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Ethanol Potentiation of 5-Hydroxytryptamine$_3$ Receptor-Mediated Ion Current in Neuroblastoma Cells and Isolated Adult Mammalian Neurons

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SUMMARY

Recent studies indicate that ethanol (EtOH) potentiates ion current through the channel associated with the 5-hydroxytryptamine$_3$ (5-HT$_3$)-type serotonin receptor. The present study was designed to determine 1) whether such potentiation occurs in adult mammalian neurons expressing 5-HT$_3$ receptors; 2) whether potentiation is selective for the 5-HT$_3$ receptor, relative to other ligand-gated ion channels; and 3) possible mechanisms by which EtOH potentiates this response. EtOH potentiated 5-HT$_3$ receptor-mediated ion current in freshly isolated nodose ganglion neurons at concentrations similar to those previously reported to be effective in neuroblastoma cells (25–100 mM). Current was blocked by the selective 5-HT$_3$ antagonist ICS 205-930 even in the presence of EtOH, and current activated by a 5-HT$_3$ agonist (2-methyl-5-HT) was potentiated by EtOH. Thus, EtOH appears to produce potentiation via an alteration in the function of 5-HT$_3$ receptors and not through an independent effect. γ-Aminobutyric acid$_A$ receptor-mediated Cl^- current was not potentiated by EtOH in neurons in which potentiation of responses to 5-HT was observed. Methanol potentiated 5-HT$_3$ receptor-mediated current with a potency lower than that of EtOH. Potentiation by EtOH decreased with increasing 5-HT concentration. In addition, EtOH increased the decay rate of current. EtOH did not alter the reversal potential of the 5-HT$_3$ receptor-mediated current. These observations indicate that intoxicating concentrations of EtOH selectively potentiate 5-HT$_3$ receptor-mediated responses by increasing the apparent potency of 5-HT for activating ion current.

The intoxicating effects of acute EtOH ingestion are well characterized, but the neural basis of these effects remains unclear. Recent investigations have focused on the cellular and molecular mechanisms of EtOH action in the central nervous system that contribute to acute intoxication. Studies in several laboratories have indicated that intoxicating concentrations of EtOH can potentiate ion current mediated by GABA$_A$ receptors in some cell types (1–4) and inhibit ion current mediated by *N*-methyl-D-aspartate-type glutamate receptors (5–9). These observations indicate that certain types of receptor-channel complexes may be especially sensitive to the actions of low concentrations of EtOH. In light of these findings, we have begun to investigate the actions of EtOH on other ligand-gated ion channels.

Initial experiments indicated that acute EtOH exposure potentiated responses mediated by the 5-HT$_3$ receptor-channel complex (10). The investigation of the actions of EtOH on this receptor is especially interesting, given the proposed relationship between serotonergic neurotransmission and EtOH abuse (11–14). On the basis of the observed potentiation by EtOH, it was proposed that potentiation of 5-HT$_3$ receptor-mediated responses may play a role in acute intoxication. However, the initial experiments were performed using clonal neuroblastoma (NCB-20) cells (10), and the EtOH sensitivity of 5-HT$_3$ receptors on these cells may differ from that of neuronal receptors. It is, thus, desirable to determine whether EtOH potentiates ion current mediated by 5-HT$_3$ receptors on mammalian neurons. Although the NCB-20 cells express 5-HT$_3$ receptors, we observed that they do not consistently express other ligand-gated ion channels (data not shown). Thus, it was not possible to determine the selectivity of the EtOH potentiation with respect to other receptors. This would be possible in a neuronal preparation that expressed other receptors. We also wished to determine the mechanism(s) by which EtOH potentiates 5-HT$_3$ receptor-mediated ion current. To address these issues, we have examined the effect of acute EtOH exposure on ion current mediated by 5-HT$_3$ receptors on freshly isolated neurons from rat nodose ganglion and continued our studies of these effects in NCB-20 cells.

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ABBREVIATIONS: EtOH, ethanol; 5-HT, 5-hydroxytryptamine; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; GABA, γ-aminobutyric acid; MetOH, methanol; *df*, degrees of freedom.

Materials and Methods

Preparation and maintenance of NCB-20 neuroblastoma cultures. NCB-20 cells were grown in culture using techniques similar to those previously described (15). Briefly, cells were dispersed using medium containing 0.085% trypsin and were plated onto 10-cm-diameter or 35-mm culture dishes (Corning). Cells were maintained in medium containing 90% Dulbecco's modified Eagle's medium that contained 3.7 g/liter sodium bicarbonate (pH adjusted to 7.4 with NaOH), 10% fetal bovine serum, 2 mM L-glutamine, and 1% hypoxanthine-aminopterin-thymidine supplement, which gave final concentrations of 13.6 mg/liter hypoxanthine, 0.178 mg/liter aminopterin, and 3.88 mg/liter thymidine. Cells were grown in an incubator containing a 90% air/10% CO_2 atmosphere. Cells grown on large culture dishes were generally dispersed one or two times/week, and some cells were plated onto 35-mm culture dishes.

