Cholesterol Antagonizes Ethanol Potentiation of Human Brain BK\(_{\text{Ca}}\) Channels Reconstituted into Phospholipid Bilayers

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ABSTRACT

The activity of large conductance, Ca\(^{2+}\)-sensitive K\(^+\) (BK\(_{\text{Ca}}\)) channels, known to control neuronal excitability, is increased by ethanol (EtOH) exposure. Moreover, brain cholesterol (CHS) is elevated after chronic exposure to EtOH, suggesting that membrane CHS may play a role in drug tolerance. Here, we use BK\(_{\text{Ca}}\) channels from human brain (hslo subunits), reconstituted into 1-palmitoyl-2-oleoyl phosphatidylethanolamine/1-palmitoyl-2-oleoyl phosphatidylserine (POPS) bilayers, to examine CHS modulation of EtOH sensitivity. Acute exposure to clinically relevant EtOH levels increases channel activity without modifying conductance. In this minimal system, increases in CHS content within the range found in neuronal membranes lead to progressive antagonism of EtOH action. Furthermore, CHS inhibits basal channel activity with an affinity similar to that of CHS blunting of the alcohol effect. Modification of channel gating by either EtOH or CHS is reduced dramatically by removal of POPS from the bilayer, suggesting a common mechanism(s) of action. Indeed, channel dwell-time analysis indicates that CHS and EtOH exert opposite actions on the stability of channel closed states. However, each agent also acts on distinct dwell states not mirrored by the other, which contribute to the opposite effects of CHS and EtOH on channel gating.

Ion channels reside in a heterogeneous lipid matrix. Lipid species partition asymmetrically both within and across biological membrane leaflets (Devaux, 1991). These nonrandom lipid associations produce domains that differ in composition and physicochemical properties from the bulk membrane (Welti and Glaser, 1994), resulting in distinct microenvironments for ion channels. An example is the cholesterol (CHS) and sphingomyelin-rich lipid raft, thought to participate in many aspects of cell function (Brown and London, 1998). Large conductance, Ca\(^{2+}\)-activated K\(^+\) (BK\(_{\text{Ca}}\)) channels cloned from human brain (hslo) and expressed in Madin-Darby canine kidney cells associate with these lipid microdomains (Bravo-Zehnder et al., 2000). The biological implications of channel association with distinct lipid domains are not well understood, but it is likely that the domain physical properties influence channel activity.

Cholesterol is a significant component of lipid rafts and a major determinant of overall membrane physical properties (Bloch, 1983), which may contribute to its effects on the activity of native ion channels (Bolotina et al., 1989; Barrantes, 1993; Chang et al., 1995; Lundbaek et al., 1996; Levitan et al., 2000). Modulation of membrane CHS content and distribution may play a role in cellular adaptation to ethanol (EtOH) (Wood et al., 1990). Increased content (Chin et al., 1978; Omodeo-Sale et al., 1995) and altered distribution of CHS between membrane leaflets (Wood et al., 1990) are observed in response to EtOH exposure in both animal and cell culture models. Ethanol differentially increases the diffusion of various lipid probes in Aplysia californica neurons, suggesting that its actions on ion channels might depend upon the existence of dissimilar lateral domains (Treistman et al., 1987). In model membranes and computer simulations, EtOH affects lateral lipid domains (Chin and Goldstein, 1981; Harris et al., 1984; Jorgensen et al., 1993; Slater et al., 1993), suggesting that specific lipid species might modify the sensitivity of a domain to EtOH action. Cholesterol, in particular, counteracts EtOH’s disordering action in mouse synapticosomal membranes and phospholipid bilayers (Chin and Goldstein, 1981). Interestingly, EtOH increases the fluidity of the extracellular leaflet of synaptic plasma membranes to a larger extent than that of the cytoplasmic leaflet, an effect

ABBREVIATIONS: CHS, cholesterol; BK\(_{\text{Ca}}\), large conductance Ca\(^{2+}\)-activated K\(^+\) channel; EtOH, ethanol; POPE, 1-palmitoyl-2-oleoyl-phosphatidylethanolamine; POPS, 1-palmitoyl-2-oleoyl-phosphatidylserine; HEK, human embryonic kidney; MOPS, 3-(N-morpholino)propanesulfonic acid; HEDTA, N-(2-hydroxyethyl) ethylene-diaminetriacetic acid; ANOVA, analysis of variance; PC, phosphatidylcholine; \(P_o\), open probability; \(N_p\), steady-state activity.
attributed to the larger CHS content in the inner leaflet. After chronic EtOH treatment, however, transbilayer differences in fluidity and CHS content are reduced in concert (Wood et al., 1990).

