Ethanol Stabilizes the Open State of Single 5-Hydroxytryptamine$_{3A}$(QDA) Receptors

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ABSTRACT

Ethanol enhancement of 5-hydroxytryptamine (5-HT)$_{3A}$ receptor-mediated responses may have important consequences in the intoxicating and addictive properties of ethanol. Although the exact mechanism is unknown, ethanol-mediated enhancement of 5-HT$_3$ receptor current has been proposed to occur due to stabilization of the open-channel state. It has not been possible to directly measure the open state of the channel due to the extremely low single-channel conductance of 5-HT$_{3A}$ channels. Recently, three arginine residues within the large intracellular loop of the 5-HT$_{3A}$ subunit were substituted by their equivalent residues (glutamine, aspartate, and alanine) of the 5-HT$_{3B}$ subunit to produce a 5-HT$_{3A}$(QDA) subunit that forms functional homomeric channels exhibiting a measurable single-channel conductance. Using whole-cell rapid-agonist application techniques and the cell-attached single-channel recording configuration, we examined human 5-HT$_{3A}$(QDA) receptors expressed in human embryonic kidney 293 cells. The agonist sensitivity, macroscopic kinetics, and modulation by ethanol were similar between mutant and wild-type channels, suggesting the substitutions had not altered these channel structure-function properties. The open time histogram for single-channel events mediated by 5-HT$_{3A}$(QDA) receptors in the presence of maximal 5-HT was best fit by three exponentials, but in the presence of ethanol a fourth open state was evident. In summary, the QDA substitution greatly enhanced single-channel conductance with little effect on 5-HT$_{3A}$ channel’s kinetic properties and ethanol enhances agonist action on 5-HT$_{3A}$ receptors by inducing a new, long-lived open-channel state. Furthermore, the 5-HT$_{3A}$(QDA) receptor appears to be suitable for pharmacological studies of 5-HT$_{3A}$ receptor modulation at a single-channel level.

The 5-hydroxytryptamine (serotonin; 5-HT) type 3 receptor is a member of the Cys-loop ligand-gated ion channel superfamily that includes the nicotinic acetylcholine, GABA$_A$, glycine, and zinc-activated channel ion channels (Davies et al., 2003). Five genes encoding for individual subunits (5-HT$_{3A–E}$) have been identified within the human genome. However, homomeric 5-HT$_{3A}$ receptors appear to be the dominant receptor in the brain, particularly the rodent brain (Jensen et al., 2008).

5-HT$_3$ receptors are ligand-gated ion channels that play important roles in substance abuse, emesis, inflammatory pain, spinal nociception, and cardiovascular reflexes (Thompson and Lummis, 2007). Activation of 5-HT$_3$ receptors enhances the release of the neurotransmitters dopamine and GABA, which are believed to be the principal neurotransmitters for addiction and intoxication, respectively (McBride et al., 2004). The role of 5-HT$_3$ receptors in the neuronal circuitry of drug dependence has been known for some time (Grant, 1995). Within the mesolimbic dopaminergic system, 5-HT$_3$ receptors located on ventral tegmental area and nucleus accumbens neurons influence dopamine release leading to an enhancement of the reward pathway (De Deurwaerdere et al., 1998). Furthermore, 5-HT$_3$ receptor-mediated dopamine release in these areas is enhanced by ethanol (Campbell et al., 1996). Antagonists to 5-HT$_3$ receptors have been shown to effectively reduce drinking behavior in both rodent and human studies. Recently, ondansetron, a selective 5-HT$_3$ receptor antagonist has been used in clinical studies to reduce the drinking behavior in adolescents and early onset alcoholics (Dawes et al., 2005).