Isolation of nodose ganglion neurons. Neuronal somata were isolated using enzymatic and mechanical means similar to those previously used (16). Adult male Sprague-Dawley rats (150–300 g) were sacrificed by decapitation; nodose ganglia were dissected from the point at which the vagus nerve enters the skull and were minced two or three times with iridectomy scissors. Ganglia were then placed in a flask containing 5 ml of Dulbecco's modified Eagle's medium in which 50,000 units/ml trypsin (Sigma type III), 1 mg/ml collagenase (Sigma type 1A), and 0.1 mg/ml DNase (Sigma type IV) had been dissolved. Cells were incubated for 50–60 min at 35°, after which soybean trypsin inhibitor (Sigma type IIs) was added in a quantity sufficient to neutralize twice the amount of added trypsin. Neurons were then aliquoted into uncoated Petri dishes and used for electrophysiological recording as needed, over a period of up to 8 hr after cell isolation.

Whole-cell patch-clamp recording. Neurons and neuroblastoma cells were viewed using an inverted microscope, and gigaohm seals were achieved using glass microelectrodes with an impedance of ~2 MΩ. Whole-cell recordings were performed at room temperature using the EPC-7 (List Electronics) patch-clamp amplifier. Cells were superfused with extracellular medium at 1.5 ml/min. Unless otherwise noted, the extracellular recording medium contained (in mM) 150 NaCl, 5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, and 10 D-glucose; pH was buffered to 7.4 using NaOH, and osmolarity was adjusted to 340 mmol/kg using sucrose. For experiments on nodose ganglion neurons, the solution in the patch pipette (which dialyzed the interior of the neuron) contained (in mM) 140 KCl, 1 $CaCl_2$, 2 $MgCl_2$, 11 EGTA, and 10 HEPES; pH was buffered to 7.4 using KOH, and osmolarity was adjusted to 310 mmol/kg using sucrose. The pipette solution in experiments on NCB-20 cells was the same, with the exception that KCl and KOH were replaced with CsCl and CsOH. Dialysis of the interior of the cell with Cs^+ (which permeates the 5-HT_3 receptor-linked channel) (17) blocked voltage-dependent K^+ currents and allowed for accurate measurement of 5-HT-activated current over a wide range of membrane potentials. Series resistance in all recordings was <8 MΩ, and compensation was 40–60%. Nodose ganglion neurons included in this study were of 25–40 μm diameter, had membrane potentials of −50 mV or greater, and fired overshooting action potentials in response to depolarization. NCB-20 cells filled with Cs^+ generally had low membrane potentials (−25 to −40 mV) and did not fire action potentials.

Neurotransmitter agonists and antagonists were dissolved in external medium and delivered from large-bore (>100 μm) pipettes placed within 50 μm of the cells under study. These pipettes were connected to solution-containing reservoirs placed above the preparation, allowing for steady gravity-induced flow of solutions from the pipettes. EtOH and other compounds were added to solutions containing agonist and delivered simultaneously to cells.

Data were statistically compared using Student's *t* test or a repeated measures *t* test, as appropriate. Ion current was considered to be potentiated if an increase in amplitude of ≥15% was observed during EtOH application.

Results

Previous studies have demonstrated that 5-HT application to nodose ganglion neurons and NCB-20 cells produces a rapid-onset depolarization (15, 18) and a rapidly activating inward current recorded under voltage-clamp (16, 19–21). These responses to 5-HT are believed to be mediated by 5-HT_3 receptors. Fig. 1 shows that the rapidly activating ion current evoked by 5-HT application in freshly isolated nodose ganglion neurons displays properties consistent with a 5-HT_3 receptor-mediated current. The ion current is rapid (rise time, hundreds of milliseconds) in onset and inward-going at negative potentials, as expected for the cationic current activated by the 5-HT_3 receptor (Fig. 1A). The range of concentrations over which 5-HT produced ion current is similar to previously reported values for 5-HT-activated current (Fig. 1B). Fig. 1C shows that the current was completely blocked in the presence of nanomolar concentrations of the selective 5-HT_3 receptor antagonist MDL 72222, whereas Fig. 1D shows that a fast inward current

