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Ethanol and trichloroethanol alter gating of 5-HT_3 receptor-channels in NCB-20 neuroblastoma cells

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Abstract

Alcohol potentiation of 5-HT_3 receptors was examined in NCB-20 neuroblastoma cells using whole-cell patch-clamp electrophysiological techniques. Activation of the receptor with the weak partial agonist dopamine (DA) was used to examine alcohol effects under conditions of full agonist occupancy, but low probability of channel opening. Dopamine activation of the receptor increased in a concentration-dependent manner (EC_{50}=0.28 mM), and on average maximal responses to DA were 8.0±0.8% of the maximal response to 5-HT. Ethanol (EtOH) and trichloroethanol (TCEt) potentiated DA-activated ion current mediated by 5-HT_3 receptors. Potentiation of responses to a maximally effective dopamine concentration averaged 52.0±8.0% for EtOH and 567±43% for TCEt, which was comparable to the potentiation observed when receptors were activated by a low concentration of 5-HT. The alcohols increased both the potency and efficacy with which dopamine activated the receptor. The observation that alcohols increase the maximal efficacy of dopamine activation of the receptor indicates that one action of alcohols on the 5-HT_3 receptor is to increase the probability of channel opening independent of any effect on agonist affinity.

Keywords: Serotonin; Alcohol; Ligand-gated ion channel; Partial agonist; Neuroblastoma cells

1. Introduction

Ethanol (EtOH) and other alcohols potentiate the function of 5-HT_3 receptors at pharmacologically relevant concentrations (Lovinger, 1991; Lovinger and Zhou, 1999). Potentiation has been observed in neuroblastoma cells, peripheral neurons isolated from adult rat, and heterologous host cells expressing recombinant receptors (Barann et al., 1995; Jenkins et al., 1996; Machu and Harris, 1994; Lovinger, 1991; Lovinger and White, 1991; Lovinger and Zhou 1993, 1994). There is also a growing body of evidence indicating that 5-HT_3 receptors participate in the intoxicating effects of EtOH, as well as in EtOH consummatory behavior and the reinforcing effects of alcohol (Fadda et al., 1991; Grant, 1995; Johnson et al., 1993; LeMarquand et al., 1994a,b; Sellers et al., 1994; Tomkins et al., 1995). Thus, it is important to understand the mechanisms by which acute alcohol exposure alters receptor function.

Alcohols potentiate the function of the receptor by favoring and stabilizing the open channel state (Zhou et al., 1998). This action is similar to that of EtOH on the nicotinic ACh (nACh) receptor (Wu et al., 1993). The potency with which 5-HT activates the 5-HT_3 receptor is enhanced in the presence of alcohols (Lovinger and White, 1991; Lovinger and Zhou, 1993). Thus, potentiation is observed only at relatively low concentrations of this agonist and not in the presence of higher agonist concentrations. This characteristic of alcohol effects is also similar to effects of short-chain alcohols on the nACh receptor (Liu et al., 1994). The increase in agonist potency could result from a few different alcohol actions, most prominent of which are an increase in the affinity of the agonist for the receptor, or an increase in the probability of channel opening (P_o) in the presence of agonist independent of changes in agonist affinity. These are somewhat difficult mechanisms to disentangle.

Abbreviations: Ethanol (EtOH); Trichloroethanol (TCEt); Dopamine (DA); Probability of opening (P_o).

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For example, one might hypothesize that alcohol effects on agonist affinity could be assayed separately from channel gating in radioligand binding assays. Indeed, EtOH does not appear to alter agonist displacement of 5-HT_3 antagonist binding (Hellevuo et al., 1991), suggesting no change in agonist affinity. However, this result is complicated by the fact that radioligand binding is performed under equilibrium conditions in which receptors are predominantly in the desensitized/high affinity state (Bartrup and Newberry, 1996; Colquhoun, 1998), and alcohols appear to have smaller effects on channel openings from this state (Lovinger and Zhou, 1993). Trichloroethanol (TCEt) does enhance the apparent affinity of agonists for the 5-HT_3 receptor (Downie et al., 1995). However, agonist binding is not independent of channel state since open and desensitized receptors are necessarily agonist bound, and thus any effect that stabilizes these states will increase apparent agonist affinity. It is therefore necessary to use different approaches to separate alcohol effects on agonist affinity and P_o.

Changes in affinity can be separated from alterations in channel gating by examination of burst patterns of single ligand-gated ion channels (Colquhoun and Hawkes, 1995). Unfortunately, this approach has proven difficult to apply to studies of the 5-HT_3 receptor in neuroblastoma cells because of the unusually small single channel-conductance of this receptor-channel (Jackson and Yakel, 1995). One approach that has been used to study nACh receptors is examination of alterations in the response to a weak partial agonist (Wu et al., 1993). If the agonist fully occupies the serotonin binding site on the 5-HT_3 receptor, but activates the associated channel with a low P_o, then one can test the hypothesis that a substance increases the P_o independent of changes in agonist affinity. In this case an increase in current amplitude in the presence of a maximally effective concentration of the partial agonist would provide evidence of an increase in P_o.

We have used dopamine (DA), a weak partial 5-HT_3 receptor agonist, to test the hypothesis that EtOH and TCEt increase P_o independent of changes in agonist affinity. Our findings indicate that both alcohols increase responses to a maximally effective concentration of DA, and thus appear to increase P_o.

2. Methods

2.1. Cell maintenance

NCB-20 neuroblastoma cells were maintained in culture as previously described (Zhou and Lovinger, 1996). Frozen cell stocks were maintained in liquid nitrogen, and thawed as needed. Cells used for electrophysiological experiments were maintained as previously described (Lovinger, 1991). Cells were seeded onto 35 mm culture dishes at least 2 days prior to electrophysiological examination.

