5-HT$_{3A}$ Receptor Subunit is Required for 5-HT$_3$ Antagonist-Induced Reductions in Alcohol Drinking

Clyde W Hodge*[,1], Stephen P Kelley[2], Alison M Bratt[3], Kimberly Iller[4], Jason P Schroeder[1] and Joyce Besheer[1]

[1]Department of Psychiatry and Bowles Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA; [2]Neurosciences Institute, Department of Pharmacology and Neuroscience, University of Dundee, Ninewells Hospital, Dundee, Scotland; [3]Pharmacology Department, Organon Laboratories Ltd., Newhouse, Lanarkshire, Scotland; [4]Ernest Gallo Clinic and Research Center, Department of Neurology, University of California San Francisco, Emeryville, California, USA

The ionotropic serotonin subtype-3 (5-HT$_3$) receptor has emerged as a potential therapeutic target in the treatment of alcohol abuse and alcoholism because selective pharmacological antagonists reduce alcohol consumption in preclinical and clinical models. 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, which indicates the presence of other receptor subunits. It is not known if specific molecular subunits of the 5-HT$_3$ receptor modulate alcohol drinking. To address this issue, we characterized acute locomotor response to alcohol and alcohol consumption in a two-bottle home-cage procedure by congenic C57BL/6J mice with a targeted deletion of the 5-HT$_{3A}$ receptor subunit gene. 5-HT$_{3A}$-null mice did not differ from wild-type littermate controls on measures of spontaneous locomotor activity, habituation to a novel environment, or locomotor response to ethanol (0, 0.5, 1, or 2 g/kg). Moreover, null mice did not differ from controls on measures of ethanol (2–10%) intake and preference during or after a two-bottle home-cage sucrose fading procedure. Systemic administration of the 5-HT$_3$ antagonist LY-278,584 (0–10 mg/kg) decreased intake of both sweetened (2% sucrose + 10% ethanol) and unsweetened (10% ethanol) ethanol in wild-type mice only. These findings indicate that reduction of alcohol drinking produced by 5-HT$_3$ antagonism is dependent on the presence of 5-HT$_{3A}$-containing receptors.
Neuropsychopharmacology (2004) **29,** 1807–1813, advance online publication, 26 May 2004; doi:10.1038/sj.npp.1300498

Keywords: serotonin; 5-HT$_3$; 5-HT$_{3A}$; alcohol drinking; LY-278,584; mice

INTRODUCTION

Serotonin (5-HT) receptors are involved in a variety of mammalian biochemical, physiological, and behavioral processes (Barnes and Sharp, 1999). 5-HT receptors are classified into seven groups (5-HT$_{1-7}$), comprising a total of at least 14 structurally and pharmacologically distinct receptor subtypes (Hoyer *et al*, 1994). The 5-HT$_3$ receptor is unique among this G-protein-coupled receptor family as the only ionotropic receptor (Derkach *et al*, 1989; Maricq *et al*, 1991). Electrophysiologically, 5-HT mediates rapid excitatory responses through ionotropic 5-HT$_3$ receptors (Derkach *et al*, 1989) and activation of this channel results in depolarization responses and subsequent desensitization (Lambert *et al*, 1989; Yakel and Jackson, 1988).

*Correspondence: Dr CW Hodge, 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: chodge@med.unc.edu

The 5-HT$_3$ receptor is comprised of five coassembled subunits that surround a centrally gated channel (Boess *et al*, 1995). The 5-HT$_{3A}$ subunit has been cloned (Maricq *et al*, 1991) and shown to be distributed peripherally and centrally in brain regions including the hippocampus, amygdala, and cortex (Tecott *et al*, 1993). 5-HT binds to the extracellular N-terminus of the 5-HT$_{3A}$ subunit (Eisele *et al*, 1993), but receptor activation can be enhanced pharmacologically at allosteric sites (Lovinger and Zhou, 1993). A novel molecular subunit (eg 5-HT$_{3B}$) was recently found to be coexpressed with the 5-HT$_{3A}$ subunit in human amygdala, caudate, and hippocampus (Davies *et al*, 1999) and heteromeric assemblies of human 5-HT$_{3A}$ and 5-HT$_{3B}$ subunits show channel conductance, calcium permeability, and current–voltage properties that closely resemble native neuronal 5-HT$_3$ channels (Davies *et al*, 1999). However, in rats 5-HT$_{3B}$ subunit transcripts are not found in brain (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 (Fletcher and Barnes, 1998).