Alcohols and volatile anesthetics of low molecular size enhance 5-HT$_{3A}$-mediated current amplitudes elicited by low concentrations of 5-HT but are substantially less effective at 5-HT$_{3AB}$ receptors (Hayrapetyan et al., 2005; Stevens et al.,

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; TM, transmembrane; HEK, human embryonic kidney.
It has been proposed that these compounds stabilize the open state of 5-HT_{3A} channels (Zhou and Lovinger, 1996; Zhou et al., 1998; Lovinger et al., 2000; Solt et al., 2005). However, this hypothesis could never be directly measured due to the small single-channel conductance of the 5-HT_{3A} receptor. This complication can be overcome by using a modified 5-HT_{3A} receptor that has a large single-channel conductance (Kelley et al., 2003; Peters et al., 2004). In this high-conducting 5-HT_{3A} channel, three arginine residues within the membrane-associated helices of the intracellular loop between transmembrane (TM)3 and TM4 have been substituted by the equivalent residues of the 5-HT_{3B} subunit; glutamine (R432Q), aspartate (R436D), and alanine (R440A). The resultant 5-HT_{3A}(QDA) receptor has a single-channel conductance of ~37 pS, which is 40-fold greater than the wild-type 5-HT_{3A} receptor (Hales et al., 2006; Deeb et al., 2007). However, these studies did not examine the effects of these mutations on channel kinetics. Recent studies on other mutant 5-HT_{3A} receptors have shown that mutant receptors can have different channel kinetic properties compared with wild type and that these differences complicate the interpretation of pharmacological data (Hu et al., 2006). If the QDA mutations do not affect channel kinetics, this 5-HT_{3A}(QDA) receptor has the potential to test the hypothesis that ethanol stabilizes the open conformation of the 5-HT_{3A} channel at a single-channel level.

Here, we assess the properties of the 5-HT_{3A}(QDA) receptor to determine its usefulness in single-channel pharmacological experiments. To demonstrate this potential, we have examined modulation by ethanol at macroscopic and single-channel levels. We determined that mutant 5-HT_{3A}(QDA) and wild-type 5-HT_{3A} receptors have similar 5-HT and alcohol sensitivities and resolve, for the first time, ethanol modulation of 5-HT_{3} receptors on a single-channel scale.

Materials and Methods

Cell Culture and Transfection

Human embryonic kidney (HEK)293 cells were cultured in media made up of Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum and 1% penicillin/streptomycin at 37°C in a humidified 5% CO_{2} atmosphere. Cells were transfected, using the calcium phosphate precipitation method, with cDNA encoding either the 5-HT_{3A} subunit (in pCDM8) or 5-HT_{3A}(QDA) subunit (in GW1) along with green fluorescent protein cDNAs (in pCDM8). Cells were used 24 to 72 h after transfection. Successful transfection of the cells was determined by fluorescence microscopy to identify green fluorescent protein-labeled cells.

Electrophysiology

Macroscopic Currents. Culture medium was replaced by an extracellular solution that was superfused into the recording chamber at a rate of 5 ml/min. The extracellular solution consisted of 140 mM NaCl, 4.7 mM KCl, 1.2 mM MgCl_{2}, 2.5 mM CaCl_{2}, 11.0 mM glucose, and 10 mM HEPES (pH 7.4 with NaOH). Patch electrodes were filled with intracellular recording solution consisting of 140 mM KCl, 2.0 mM MgCl_{2}, 11 mM EGTA, and 10 mM HEPES (pH 7.4 with KOH), giving a patch electrode resistance of 2 to 4 MΩ. Currents were recorded using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA), low-pass filtered at 1 to 2 kHz, and digitized at 10 to 20 kHz with a Digidata 1200 A/D converter (Molecular Devices). For macroscopic current kinetic studies, 5-HT was rapidly applied to cells via a piezo-driven pipette that achieves whole-cell solution exchange times of 1 to 2 ms as measured using the approach described by Li et al., 2000. For alcohol modulation of 5-HT-mediated currents, 5-HT (100 μM) was applied by pressure (70-kPa) ejection (Picospritzer II; General Valve, Fairfield, NJ) from modified patch pipettes for a duration sufficient to evoke ~10% of the maximal 5-HT (100 μM)-evoked current amplitude. Experiments were performed at room temperature with the cells voltage-clamped at ~60 mV.

