Antagonists of alcohol inhibition of cell adhesion

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Increasing evidence suggests that alcohols act within specific binding pockets of selective neural proteins; however, antagonists at these sites have not been identified. 1-Alcohols from methanol through 1-butanol inhibit with increasing potency the cell–cell adhesion mediated by the immunoglobulin cell adhesion molecule L1. An abrupt cutoff exists after 1-butanol, with 1-pentanol and higher 1-alcohols showing no effect. Here, we demonstrate surprisingly strict structural requirements for alcohol inhibition of cell–cell adhesion in L1-transfected NIH 3T3 fibroblasts and in NG108–15 neuroblastoma × glioma hybrid cells treated with BMP-7, an inducer of L1 and neural cell adhesion molecule. The target site discriminates the tertiary structure of straight-chain and branched-chain alcohols and appears to comprise both a hydrophobic binding site and an adjacent hydrophilic allosteric site. Modifications to the 2- and 3-carbon positions of 1-butanol increased potency, whereas modifications that restrict movement about the 4-carbon abolished activity. The effects of ethanol and 1-butanol on cell–cell adhesion were antagonized by 1-pentanol (IC_{50} = 715 μM) and 1-octanol (IC_{50} = 3.6 μM). Antagonism by 1-octanol was complete, reversible, and noncompetitive. 1-Octanol also antagonized ethanol inhibition of BMP-7 morphogenesis in NG108–15 cells. 1-Octanol and related compounds may prove useful in dissecting the role of altered cell adhesion in ethanol-induced injury of the nervous system.

Ethanol causes serious injury to both the developing and mature nervous systems (1). Recent evidence suggests that alcohols alter nervous system function by interacting directly with selective neural proteins, including ion channels, kinases, and transporters (2, 3). Experiments with the homologous series of 1-alcohols reveal different cutoffs for alcohol effects on diverse native and purified proteins (4–6). For alcohols below the cutoff, potency increases as a function of increasing hydrophobicity; alcohols above the cutoff are less potent or inactive. The inactivity of 1-alcohols of greater hydrophobicity than those below the cutoff has been taken as evidence that the active 1-alcohols interact with protein rather than lipid sites. The size of the alcohol cutoff for the γ-aminobutyric acid type A and glycine receptors can be manipulated by substituting single amino acids within the transmembrane region of a protein subunit (7), indicating a striking degree of target specificity. Diverse alcohol targets appear to comprise a hydrophobic crevice that binds methyl groups and a hydrophilic allosteric site that interacts with the hydroxyl group (8). The observation that alcohols interact specifically with selective neural proteins suggests that one might discover specific alcohol antagonists; however, none has yet been identified.

L1 is an immunoglobulin cell adhesion molecule that regulates neuronal migration, axon fasciculation, and growth cone guidance, through homophilic and heterophilic interactions (9). We have shown that clinically relevant concentrations of ethanol inhibit cell–cell adhesion mediated by L1 in transfected fibroblasts and in the NG108–15 neuroblastoma × glioma cell line (10–13). In NG108–15 cells, ethanol also inhibits morphogenetic changes induced by BMP-7, a powerful inducer of L1 and neural cell adhesion molecule (N-CAM) gene expression (10). Because of the similarity in brain lesions in children with fetal alcohol syndrome and those with mutations in the gene for L1, we have speculated that ethanol effects on L1 could play a role in the pathophysiology of fetal alcohol syndrome (11). Interestingly, ethanol potently inhibits L1-mediated neurite extension in cerebellar granule cells (14).

1-Alcohol inhibition of cell–cell adhesion demonstrates an abrupt cutoff effect between 1-butanol and 1-pentanol (10, 11), consistent with a direct effect on L1 or an associated protein. Here, we show that straight-chain and branched-chain alcohols have highly specific structural requirements for inhibition of cell–cell adhesion. Moreover, 1-pentanol and 1-octanol abolish the effects of ethanol and 1-butanol on cell–cell adhesion and the effects of ethanol on the morphogenetic actions of BMP-7.

Materials and Methods

Reagents. Alcohols were purchased from Sigma; all other chemicals were purchased from Sigma, or as indicated. The values for membrane/buffer partition coefficients ($P_{m/b}$) of the alcohols were derived from a published source (15) or calculated by dividing the octanol/water partition coefficient by 5.