Ethanol alters the function of 5-HT$_3$ receptors (Barann et al, 1995; Jenkins et al, 1996; Lovinger et al, 2000; Lovinger and Zhou, 1993; Lovinger and Zhou, 1994; Lovinger and Zhou, 1998) with the most consistent finding being potentiation of receptor function by ethanol (Lovinger, 1999). Biochemically, infusion of the 5-HT$_3$ antagonist tropisetron in the nucleus accumbens decreases elevation of extracellular dopamine produced by local application of ethanol (Yoshimoto et al, 1992). Accordingly, a number of studies have shown that systemic administration of 5-HT$_3$ antagonists decreases voluntary alcohol drinking by rats (Fadda et al, 1991; Knapp and Pohorecky, 1992; Kostowski et al, 1993; Tomkins et al, 1995) and mice (Tomkins et al, 1995), and reduce operant ethanol self-administration by rats (Hodge et al, 1993). Site-specific injection of 5-HT$_3$ antagonists in the nucleus accumbens also decreases alcohol consumption (Jankowska and Kostowski, 1995). In humans, the 5-HT$_3$ antagonist ondansetron reduces alcohol-induced craving in social drinkers (Johnson et al, 1993) and increases abstinence among alcoholics with a biological predisposition (Johnson et al, 2000).

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-HT$_{3A/B}$ receptors (Brady et al, 2001), the functional significance of specific 5-HT$_3$ receptor subunits in alcohol drinking has not been determined. To address this issue, we studied voluntary alcohol drinking both during and after a sucrose fading procedure in mice with a targeted deletion of the 5-HT$_{3A}$ receptor subunit (Kelley et al, 2003; Zeitz et al, 2002b). Moreover, to determine if the 5-HT$_3$ antagonists reduce alcohol drinking by inhibiting 3A containing receptors, we tested the effects of the 5-HT$_3$ antagonist LY-278,584 on alcohol drinking by 5-HT$_{3A}$-null mice and controls.

METHODS

Mice

5-HT$_{3A}$ receptor-null mice were derived by homologous recombination as previously reported (Zeitz et al, 2002a). F1 hybrid C57BL/6J × 129vJ heterozygous progeny were backcrossed to C57BL/6J mice to produce F9 generation congenics. Heterozygotes from the F9 generation were bred to generate male 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 San Francisco as described in Zeitz et al (2002a). All experiments were conducted in male mice. 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 06:00) 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.

Locomotor Activity

Spontaneous locomotor activity and habituation of naive 5-HT$_{3A}$ (−/−, $n = 16$) and 5-HT$_{3A}$ (+/+, $n = 16$) mice was measured in Plexiglas chambers (43 cm^2) located in sound-attenuating cubicles equipped with exhaust fans that masked external noise (Med Associates, St Albans, VT). Two sets of 16 pulse-modulated infrared photobeams were placed on opposite walls at 1-in centers to record $x–y$ ambulatory movements. Activity chambers were computer interfaced (Med Associates) for data sampling at 100-ms resolution. Mice were handled and weighed daily for 1 week before activity testing. Prior to each session, the activity chamber was wiped clean with 2.5% glacial acetic acid to limit any confounding odors. The mice were placed in the corner of the chamber and left to behave freely for 60 min.

At 1 week after baseline locomotor testing, the mice were used to determine the effects of ethanol on locomotor activity. The same procedure was used to test ethanol-induced locomotor activity except that mice were administered ethanol (0. 0.5, 1, or 2 g/kg, i.p.) in a Latin-Square randomized dose order immediately before the start of activity monitoring. Horizontal distance traveled (cm) was recorded for 60 min after ethanol injection. Tests sessions were separated by 3 days.

Alcohol Drinking

Oral alcohol drinking and preference were examined using a two-bottle choice protocol (Hodge et al, 1999). Eight experimentally naïve male 5-HT$_{3A}$ (+/+) and six 5-HT$_{3A}$ (−/−) mice were tested in parallel. Mice were given 1 week to acclimatize to individual housing conditions and handling. During this period, water was the only fluid available. Subsequently, two-bottle drinking sessions were then conducted 24 h per day, 7 days per week, in the home-cage. Ethanol and water solutions were in 50-ml plastic bottles (0.5 ml graduates) equipped with ball-bearing stoppers to limit spillage. The location (left or right side of the cage) of ethanol and water solutions was counter-balanced between animals to control for side preference. Volume was recorded at the beginning and end of access periods. No significant spillage was noted throughout the experiment.

To evaluate potential involvement of the 5-HT$_{3A}$ receptor in the initiation of alcohol drinking, mice were given access to a series of sweetened ethanol solutions vs water (eg Samson, 1986). The sucrose (% w/v) and ethanol (% v/v) content of the solutions were as follows: 10S/2E; 10S/5E; 10S/10E; 5S/10E; 2S/10E; 10E. Mice were exposed to each sweetened ethanol solution for 3 days and 10% ethanol at the end for a 6-day period.