Ethanol reversibly potentiates BK_{Ca} channels in excised membrane patches from rat neurohypophysial terminals, an action that may contribute to EtOH inhibition of neuropeptide release (Dopico et al., 1996). EtOH potentiation persists after expression of BK_{Ca} (mslo) channels in Xenopus laevis oocytes (Dopico et al., 1998) and incorporation of native BK_{Ca} channels into 1-palmitoyl-2-oleoyl phosphatidylethanolamine (POPE)/1-palmitoyl-2-oleoyl phosphatidylserine (POPS) bilayers (Chu et al., 1998). Thus, neither cytosolic second messengers nor complex cytoskeletal architecture is required for EtOH action on BK_{Ca} channels.

Here, we use hslo channels expressed in HEK 293 cells and incorporated into bilayers made of only one or two phospholipid species to study CHS modulation of EtOH action on channel function. This preparation allows near complete control of both protein and lipid constituents. Results indicate that increased bilayer CHS antagonizes EtOH potentiation of channel activity in a concentration-dependent manner. The reciprocal actions of EtOH and CHS on commonly targeted channel dwell states are the major determinants of CHS antagonism of EtOH effect on channel activity. Furthermore, the effect of each of these modulators on channel activity is drastically reduced in the absence of phosphatidylserine in the bilayer.

**Materials and Methods**

**HEK 293 Membrane Preparation.** HEK 293 membrane fragments were isolated using a protocol for COS cells (Sun et al., 1994), modified slightly. Briefly, HEK 293 cells stably transfected with hslo cDNA (a gift from Dr. P. Ahring, NeuroSearch A/S, Ballerup, Denmark) were grown to confluence, pelleted, and resuspended on ice in 10 ml of buffer: 30 mM KCl, 2 mM MgCl₂, 10 mM HEPES, and 5 mM EGTA, pH 7.2. The cell suspension was forced through a 27-gauge needle four times and sonicated at 30% maximum power for 30 s, twice. The suspension was layered on a 20 to 38% sucrose density gradient, with the sucrose gradient, with the sucrose density gradient (in 20 mM MOPS, pH 7.1) and centrifuged at 25,000 rpm for 20 min. The resultant supernatant was layered on a 20 to 38% sucrose density gradient, with the sucrose gradient, and centrifuged at 25,000 rpm for 60 min at 4°C. The resulting pellet was resuspended in 200 μl of buffer: 250 mM sucrose and 10 mM HEPES, pH 7.3. Aliquots were stored at −80°C.

**Electrophysiology.** Channels were incorporated by dropping 0.5 μl of the membrane preparation onto bilayers consisting of POPE/POPS and differing concentrations of CHS. Lipids were dried under hyperosmotic to the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. In the cis chamber was adjusted using aliquots from a 1 M stock solution of HEDTA, pH 7.2. 

**Data Analysis.** Data were acquired and analyzed using pClamp 6.0.2 (Axon Instruments, Inc., Union City, CA). As an index of steady-state channel activity, we used the product of the number of channels in the bilayer during recording (N) and the open channel probability (P_o). N was monitored pre- and post-EtOH by stepping to positive potentials to maximize P_o. Experiments showing an increase in N after EtOH addition were discarded. N_P was determined as described elsewhere (Dopico et al., 1996) from periods of at least 20 s of continuous recording. Dwell-time histograms were constructed using the half-amplitude threshold criterion, events shorter than 0.3 ms being excluded. An F ratio (p < 0.01) was used to determine the minimum number of exponential components to appropriately fit the dwell-time histogram data. Fifty percent of maximal change (EC50 or IC50) was obtained from concentration-response curves by extrapolation. Data are shown as mean ± S.E.M. The significance of the difference between means was determined by ANOVA and a posteriori test (Dunnett’s).

**Chemicals.** All solutions were prepared with Milli-Q water and ultrapure grade salts. Ethanol (100%, anhydrous) was purchased from Sigma-Aldrich Chemical Co. (Milwaukee, WI), and POPE, POPS, and CHS from Avanti Polar Lipids (Alabaster, AL).