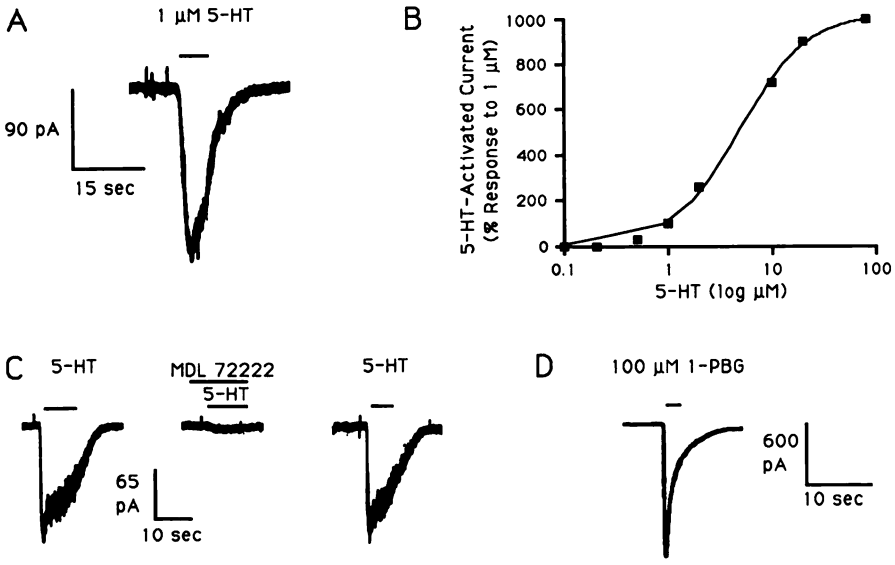

Fig. 1. Properties of 5-HT_3 receptor-mediated ion current. A, Inward current activated by application of 1 μM 5-HT. B, Graph plotting the relative amplitude of 5-HT-activated current as a function of 5-HT concentration. Amplitude was normalized to the average current activated by 1 μM 5-HT. Data are mean ± standard error from at least four neurons, with standard error smaller than the symbol in all cases. Data were fit using Kaleidagraph software. The fitting algorithm was a modified single-site binding isotherm, which corrects for slope factors unequal to 1 (34). ED_{50} for current activation = 4.95 μM 5-HT; slope factor = 1.32. C, Ion current activated by 1 μM 5-HT before, during, and 20 min after application of the selective 5-HT_3 receptor antagonist MDL 72222 (500 nM). The long time period needed for recovery from block is common when these potent antagonists are used (17). D, Activation of current by the 5-HT_3 receptor agonist 1-phenylbiguanide (*1-PBG*). The fact that the current is larger and decays faster than the 5-HT-activated currents in A and C is due to the fact that a maximal concentration of 1-phenylbiguanide was used, whereas relatively low concentrations of 5-HT were employed. Data and recordings are from neurons freshly isolated from adult rat nodose ganglion.

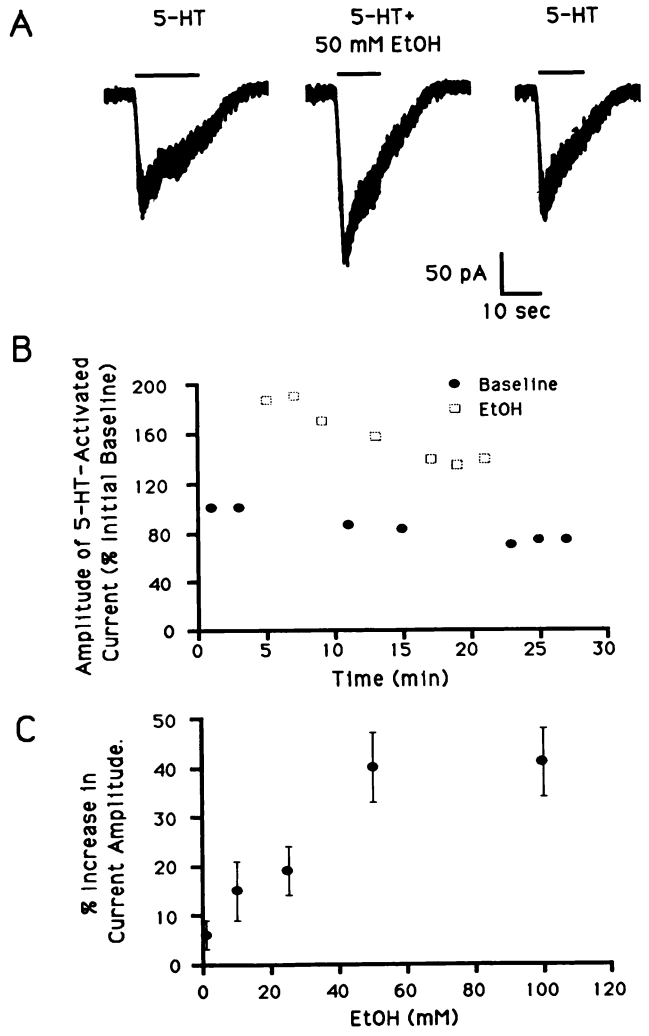

Fig. 2. EtOH potentiation of 5-HT$_3$ receptor-mediated ion current. A, Current activated by application of 1 μM 5-HT to an isolated nodose ganglion neuron before, during, and after application of 50 mM EtOH. B, Graph plotting the relative magnitude of individual responses to application of 1 μM 5-HT during the time course of an experiment in which successive applications of 5-HT were given in the absence or presence of 50 mM EtOH. Note the consistent potentiation of 5-HT-activated current in the presence of EtOH. Current amplitude was normalized to the amplitude of responses during the first 5-HT application in the absence of EtOH. Potentiation averaged 83, 80, and 67% of the previous baseline level during the first, second, and third applications of EtOH, respectively. C, Graph plotting percentage of potentiation of the amplitude of current activated by 1 μM 5-HT as a function of EtOH concentration. Values are mean ± standard error (number of neurons examined: 1 mM, 4; 10 mM, 12; 25 mM, 13; 50 mM, 20; 100 mM, 6).

could be evoked in these neurons by application of the 5-HT$_3$ receptor agonist 1-phenylbiguanide. Similar results were observed when the 5-HT$_3$ agonist 2-methyl-5-HT (10–100 μM) was used (data not shown). These observations indicate that the 5-HT-activated current in freshly isolated nodose ganglion neurons is mediated predominantly, if not exclusively, by 5-HT$_3$ receptors. Responses to 5-HT application were observed in 57% of the nodose ganglion neurons examined. Previous studies have shown that >80% of NCB-20 cells respond to 5-HT and that these electrophysiological responses are mediated by 5-HT$_3$ receptors (10, 17).