After evaluating initiation of ethanol intake, the drinking solutions were returned to 2S/10E vs water for a 3-day baseline period. Then, the effects of the 5-HT$_3$ antagonist LY-278,584 (0–1 mg/kg) were evaluated on sweetened ethanol vs water intake. After determining the LY-278,584 dose–response curve, mice were re-exposed to 10E vs water for a 3-day baseline period. The effects of LY-278,584 were then redetermined on unsweetened ethanol vs water intake. Drug dosing was conducted in a randomized Latin-Square manner. Injections occurred immediately prior to 24-h drinking sessions no more than twice per week. The day immediately prior to saline vehicle treatment was used as a noninjection control in each experiment.

RESULTS

Locomotor Activity

To determine if any overt motor changes were produced by the targeted mutation, we first tested 5-HT_{3A}-null mice and wild-type littermates in a novel open-field environment for 1 h. The null mutation produced no effect on spontaneous locomotor activity (Figure 1a). Moreover, locomotor activity by both genotypes decreased as a function of time in the open-field environment ($F_{time}(5,150) = 13.55$, $p < 0.001$), which indicates normal habituation to the environment. This finding is consistent with our previous report (Kelley et al, 2003) and supports pharmacological evidence, which indicates that 5-HT_3 receptor antagonists have no effects on locomotor behavior (Jones et al, 1988).

The effects of ethanol on locomotor activity of the 5-HT_{3A}-null mice and wild-type littermates are illustrated in Figure 1b. Ethanol significantly reduced locomotor activity ($F_{dose}(3,90) = 5.08$, $p = 0.003$), which was due to reduced activity at the 2 g/kg ethanol dose as compared to the 1 g/kg dose (Tukey, $p = 0.001$). However, the ethanol-induced

activity of the two genotypes did not differ. Further, planned comparisons revealed that locomotor activity after ethanol injections did not differ from the saline injection for the 5-HT_{3A}-null mice and wild-type littermates. These findings are consistent with pharmacological evidence showing no effect of 5-HT_3 receptor antagonists on ethanol-induced locomotor activity (Le et al, 1997), and the absence of ethanol-induced locomotor stimulation in C57BL/6J mice (Liljequist and Ossowska, 1994).

Alcohol Drinking

When examined through the course of a sucrose fading procedure (eg Samson, 1986) conducted during daily 24-h periods in the home cage 5-HT_{3A} receptor-null mice did not differ from wild-type controls on measures of ethanol intake or preference (Figure 2a and 2b). Repeated measures ANOVA showed that intake and preference decreased as a function of reduced sucrose and increased ethanol concentration in both genotypes ($F_{dose}(6,72) = 45.57$, $p < 0.001$). Although ethanol intake was relatively low for C57BL/6J mice (Middaugh et al, 1999), no differences were observed in ethanol, water, or total fluid intake between genotypes. These data indicate that deletion of the 5-HT_{3A} receptor subunit does not influence the acquisition or
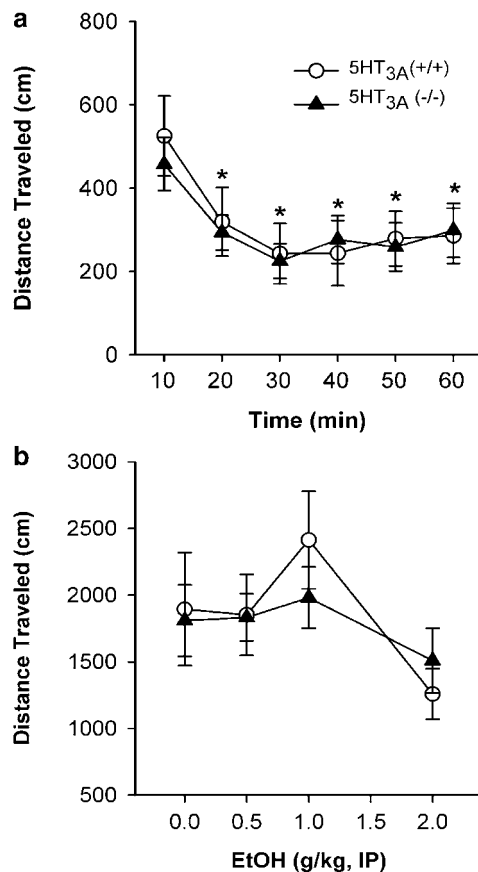