2.2. Whole-cell patch-clamp recording

Whole-cell patch-clamp recordings were performed in 35 mm culture dishes at room temperature on the stage of an inverted microscope as described previously (Zhou and Lovinger, 1996; Zhou et al., 1998). The extracellular solution bathing cells contained (mM): 150 NaCl, 2.5 KCl, 2.5 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 10 D-glucose (pH adjusted to 7.4 with NaOH and osmolality adjusted to 340 mosm/kg with sucrose). This solution constantly superfused cells at a rate of 2–3 ml/min. Patch pipettes had resistances of 1–2 MΩ when filled with (mM): 140 CsCl, 10 Cs-EGTA and 10 HEPES (pH adjusted to 7.4 with CsOH, osmolality adjusted to 310 with sucrose). In the majority of experiments, whole-cell current was amplified using an Axopatch 200 or Axopatch 1D patch-clamp amplifier (Axon Instruments, Foster City, CA) and saved on a 486 or pentium microcomputer using pClamp v5.5 or v6.0 software (Axon Instruments, Foster City, CA). In experiments examining receptor kinetics, currents were recorded using an Axopatch 200 amplifier, digitized using an ITC-16 analog/digital interface and stored on a Macintosh IIfx computer using IGOR software (Wavemetrics Inc., Lake Oswego, OR). Currents were low-pass filtered at 5 kHz using a 3 pole Bessel filter, and digitized at up to 10 kHz.

Current amplitude was measured using pClamp v5.5 or v6.0 software by determining the difference in current at time points set by cursors positioned to measure current prior to the onset of agonist application, and at the peak of current during agonist application. Current amplitude was measured in a similar manner using IGOR software. The peak amplitudes of the responses to DA were used to construct DA concentration response curves. The 10–20% slope of current (initial slope, a measure of channel activation, Zhou et al., 1998) was measured using IGOR software. First, the peak current amplitude was calculated as described. Then cursors were positioned to determine time points at which current amplitude was 10 and 20% of maximal. Linear regression was then performed using routines in IGOR to estimate the slope of current between the two cursors ($I_{10–20\%}/t_{10–20\%}$). The time constant (τ) of current decay after agonist removal was measured using IGOR software as previously described (Zhou et al., 1998). Averaged data presented in the text and figures are mean±SEM values.

2.3. Drug application

In the majority of experiments, agonists and other drugs were applied using methodology similar to that

employed in past studies (Zhou and Lovinger, 1996). Drugs were delivered from individual HPLC tubes of ~300 μm inner diameter, which were arranged in a linear array. Solution flow was driven by gravity from wells placed above the preparation. Solution application was gated by valves placed upstream of the drug-containing tubing. The tips of the drug application tubes were placed within 50 μm of the cell under study, allowing for solution exchange times on the order of 150–200 ms. Agonists and other drugs were diluted in extracellular solution from stock solutions in DMSO or dH_20. The high affinity 5-HT_3 receptor antagonist zacopride was delivered for 5–10 s in the absence of agonist, and this application procedure was sufficient to produce receptor antagonism lasting for tens of minutes, as previously reported (Lovinger and Zhou, 1994). In experiments designed to examine alcohol actions, the alcohols were diluted into agonist-containing extracellular solution, and were delivered simultaneously with agonist. We have previously determined that pre-exposure to alcohols does not increase potentiation of receptor function relative to simultaneous drug and agonist application (Lovinger and Zhou, 1993). Presumably, alcohol equilibrates at its site of action well before full current activation.

Experiments examining the initial slope and post-agonist decay of agonist-induced current were performed using a system that allows for more rapid drug application, as previously described (Zhou et al., 1998). Extracellular solution and agonist-containing extracellular solution flowed through different sides of theta tubing pulled to an inner diameter of >500 μm. After successfully obtaining a whole-cell recording from an NCB-20 cell, the cell was lifted from the bottom of the dish and placed in front of the stream of extracellular solution coming from one side of the theta tubing. Solution was rapidly switched around the cell using a piezoelectric-driven micromanipulator that displaced the tubing laterally so that the cell could be bathed for a defined period of time in drug-containing solution, and then rapidly returned to drug-free solution. Switching times in the whole-cell mode ranged from 10 to 30 ms depending primarily on the cell circumference. We have previously demonstrated that such switching times allow for adequate resolution of the initial slope of current activated by low to moderate concentrations of 5-HT and current decay following agonist removal (Zhou et al., 1998). Agonist flow was driven through the theta tubing by gravity from wells placed above the preparation.

3. Results

3.1. Dopamine is a partial agonist of the 5-HT_3 receptor

We have confirmed in NCB-20 neuroblastoma cells, as previously demonstrated in other cells, that DA at high concentrations acts as a partial 5-HT_3 receptor agonist. Application of DA (0.1–1 mM) to NCB-20 cells voltage-clamped at −60 mV produced an inward current [Fig. 1(A)]. Current amplitude increased with ascending concentrations of DA, with maximal current observed at 1 mM DA [Fig. 1(B)]. The potency of DA and the fact that a maximal effect was observed at 1 mM DA in our cells was similar to observations made in N1E-115 cells (Van Hooft and Vijverberg, 1996). The amplitude of the maximal response to DA was consistently much smaller than the response to an $EC_{95–100}$ concentration of 5-HT with the current evoked by 1 mM DA averaging 8±0.8% of the current activated by 40 μM 5-HT. This relative maximal effect was somewhat less than that previously reported in N1E-115 cells (Neijt et al., 1986; Van Hooft and Vijverberg, 1996). Current activated by 1 mM DA exhibited a reasonably slow rise time and either no decay or a small, slow decay during agonist application lasting 10 s or more. In this respect, the DA-activated current resembled current activated by a low concentration of 5-HT.

