolytic activity in the social interaction test in the rat and light/dark exploration test in the mouse but was without effect in a rat water-lick conflict test (Jones et al., 1988). Results of conflict tests vary, with some studies finding anxiolytic-like effects (Artaiz et al., 1995) and others finding no effect (Cervo and Samanin, 1995). Anxiolytic activity has been reported in the Vogel-lick test for some (ondansetron, granisetron, zacopride, tropisetron) but not other (bemesetron, DAU 6215) 5-HT_3 receptor antagonists tested in a single study (Filip et al., 1992). At present, it is unclear why 5-HT_3 receptor antagonists show differential efficacy across and within experiments, but experimental methods and pharmacological specificity are among potential reasons (Olivier et al., 2000). The consistent anxiolytic-like results observed in the present study suggest that differential pharmacological activity at 5-HT_{3A}-containing receptors may be a factor. Additional studies examining the anxiolytic effects of various 5-HT_3 antagonists in 5-HT_{3A} null mice could address this issue.

The molecular mechanism(s) responsible for the anxiolytic phenotype produced by the deletion of the 5-HT_{3A} receptor subunit remains to be studied. The absence of the 5-HT_{3A} subunit may functionally disable or significantly alter the 5-HT_3 cation channel, producing changes similar to 5-HT_3 pharmacological blockade. Channel conductance studies need to be completed in order to determine the exact nature of the changes produced by the deletion of the 5-HT_{3A} subunit or receptor blockade. Future studies may also examine the structural and functional integrity of 5-HT_3 receptors following 5-HT_{3A} deletion. Moreover, since the mice used in the present study were derived by traditional gene knockout techniques, it is possible that developmental compensation in 5-HT_3 or related systems may have influenced the results. This is unlikely, however, given the high degree of similarity between the anxiolytic-like results obtained in this study and results from experiments that tested 5-HT_3 antagonists.

In conclusion, anxiety disorders are the most prevalent psychiatric conditions and account for benzodiazepines becoming the most prescribed medications worldwide. In spite of the effectiveness of benzodiazepine therapy for

anxiety, these compounds produce a number of undesirable side effects including motor impairment and addiction liability. For this reason, there is much interest in the development of new drug treatments for anxiety. The results of this study showed that targeted gene deletion of the 5-HT_{3A} subunit produced anxiolytic-like effects similar to pharmacological blockade of 5-HT_3 receptors. Also consistent with pharmacological blockade of 5-HT_3 receptors, there were no changes in locomotor behavior in the null mutants. This suggests that the anxiolytic effects of 5-HT_3 antagonists may be mediated, in part, by the 5-HT_{3A} subunit.

Acknowledgements

This work, including mouse breeding and genotyping, was supported by funds provided by the State of California for medical research on alcohol and substance abuse through the University of California at San Francisco. We are grateful to David Julius, PhD, for initial contribution of the mice.

References

Artaiz, I., Romero, G., Zazpe, A., Monge, A., Caldero, J.M., Roca, J., Lasheras, B., Del Rio, J., 1995. The pharmacology of VA21B7: an atypical 5-HT_3 receptor antagonist with anxiolytic-like properties in animal models. Psychopharmacology (Berlin) 117, 137–148.

Barnes, N.M., Sharp, T., 1999. A review of central 5-HT receptors and their function. Neuropharmacology 38, 1083–1152.

Barnes, J.M., Barnes, N.M., Costall, B., Domeney, A.M., Johnson, D.N., Kelly, M.E., Munson, H.R., Naylor, R.J., Young, R., 1990. The differential activities of *R*(+)- and *S*(−)-zacopride as 5-HT_3 receptor antagonists. Pharmacol. Biochem. Behav. 37, 717–727.

Barnes, N.M., Costall, B., Ge, J., Kelly, M.E., Naylor, R.J., 1992a. The interaction of *R*(+)- and *S*(−)-zacopride with PCPA to modify rodent aversive behaviour. Eur. J. Pharmacol. 218, 15–25.

Barnes, N.M., Cheng, C.H., Costall, B., Ge, J., Kelly, M.E., Naylor, R.J., 1992b. Profiles of interaction of *R*(+)/*S*(−)-zacopride and anxiolytic agents in a mouse model. Eur. J. Pharmacol. 218, 91–100.

Bill, D.J., Fletcher, A., Glenn, B.D., Knight, M., 1992. Behavioural studies on WAY100289, a novel 5-HT_3 receptor antagonist, in two animal models of anxiety. Eur. J. Pharmacol. 218, 327–334.

Boess, F.G., Beroukhim, R., Martin, I.L., 1995. Ultrastructure of the 5-hydroxytryptamine3 receptor. J. Neurochem. 64, 1401–1405.