Figure 1 Deletion of the 5-HT_{3A} receptor subunit produced no significant effects on motor activity or locomotor response to ethanol. (a) Spontaneous locomotor activity and habituation to a novel environment. Both null mice ($-/-$, $n = 16$) and wild-type controls ($+/+$, $n = 16$) showed normal levels of locomotor activity that decreased as a function of time in the environment. Horizontal distance traveled (cm ± standard error) was averaged for each 10 min of a 60 min exposure. (b) Locomotor response to acute ethanol administration. All data are expressed as mean (±SEM). * – indicates significantly different from initial 10-min period irrespective of genotype, $p < 0.05$, Tukey test.
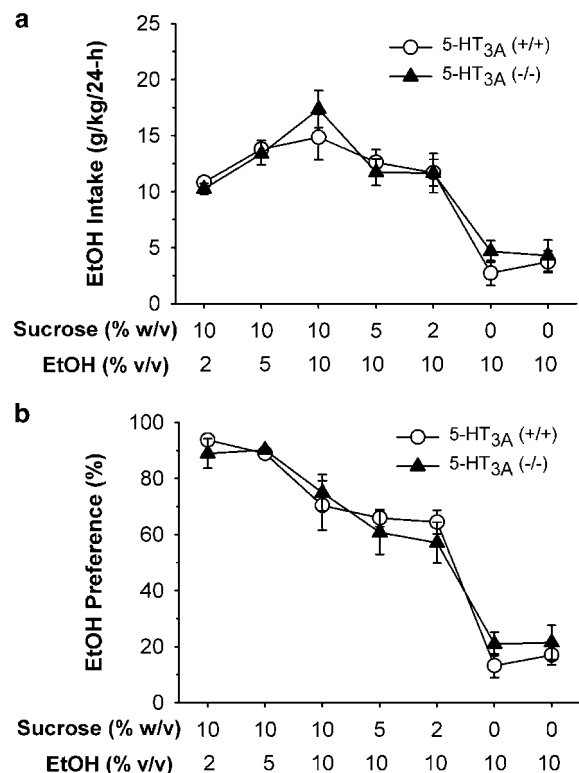

Figure 2 Initiation and maintenance of alcohol drinking by mice lacking the 5-HT_{3A} receptor gene. (a) Null mice ($-/-$, $n = 6$) consumed the same amount of sweetened and unsweetened alcohol in a home-cage two-bottle preference test as wild-type mice ($+/+$, $n = 8$). (b) The null mutation had no effect on ethanol preference expressed as a percentage of total fluid intake (ethanol mls plus water mls). Each animal's alcohol intake (g/kg/24-h) or preference data were averaged over a 3-day exposure period at each ethanol/sucrose combination and then plotted as a group mean (±SEM).

maintenance of alcohol drinking under these experimental conditions.

Figure 3a shows the effects of the 5-HT₃ antagonist LY-278,584 on sweetened ethanol (2% sucrose + 10% EtOH)
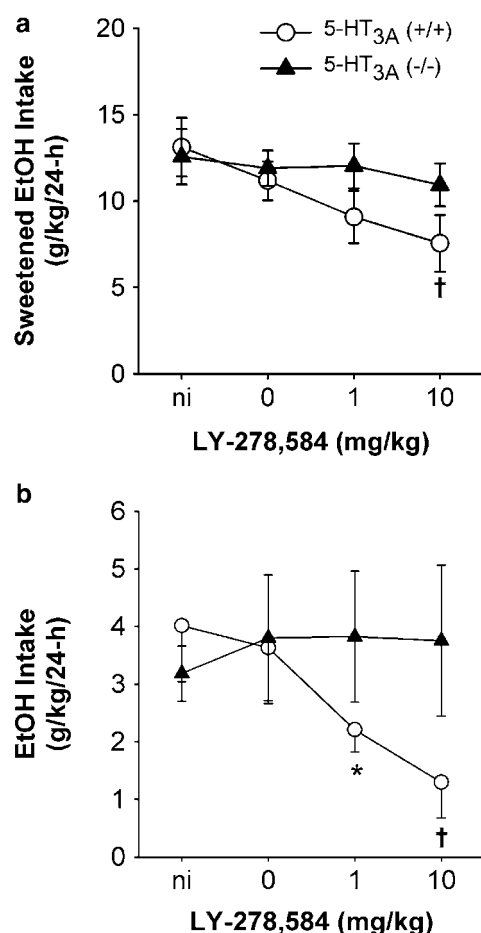

a

b

intake. LY-278,584 dose dependently decreased intake of sweetened ethanol (F_{dose} (3,33) = 4.6, $p = 0.009$), which was primarily due to a significant difference between the no-injection control and 10 mg/kg dose of LY-278,584 (Tukey, $p = 0.001$). There was no genotypic difference or interaction between LY-278,584 and genotype. Planned comparisons within the wild-type group showed that ethanol intake following no-injection differed from the 1 and 10 mg/kg doses of LY-278,584 ($p = 0.009$ and 0.01, respectively), whereas intake after saline differed only from the 10 mg/kg dose ($p = 0.013$) (Figure 3a). LY-278,584 produced no significant effects on alcohol drinking by 5-HT₃A-null mice. There was also a main effect of dose of LY-278,584 on preference for sweetened ethanol relative to water (F_{dose} (3,33) = 4.7, $p = 0.008$), which was attributable to a significant reduction in preference only in the wild-type group (Table 1). There was no effect of genotype, or a genotype by dose interaction, on sweetened ethanol preference. No differences were observed in water, or total fluid, intake.