The current activated by DA was indeed mediated by

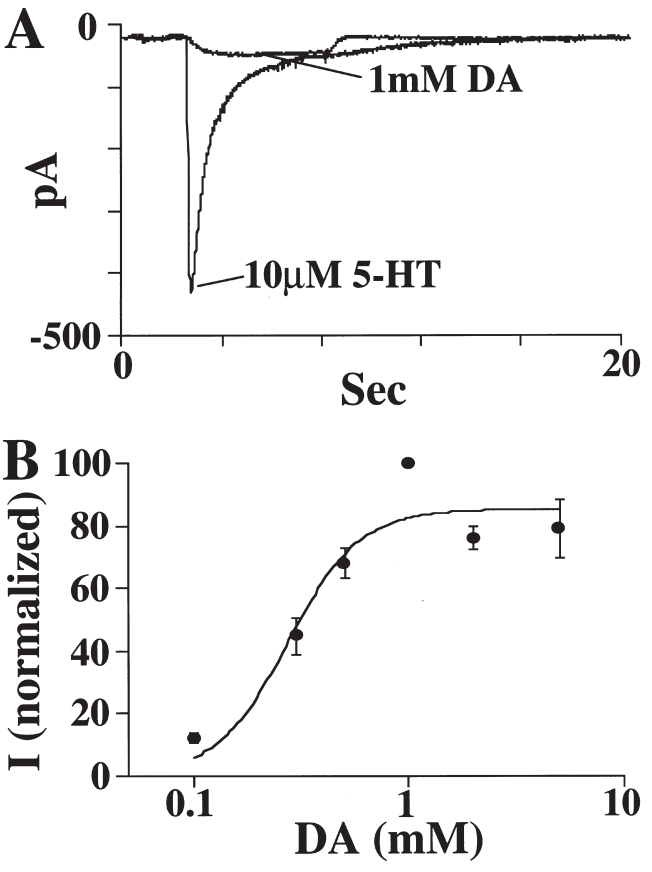

Fig. 1. Dopamine (DA) activation of ion current in NCB-20 cells. (A) Inward current activated in a single NCB-20 cell by the indicated concentrations of 5-HT and DA. Note the relatively small amplitude of DA-activated current. (B) Amplitude of inward current as a function of DA concentration (EC_{50}=0.28 mM, Hill slope=2.63, *n*=6–10 cells per DA concentration). Note that 1 mM DA produces maximal current.

the 5-HT_3 receptor since DA-induced responses were completely blocked in the in the presence of 50 nM zacopride, a potent and highly selective 5-HT_3 antagonist [Fig. 2(A)]. Furthermore, DA and 5-HT showed evidence of competition for the same agonist site on the receptor. Fig. 2(B) plots normalized ion current amplitude in the presence of a given concentration of 5-HT (3 μM, left) or DA (1 mM, right) as the concentration of the other agonist was increased. It is clearly demonstrated that the response to 5-HT is completely occluded in the presence of a concentration of DA (1 mM) that produces the maximal response on the DA concentration–response curve [see Fig. 1(B)]. This finding indicates that DA can fully compete with 5-HT in producing receptor activation. It can also be seen that current amplitude increases as the concentration of 5-HT is increased in the presence of a fixed concentration of DA. This finding indicates that inhibition by DA is competitive and can be overcome by increasing the concentration of 5-HT. Thus, DA satisfies the criteria for a weak partial agonist and can be used to test the hypothesis we have proposed.

Many partial agonists of the nACh receptor are low potency channel blockers as well (Wu et al., 1993). We wanted to determine if there was any evidence consistent with a DA channel blocking action on the 5-HT_3 receptor. DA should be positively charged at pH 7.4, and thus any open channel block would likely be stronger at nega-