Single-Channel Recordings. Cell-attached patches were formed on HEK293 cells expressing 5-HT_{3A}(QDA) receptors. The cell-attached patch configuration allows for a stable, long-lasting patch to be exposed to a constant concentration of agonist and modulator. Cells were bathed in an extracellular recording solution consisting of 140 mM KCl, 4.7 mM NaCl, 1.2 mM MgCl_{2}, 2.5 mM CaCl_{2}, 11.0 mM glucose 11.0, and 10 mM HEPES (pH 7.4 with NaOH). Sylgard-coated electrodes were filled with extracellular solution containing 5-HT (100 μM) with or without ethanol (25 mM). Single-channel currents were low pass filtered at 2 to 5 kHz and digitized at 40 kHz.

Data Analysis

For analysis of the 5-HT concentration-response relationship, all currents were normalized to the average maximal current elicited by 100 μM 5-HT immediately before and after each measurement. Normalized data were plotted as mean ± S.D. The 5-HT concentration-response curves were fitted to the following Hill equation: \( I = I_{\text{max}} /[1 + (\text{EC}_{50}/[\text{5-HT}])^{n} \] \), where \( I \) is the peak current at a certain concentration of 5-HT, \( I_{\text{max}} \) is the maximum 5-HT-evoked current, \( \text{EC}_{50} \) is the concentration of 5-HT that elicits 50% of the maximal response, and \( n \) is the Hill coefficient.

The activation phase of whole-cell macroscopic current (from 10 to 90% of peak current amplitude) was fitted by a single exponential function. Measurements of time constants for deactivation and desensitization were determined by fitting the decay of currents (90–10% of peak current amplitude) to exponential functions using the Levenberg-Marquardt algorithm with least-squares minimization (Clampfit version 9.2; Molecular Devices). The number of exponentials that best described the decay of currents was determined from an F-test. Most decay components were best fit to two exponentials, but some were fitted by one or more than two exponentials. To allow for comparisons, a weighted time constant (Στ_{A}/ΣA_{τ}) was used.

Single-channel currents were analyzed using the single-channel analysis software in Clampfit 9.2 (Molecular Devices). Records were leak subtracted before analysis. All-points histograms were fitted with multiple Gaussians (least-squares minimization) to determine the unitary current amplitudes. The slope conductance was established by determining the unitary current amplitudes from patches were the pipette potential ranged from 40 to 100 mV.

Single-channel events were detected using the 50% threshold detection method (Colquhoun and Sigworth, 1995). From the single-channel events list, histograms of channel open dwell time distributions were plotted and fitted using a maximum likelihood procedure with correction for missed events (Colquhoun and Sakmann, 1985). The minimal number of exponential components required to fit the distribution was determined by chi-square statistics. A dead time of 90 μs was imposed on the fitting routine. Mean open durations were calculated, from the open dwell time fitted components, as a weighted mean from the open durations and proportions of each component (Krzywykowski et al., 2008).

Data were analyzed post hoc using Prism version 4 software (GraphPad Software Inc., San Diego, CA). Statistical analysis was performed by using an unpaired Student’s t test with statistical significance set at \( p < 0.05 \).
Drugs and Chemicals

5-Hydroxytryptamine (serotonin) was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol was purchased from American Bioanalytical (Natick, MA).

Results

Agonistic Profiles of 5-HT₁₃α(QDA) Receptors Are Similar to Wild-Type 5-HT₁₃α Receptors. Serotonin (5-HT) elicited a concentration-dependent inward current when applied to HEK293 cells expressing 5-HT₁₃α and 5-HT₁₃α(QDA) receptors (Fig. 1). A fit of the peak current data to a Hill equation yielded EC₅₀ values of 2.5 ± 0.13 and 1.8 ± 0.08 µM for 5-HT₁₃α and 5-HT₁₃α(QDA) receptors, respectively, with Hill coefficients of 1.8 ± 0.16 and 2.2 ± 0.16, respectively (3–8 cells).