Brady, C.A., Stanford, I.M., Ali, I., Lin, L., Williams, J.M., Dubin, A.E., Hope, A.G., Barnes, N.M., 2001. Pharmacological comparison of human homomeric 5-HT_{3A} receptors versus heteromeric 5-$HT_{3A/3B}$ receptors. Neuropharmacology 41, 282–284.

Cervo, L., Samanin, R., 1995. 5-HT_{1A} receptor full and partial agonists and 5-HT_{2C} (but not 5-HT_{3C}) receptor antagonists increase rates of punished responding in rats. Pharmacol. Biochem. Behav. 52, 671–676.

Cheng, C.H., Costall, B., Kelly, M.E., Naylor, R.J., 1994. Actions of 5-hydroxytryptophan to inhibit and disinhibit and mouse behaviour in the light/dark test. Eur. J. Pharmacol. 255, 39–49.

Costall, B., Naylor, R.J., 1991. Pharmacological properties and functions of central 5-HT_3 receptors. Therapie 46, 437–444.

Costall, B., Naylor, R.J., 1992a. Astra Award Lecture. The psychopharmacology of 5-HT_3 receptors. Pharmacol. Toxicol. 71, 401–415.

Costall, B., Naylor, R.J., 1992b. Anxiolytic potential of 5-HT_3 receptor antagonists. Pharmacol. Toxicol. 70, 157–162.

Costall, B., Jones, B.J., Kelly, M.E., Naylor, R.J., Tomkins, D.M., 1989a. Exploration of mice in a black and white test box: validation as a model of anxiety. Pharmacol. Biochem. Behav. 32, 777–785.

Costall, B., Kelly, M.E., Naylor, R.J., Onaivi, E.S., Tyers, M.B., 1989b. Neuroanatomical sites of action of 5-HT_3 receptor agonist and antagonists for alteration of aversive behaviour in the mouse. Br. J. Pharmacol. 96, 325–332.

Costall, B., Naylor, R.J., Tyers, M.B., 1990. The psychopharmacology of 5-HT_3 receptors. Pharmacol. Ther. 47, 181–202.

Costall, B., Domeney, A.M., Kelly, M.E., Tomkins, D.M., Naylor, R.J., Wong, E.H., Smith, W.L., Whiting, R.L., Eglen, R.M., 1993. The effect of the 5-HT_3 receptor antagonist, *RS*-42358-197, in animal models of anxiety. Eur. J. Pharmacol. 234, 91–99.

Cutler, M.G., Rodgers, R.J., Jackson, J.E., 1997. Behavioural effects in mice of subchronic buspirone, ondansetron and tianeptine: I. Social interactions. Pharmacol. Biochem. Behav. 56, 287–293.

Davies, P.A., Pistis, M., Hanna, M.C., Peters, J.A., Lambert, J.J., Hales, T.G., Kirkness, E.F., 1999. The 5-HT_{3B} subunit is a major determinant of serotonin-receptor function. Nature 397, 359–363.

Derkach, V., Surprenant, A., North, R.A., 1989. 5-HT_3 receptors are membrane ion channels. Nature 339, 706–709.

Eisele, J.L., Bertrand, S., Galzi, J.L., Devillers-Thiery, A., Changeux, J.P., Bertrand, D., 1993. Chimeric nicotinic–serotonergic receptor combines distinct ligand binding and channel specificities. Nature 366, 479–483.

File, S.E., Johnston, A.L., 1989. Lack of effects of 5-HT_3 receptor antagonists in the social interaction and elevated plus-maze tests of anxiety in the rat. Psychopharmacology 99, 248–251.

Filip, M., Baran, L., Siwanowicz, J., Chojnacka-Wojcik, E., Przegalinski, E., 1992. The anxiolytic-like effects of 5-hydroxytryptamine3 (5-HT_3) receptor antagonists. Pol. J. Pharmacol. Pharm. 44, 261–269.

Fletcher, S., Barnes, N.M., 1998. Desperately seeking subunits: are native 5-HT_3 receptors really homomeric complexes? Trends Pharmacol. Sci. 19, 212–215.

Freeman III, A.M., Westphal, J.R., Norris, G.T., Roggero, B.A., Webb, P.B., Freeman, K.L., Rush, J.A., Hearne III, E.M., Evoniuk, G., 1997. Efficacy of ondansetron in the treatment of generalized anxiety disorder. Depress. Anxiety 5, 140–141.

Gao, B., Cutler, M.G., 1992a. Effects of sub-chronic treatment with chlordiazepoxide, buspirone and the 5-HT_3 receptor antagonist, BRL 46470, on the social behaviour of mice. Neuropharmacology 31, 207–213.

Gao, B., Cutler, M.G., 1992b. Effects of acute administration of the 5-HT_3 receptor antagonist, BRL 46470A, on the behaviour of mice in a two compartment light–dark box and during social interactions in their home cage and an unfamiliar neutral cage. Neuropharmacology 31, 743–748.