The observations presented in Fig. 2 show the effect of intoxicating concentrations of EtOH on 5-HT-activated current in isolated nodose ganglion neurons. We initially observed that application of 5-HT in the presence of 50 mM EtOH (Fig. 2A, *middle*) resulted in an increase in the amplitude of the 5-HT-activated current, relative to the amplitude observed before (Fig. 2A, *left*) or several minutes after (Fig. 2A, *right*) EtOH application. Potentiation by 50 mM EtOH was observed in 16 of 20 neurons examined and averaged 40 ± 7% (n = 20) of control amplitude of current activated by 1 μM 5-HT. Fig. 2B plots the amplitude of individual 5-HT-activated currents during three cycles of EtOH application and wash. It can be seen that the magnitude of potentiation was relatively stable during repeated exposures to EtOH. Fig. 2C shows the concentration dependence of the EtOH potentiation of 5-HT-activated current amplitude in these neurons. The threshold for significant potentiation was 25 mM EtOH (repeated measures t test = 3.19, df = 12, $p < 0.005$). Potentiation was seen in two of 12 neurons at 10 mM but, on average, responses in 10 mM EtOH did not differ in amplitude from baseline responses (repeated measures $t = 1.53$, $df = 11$, $p > 0.05$). Potentiation was observed in 77% of neurons in which higher EtOH concentrations (50 and 100 mM) were applied, and potentiation was of greater magnitude at these concentrations than at lower concentrations, in cells in which the effect of the different concentrations was directly compared (n = 13). In these neurons, 25 mM EtOH produced 19 ± 5% potentiation, whereas 50 and 100 mM EtOH produced 39 ± 8% potentiation (paired t = 2.41, df = 12, $p <$ 0.025).

Although the 5-HT-activated current in nodose ganglion neurons is mediated by 5-HT$_3$ receptors, it is possible that EtOH might bring about a current on its own or that 5-HT might activate some additional current in the presence of EtOH. However, the ion current activated by 5-HT in the presence of EtOH was blocked by the 5-HT$_3$ receptor antagonist ICS 205-930 (Fig. 3A). Similar results were observed in three of three neurons in which this experiment was performed. EtOH also potentiated ion current activated by the 5-HT$_3$ receptor agonist 2-methyl-5-HT in eight of eight NCB-20 cells examined (Fig. 3B). Furthermore, we observed no ion current in the presence of EtOH alone, even at concentrations as high as 100 mM (Fig. 3C). It is, therefore, quite likely that the potentiation of 5-HT-activated current by EtOH is mediated by an action of EtOH that alters the function of the 5-HT$_3$ receptor, rather than an action on another receptor or ion channel that sums with the 5-HT$_3$ receptor-mediated current.

To determine whether the EtOH effect was selective for 5-HT$_3$ receptors among the ligand-gated ion channels consistently expressed in the nodose ganglion preparation, we examined GABA$_A$ receptor-mediated Cl^- current in the absence and presence of EtOH using these neurons. The records in Fig. 4 show ion current activated by 5-HT and GABA in the same neuron before and during application of 50 mM EtOH. It can be seen from this figure that the amplitude of the 5-HT-activated current was potentiated in the presence of EtOH, whereas the amplitude of the GABA-activated current was little altered. The amplitude of GABA-activated current was not significantly altered by 50 mM EtOH in six of six neurons tested (1 ± 1% potentiation of current, repeated measures t = 1.56, $p > 0.05$). Of these neurons, five exhibited 5-HT-activated current, which was potentiated by 63 ± 15% in the presence of 50 mM EtOH, whereas EtOH potentiated GABA-activated