**Results**

**Ethanol Increases hslo Channel Activity in Binary Bilayers.** We first determined whether EtOH modifies hslo steady-state activity (N_P) in a binary phospholipid mixture. We chose a 3:1 POPE/POPS (w/w) planar bilayer, where EtOH sensitivity of native skeletal muscle BK_{Ca} channels was initially explored (Chu et al., 1998). Thus, cloned hslo subunits were incorporated into this bilayer type, where they displayed characteristic features of BK_{Ca} channels: large unitary current amplitude (Fig. 1) and increases in P_o as the applied voltage is made more positive (9.8 mV ± 0.4 mV/e-fold change in N_P) and/or [Ca^{2+}]_o at the cytosolic side of the channel is increased (not shown). Figure 1 shows traces of hslo activity before and during application of 50 mM EtOH to the “intracellular” side of the POPE/POPS bilayer. The EtOH-induced increase in P_o shown in the figure, which occurred within 1 to 5 min of drug exposure, was observed in 8 of 10 bilayers, the average N_P showing a 5.2 ± 1.5-fold increase over pre-EtOH values. EtOH-induced potentiation of hslo channel activity in the POPE/POPS bilayer is similar to our previously reported results in complex lipid/protein systems with mslo subunits expressed in oocytes (Dopico et al., 1998), native BK_{Ca} channels studied in situ (Dopico et al., 1996), and rat skeletal muscle t-tubule BK_{Ca} channels recon-
stitioned into this bilayer type (Chu et al., 1998). Thus, hslo subunits, POPE, POPS, and the system interfaces are sufficient for EtOH potentiation of BKca channel activity.

In contrast to its action on steady-state activity, 50 mM EtOH consistently failed to modify other parameters of hslo function, such as unitary amplitude (14.9 versus 14.8 pA, recorded at 0 mV and 300/30 mM [K+]i/[K+]o in the presence and absence of EtOH, respectively) (Fig. 1), suggesting that EtOH actions on hslo channels are restricted to modification of channel gating. This is also in agreement with data obtained with BKca channels in more complex lipid/protein systems (Dopico et al., 1996, 1998; Jakab et al., 1997; Chu et al., 1998), validating our minimal system for studies on CHS modulation of EtOH sensitivity.

**Cholesterol Blunts Alcohol Potentiation of hslo Channel Activity.** We next tested whether CHS incorporation into this binary bilayer could modify EtOH-induced increases in hslo activity. Figure 2 shows that CHS incorporation (11–33 mol%) into POPE/POPS bilayers diminishes EtOH-induced potentiation in a concentration-dependent manner (IC50 = 15 mol%). CHS content of 23 mol% resulted in almost total prevention of channel potentiation by 50 mM EtOH. Under these conditions, EtOH slightly increased channel activity in two of five experiments, and slightly inhibited activity in the remaining three cases, yielding no net effect on channel activity (average NPo value = 0.96 ± 0.38 of controls). This lack of a major alcohol effect in the presence of CHS may be explained by 1) reduced EtOH partitioning into CHS-containing bilayers; 2) CHS antagonism of EtOH at EtOH’s recognition site(s) on the hslo subunit or its immediate phospholipid microenvironment (see Discussion); and/or 3) the actions of CHS and EtOH on channel kinetic states are of opposite sign, and balanced, which results in reciprocal modulation of basal channel activity.