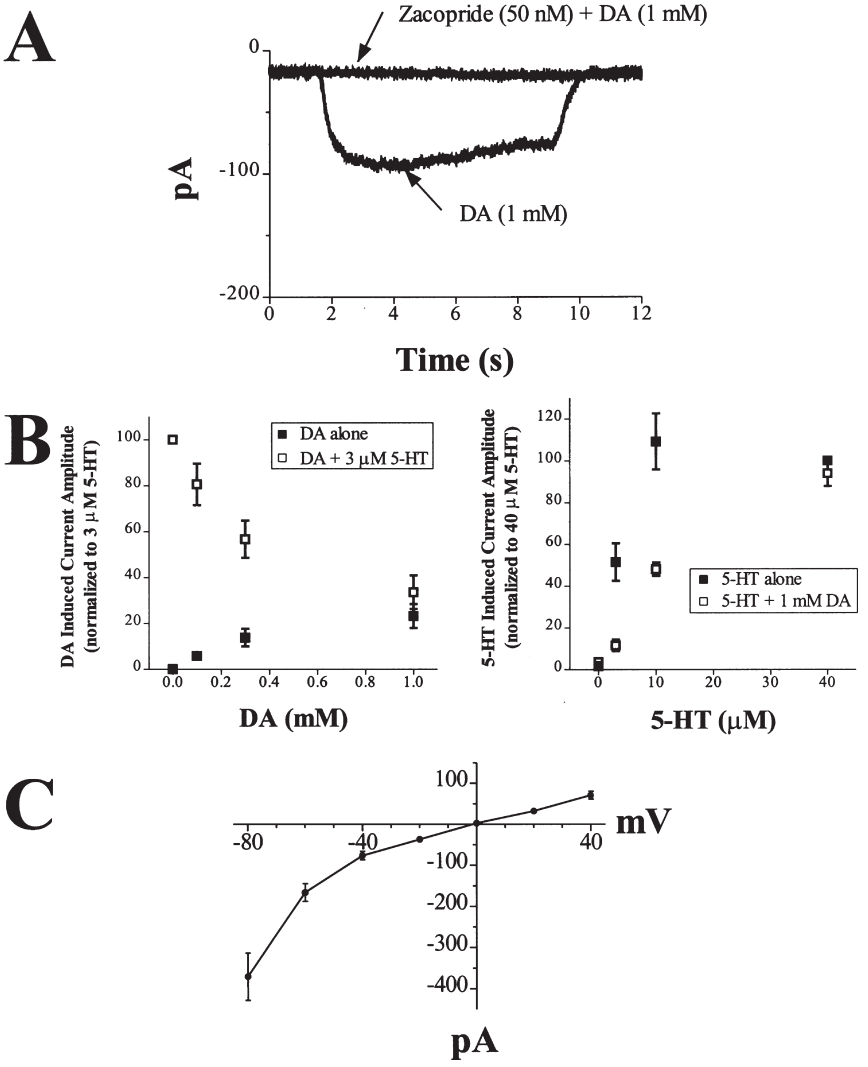

Fig. 2. DA activates 5-HT_3 receptors on NCB-20 cells. (A) Current activated by 1 mM DA in the absence of antagonist and immediately following a 10 s exposure to 50 nM zacopride. Note the complete block of DA activated current by zacopride. (B) Lefthand graph plots the amplitude of current activated by 3 μM 5-HT as the concentration of DA is increased over the indicated range. Current amplitude was normalized to that produced by 3 μM 5-HT alone in each cell. Note the decrease in amplitude of 5-HT activated current indicative of DA antagonism of 5-HT actions (n=5 cells). Righthand graph plots the amplitude of current activated by 1 mM DA as the concentration of 5-HT is increased over the indicated range. Current was normalized to that produced by 40 μM 5-HT alone in each cell. Note the increase in current amplitude as the 5-HT concentration increases, indicative of 5-HT competition with DA for the agonist binding site on the receptor. (C) Amplitude of current activated by 1 mM DA plotted as a function of membrane potential. Note the evidence for inward rectification, as described in the text (n=8–12 cells at each voltage).

tive membrane potentials than at more positive potentials. We examined the current/voltage relationship for 1 mM DA-activated current in NCB-20 cells to determine if any evidence of such a voltage-dependent blocking action could be observed. The current activated by DA showed inward rectification as evidenced by the fact that inward current at negative holding potentials was much stronger than the outward current at positive potentials [Fig. 2(C)]. This voltage-sensitivity is similar to that of channel activation by 5-HT (Jackson and Yakel, 1995). The pattern is not consistent with channel block by 1 mM DA which would lead to small current amplitudes at strongly negative membrane potentials at which DA should be attracted into the channel pore.

3.2. Alcohols potentiate dopamine activation of the 5-HT_3 receptor

We next examined the effect of EtOH and TCEt on DA-activated, 5-HT_3 receptor-mediated current. A maximally effective concentration of 1 mM DA was used in these experiments. Application of 10–100 mM EtOH potentiated the peak amplitude of DA-activated current. Maximal potentiation averaged 52±8% above baseline [Fig. 3(A)(B)]. Current activated by 1 mM DA was also potentiated in the presence of 0.3–10 mM TCEt [Fig. 3(C)(D)]. Potentiation by TCEt was greater in magnitude than that produced by EtOH (maximal increase in current amplitude was 567±43% above baseline). The decay of DA-activated current in the presence of 10 mM TCEt was markedly enhanced relative to decay observed in the presence of DA alone or with combinations of DA and lower TCEt concentrations. This is consistent with our previous observation that high concentrations of TCEt (10 and 25 mM) produce a slowly developing inhibition of channel function in addition to the more rapid potentiating effect of the drug (Zhou and Lovinger, 1996; Zhou et al., 1998). Responses to DA in the presence of either 100 mM EtOH or 3 mM TCEt were completely blocked in the presence of 50 nM zacopride. These observations indicate that potentiated current resulted from an increase in 5-HT_3 receptor function and not from an additional effect of the agonist in combination with the alcohols. All of these data indicate that alcohols potentiate 5-HT_3 receptor function when the receptor is activated by the weak partial agonist DA.

Fig. 4 shows concentration response curves for DA in the absence and presence of 3 mM TCEt and 100 mM EtOH. The potency with which DA activated the receptor was increased slightly (less than a 2-fold decrease in EC_{50}) in the presence of each of the alcohols, and the maximal response to DA was also increased by both alcohols, as expected from the data presented above.

To determine if potentiation by alcohols differed when the receptor was activated by different agonists, we examined current activated by low concentrations of 5-HT (0.75–1 μM) or 1 mM DA in the presence and absence of 100 mM EtOH or 3 mM TCEt. Peak currents activated by DA or the low 5-HT concentrations were compared to each other and to current activated by 40