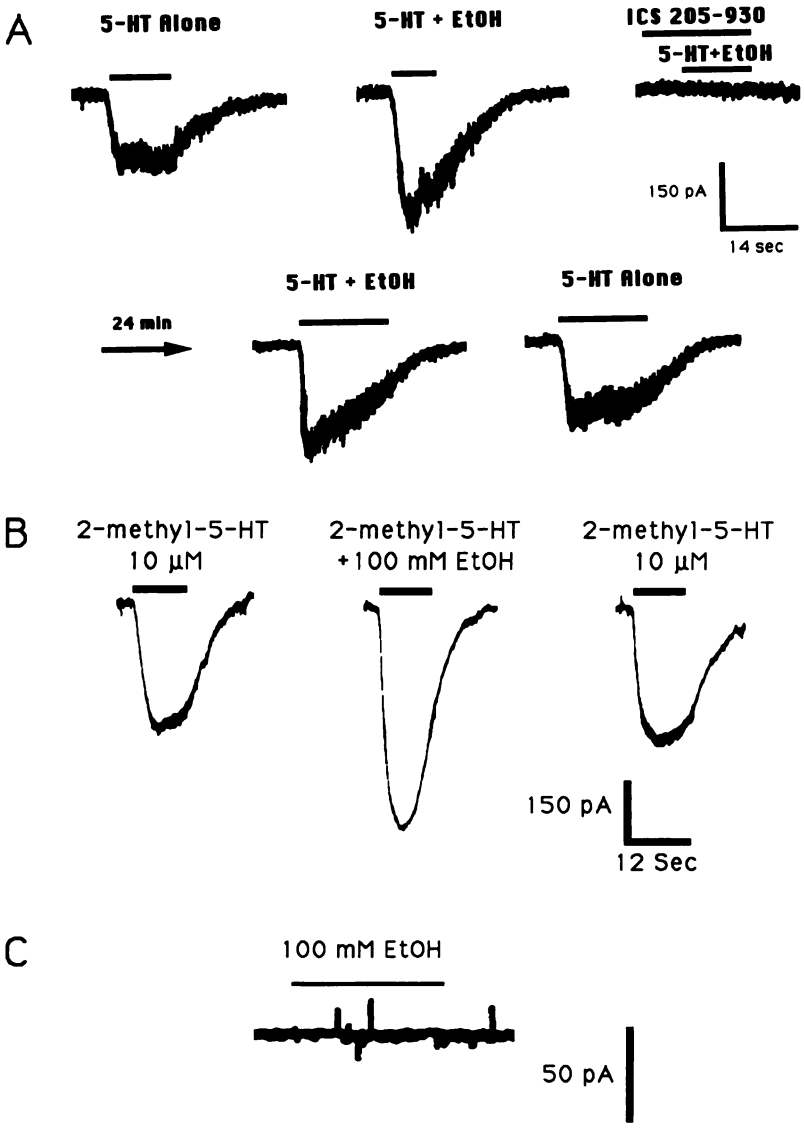

Fig. 3. EtOH potentiation is due to an alteration in 5-HT_3 receptor function. A, Current activated by 1 μM 5-HT before EtOH application, during application of 50 mM EtOH, during application of EtOH in the presence of the 5-HT_3 receptor antagonist ICS 205-930 (50 nM), during application of EtOH after removal of ICS 205-930, and after removal of EtOH. Note the complete block of the response in the presence of the antagonist. This experiment was performed in an isolated nodose ganglion neuron. B, Current activated by the 5-HT_3 receptor agonist 2-methyl-5-HT (10 μM) before, during, and after application of 100 mM EtOH. Recordings are from an NCB-20 neuroblastoma cell. C, Current record during recording from a voltage-clamped nodose ganglion neuron before, during, and after application of 100 mM EtOH in the absence of agonist.

current by only 2 ± 1% (paired $t = 4.28$, $df = 4$, $p > 0.01$). The other neuron did not respond to 5-HT.

It has generally been observed that alcohols of varying carbon chain length have similar behavioral effects but vary in their potency, as predicted by the Meyer-Overton relation (22–24). This relation shows that the potency of alcohols for producing intoxication or anesthesia increases with increasing carbon chain length. We thus wished to determine whether MetOH would potentiate 5-HT-activated ion current in nodose ganglion neurons and whether the potency of this alcohol differed from that of EtOH in a manner consistent with the Meyer-Overton relation. The records in Fig. 5A show 5-HT-activated ion current before, during, and after the application of 100 and 200 mM MetOH. It can be seen from these records that the higher concentration of MetOH produced potentiation of 5-HT-activated current amplitude, whereas the lower concentration had little effect. Potentiation by 200 mM MetOH was observed in seven of nine neurons examined (repeated measures $t = 4.07$, $df = 8$, $p < 0.005$). Fig. 5B plots percentage of potentiation of 5-HT-activated current in the presence of different concentrations (50, 100, and 200 mM) of MetOH. Significant potentiation was not observed in the presence of 50 or 100 mM MetOH (50 mM: repeated measures $t = 1.75$, $p > 0.10$; $df = 3$, 100 mM: $t = 1.95$, $p > 0.05$, $df = 3$). These observations indicate that 5-HT-activated current is potentiated in the presence of MetOH but that the effective concentrations are higher than the effective concentrations of EtOH.

EtOH might produce potentiation by increasing the apparent potency of 5-HT for activation of current. To determine the potency of 5-HT, we measured current activated by different concentrations of 5-HT (1, 2, and 10 μM), in the presence and absence of 50 mM EtOH, using NCB-20 neuroblastoma cells. The records in Fig. 6A show ion current activated by 1 μM 5-HT before, during, and after application of 50 mM EtOH (Fig. 6A, *upper*) and current activated by 10 μM 5-HT under the same conditions in the same cell (Fig. 6A, *lower*). It can be seen that EtOH potentiated current activated by the lower but not the higher concentration of 5-HT. Fig. 6B plots the percentage of potentiation of current by 50 mM EtOH as a function of 5-HT concentration. The magnitude of potentiation by EtOH decreased with increasing agonist concentration, and the ED_{50} for 5-HT activation of current (estimated from log concentra-

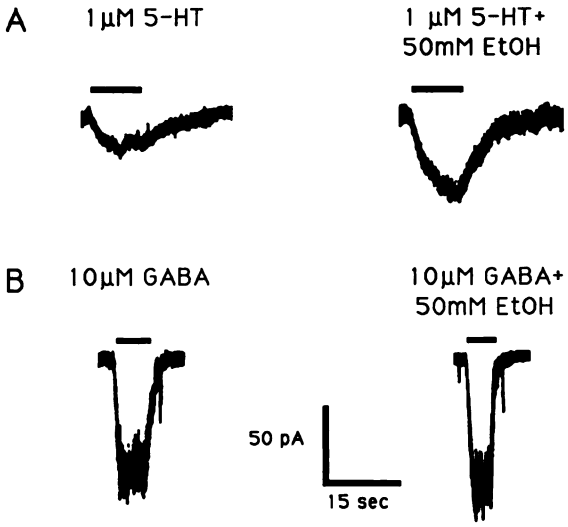

Fig. 4. EtOH potentiation is selective for 5-HT$_3$ receptors relative to GABA$_A$ receptors. A, Current activated by application of 1 μM 5-HT to an isolated nodose ganglion neuron before and during application of 50 mM EtOH. B, Current activated by application of 10 μM GABA to the same neuron before and during EtOH treatment. Calibrations in B apply to both traces.

tion-effect curves in the absence and presence of EtOH) decreased from 3.2 μM to 2.3 μM in the presence of 50 mM EtOH.