**Cholesterol Reduces Basal hslo Channel Activity in POPE/POPS Bilayers.** A reduction in hslo activity caused by the presence of CHS in the bilayer could explain part or all of the CHS antagonism of EtOH potentiation of hslo activity in POPE/POPS bilayers. Figure 3A shows hslo single channel activity recorded under identical conditions of voltage and free [Ca2+]i at the cis side of POPE/POPS bilayers in the absence and presence of CHS in this bilayer. Increases from 11 to 49 mol% inhibit hslo activity in a concentration-dependent manner. Maximal inhibition is reached at 33 to 49 mol% CHS with an IC50 = 15.5 mol% (Fig. 3B). Both the concentration for maximal effect and the IC50 are similar to those for CHS blunting of EtOH-induced increases in hslo activity (see above), which suggests that CHS inhibition of channel basal activity contributes to the sterol modulation of alcohol-induced potentiation of hslo activity.
Inhibition of basal $P_o$ could result from a CHS-induced decrease in channel mean open time ($t_o$), an increase in channel mean closed time ($t_c$), or a combination of both. Figure 3, C and D, clearly demonstrates that the third possibility is the case. Furthermore, single channel analysis reveals differential CHS effects on $t_o$ and $t_c$, dependent upon concentration. Although inhibition of channel $t_c$ is maximal at 23 mol% CHS (IC$_{50}$ = 4.3 mol%) (Fig. 3C), channel $t_o$ failed to reach a well defined maximum at the concentrations tested (Fig. 3D). If a maximal effect is assumed at the highest [CHS] tested (49 mol%), $t_o$ data extrapolation yields an EC$_{50}$ = 36.1 mol%, representing a minimum for this value. Thus, at concentrations of CHS below the IC$_{50}$ for the reduction in $P_o$, decreases in channel activity are determined by a major reduction in $t_o$ and a minor increase in $t_c$. In contrast, above the IC$_{50}$, further reduction in $P_o$ is caused primarily by a progressive increase in $t_c$.

As with ETOH (see above), CHS-induced modifications of hslo channel $P_o$ were not accompanied by significant changes in conductance in (pS): 323.3 ± 5 in POPE/POPS (n = 12), 33.9 ± 4 in POPE/POPS + 13% CHS (n = 11), and 335.3 ± 7.3 in POPE/POPS + 33% CHS (n = 4); all measurements obtained in 300/30 mM [K$^+$]/[K$^+$]o ($p^2 > 0.95$ for linear fits of unitary current amplitude/voltage data). This result is in agreement with studies of CHS action on BK$_{Ca}$ channels in rabbit aorta (Bolotina et al., 1989). A subtle 7% change in slope conductance has been reported for rat brain BK$_{Ca}$ channels displayed changes in $P_o$ values were calculated from hslo channel reconstituted into bilayers with varying weight percentages of POPS in a binary mixture with POPE. Current records were obtained at 0 mV and [Ca$^{2+}$]free = 50 μM on the intracellular side of the bilayer. Data were low-pass filtered at 1 kHz and sampled at 10 kHz. $P_o$ values were obtained from continuous recording (see Materials and Methods). The number of experiments performed for each condition is shown in parentheses above each point. *, significantly different from POPE/POPS [3:1, or 25% w/w] values ($p < 0.05$).
mined by the amount of POPS in the bilayer (Fig. 5). Thus, we considered the possibility that CHS and EtOH actions on 
hslo channels are mediated by a single, common mechanism. In this case, we might expect channel dwell-time histograms in the presence of each agent to show a mirroring profile of actions.

Dwell times in the absence and presence of 50 mM EtOH were evaluated in several POPE/POPS (3:1) bilayers (Fig. 6A), indicating the existence of at least two open states. Although 50 mM EtOH characteristically increases channel NPo to ~5-fold of control values, on average, it mildly increased the duration of short and long openings and slightly shifted the channel population from long to short openings. These drug-induced changes in open channel populations result only in a minor change in mean open time. Thus, a major increase in hslo steady-state activity induced by acute EtOH could be obtained in the absence of a significant increase in channel mean open time.

The closed times distribution was also well fitted with a double exponential function, in both the absence and presence of EtOH (Fig. 6B), indicating the existence of at least two closed states. Two actions of EtOH are evident: a decrease in the average duration of long closures, and a marked shift in the closed channel population from long to brief events. EtOH-induced changes in the closed times distribution result in a significant change in mean closed time (~60% of control), the major contributor to EtOH-induced increase in channel Poc. In summary, EtOH markedly increases hslo steady-state activity by producing a marked reduction in the average duration of channel long closures and their relative contribution to the total time spent in closed states, without causing a major change in the channel mean open time.

Were CHS and EtOH having opposite effects on channel Poc solely by targeting common kinetic states in opposite manner, we might expect a profile of changes in open and closed times distributions by CHS mirroring those caused by EtOH. This complementarity was indeed present, but each of these agents also had unique effects not mirrored by the other. The dwell-time data for hslo channels in POPE/POPS (3:1) bilayers with increasing amounts of CHS (n = 2–8 for each CHS concentration) were obtained under identical conditions of voltage (0 mV) and [Ca2+]free (50 μM). The closed times distributions in the presence and absence of CHS could be well fitted with two exponentials in the representative example shown (Fig. 7B), which is particularly useful for a comparison with the closed time distribution in the presence of EtOH (Fig. 6B, right). These data demonstrate that CHS increases the average duration of longer closures and shifts the closed channel population from short to longer closures, these two changes being a mirror of EtOH actions that lead to channel activation. However, CHS also increases the average duration of short closures, an action not mirrored by EtOH.