Statistical analysis of unsweetened ethanol intake (g/kg) found no main effect of genotype or LY-278,584 (Figure 3b). However, the effects of LY-278,584 on ethanol intake (g/kg) depended on genotype as evidenced by a significant interaction ($F_{dose \times genotype}$ (3,33) = 2.9, $p = 0.04$). Planned comparisons showed that LY-278,584 (10 mg/kg) significantly decreased ethanol intake as compared to no-injection and saline controls ($p = 0.009$ and 0.01, respectively) in wild-type mice only (Figure 3b). Ethanol intake by 5-HT₃A-null mice was unchanged by any dose of the antagonist (Figure 3b). Thus, the significant interaction was due to antagonist-induced decreases in ethanol intake only within the wild-type group. RM ANOVA showed a trend toward a significant decrease in preference for unsweetened ethanol produced by LY-278,584 (F_{dose} (3,33) = 2.5, $p = 0.07$). There was no effect of genotype, and no interaction among the variables. Planned comparisons across genotype and dose of the 5-HT₃ antagonist showed that the 10 mg/kg dose of LY-278,584 significantly decreased preference only in the wild-type group (Table 1). No differences were observed in water, or total fluid, intake.

Figure 3 The 5-HT₃ antagonist LY-278,584 significantly decreased intake (g/kg/24 h) of sweetened (2S/10E) (a) or unsweetened (10E) (b) alcohol in a 24-h home-cage two-bottle test in 5-HT₃A wild-type mice (+/+, n = 8). Data represent mean (±SEM) during a single 24-h period following i.p. administration of LY-278,584. Variability in ethanol intake by 5-HT₃A-null mice (−/−, n = 6) was due to a single outlier (b). Note the different y-axis scales on the two panels. * – indicates significantly different from no-injection control within genotype; † – indicates significantly different from both no-injection and saline vehicle control within genotype, $p < 0.05$, Tukey test.

DISCUSSION

The 5-HT₃ receptor has emerged as a potential therapeutic target for the medical management of alcohol abuse and alcoholism because inhibition of the receptor by selective antagonists produces positive effects in preclinical (Costall

Table 1 Effect of LY-278,584 on Preference (mean ± SEM) for Sweetened and Unsweetened Ethanol

		LY-278,584 (mg/kg, i.p.)			
Solution	Genotype	No injection	Saline	1.0	10.0
2S/10E	5-HT₃A (+/+)	0.71 (0.03)	0.74 (0.09)	0.59 (0.06)	0.46 (0.11)[a]
	5-HT₃A (−/−)	0.74 (0.08)	0.78 (0.06)	0.70 (0.06)	0.69 (0.07)
10E	5-HT₃A (+/+)	0.21 (0.04)	0.20 (0.04)	0.17 (0.009)	0.10 (0.04)[b]
	5-HT₃A (−/−)	0.27 (0.06)	0.21 (0.06)	0.23 (0.06)	0.23 (0.08)

[a]significantly different from no-injection and saline controls within 5-HT₃A (+/+) group, and from 5-HT₃A(−/−) at corresponding dose.
[b]significantly different from no-injection control.

et al, 1993) and clinical (Sellers et al, 1994) studies. A common finding from preclinical studies is that 5-HT₃ antagonists selectively decrease alcohol drinking and reinforcement (Fadda et al, 1991; Hodge et al, 1993; Jankowska and Kostowski, 1995; Knapp and Pohorecky, 1992; McKinzie et al, 1998; Silvestre et al, 1998; Tomkins et al, 1995), suggesting that alcohol self-administration is at least partly maintained by 5-HT₃ receptor activity (but see Beardsley et al, 1994).

Ionotropic 5-HT₃ receptors are thought to consist of five coassembled subunits that surround a centrally gated channel (Boess et al, 1995). The 5-HT₃A subunit (Maricq et al, 1991) is the only known 5-HT₃ subunit that occurs in rodent brain (Morales and Wang, 2002). It is not known, however, if the effects of 5-HT₃ antagonists on alcohol drinking are mediated by this subunit or other(s), which may coexpress with the 3A subunit (eg, Fletcher and Barnes, 1998). To address this question, we examined alcohol drinking by mutant mice lacking the 5-HT₃A receptor subunit (Zeitz et al, 2002a). One of the primary findings of this study is that 5-HT₃A-null mice did not differ from wild-type controls on measures of voluntary alcohol drinking either during or after a sucrose fading initiation procedure. These data are in contrast with a number of studies showing that 5-HT₃ antagonists decrease alcohol drinking and operant self-administration (Hodge et al, 1993; Knapp and Pohorecky, 1992).