Because of the higher single-channel conductance of 5-HT₁₃α(QDA) channels, it is presumed that the 5-HT-evoked currents will be larger than that evoked from 5-HT₁₃α channels. When the whole-cell current densities of the maximally evoked 5-HT response (100 µM) were examined, there was a more than 2-fold increase in whole-cell current density for the QDA containing receptors: 150 ± 22 and 355 ± 67 pA/pF (p = 0.01; n = 9) for 5-HT₁₃α and 5-HT₁₃α(QDA) receptors, respectively.

Macroscopic Kinetics of 5-HT₁₃α(QDA) Receptors Is Similar to Wild-Type 5-HT₁₃α Receptors. Receptors were activated by rapidly applying 5-HT using a piezo-driven capillary pipette. Figure 2A (left) shows typical currents evoked by the rapid application of 100 µM 5-HT to either 5-HT₁₃α (Fig. 2A, top) or 5-HT₁₃α(QDA) receptors (Fig. 2A, bottom) expressed in HEK293 cells. There was no significant difference in the time for activation between the receptor types at maximal (100 µM) concentrations of 5-HT (Fig. 2A, right). Time constants for receptor activation, at 100 µM 5-HT, were 3.9 ± 0.4 ms (n = 7) for wild-type 5-HT₁₃α receptors and 2.9 ± 0.3 ms (n = 6) for mutant 5-HT₁₃α(QDA) receptors (t test, p = 0.08).

To determine deactivation characteristics of the receptors, a 5-ms pulse of 100 µM 5-HT was rapidly applied to HEK293 cells expressing either 5-HT₁₃α or 5-HT₁₃α(QDA) receptors. The current decay upon agonist termination was fit with an exponential function. The deactivation time constant of 5-HT₁₃α(QDA) receptors was 1900 ± 80 ms (n = 3) compared with 1300 ± 290 ms (n = 4) for wild-type 5-HT₁₃α receptors (t test, p = 0.11; Fig. 2B).

An exponential fit of the current decay recorded during prolonged exposure to 100 µM 5-HT rapidly applied to HEK293 cells expressing 5-HT₁₃α receptors (gray trace) and by 5-HT₁₃α(QDA) receptors (black trace). The color-contrasted line during the decay phase is the exponential fit. No difference to the mean desensitization time constant was detected. Data are mean ± S.E.M. from three cells.

Deactivation

Activation

Fig. 2. The macroscopic kinetics of the 5-HT₁₃α(QDA) receptors are similar to that wild-type 5-HT₁₃α receptors. A, QDA substitutions minimally affect macroscopic current recordings. Left, current traces produced by activation of wild-type 5-HT₁₃α receptors (top; gray trace) and by 5-HT₁₃α(QDA) receptors (bottom; black trace). Currents were evoked by rapidly (solution exchange time, ~1–2 ms) applying 100 µM 5-HT to HEK293 cells expressing the indicated receptor. Right, mean activation time constants (data are mean ± S.E.M. from three to four cells). B, deactivation. A 5-ms pulse of 100 µM 5-HT was rapidly applied to HEK293 cells expressing 5-HT₁₃α receptors (gray trace) and 5-HT₁₃α(QDA) receptors (black trace). The current decay between 90 and 10% of maximal current amplitude) immediately after the agonist pulse was fit to an exponential equation; the best fit is shown as a color-contrasted line. The two receptors had similar time constants for deactivation. Data are mean ± S.E.M. from three to four cells. C, desensitization. An exponential fit of the current decay recorded during prolonged exposure to 100 µM 5-HT rapidly applied to HEK293 cells expressing 5-HT₁₃α receptors (gray trace) and 5-HT₁₃α(QDA) receptors (black trace). The color-contrasted line during the decay phase is the exponential fit. No difference to the mean desensitization time constant was detected. Data are mean ± S.E.M. from three cells.

Fig. 1. Serotonin sensitivity of 5-HT₁₃α(QDA) receptors is similar to wild-type 5-HT₁₃α receptors. 5-HT concentration-response relationship for wild-type 5-HT₁₃α receptors (open circles) and 5-HT₁₃α(QDA) receptors (closed circles). Data fit to a Hill equation. For EC₅₀ and Hill coefficient values, see text. Data are from three to eight cells.