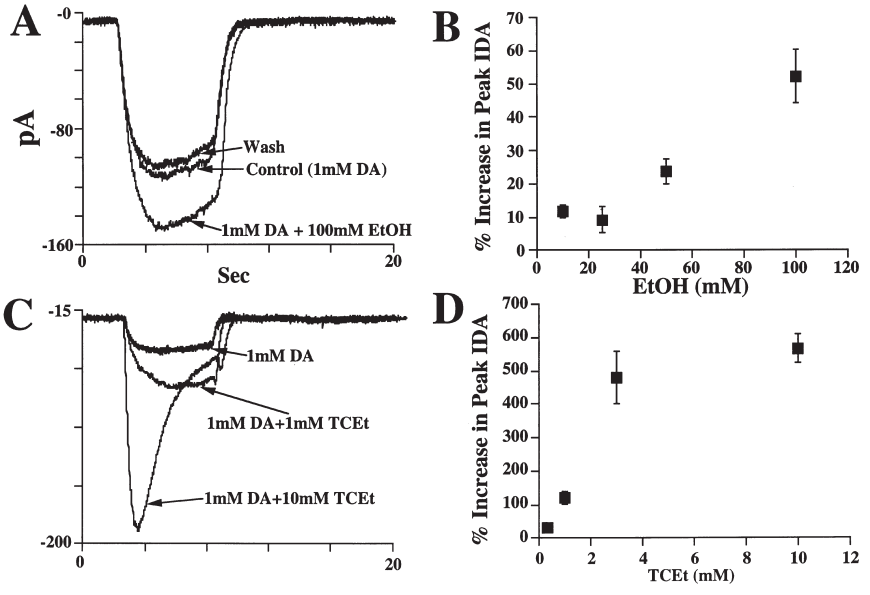

Fig. 3. Alcohols potentiate receptor-channels activated by DA. (A) Current activated by application of DA before (Control), during (1 mM DA+100 mM EtOH) and after (Wash) exposure to EtOH. (B) Potentiation of current activated by 1 mM DA plotted as a function of EtOH concentration (*n*=4–43 cells per EtOH concentration). (C) Current activated by application of DA in the absence of TCEt (1 mM DA), and in the presence of 1 and 10 mM TCEt as indicated. Note the nearly complete overlap of responses to 1 mM DA recorded before beginning TCEt exposure and after washing TCEt away. (D) Potentiation of current activated by 1 mM DA plotted as a function of TCEt concentration (*n*=5–34 cells per TCEt concentration).

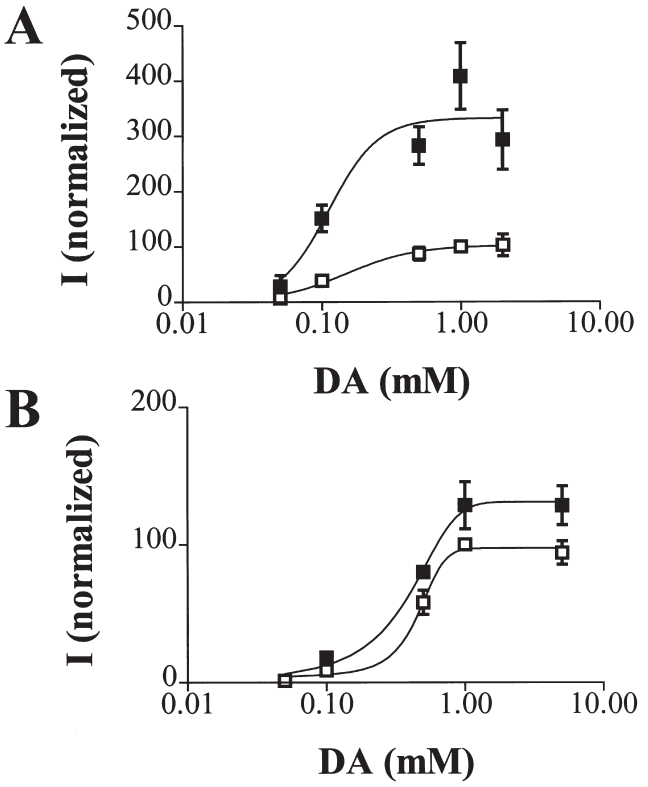

Fig. 4. Effect of alcohols on the DA concentration–response relationship. (A) Relative amplitude of ion current activated by the indicated concentrations of DA in the presence and absence of 3 mM TCEt (n=4–5 cells per data point). (B) Relative amplitude of ion current activated by the indicated DA concentrations in the absence and presence of 100 mM EtOH. Values in both graphs are normalized to the response to 1 mM DA alone (n=4–5 cells per data point).

μM 5-HT to determine the percent of maximal response of each agonist on a cell-by-cell basis. Percent of maximal response was calculated by dividing current activated by a given concentration of agonist by current activated by 40 μM 5-HT. On average, the percent of maximal response to 0.75 μM 5-HT was 4±0.7% while that in the presence of 1 μM 5-HT averaged 8±1.1%. The percent of maximal response averaged 8±0.8% in the presence of 1 mM DA as mentioned above. There was considerable variability between cells and individual 35 mm dishes of cells with respect to this value. Thus, in some cells percent of maximal response produced by DA was similar to that of 0.75 μM 5-HT whereas in other cells the responses of DA and 1 μM 5-HT were more closely matched. Thus, we compared potentiation by DA to the concentration of 5-HT that was closest in percent of maximal response within a given cell. This analysis indicated that neither EtOH nor TCEt potentiation were different in magnitude when DA was used to activate the receptor compared to activation by 5-HT. The percent potentiation by 100 mM EtOH in this subset of cells averaged 22±8% above baseline in the presence of 1 mM DA and 21±7% above baseline in the presence of a concentration of 5-HT with a response similar in magnitude to the DA response in that cell (either 1 or 0.75 μM depending on the cell, t=0.183, P>0.4, n=9 cells). Potentiation by 3 mM TCEt in all cells in which a direct comparison of effects was made averaged 264±48% above baseline in the presence of DA compared to 266±66% in the presence of 5-HT (paired t=0.323, P>0.1 for TCEt, n=14). Thus, the magnitude of potentiation by alcohols does not vary when different agonists are used to activate the receptor when the agonists are applied at concentrations giving similar percent of maximal responses.