To determine, therefore, if decreases in alcohol drinking produced by 5-HT₃ antagonists might be dependent on the 5-HT₃A receptor subunit, we tested the effects of the 5-HT₃ antagonist LY-278,584 on intake (g/kg/24-h) of sweetened and unsweetened ethanol by null mice and wild types. LY-278,584 decreased intake of, and preference for, both ethanol solutions only in wild-type mice. This indicates that the 5-HT₃A receptor subunit is required for 5-HT₃ antagonist-induced reductions in alcohol drinking, and strongly suggests that 5-HT₃ antagonist-induced reductions in ethanol drinking (Fadda et al, 1991; Hodge et al, 1993; Jankowska and Kostowski, 1995; Knapp and Pohorecky, 1992; McKinzie et al, 1998; Silvestre et al, 1998; Tomkins et al, 1995) are likely due to pharmacological blockade of the 5-HT₃ receptor subunit. Moreover, these data indicate that other 5-HT₃ receptor subunits, which may be expressed in brain (Fletcher and Barnes, 1998; Morales and Wang, 2002; Niesler et al, 2003), are not able to form functional channels that are as sensitive to 5-HT₃ antagonists as native receptors containing the 3A subunit.

A question that emerges from these data is why deletion of the 5-HT₃A receptor had no effect on alcohol drinking even though the 5-HT₃ antagonist decreased drinking in wild-type mice. One possible explanation for the lack of agreement between targeted gene deletion and pharmacological inhibition of the 5-HT₃ receptor is that developmental compensation occurred in the null mutants, which may have masked any functional involvement of the receptor subunit in alcohol drinking. Compensation may be due to genetic redundancy whereby so-called 'helper' genes can take over the function of the targeted gene (Gerlai, 1996; Gerlai, 2001). Thus, it is possible that the 5-HT₃A receptor subunit is an important mediator of alcohol drinking, but compensation by another unknown pathway concealed this involvement. In this regard, gene targeting missed the mark of revealing new information about 5-HT₃ receptor regulation of alcohol drinking. Whether new information could be gained by investigating the mechanisms of developmental compensation, such as uncovering novel interactions between the 5-HT₃A receptor subunit and other genes, is a matter of debate (eg Gerlai, 2001; Routtenberg, 1995; Routtenberg, 1996).

Compensation, however, may not occur in all phenotypes associated with the targeted gene. For instance, we recently reported that 5-HT₃A-null mice exhibit less anxiety-like behavior on three validated animal models of anxiety (Kelley et al, 2003). This phenotypic profile corresponds with the known anxiolytic properties of pharmacological antagonists of 5-HT₃ receptors (eg Costall and Naylor, 1991) and suggests that the 5-HT₃A receptor subunit is an important mediator of anxiety. One might conclude, therefore, that deletion of the 5-HT₃A receptor subunit did not produce compensatory changes in neurobiological pathways associated with anxiety. Moreover, it seems reasonable to conclude that compensatory changes that might have masked 5-HT₃A receptor involvement in alcohol drinking are not associated with anxiety-like behavior.

A final remark regarding potential compensatory factors merits discussion. Genetic background may interact with the mutated gene in ways that can alter phenotypic expression. For instance, protein kinase C gamma (PKCγ)-null mutants from a C57BL/6J × 129/SvJ mixed genetic background exhibited reduced ethanol sensitivity and the absence of ethanol tolerance, however, expression of the null mutation on a C57BL/6J background eliminated the phenotypes (Bowers 1999). Mice used in the present study were congenic C57BL/6J, which are widely known as a model of high alcohol preference (Belknap, 1993). Thus, it is plausible that background genes driving alcohol drinking in this mouse strain might blunt the effects of some null mutations, such as the 5-HT₃A receptor.

REFERENCES

Barann M, Ruppert K, Gothert M, Bonisch H (1995). Increasing effect of ethanol on 5-HT3 receptor-mediated 14C-guanidinium influx in N1E-115 neuroblastoma cells. *Naunyn-Schmiedebergs Arch Pharmacol* 352: 149–156.

Barnes NM, Sharp T (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* 38: 1083–1152.

Beardsley PM, Lopez OT, Gullikson G, Flynn D (1994). Serotonin 5-HT3 antagonists fail to affect ethanol self-administration of rats. *Alcohol* 11: 389–395.

Belknap JK, Crabbe JC, Young ER (1993). Voluntary consumption of ethanol in 15 inbred mouse strains. *Psychopharmacology* 112: 503–510.