In addition, representative open times distributions (Fig. 7A), fitted by double exponential functions, indicate that CHS at all concentrations tested decreased the average duration of long openings, a dwell state basically unmodified by EtOH. Figure 7A also shows that CHS produces a minor shift

Fig. 5. Both ethanol and cholesterol fail to markedly modify the activity of hslo channels incorporated into a single (POPE) bilayer. A, EtOH concentrations (50 and 100 mM) that increase hslo channel activity in POPE/POPS bilayers fail to potentiate activity in 100% POPE bilayers. Ratios of NPo values obtained in the presence and absence of EtOH (left, 50 mM; right, 100 mM) are shown in a scatter graph, where each data point represents an individual bilayer (n). Mean ± S.E.M. of data are shown at the top of the graph. The dotted line highlights the point at which NPo EtOH/NPo control = 1. The potential at the cis side of the bilayer was set between −20 and +60 mV and free [Ca2+]free = 10 μM. Data were low-pass filtered at 1 kHz and sampled at 10 kHz. NPo values were obtained from continuous recording (see Materials and Methods). B, significant inhibition of hslo activity by CHS in POPE/POPS bilayers is not observed when CHS action is evaluated in POPE bilayers. Scatter graph of hslo NPo from POPE bilayers in the absence (left) and presence of 23 mol% (middle) or 32 mol% (right) CHS. Data points represent individual bilayers. Mean ± S.E.M. of data are shown at the top of the graph. The potential at the cis side of the bilayer was set at 0 mV and free [Ca2+]free = 50 μM. Data were low-pass filtered at 1 kHz and sampled at 10 kHz. NPo values were obtained from continuous recording (see Materials and Methods).
from long to short openings, this shift being another contributor to the decrease in mean open time induced by CHS. In summary, overall antagonism between CHS and EtOH on *hslo* steady-state activity results from the targeting of both common and distinct channel dwelling states by these modulators, probably reflecting their common and distinct sites of action (see Discussion).

**Discussion**

The role of the lipid environment in the function of embedded ion channel proteins and their drug sensitivity is difficult to assess in complex biological systems but is more approachable in reduced preparations such as with cloned channels incorporated into planar lipid bilayers. Our data demonstrate the feasibility of this approach, because *hslo* channels in POPE and POPE/POPS bilayers retain base BK$_{ca}$ channel characteristics and respond to the change in bulk phospholipid composition. Of course, this reductionist approach ignores many of the potential interactions present in a rich, heterogeneous natural membrane. However, the model used successfully addresses the specific questions that are being asked.

We demonstrated that both CHS and EtOH modulation of basal *hslo* activity are dramatically impaired in the absence of POPS in the bilayer. The fact that these agents modulate *hslo* channel activity in POPE/POPS, but fail to do so in pure POPE bilayers, could be attributed to the loss of headgroup negative charge, differing headgroup size, and/or altered headgroup interactions. Neutral phosphatidylethanolamine bilayers have a high propensity to transition from the lamellar phase into the H$_{II}$ (inverted hexagonal) phase, a transition directly attenuated by negative membrane surface charge carried by PS (Lewis and McElhaney, 2000). Although this transition for POPE generally occurs at higher temperatures (70°C; Epand, 1985) than those used in this study, we cannot rule out that such a tendency may mask or alter the actions of CHS or EtOH under the exact conditions used in our system. Both PE and CHS are nonlamellar phase-prefering lipids, which can presumably increase curvature stress when incorporated into a lamellar bilayers and, eventually, modify channel function (Lundbaek et al., 1996). It is possible that a pure POPE bilayer with a high initial degree of curvature stress masks sterol modulation of this parameter. The inability of CHS to modify *hslo* function when added to POPE bilayers might also reflect a relatively low miscibility of CHS in pure PE (McMullen et al., 1999), perhaps alleviated by the headgroup structure and charge in the POPE/POPS mixture.