Fig. 3. QDA Mutations Do Not Alter Ethanol Action on 5-HT₁₃α Receptors. To test whether the QDA mutations alter the action of ethanol on 5-HT₁₃α receptors, we measured the effect of an intoxicating concentration of ethanol on the 5-HT-mediated current that was approximately 10% of the maximal response. The application of 25 mM ethanol by itself did not elicit any current from cells expressing either 5-HT₁₃α receptors or 5-HT₁₃α(QDA) receptors. Figure 3 demonstrates that ethanol similarly enhanced currents mediated by wild-type versus QDA mutant 5-HT₁₃α receptors. At 25 mM, eth-
Ethanol reversibly increased currents mediated by wild-type 5-HT$_{3A}$ receptors by 165 ± 34% and those by 5-HT$_{3A}$(QDA) receptors by 139 ± 28% ($p = 0.66$).

**Ethanol Does Not Modulate the Single-Channel Conductance of 5-HT$_{3A}$ Receptors Containing the QDA Mutation.** Single-channel currents mediated by 5-HT$_{3A}$(QDA) receptors expressed in HEK293 cells were recorded using the cell-attached configuration of the patch-clamp technique, where the pipette solution contained 5-HT in the absence or presence of ethanol (25 mM). We used a concentration of 5-HT (100 µM) that in our macroscopic experiments resulted in maximal agonist-evoked current.

Single-channel currents were initially analyzed by creating all-points amplitude histograms and fitting the distribution with multiple Gaussians (least-squares minimization) to determine the unitary current amplitude. Single-channel events were clearly detected in 5-HT$_{3A}$(QDA) receptor-expressing cells, which is in marked contrast to our previous recordings from cells expressing 5-HT$_{3A}$ receptors where no single-channel events were detected upon 5-HT application (Davies et al., 1999). To investigate whether ethanol modulated single-channel conductance, we determined unitary current amplitudes from patches held at different membrane potentials (−40 to −80 mV) in the absence and presence of ethanol (25 mM). The slope conductance of 5-HT$_{3A}$(QDA) channels was similar in the absence (28 ± 11 pS; $n = 3$) and presence (27 ± 2 pS; $n = 3$) of ethanol (data not shown).

**Ethanol Increases the Open State of the 5-HT$_{3A}$(QDA) Channel.** In all patches examined, distinct bursts of channel activity were clearly evident that were terminated by long periods of closure that are thought to represent entry into the desensitized state. A substantial increase in single-channel activity was observed in the presence of ethanol (25 mM) with bursts forming clusters. Figure 4 shows 20-s sweeps of exemplar single-channel activity from two cell-attached patches in the absence and presence of ethanol (25 mM).

The ethanol-mediated increase in single-channel activity was not accompanied by a change in unitary current amplitude. At a predicted membrane potential of −80 mV, the unitary current amplitudes evoked by 100 µM 5-HT in the absence and presence of ethanol were 3.1 ± 0.8 and 2.5 ± 0.2 pA, respectively (three patches).

**Ethanol Modulates the Channel Open Time Distribution.** Changes to the 5-HT$_{3A}$(QDA) channel open state were further characterized by examining the open time distribution. Single-channel events were analyzed by a maximal likelihood minimization procedure to fit exponentials to the distribution of open times. The minimal number of exponentials required to fit the distribution was determined by Chi-square statistics.

The open time histogram generated from single-channel events mediated by 5-HT$_{3A}$(QDA) receptors in the presence of 100 µM 5-HT was best fit by three exponentials (Fig. 5B). The three resulting time constants ($\tau_1$–$\tau_3$) represent channel open times of brief, medium and long-lasting durations (Table 1).

In the presence of ethanol (25 mM), a fourth open state ($\tau_4$) was evident (Fig. 5B). Its open time was 11-fold longer than even the longest open state in ethanol’s absence. In addition,
the proportion of channels with brief openings was greatly reduced by 25 mM ethanol (Table 1). These changes resulted in the mean open duration of 5-HT$_{3A}$A(QDA) receptors (4.8 ms) to be increased approximately 10-fold in the presence of ethanol (47 ms).