The records in Fig. 7 show ion current activated by a prolonged (35-sec) application of 2 μM 5-HT before (Fig. 7, *left*), during (Fig. 7, *center*), and after (Fig. 7, *right*) exposure to 100 mM EtOH. It can be seen from these records that the rate of decay of ion current in the presence of a given concentration of agonist increased in the presence of EtOH. Similar results were observed in three of three cells in which the effects of such prolonged applications of 5-HT were examined.

We also examined the effect of EtOH on the reversal potential of 5-HT$_3$ receptor-mediated ion current, to determine whether EtOH alters the ion selectivity of the receptor-linked ionophore. The records in Fig. 8A show ion current activated by 5-HT application to an NCB-20 cell before, during, and after application of 50 mM EtOH. In each case, a voltage ramp was applied to the recording pipette to change the membrane potential over a range from −60 to +60 mV during 5-HT application. Fig. 8B plots the current elicited by the voltage ramp as a function of membrane potential, for responses recorded before and during EtOH application. It can be seen from this graph that the polarity of 5-HT-activated current reversed at approximately the same membrane potential in the absence and presence of EtOH. Similar results were observed in three of three cells tested. It is also worth noting that the percentage of potentiation of current in the presence of EtOH did not differ at different membrane potentials. These data are in accord with the results of experiments in which the effect of EtOH on 5-HT-activated ion current was examined while the membrane potential was held at different levels for minutes (data not shown). Namely, EtOH potentiation was of similar magnitude at membrane potentials negative and positive to 0 mV. Thus, EtOH potentiation of 5-HT$_3$ receptor-mediated ion current seems to be independent of membrane potential.

Discussion

The present study confirms the previous observation that 5-HT$_3$ receptor-mediated current is potentiated in the presence of intoxicating concentrations of EtOH (10) and extends these observations by demonstrating 1) that EtOH produces an apparent increase in the potency of 5-HT for activation of current, 2) that EtOH potentiates 5-HT$_3$ receptor-mediated current in isolated neurons from adult mammals, and 3) that this effect of EtOH is selective for 5-HT$_3$ receptors, relative to GABA$_A$ receptors on the same neurons. The observation that EtOH potentiates 5-HT$_3$ receptor-mediated current in isolated neurons from adult rat nodose ganglion indicates that this phenomenon occurs in mammalian neurons and is, thus, not restricted to neuroblastoma cells. EtOH potentiation of 5-HT$_3$ receptor-mediated ion current occurs over a similar range of EtOH concentrations in both cell types. In light of recent observations indicating differences in the biophysical and pharmacological

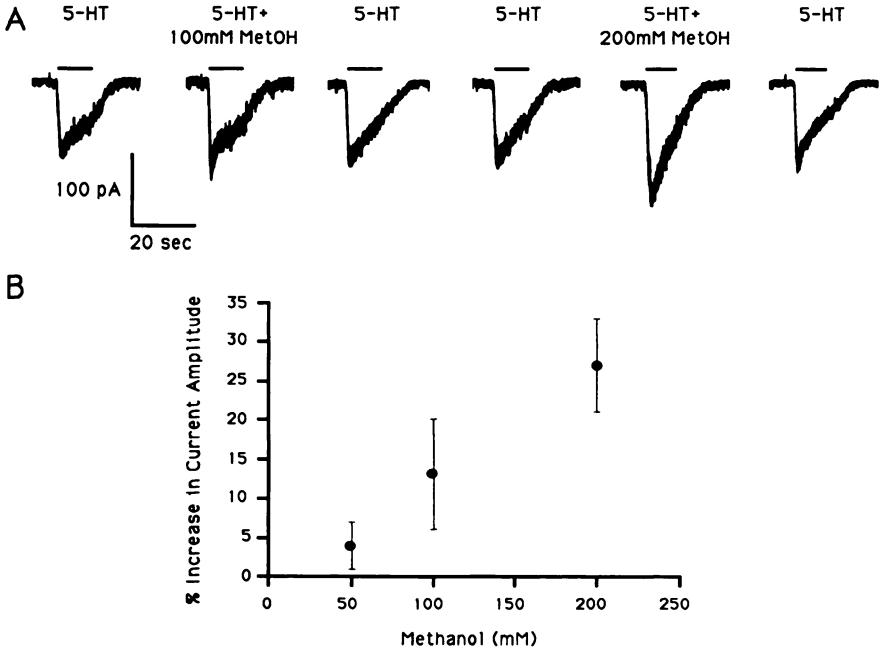

Fig. 5. MetOH potentiation of 5-HT$_3$ receptor-mediated current. A, Current activated by 1 μM 5-HT in a single nodose ganglion neuron before, during, and after application of 100 mM (*left three traces*) and 200 mM (*right three traces*) MetOH. B, Graph plotting the percentage of potentiation of current amplitude as a function of MetOH concentration. Note the lower apparent potency of MetOH, relative to EtOH (compare with Fig. 2C). Values are mean ± standard error. Data are from isolated nodose ganglion neurons (number of neurons: 50 mM, 4; 100 mM, 4; 200 mM, 9).