Boess FG, Beroukhim R, Martin IL (1995). Ultrastructure of the 5-hydroxytryptamine3 receptor. *J Neurochem* **64**: 1401–1405.

Bowers BJ, Owen EH, Collins AC, Abeliovich A, Tonegawa S, Wehner JM (1999). Decreased ethanol sensitivity and tolerance development in gamma-protein kinase C null mutant mice is dependent on genetic background. *Alcohol: Clin Exp Res* **23**: 387–397.

Brady CA, Stanford IM, Ali I, Lin L, Williams JM, Dubin AE *et al* (2001). Pharmacological comparison of human homomeric 5-HT3A receptors versus heteromeric 5-HT3A/3B receptors. *Neuropharmacology* **41**: 282–284.

Costall B, Domeney AM, Kelly ME, Naylor RJ (1993). The effects of 5-HT3 receptor antagonists in models of dependency and withdrawal. *Alcohol Alcohol Suppl* **2**: 269–273.

Costall B, Naylor RJ (1991). Pharmacological properties and functions of central 5-HT3 receptors. *Therapie* **46**: 437–444.

Davies PA, Pistis M, Hanna MC, Peters JA, Lambert JJ, Hales TG *et al* (1999). The 5-HT3B subunit is a major determinant of serotonin-receptor function. *Nature* **397**: 359–363.

Derkach V, Surprenant A, North RA (1989). 5-HT3 receptors are membrane ion channels. *Nature* **339**: 706–709.

Eisele JL, Bertrand S, Galzi JL, Devillers-Thiery A, Changeux JP, Bertrand D (1993). Chimaeric nicotinic-serotonergic receptor combines distinct ligand binding and channel specificities. *Nature* **366**: 479–483.

Fadda F, Garau B, Marchei F, Colombo G, Gessa GL (1991). MDL 72222, a selective 5-HT3 receptor antagonist, suppresses voluntary ethanol consumption in alcohol-preferring rats. *Alcohol Alcohol* **26**: 107–110.

Fletcher S, Barnes NM (1998). Desperately seeking subunits: are native 5-HT3 receptors really homomeric complexes? *Trends Pharmacol Sci* **19**: 212–215.

Gerlai R (1996). Gene-targeting studies of mammalian behavior: is it the mutation or the background genotype? (comment) (erratum appears in *Trends Neurosci* 1996 Jul; 19(7): 271). *Trends Neurosci* **19**: 177–181.

Gerlai R (2001). Gene targeting: technical confounds and potential solutions in behavioral brain research. *Behav Brain Res* **125**: 13–21.

Hodge CW, Mehmert KK, Kelley SP, McMahon T, Haywood A, Olive MF *et al* (1999). Supersensitivity to allosteric GABA(A) receptor modulators and alcohol in mice lacking PKCepsilon. *Nat Neurosci* **2**: 997–1002.

Hodge CW, Samson HH, Lewis RS, Erickson HL (1993). Specific decreases in ethanol – but not water – reinforced responding produced by the 5-HT3 antagonist ICS 205-930. *Alcohol* **10**: 191–196.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ *et al* (1994). International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). *Pharmacol Rev* **46**: 157–203.

Jankowska E, Kostowski W (1995). The effect of tropisetron injected into the nucleus accumbens septi on ethanol consumption in rats. *Alcohol* **12**: 195–198.

Jenkins A, Franks NP, Lieb WR (1996). Actions of general anaesthetics on 5-HT3 receptors in N1E-115 neuroblastoma cells. *Br J Pharmacol* **117**: 1507–1515.

Johnson BA, Ait-Daoud N, Prihoda TJ (2000). Combining ondansetron and naltrexone effectively treats biologically predisposed alcoholics: from hypotheses to preliminary clinical evidence. *Alcohol: Clin Exp Res* **24**: 737–742.

Johnson BA, Campling GM, Griffiths P, Cowen PJ (1993). Attenuation of some alcohol-induced mood changes and the desire to drink by 5-HT3 receptor blockade: a preliminary study in healthy male volunteers. *Psychopharmacology* **112**: 142–144.

Jones BJ, Costall B, Domeney AM, Kelly ME, Naylor RJ, Oakley NR *et al* (1988). The potential anxiolytic activity of GR38032F, a 5-HT3-receptor antagonist. *Br J Pharmacol* **93**: 985–993.

Kelley SP, Bratt AM, Hodge CW (2003). Targeted gene deletion of the 5-HT3A receptor subunit produces an anxiolytic phenotype in mice. *Eur J Pharmacol* **461**: 19–25.

Knapp DJ, Pohorecky LA (1992). Zacopride, a 5-HT3 receptor antagonist, reduces voluntary ethanol consumption in rats. *Pharmacol, Biochem Behav* **41**: 847–850.