In both cell culture and animal models of chronic EtOH exposure, alterations in both content (Chin et al., 1978; Omodeo-Sale et al., 1995) and distribution (Wood et al., 1990) of membrane CHS have been reported. These alterations might represent a compensatory response (i.e., “tolerance”) to counteract the effects of EtOH on relevant targets, such as defined ion channel populations. Here, we demonstrate that increases in the CHS content of POPE/POPS bilayers, indeed, reduce EtOH potentiation of *hslo* channel P$_o$.

Increases in bilayer/membrane CHS content diminish the lipid/membrane partition coefficient of a variety of small analytes, such as halothane (Lechleiter et al., 1986), uncharged pentobarbitone (Miller and Yu, 1977), and benzyl alcohol (Colley and Metcalfe, 1972). Isothermal titration calorimetry data show that EtOH partitioning into phosphatidylcholine (PC) liposomes is also reduced by CHS, when present in the bilayer at concentrations >10 mol% (Trandum et al., 2000). Consistent with these findings, Fig. 2 demonstrates that CHS effects on EtOH sensitivity of *hslo* channels...
is largely absent at 10 mol% CHS but evident at concentrations above 23 mol%.

The effect of CHS on EtOH partitioning may be explained by bilayer phase behavior. Isothermal titration calorimetry (Trandum et al., 1999) and computer stimulation (Jorgensen et al., 1993) studies strongly suggest that EtOH preferentially partitions into bilayers at the interfaces between the gel and the liquid crystalline domains that form as the bilayer approaches the transition temperature. High bilayer CHS concentrations abolish the gel-to-liquid crystalline transition, causing the bilayer to exist in a liquid-ordered state (Trandum et al., 2000). The resulting disappearance of the gel/liquid crystalline interfaces preferentially targeted by EtOH would serve to decrease its partitioning into the membrane. In fact, X-ray diffraction studies of POPE/POPS mixtures suggest that at 25°C, multilayer samples exist as a combination of both gel and liquid crystalline phases (Chang et al., 1999). The coexistence of these lateral domains would support the partitioning of EtOH into this lipid mixture. We show here that channels are sensitive to EtOH in POPE/POPS bilayers (Fig. 1). However, in bilayers containing >20 mol% CHS that lack these domain interfaces (Chang et al., 1999), EtOH potentiation of hslo activity is markedly reduced (Fig. 2). Thus, our data are consistent with a CHS-induced reduction of EtOH partitioning in the bilayer. In our system, however, the hydrocarbon interior of the bilayer probably contains contaminant decane in equilibrium with that in the torus (Gruen, 1981), so we cannot rule out some contribution of this solvent to the phase behavior of the bilayers used in our study. The manipulation of parameters such as temperature and acyl chain saturation will yield further insight into the importance of bilayer phase behavior on BKca ethanol sensitivity.

Further evidence that CHS reduces EtOH interaction with the membrane comes from NMR spectroscopy data demonstrating that EtOH resides at the lipid-water interface in phospholipid bilayers. The carbonyl groups in the glycerol backbone are specifically favored hydrogen bonding sites for EtOH (Barry and Gawrisch, 1994), this binding being decreased by increasing amounts of CHS (Barry and Gawrisch, 1995). Because CHS interacts with PC at the same carbonyl groups in the glycerol backbone (Worcester and Franks, 1976), this was interpreted as CHS directly competing for EtOH’s favored binding sites (Barry and Gawrisch, 1995). CHS location at the phospholipid carbonyl groups would also increase the packing density of the phospholipids and antagonize the increase in acyl chain motion (“disordering”) introduced by EtOH (Sun and Sun, 1985), which may contribute to functional antagonism on hslo kinetics (see below).

Apart from effects on EtOH partitioning, CHS may directly antagonize the action of the drug on the bilayer or the hslo channel itself. If CHS and EtOH act through a single, common mechanism, we might expect them to exert reciprocal actions on common dwell states of the channel. Indeed, EtOH and CHS produce a mirrored shift between the long and short channel closed states and have opposite actions on the mean duration of long closures. This suggests that CHS and EtOH share a common target on the channel protein or in the phospholipid bilayer, which is important in determining the stability of the channel closed state(s). A similar increase in the average duration of native BKca channel long closures after CHS enrichment of myocyte membranes has been reported (Bohotina et al., 1989). Channel P, was approximately halved, coincident with a similar decrease in the rotational diffusion coefficient of DPH (Bohotina et al., 1989), indicating that a decrease in both rate and range of motion of phospholipid acyl chains accompanies the reduction in channel activity. Spin-labeling experiments also demonstrate that CHS increases, whereas EtOH decreases bilayer order (Chin and Goldstein, 1981). Interestingly, the magnitude of CHS and EtOH effects on multilayer PC vesicle order are similar to those in brain synaptosomal membranes (Chin and Goldstein, 1981). Thus, we postulate that opposite actions of CHS and EtOH on acyl chain order may underlie or, at least, contribute to CHS and EtOH opposing effects on common channel dwell states, such as the long closed state.