### Discussion

We have shown, for the first time, the modulation of single-channel 5-HT$_{3A}$ receptors by ethanol. This was achieved by using a high-conducting mutant subunit that has minimal effect upon the apparent affinity of the receptor for 5-HT, the same kinetic properties compared with wild-type and maintains macroscopic ethanol sensitivity. It is well documented that macroscopic currents mediated by homomeric 5-HT$_{3A}$ receptors are modulated by alcohols both in recombinant expressed receptors and naturally occurring receptors in neuroblastoma cells (Machu and Harris, 1994; Downie et al., 1995; Jenkins et al., 1996; Zhou and Lovinger, 1996; Zhou et al., 1998; Stevens et al., 2005a).

We have shown that the small halogenated volatile anesthetics chloroform and halothane enhanced currents evoked by saturating concentrations of dopamine (a partial agonist at the 5-HT$_3$ receptor) without affecting the dopamine concentration-response relationship (Solt et al., 2005). These data imply that channel gating is enhanced but agonist binding affinity is unaltered. A similar conclusion was drawn by Lovinger et al. (2000) examining ethanol modulation of dopamine activated rodent 5-HT$_{3A}$ receptors.

The increase in channel gating by alcohols strongly suggests that the open state of the channel has been stabilized. However, due to the low single-channel conductance of the 5-HT$_{3A}$ receptor, the direct observation of single open channels is not possible. For this study, we have used a previously described triple mutation in which three arginines in the intracellular loop between TM3 and TM4 of the 5-HT$_{3A}$ subunit have been replaced with equivalent residues from the 5-HT$_{3B}$ subunit (R432Q, R436D, and R440A). The mutant homomeric receptor [5-HT$_{3A}$A(QDA)] has a large measurable single-channel conductance (Kelley et al., 2003; Hales et al., 2006). Although single-channel activity can now be recorded from 5-HT$_{3A}$A(QDA) receptors a potential problem is that such residue substitutions could alter pharmacological and kinetic properties compared with wild-type receptors.

The 5-HT$_{3A}$A(QDA) receptor had a slightly left-shifted 5-HT concentration-response relationship compared with the wild-type 5-HT$_{3A}$A receptors. In addition to similar 5-HT sensitivity, we observed no differences in the rates of activation, deactivation, and desensitization between mutant and wild-type 5-HT$_{3A}$A receptors, indicating that agonist affinity and channel gating have not been drastically altered by the mutations. In support of our observations, a recent study of the nicotinic $\alpha7$/5-HT$_{3A}$ chimera demonstrated that the QDA
substitutions did not alter the affinity of acetylcholine (Rayes et al., 2005).

Data from whole-cell macroscopic recordings have previously shown that enhancement of 5-HT_{3A} receptor-mediated current amplitude by alcohols is dependent upon the concentration of agonist (Zhou et al., 1998). At high concentrations of a highly efficacious agonist (5-HT), alcohols tend to minimally modulate current amplitude (Lovinger and White, 1991; Machu and Harris, 1994; Stevens et al., 2005a,b), but they do slow desensitization (Zhou et al., 1998; Hu et al., 2006). Our data explain these macroscopic observations on a microscopic scale. In our single-channel recordings with maximal agonist concentration, we see three open states in control but an additional, prolonged fourth open event in the presence of ethanol. At saturating 5-HT concentrations, open channels close by entering a period of desensitization, and in the presence of ethanol the extra prolonged open state appears to come at the expense of desensitization because the brief open states do not change in duration between the absence and presence of ethanol (Table 1). This prolongation of the open state of the channel at the expense of the desensitized state is in agreement with the prolonged desensitization effect of ethanol upon macroscopic currents.

Using the cell-attached patch method, we have examined ethanol effects at steady state that favors the transitions between the open and desensitized states. Ethanol may increase open-channel duration by increasing the activation rate or slowing the rate at which the channel deactivates. Having established the usefulness of the 5-HT_{3A}(QDA) receptor in examining the pharmacological properties of 5-HT_{3A} receptors at a single-channel level, future experiments will examine the effects of ethanol upon activation and deactivation kinetics.