Potentiation by EtOH was quite variable in magnitude among different cells, with the magnitude of current in the presence of 100 mM of this alcohol ranging from 95 to 254% of baseline peak current amplitude. This was true whether DA or a low concentration of 5-HT was used to activate the receptor. In some cells EtOH potentiated responses to both agonists but with variable magnitude, while in other cells potentiation was similar for current activated by both agonists. Of the nine cells in which EtOH effects were examined in the presence of both agonists 3 cells showed greater potentiation when DA was the agonist 2 exhibited greater potentiation when activated by 5-HT and the remaining cells showed percent of control values that were similar (i.e. within 5%) in the presence of either agonist. Interestingly, in three cells potentiation by EtOH of greater than 10% above baseline peak current was observed when the receptor was activated by one agonist but not the other. These findings are intriguing in that they indicate that one source contributing to the variability in EtOH potentiation within a given cell is the gating of the channel. As previously observed, TCEt was much more consistent in potentiating 5-HT_3 receptor function. Robust potentiation (150–1053% above baseline values at 3 mM TCEt) was observed in all cells regardless of the agonist used to activate the receptor-channel.

It is possible that alcohol potentiation of DA induced 5-HT_3 receptor function was observed because measurements were made at a time point at which DA-mediated current had not reached steady-state. This could occur if, for example, DA association was considerably slower than association of a low concentration of 5-HT. In this case, potentiation might reflect increased agonist association rate rather than transitions between agonist-bound closed and open states, which would be expected with maximal occupancy at steady-state. However, in rapid application experiments we observed that the initial slope (10–20% rise time) of DA activated current was on average 7.8 times greater than that observed for current activated by 1 μM 5-HT in the same cell (n=9 cells). This result suggests that current activates faster in the presence of DA. We also observed that steady-state current levels were achieved within a few seconds in the presence of either agonist (Fig. 5). This finding, along

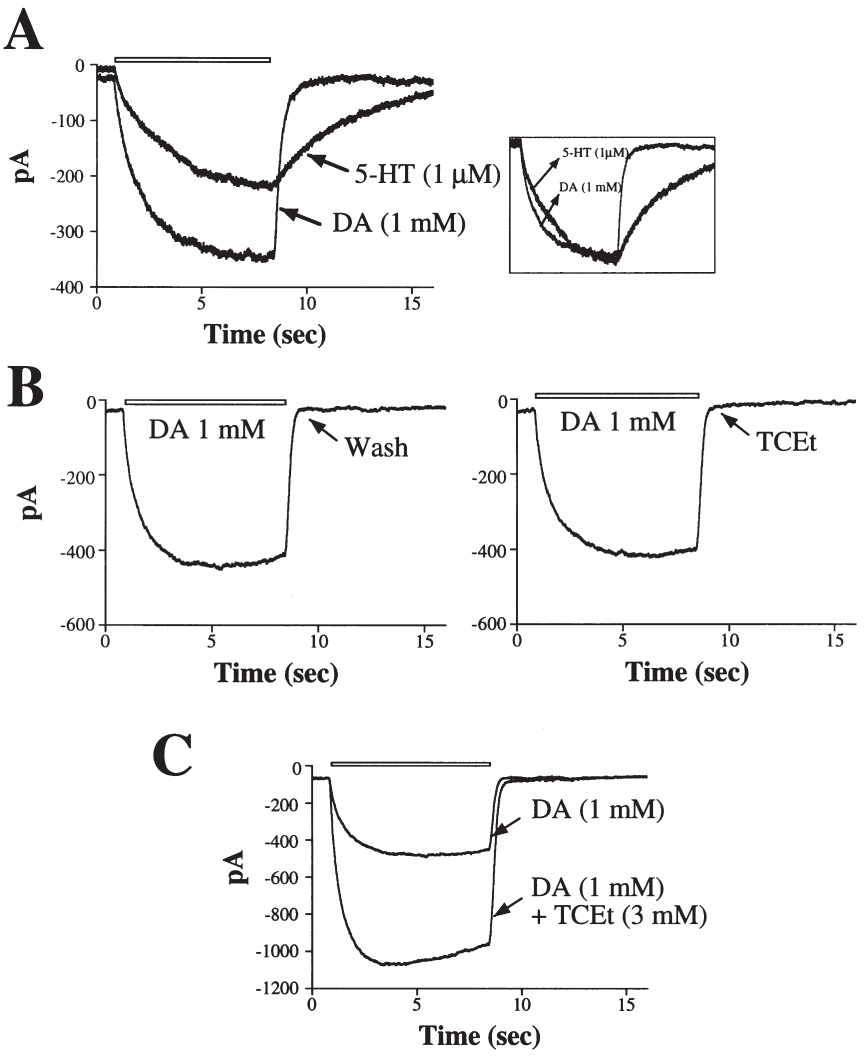

Fig. 5. Receptor activation and deactivation examined using rapid agonist application. (A) Currents activated in the same cell by 1 μM 5-HT and 1 mM DA using rapid solution exchange. Inset shows the two currents overlapped after expansion of the amplitude of the response to 5-HT to allow for better comparison of the current time courses. Note the slower onset of current when 5-HT is the agonist is applied, and the faster deactivation following application of the lower affinity agonist DA. (B) Currents elicited by application of 1 mM DA followed by exposure to standard extracellular solution (Wash, left) or extracellular solution containing 3 mM TCEt (TCEt, right). Note the absence of any apparent tail current when TCEt is present in the wash phase, and the slowing of current decay in the presence of TCEt. (C) Currents elicited by rapid application of DA in the absence and presence of TCEt, as indicated.

with the ability of DA to compete with 5-HT at steady-state, strongly argues that the steady state current, which is potentiated by alcohols, reflects a situation in which the receptor is fully occupied by DA.