In addition to their common modulation of channel long closures, CHS and EtOH exhibit individual effects on channel dwell times, which contribute to their opposite actions on P. These distinct effects on channel dwell states may represent independent actions of these modulators on specific bilayer characteristics that modify channel function. For example, EtOH increases the rate of phospholipid desorption, displacing water from the hydrogen-bonded network of water molecules in the hydration layer, whereas CHS has little, if any, effect on phospholipid desorption (Slater et al., 1993). Changes in phospholipid desorption alter not only lipid-lipid interactions but also protein-lipid interactions, with eventual modification of ion channel function. Interestingly, EtOH desorption is more marked in PS than PE (Slater et al., 1993), consistent with the EtOH activation of hslo channels (Figs. 1 and 5).

Monolayer or bilayer properties modified by CHS, but not EtOH, include a broadening and eventual elimination of the gel-to-liquid crystalline phase transition, a decrease in the cross-sectional area occupied by the phospholipid in the liquid crystalline state, increases in both bilayer thickness and mechanical strength, and increases in the lateral stress and stiffness of the phospholipid monolayer or bilayer in the physiologically relevant fluid phase (McMullen et al., 1999; Nielsen et al., 1999). In particular, changes in lateral stress and bilayer stiffness have been causally related to modification of ion channel function. Cholesterol and other compounds promoting negative monolayer curvature increase stiffness and decrease channel activity, whereas compounds promoting positive monolayer curvature have opposite effects on both stiffness and channel activity (Lundbaek et al., 1996; Bezrukov et al., 1998). Furthermore, CHS inhibition of native BKca channels in PE/PS bilayers has been linked to an increase in bilayer lateral stress caused by the presence of the steroid (Chang et al., 1995). A major consequence of increases in lateral stress is a reduction in the activation energy for the transition from open to closed state(s). This reduces the average duration of long openings, as we report here (Fig. 7A). Thus, we postulate that the distinct decrease in the average duration of long openings observed with CHS, an effect not mirrored by EtOH, may be related to the increase in lateral stress caused by the steroid.

In our demonstration of CHS blunting of alcohol potentiation of hslo activity we used 50 mM EtOH, close to legal intoxication (~20 mM) and below lethal blood levels in naive subjects (>90 mM), and a range of CHS content similar to that found in cell membranes (5.6–44 mol%). Thus, modification of hslo function by EtOH may depend on the mem-
brane CHS content where the channel resides. Membrane CHS content and/or distribution might contribute to differential EtOH sensitivity of BK$_{ca}$ in different cell types and in different channel subtypes from different neuronal domains, as in supraoptic neurons (Dopico et al., 1999). Present results might also help to explain recent findings that rats undergoing extending feeding with EtOH display not only reduced BK$_{ca}$ current density in neurohypophysial terminals but also reduced BK$_{ca}$ sensitivity to acute EtOH (Knott et al., 2002).

Our findings suggest that manipulation of membrane lipid composition may represent a mechanism for plasticity responsible for alteration of channel basal $P_e$, as well as sensitivity to small amphiphiles such as EtOH. This hypothesis becomes particularly attractive when coupled with data demonstrating alterations in lipid composition after EtOH exposure, and more generally with the emerging theme of lipid domains. Here, we demonstrate that alcohol action on the activity of a human neural ion channel depends on the lipid environment of the channel protein.

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Chu B, Dopico AM, Lemos JR, and Treistman SN (1998) Ethanol content of apical membranes of rat supraoptic neurons (Dopico et al., 1999). Present results might also help to explain recent findings that rats undergoing extending feeding with EtOH display not only reduced BK$_{ca}$ current density in neurohypophysial terminals but also reduced BK$_{ca}$ sensitivity to acute EtOH (Knott et al., 2002).

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