Hoyer, D., Clarke, D.E., Fozard, J.R., Hartig, P.R., Martin, G.R., Mylecharane, E.J., Saxena, P.R., Humphrey, P.P., 1994. International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol. Rev. 46, 157–203.

Hussy, N., Lukas, W., Jones, K.A., 1994. Functional properties of a cloned 5-hydroxytryptamine ionotropic receptor subunit: comparison with native mouse receptors. J. Physiol. 481, 311–323.

Jones, K.A., Surprenant, A., 1994. Single channel properties of the 5-HT_3 subtype of serotonin receptor in primary cultures of rodent hippocampus. Neurosci. Lett. 174, 133–136.

Jones, B.J., Costall, B., Domeney, A.M., Kelly, M.E., Naylor, R.J., Oakley, N.R., Tyers, M.B., 1988. The potential anxiolytic activity of GR38032F, a 5-HT_3-receptor antagonist. Br. J. Pharmacol. 93, 985–993.

Klebaur, J.E., Bardo, M.T., 1999. Individual differences in novelty seeking on the playground maze predict amphetamine conditioned place preference. Pharmacol. Biochem. Behav. 63, 131–136.

Kshama, D., Hrishikeshavan, H.J., Shanbhogue, R., Munonyedi, U.S., 1990. Modulation of baseline behavior in rats by putative serotonergic agents in three ethoexperimental paradigms. Behav. Neural Biol. 54, 234–253.

Lambert, J.J., Peters, J.A., Hales, T.G., Dempster, J., 1989. The properties

of 5-HT_3 receptors in clonal cell lines studied by patch-clamp techniques. Br. J. Pharmacol. 97, 27–40.

Lovinger, D.M., Zhou, Q., 1993. Trichloroethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in nodose ganglion neurons from the adult rat. J. Pharmacol. Exp. Ther. 265, 771–776.

Malleret, G., Hen, R., Guillou, J.L., Segu, L., Buhot, M.C., 1999. 5-HT_{1B} receptor knock-out mice exhibit increased exploratory activity and enhanced spatial memory performance in the Morris water maze. J. Neurosci. 19, 6157–6168.

Maricq, A.V., Peterson, A.S., Brake, A.J., Myers, R.M., Julius, D., 1991. Primary structure and functional expression of the 5-HT_3 receptor, a serotonin-gated ion channel. Science 254, 432–437.

Morales, M., Wang, S.D., 2002. Differential composition of 5-hydroxytryptamine3 receptors synthesized in the rat CNS and peripheral nervous system. J. Neurosci. 22, 6732–6741.

Olivier, B., van Wijngaarden, I., Soudijn, W., 2000. 5-HT(3) receptor antagonists and anxiety; a preclinical and clinical review. Eur. Neuropsychopharmacol. 10, 77–95.

Pellow, S., File, S.E., 1986. Anxiolytic and anxiogenic drug effects on exploratory activity in an elevated plus-maze: a novel test of anxiety in the rat. Pharmacol. Biochem. Behav. 24, 525–529.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:-closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Rodgers, R.J., Dalvi, A., 1997. Anxiety, defence and the elevated plus-maze. Neurosci. Biobehav. Rev. 21, 801–810.

Rodgers, R.J., Cutler, M.G., Jackson, J.E., 1997. Behavioural effects in mice of subchronic buspirone, ondansetron and tianeptine: II. The elevated plus-maze. Pharmacol. Biochem. Behav. 56, 295–303.

Sanchez, C., 1995. Serotonergic mechanisms involved in the exploratory behaviour of mice in a fully automated two-compartment black and white text box. Pharmacol. Toxicol. 77, 71–78.

Schneier, F.R., Garfinkel, R., Kennedy, B., Campeas, R., Fallon, B., Marshall, R., O'Donnell, L., Hogan, T., Liebowitz, M.R., 1996. Ondansetron in the treatment of panic disorder. Anxiety 2, 199–202.

Tecott, L.H., Maricq, A.V., Julius, D., 1993. Nervous system distribution of the serotonin 5-HT_3 receptor mRNA. Proc. Natl. Acad. Sci. U. S. A. 90, 1430–1434.

van Gaalen, M.M., Steckler, T., 2000. Behavioural analysis of four mouse strains in an anxiety test battery. Behav. Brain Res. 115, 95–106.

Yakel, J.L., Jackson, M.B., 1988. 5-HT_3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. Neuron 1, 615–621.

Yang, J., Mathie, A., Hille, B., 1992. 5-HT_3 receptor channels in dissociated rat superior cervical ganglion neurons. J. Physiol. 448, 237–256.

Zeitz, K.P., Guy, N., Malmberg, A.B., Dirajlal, S., Martin, W.J., Sun, L., Bonhaus, D.W., Stucky, C.L., Julius, D., Basbaum, A.I., 2002. The 5-HT_3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. J. Neurosci. 22, 1010–1019.