Another possible interpretation of our findings with alcohols and DA is that potentiation results from a decrease in the rate of receptor desensitization. Indeed, previous experiments suggested that slowing of desensitization contributes to alcohol potentiation when the receptor is activated by 5-HT (Zhou et al., 1998). Despite the fact that 5-HT_3 receptor-mediated current activated by DA exhibits little decay during 5–10 s application of this agonist, it is possible that steady-state current could reflect an equilibrium condition in which channels open from desensitized as well as "closed" states. The contribution of desensitization to steady-state DA-activated current can be determined using a protocol developed in our previous study (Zhou et al., 1998) in which TCEt is applied only after removal of agonist. Since TCEt promotes channel opening from desensitized states, application of the alcohol following the end of agonist application will result in a "tail" current upon TCEt application since channels will be driven into the open state prior to closing. We previously observed a tail current with a relatively long duration (s) when examining TCEt actions following 5-HT application (Zhou et al., 1998). When this protocol was applied using DA as the agonist we observed either no measurable tail current (8 of 12 cells) when washing away DA using either standard external solution or TCEt [Fig.

5(B)], or a very small, rapidly decaying tail current (4 cells) under both wash conditions. The fact that the small, rapidly decaying current sometimes occurs during agonist removal in standard external solution indicates that this current is probably due to mixing of solutions and is distinct from the slower tail current observed with TCEt exposure after 5-HT application. These observations indicate that steady-state DA-induced current after seconds of agonist application does not involve a large proportion of receptor/channels opening from desensitized states. If a large proportion of receptors was opening in this manner we would have expected a consistent, slow time course tail current following agonist removal in the presence of TCEt.

The decay of current following DA removal was slower in the presence of TCEt than in the presence of standard external solution (τ=0.32±0.03 ms in standard external, and 0.47±0.03 ms in TCEt, paired t=3.82, $P<0.005$, d.f.=9). This finding is consistent with our previous observation that TCEt slows the deactivation/unbinding process of the 5-HT_3 receptor (Zhou et al., 1998). In addition, the initial slope of current (10–20% rise time) was increased in the presence of DA+TCEt relative to that observed in DA alone [Fig. 5(C)] (repeated measures t=4.36, $P<0.005$, d.f.=9), as previously observed using low concentrations of 5-HT (Zhou et al., 1998). This observation is consistent with our previous finding that TCEt increases the rate of activation of the receptor-channel (Zhou et al., 1998).

4. Discussion

We have used a partial 5-HT_3 receptor agonist to begin to separate alcohol effects on agonist occupancy versus gating of the receptor-channel. We selected DA as the partial agonist to be used in these experiments since DA activates the receptor with a low efficacy at a concentration that competes with 5-HT. Furthermore, 5-HT competition with a saturating concentration of DA was demonstrated. These observations indicate that DA interacts with the agonist site on the 5-HT_3 receptor in a competitive manner and only activates the agonist site. We are not certain why DA appears to have a lower efficacy in our studies as compared to the findings of Van Hooft and Vijverberg (1996). This difference may relate to subtle differences in receptors in different neuroblastoma cell lines or in cells in the undifferentiated state (present study) versus differentiated cells (Van Hooft and Vijverberg, 1996).

DA shows no evidence of a noncompetitive or channel blocking action at concentrations up to 1 mM. It is possible that the slight decrease in current observed at DA concentrations higher than 1 mM might be due to channel block. However, these concentrations are higher than those that appear to produce maximal receptor occupancy as judged by concentration–response curves and competition with 5-HT. Likewise, inhibition of the response to 5-HT could not be attributed to desensitization of the current because the current peak was measured a few seconds after the onset of agonist application. At this early time point the magnitude of desensitization is relatively small when DA activates the receptor (Van Hooft and Vijverberg, 1996). Responses to 1 mM DA showed no evidence of a surge current following agonist application, and no evidence of voltage-dependent channel block was observed in the current/voltage relationship of DA-activated current. Current activated by DA was completely blocked in the presence of 50 nM zacopride. This finding is in accord with the previously reported 5-HT_3 antagonist block of DA responses in neuroblastoma cells (Neijt et al., 1986). Antagonism by zacopride was also complete in the presence of alcohols. Thus, the effects of DA and alcohols all take place on the 5-HT_3 receptor and are not the result of nonspecific alcohol effects that sum with the actions of DA. Our findings indicate that EtOH and TCEt potentiate receptor mediated current even when the receptor is fully bound by the weak partial agonist DA.

This finding supports the idea that alcohols potentiate 5-HT_3 receptor function, at least in part, by increasing P_o at a fixed level of receptor occupancy. Since a saturating concentration of DA was used in the experiments reported at present, potentiation by alcohols could not have resulted from an increase in agonist affinity under these conditions. Some caution must be exercised in the interpretation of these findings. First, we cannot exclude an additional effect of alcohols on agonist affinity. This may occur under pre-steady-state conditions or conditions in which receptor occupancy is low, such as in the presence of relatively low concentrations of 5-HT. This mechanism could work in concert with increased P_o to potentiate receptor-channel function. Second, it is possible that the mechanism of potentiation when the receptor is activated by DA is different from that occurring when 5-HT activates the receptor. This has to be true to some extent since P_o is low even when the receptor is fully occupied by DA and is quite a bit higher when 5-HT activates the receptor-channel. However, the macroscopic kinetics of the receptor when activated by DA are not slower than when activated by a low concentration of 5-HT. Thus, it is likely that receptor desensitization/resensitization rates are similar for both agonists, and that activation and deactivation rates are similar when comparing current activated by the low 5-HT concentration and the saturating DA concentration. Furthermore, the experiments with post-agonist application of TCEt, and past studies by Van Hooft and Vijverberg (1996), indicate that little receptor desensitization takes place during applications of DA for the durations used in our experiments. Thus, it is unlikely that potentiation by alcohols in the presence of DA can be

accounted for solely by slowing of the desensitization process. The major difference would be in the fast opening and closing rate constants of the agonist bound receptor that govern microscopic probability of opening. These transitions could well be altered when the receptor is activated by DA and not when 5-HT is the agonist. Resolution of this issue can best be gained with single channel kinetic analysis which is not possible in NCB-20 cells due to the extremely low single channel conductance of 5-HT_3 receptors in this preparation (<1 pS, Jackson and Yakel, 1995). The magnitude of potentiation by alcohols in the presence of DA was similar to that observed in the presence of 0.75–1 μM 5-HT. This observation suggests that increased P_o can account for a substantial proportion of the potentiation by alcohols.