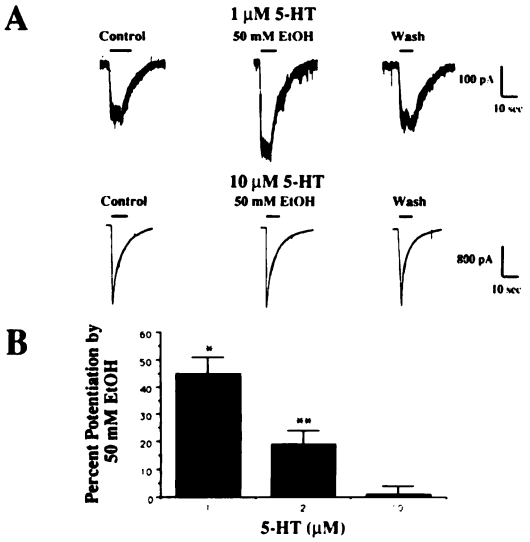

Fig. 6. Potentiation by EtOH decreases with increasing agonist concentration. A, Current activated by 1 μM (*upper*) or 10 μM (*lower*) 5-HT before, during, and after application of 50 mM EtOH. Records are from the same cell. B, Graph plotting percentage of potentiation of 5-HT-activated ion current by 50 mM EtOH as a function of 5-HT concentration. Values are mean ± standard error. All recordings and data in this figure are from NCB-20 neuroblastoma cells (number of cells: 1 μM, 29; 2 μM, 17; 10 μM, 8). $^{*}p < 0.01$, $^{**}p < 0.05$ relative to baseline by repeated measures.

properties of 5-HT_3 receptors in different cell types (17, 21, 25), this is an important observation.

The observation that EtOH potentiates ion current activated by low but not high concentrations of 5-HT provides the first evidence of an effect of EtOH on agonist potency at this receptor. The molecular mechanisms underlying this change in potency may be explained in a number of ways. First, it is possible that EtOH may increase the affinity of 5-HT for its recognition site. However, recent studies of EtOH effects on radioligand binding to the 5-HT recognition site on this receptor suggest that EtOH has little effect on antagonist affinity or the potency of agonists for displacing antagonist binding at EtOH concentrations up to 100 mM (26). Second, EtOH may increase the probability of opening of 5-HT_3 receptor-associated ion channels. Such a change could account for the larger increase in current amplitude at lower agonist concentrations, where receptor occupancy and probability of channel opening are low under basal conditions. Third, EtOH may increase the rate of receptor desensitization. Indeed, faster decay of current activated by low concentrations of 5-HT was often observed in the presence of EtOH, as shown. Thus, current activated by high concentrations of 5-HT in the presence of EtOH might desensitize at a rate faster than our present experimental system is able to detect. The EtOH-induced increase in current amplitude may be undetectable under such conditions.

The magnitude of EtOH potentiation remained stable over repeated applications in both cells types. Thus, this effect of EtOH exhibits little "short term" tolerance.

It is important to note that EtOH did not alter 5-HT-activated current in a subset of neuroblastoma cells and isolated neurons. This observation suggests that certain subtypes or states of the receptor may be insensitive to the actions of EtOH. The heterogeneity of EtOH sensitivity of 5-HT_3 receptors may, thus, be similar to that already described for $GABA_A$ receptors (2, 4).

The observation that EtOH potentiates 5-HT-activated ion current without altering current mediated by $GABA_A$ receptors indicates that there is some selectivity to the effect of EtOH on ligand-gated ion channels in nodose ganglion neurons. The selectivity of this action of EtOH is further emphasized by previous observations that EtOH has little effect on the properties of voltage-activated Ca^{2+} currents in isolated nodose ganglion neurons, even at a 100 mM concentration.[2] Furthermore, selective EtOH effects on receptor-activated ion currents in hippocampal (6, 27) and dorsal root ganglion neurons (28) have been observed. These observations indicate that EtOH does not nonselectively alter the properties of ion currents in neurons. Indeed, alterations in the activity of individual neurons in the presence of EtOH likely reflect the net effect of one or a few actions of EtOH on neuronal ion channels and/or synaptic transmission.

The observation that EtOH does not alter $GABA_A$ receptor-mediated ion current in nodose ganglion neurons is consistent with our previous observations that $GABA_A$ receptors on sensory neurons from adult rat are insensitive to EtOH (28). In one recent study, EtOH potentiation of $GABA_A$ receptor-mediated ion current was observed in fetal rat sensory neurons grown in culture (29). This apparent difference in EtOH sensitivity might reflect differences in the subunit composition of $GABA_A$ receptors expressed in fetal and adult sensory neurons or differences in the development of these receptors in culture and *in vivo*. It should be noted that intoxicating concentrations of EtOH potentiate responses activated by $GABA_A$ receptors in certain neural preparations (1–4). One recent study indicates that EtOH potentiates $GABA_A$ receptor-mediated current in *Xenopus laevis* oocytes injected with brain mRNA from alcohol-sensitive but not alcohol-insensitive mice (4). This important finding strongly suggests that molecular differences in $GABA_A$

[2] S. R. Ikeda, personal communication to D.M.L.

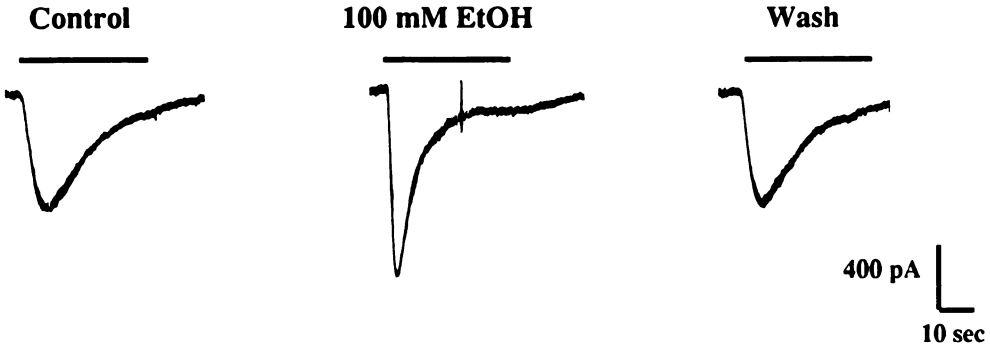

Fig. 7. Increased decay rate of 5-HT-activated current in the presence of EtOH. Current activated by a prolonged (35-sec) application of 2 μM EtOH to an NCB-20 neuroblastoma before, during, and after application of 100 mM EtOH. Note the increase in current offset in the presence of 5-HT plus EtOH.