Kostowski W, Dyr W, KrzaScik P (1993). The abilities of 5-HT3 receptor antagonist ICS 205-930 to inhibit alcohol preference and withdrawal seizures in rats. *Alcohol* **10**: 369–373.

Lambert JJ, Peters JA, Hales TG, Dempster J (1989). The properties of 5-HT3 receptors in clonal cell lines studied by patch- clamp techniques. *Br J Pharmacol* **97**: 27–40.

Le AD, Tomkins D, Higgins G, Quan B, Sellers EM (1997). D1 and D2 receptor antagonists on ethanol- and cocaine-induced locomotion. *Pharmacol Biochem Behav* **57**: 325–332.

Liljequist S, Ossowska K (1994). Genotypic differences in locomotor stimulation and dopaminergic activity following acute ethanol administration. *Eur Neuropsychopharmacol* **4**: 31–38.

Lovinger DM (1999). 5-HT3 receptors and the neural actions of alcohols: an increasingly exciting topic. *Neurochem Int* **35**: 125–130.

Lovinger DM, Sung KW, Zhou Q (2000). Ethanol and trichloro-ethanol alter gating of 5-HT3 receptor-channels in NCB-20 neuroblastoma cells. *Neuropharmacology* **39**: 561–570.

Lovinger DM, 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.

Lovinger DM, Zhou Q (1994). Alcohols potentiate ion current mediated by recombinant 5-HT3RA receptors expressed in a mammalian cell line. *Neuropharmacology* **33**: 1567–1572.

Lovinger DM, Zhou Q (1998). Alcohol effects on the 5-HT3 ligand-gated ion channel. *Toxicol Lett* **100–101**: 239–246.

Maricq AV, Peterson AS, Brake AJ, Myers RM, Julius D (1991). Primary structure and functional expression of the 5HT3 receptor, a serotonin-gated ion channel. *Science* **254**: 432–437.

McKinzie DL, Eha R, Cox R, Stewart RB, Dyr W, Murphy JM *et al* (1998). Serotonin3 receptor antagonism of alcohol intake: effects of drinking conditions. *Alcohol* **15**: 291–298.

Middaugh LD, Kelley BM, Bandy AE, McGroarty KK (1999). Ethanol consumption by C57BL/6 mice: influence of gender and procedural variables. *Alcohol* **17**: 175–183.

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

Niesler B, Frank B, Kapeller J, Rappold GA (2003). Cloning, physical mapping and expression analysis of the human 5-HT3 serotonin receptor-like genes HTR3C, HTR3D and HTR3E. *Gene* **310**: 101–111.

Routtenberg A (1995). Knockout mouse fault lines (comment). *Nature* **374**: 314–315.

Routtenberg A (1996). Reverse piedpiperase: is the knockout mouse leading neuroscientists to a watery end? (comment). *Trends Neurosci* **19**: 471–472.

Samson HH (1986). Initiation of ethanol reinforcement using a sucrose-substitution procedure in food- and water-sated rats. *Alcohol: Clin Exp Res* **10**: 436–442.

Sellers EM, Toneatto T, Romach MK, Somer GR, Sobell LC, Sobell MB (1994). Clinical efficacy of the 5-HT3 antagonist ondansetron in alcohol abuse and dependence. *Alcohol: Clin Exp Res* **18**: 879–885.

Silvestre JS, Palacios JM, Fernandez AG, O'Neill MF (1998). Comparison of effects of a range of 5-HT receptor modulators on consumption and preference for a sweetened ethanol solution in rats. *J Psychopharmacol* **12**: 168–176.

Tecott LH, Maricq AV, Julius D (1993). Nervous system distribution of the serotonin 5-HT3 receptor mRNA. *Proc Natl Acad Sci USA* **90**: 1430–1434.

Tomkins DM, Le AD, Sellers EM (1995). Effect of the 5-HT3 antagonist ondansetron on voluntary ethanol intake in rats and mice maintained on a limited access procedure. *Psychopharmacology* 117: 479–485.

Yakel JL, Jackson MB (1988). 5-HT3 receptors mediate rapid responses in cultured hippocampus and a clonal cell line. *Neuron* 1: 615–621.

Yoshimoto K, McBride WJ, Lumeng L, Li TK (1992). Alcohol stimulates the release of dopamine and serotonin in the nucleus accumbens. *Alcohol* 9: 17–22.

Zeitz KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L et al (2002a). The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *J Neurosci* 22: 1010–1019.

Zeitz KP, Guy N, Malmberg AB, Dirajlal S, Martin WJ, Sun L et al (2002b). The 5-HT3 subtype of serotonin receptor contributes to nociceptive processing via a novel subset of myelinated and unmyelinated nociceptors. *J Neurosci* 22: 1010–1019.