Our observations are consistent with the results of similar studies performed on the nACh receptor. These studies clearly demonstrated EtOH potentiation of receptor activation by a weak partial agonist. These observations, along with other mechanistic similarities noted in a previous paper (Zhou et al., 1998), strongly suggest that the actions of alcohols on the 5-HT_3 and nACh receptors share a common mechanism. It will be interesting to determine if the structural basis of the alcohol effects are similar for the two receptors.

It is possible that potentiation by alcohols could be the result of increased single channel conductance. However, we believe that this mechanism does not contribute to potentiation when 5-HT activates the receptor because potentiation is not observed at all agonist concentrations (as it would be if conductance increased, Zhou et al., 1998), and non-stationary noise analysis indicates no change in single channel conductance in the presence of EtOH (Lovinger and Zhou, 1999). Thus, it does not appear that alcohols increase single channel conductance and it is unlikely that such an increase would be observed only when the receptor is activated by DA.

It should be noted that a previous study described TCEt potentiation of activation of the 5-HT_3 receptor by the partial agonist 2-methyl-5-HT in *Xenopus* oocytes (Downie et al., 1995). This observation is consistent with our findings. However, the properties of this partial agonist were not fully tested in the previous study. We have found that 2-methyl-5-HT has an apparent inhibitory action on 5-HT_3 receptors in NCB-20 neuroblastoma cells. The evidence for this includes the presence of a "surge" current following removal of 200–500 μM of this agonist. This surge current is greater in magnitude at negative than at positive membrane potentials (data not shown). This current is consistent with a voltage-dependent channel block by 2-methyl-5-HT. This blocking mechanism is not too surprising, since the agonist is positively charged at neutral pH, and thus could enter the ion pore. Interestingly, we observed similar evidence of channel block when the receptor was activated by tryptamine (100 μM–1 mM). Thus, these agonists could not be used in these experiments, since they did not behave as pure partial agonists over a relevant concentration range. Dopamine is also positively charged at neutral pH, and thus would be attracted into the channel pore at negative holding potentials. Therefore, the lack of channel block by DA is most likely due to a lower affinity of this compound for the pore-blocking site relative to 2-mehtyl-5-HT and tryptamine. Likewise, we could not employ quipazine in these experiments since whole-cell recordings in NCB-20 cells revealed that this agent appeared to act as a pure antagonist with no discernible partial agonist activity (data not shown). The only agonist that met the criteria for clear partial agonism over a range of concentrations up to receptor saturation was DA.

During the course of these experiments we observed variability of alcohol, and particularly EtOH, effects on current when the receptor was activated by different agonists, even within the same cell. In many of the cells, EtOH produced greater potentiation of current activated by DA than that activated by 5-HT. This was true even when the relative efficacies of the two agonists were approximately the same or when the efficacy of DA was greater than that of 5-HT or vice versa. Thus, the differential alcohol effects in the presence of different agonists could not be accounted for by differences in basal P_o. It is not clear what underlies this difference in sensitivity of the same receptor under different conditions. However, this observation rules out contributions from a number of factors. The most obvious is cell to cell variability. We have noted in the past that in a percentage of cells, we were not able to observe potentiation by EtOH of receptor activation by 5-HT (Lovinger, 1991; Lovinger and White, 1991; Lovinger and Zhou, 1994). The observation that potentiation by EtOH can be observed in some of these cells upon receptor activation by DA suggests that the lack of potentiation is not a function of the cellular context. Furthermore, this observation suggests that posttranslational modifications such as phosphorylation/dephosphorylation do not play a major role in determining EtOH sensitivity of the receptor since the receptor is not likely to be in a differentially modified state when activated by different agonists during a given recording from a single cell. This observation is consistent with recent findings from Coultrap and Machu (1997) suggesting that the presence of phosphorylation sites is not required for EtOH potentiation of receptor function. We favor the idea that differential sensitivity to a weak allosteric agent such as EtOH is more likely to be a result of small differences in the configuration of the channel and the transitions in configuration upon channel opening. These differences are likely to be subtle, and may require structural analysis of the receptor-channel to be fully elucidated.

Our observations with the 5-HT_3 receptor have implications for other receptors as well. The variability in

EtOH potentiation of $GABA_A$ receptor-mediated current in whole-cell recordings is notoriously large. Factors such as cellular context and posttranslational modification have been invoked to explain this variability (Harris et al., 1995; Wafford and Whiting, 1992; Whatley et al., 1996). However, it is worth considering the possibility that subtle differences in the resting or ligand-bound conformation of the receptor-channel might account for variable EtOH effects on this receptor.

In summary, our findings indicate that EtOH and TCEt potentiate 5-HT_3 receptor function, at least in part, by increasing P_o. This mechanism is similar to that previously described for EtOH potentiation of the *Torpedo* nicotinic ACh receptor. It will be interesting to examine the impact of changes in receptor amino acid structure on this mechanism of potentiation.

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