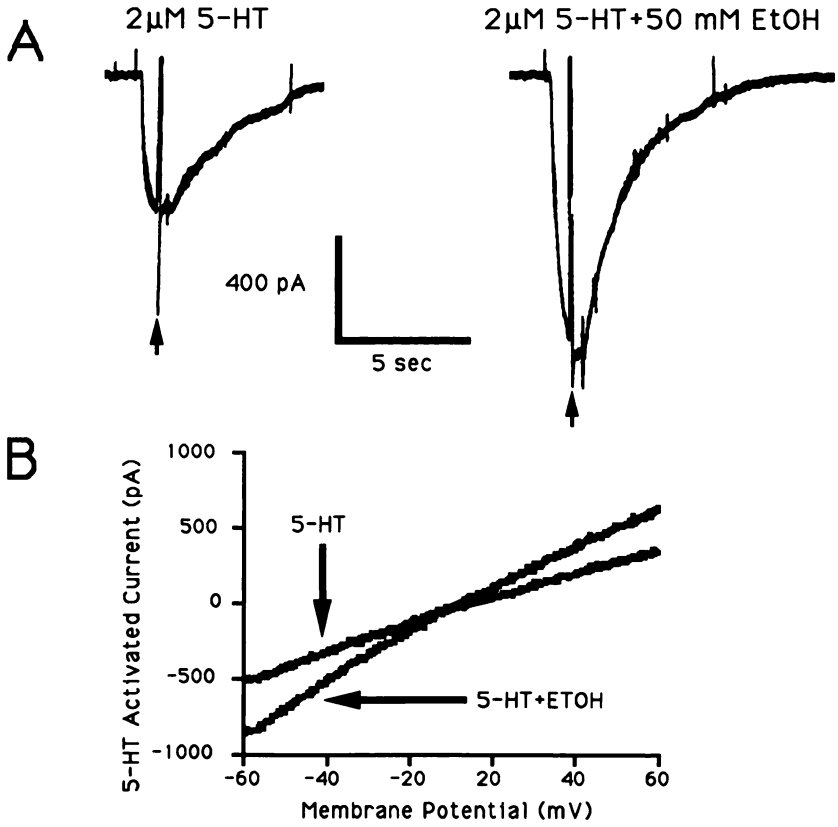

Fig. 8. EtOH does not alter the reversal potential of 5-HT-activated current. A, Current activated by application of 2 μM 5-HT to an NCB-20 neuroblastoma cell before and during application of 50 mM EtOH. At the peak of current, a voltage ramp (125 mV/sec rate) was applied to change the cell membrane potential from −60 to +60 mV. B, Graph plotting the amplitude of 5-HT-activated current as a function of membrane potential in the presence and absence of EtOH. Data are from the cell in A. Note that current reverses from inward- to outward-going at a similar membrane potential under both conditions.

receptors account for their differential EtOH sensitivity. Such variations in sensitivity could arise from differences in subunit composition of receptors expressed *in vivo*.

The increased decay rate of current in the presence of EtOH could result from a direct effect of EtOH on "fast desensitization" of the receptor or an indirect effect due to increased current amplitude. The contribution of current amplitude to rate of decay is not well described for these receptors, and thus it is difficult to choose between these hypotheses. It should be noted, however, that the decay rate of 5-HT-activated current was not increased in the presence of EtOH in cells in which EtOH did not potentiate current amplitude. Furthermore, the increase in decay rate varied in magnitude even among cells in which clear potentiation of current amplitude was observed (compare EtOH responses in Fig. 6A with those in Fig. 7). The increase in decay rate may be a secondary consequence of the increase in current amplitude or a separate effect of EtOH that is observed less consistently than amplitude potentiation.

MetOH appears to potentiate 5-HT$_3$ receptor-mediated ion current with a potency lower than that of EtOH. The observed order of potency of these alcohols is consistent with the idea that the potency of alcohols for potentiating current increases with increasing hydrophobicity. It is well known that the potency of alcohols for producing intoxication increases with increasing hydrophobicity (22). Thus, the order of potency of EtOH and MetOH for potentiating 5-HT$_3$ receptor-mediated ion current is consistent with a role for this effect of EtOH in intoxication. Such a role is supported by evidence from a recent study of EtOH discrimination (30). This study indicates that selective 5-HT$_3$ receptor antagonists can block the subjective recognition of EtOH intoxication, with the relative potencies of the drugs indicating that the effect takes place at the 5-HT$_3$ receptor. It has also been suggested that 5-HT$_3$ receptors may play a role in the reinforcing effects of EtOH, based on their ability to regulate dopamine release in the nucleus accumbens (31, 32). Preliminary results also suggest that 5-HT$_3$ receptor antagonists can reduce EtOH consumption in humans (33). Further studies are needed to clarify the contribution of these receptors to intoxication and long term alcohol abuse.

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