The modulation of 5-HT_{3} receptors by ethanol has a significant role in the plasticity of pathways mediating alcohol addiction and intoxication. Activation of 5-HT_{3} receptors enhances the release of the neurotransmitters dopamine and GABA, which are thought to be the primary mediators for addiction and intoxication, respectively (McBride et al., 2004). Presynaptic 5-HT_{3} receptors are known to cause release of GABA from some inhibitory GABAergic interneurons in the amygdala (Koyama et al., 2000), cortex (Zhou and Hablitz, 1999; Puig et al., 2004), and hippocampus (McMahon and Kauer, 1997; Turner et al., 2004), resulting in an increased inhibition of postsynaptic neurons. Such increased GABA release by 5-HT_{3} receptor activation in the hippocampus is a potential mechanism to explain alcohol-induced memory impairment. The hippocampal tonic current, mediated by extrasynaptic GABA_A receptors, has been suggested to be enhanced by ethanol (Choi et al., 2008; Liang et al., 2008; but see Borghese et al., 2006). Consequently, ethanol enhancement of presynaptic 5-HT_{3}-mediated GABA release could result in more GABA release and thus an increase both phasic (synaptic) and tonic (extrasynaptic) inhibition. The tonic inhibition could be further enhanced by the presence of ethanol.

The role of 5-HT_{3} receptors in the neuronal circuitry of drug dependence has been known for some time (Grant, 1995). Within the mesolimbic dopaminergic system, 5-HT_{3} receptors located on the ventral tegmental area and nucleus accumbens neurons have been shown to control dopamine release leading to an enhancement of the reward pathway (De Deurwaerdere et al., 1998). Furthermore, 5-HT_{3} receptor-mediated dopamine release in these areas is enhanced by ethanol (Campbell et al., 1996). By stabilizing the open state of the 5-HT_{3A} channel, ethanol would allow an increase in calcium permeating through the channel, enhancing the calcium cascade that result in increased neurotransmitter release.

Macroscopic currents mediated by heteromeric 5-HT_{3A} receptors have reduced sensitivity to alcohols compared with homomeric 5-HT_{3A} receptors (Hayrapetyan et al., 2005; Stevens et al., 2005b). A recent study found an association with a variant within HTR3B, the gene encoding for 5-HT_{3B} subunit, and subjects diagnosed for alcohol use disorder with antisocial behavior (Ducci et al., 2009). Alcoholism with antisocial behavior is a characteristic of early onset alcoholics, a subset of alcoholics with which the 5-HT_{3} receptor antagonist ondansetron was shown in several clinical trials to be effective in reducing the craving for alcohol; reducing the number of drinking episodes (Dawes et al., 2005); and reducing symptoms of anxiety, depression, and hostility in early onset alcoholics (Johnson et al., 2003). The intronic variant found within HTR3B may result in reduced expression of the 5-HT_{3B} subunit and hence an increase in the number of ethanol-sensitive 5-HT_{3A} receptors. This illustrates the need to better understand the interaction between ethanol and 5-HT_{3A} receptors so that novel therapies can be developed to prevent such interactions.

In addition to its treatment for addiction, antagonists of the 5-HT_{3} receptor are frequently used to treat emesis and irritable bowel syndromes. Modulators of 5-HT_{3} receptors have also been suggested to have potential therapeutic promise for schizophrenia, anxiety, cognition, and nociception (Thompson and L lumis, 2007). Having demonstrated that 5-HT_{3A}(QDA) receptors closely resemble 5-HT_{3A} receptors except for the ability to have distinguishable single-channel currents makes 5-HT_{3A}(QDA) receptors a valuable recombinant channel to determine the properties of potential new therapeutic agents at a molecular level.

In summary, the QDA mutations within the membrane-associated stretch of the intracellular loop between transmembrane domains III and IV form receptors with high single-channel conductance but with wild-type kinetic properties and alcohol sensitivity. We have, for the first time, directly shown that ethanol stabilizes the open state of 5-HT_{3A} channels.

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