Cell Culture. NIH 3T3 cells were cultured in DMEM (Life Technologies, Gaithersburg, MD) supplemented with 10% normal calf serum (Intergen, Purchase, NY) and 400 μg/ml G418 (Life Technologies). NG108–15 neuroblastoma × glioma cells (passages 21 to 30) were plated in serum-free, defined medium (16). At the start of the morphogenetic and cell adhesion assays, serum-free medium containing BMP-7 (Creative Biomolecules, Hopkinton, MA) (1–40 ng/ml, final) was added daily to the NG108–15 cells. Both cell lines were cultured at 37°C, in an atmosphere of 90% air and 10% CO_2. Three NIH 3T3 subclones were used in these studies: 2B2-L1, 2A2-L1, and Vec-1A5. The 2B2-L1 and 2A2-L1 cell lines are subclones derived from a stable transfection of NIH 3T3 cells with the human L1 cDNA, and Vec-1A5 is a subclone from a transfection with the empty expression vector (12).

Morphogenetic Actions of BMP-7 in NG108 Cells. NG108–15 cells were plated from suspensions of single cells at a density of 50,000 cells per well in poly-D-lysine-coated, six-well plates containing serum-free medium in the absence or presence of BMP-7, as described (17, 18). After the addition of ethanol (50 mM, final), the plates were sealed with Parafilm to prevent evaporation. Control cultures were treated similarly. The medium for all cells was replaced daily after the addition of ethanol. At 1–3 days after the addition of ethanol, two randomly selected, subconfluent (<50%) fields of cells were viewed at ×100–200 magnification and evaluated for the presence of cell clusters. A cell cluster was defined as a group of three or more cells that adhered to each

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Abbreviation: N-CAM, neural cell adhesion molecule.

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Table 1. Inhibition of cell–cell adhesion, membrane/buffer partition coefficient, and molar volume for a series of alcohols and a nonvolatile anesthetic

Alcohol	Conc., mM	Inhibition of cell–cell adhesion		$P_{m/b}$	V_m, ml/mol
		NG108-15	NIH 3T3-L1		
Ethanol	100.0	61 ± 3	58 ± 5	0.10	58
Cyclopropylmethanol	13.5	55 ± 5	64 ± 10	0.71	71
Cyclopropylethanol	11.6	0 ± 1	4 ± 1	0.83	91
Cyclobutanol	10.5	44 ± 12	46 ± 6	0.91	78
3-Buten-1-ol	9.6	3 ± 3	2 ± 6	1.00	86
2-Methyl-1-propanol	8.7	41 ± 7	51 ± 1	1.10	90
1-Butanol	2.0	52 ± 4	40 ± 4	1.52	92
2-Methyl-2-butanol	6.2	47 ± 8	47 ± 4	1.55	109
Cyclopentanol	4.3	2 ± 1	0 ± 7	2.24	92
Benzyl alcohol	3.8	0 ± 4	4 ± 4	2.52	104
3-Methyl-1-butanol	3.3	57 ± 6	51 ± 5	2.89	109
2-Pentanol	2.2	5 ± 6	11 ± 6	4.38	109
3-Pentanol	2.1	5 ± 9	10 ± 4	4.69	107
1-Pentanol	5.0	0 ± 3	7 ± 5	5.02	108
2-Methyl-2-pentanol	1.8	0 ± 2	2 ± 7	5.20	122
3,3-Dimethyl-1-butanol	1.7	42 ± 4	64 ± 20	5.77	121
2-Ethyl-1-butanol	1.1	43 ± 3	63 ± 12	9.14	123
4-Methyl-1-pentanol	1.1	7 ± 10	5 ± 3	9.36	126
1-Octanol	0.05	5 ± 4	3 ± 6	189	158
Propofol	0.002	2 ± 6	5 ± 6	1.00	86

Cells were cultured and cell adhesion assays were performed in the absence and presence of the indicated alcohols as described in the text. Percent inhibition is presented as mean ± SEM. The concentrations of alcohols used in the adhesion assays were calculated to produce membrane concentrations equivalent to 50–100 mM ethanol. Membrane/buffer partition coefficients ($P_{m/b}$) were obtained or were calculated from published octanol/water partition coefficients (15). Molar volumes (V_m) were from published sources (15) or were calculated from molecular weights and densities at 20°C. The alcohols are sorted in the table by increasing membrane/buffer partition coefficients (with the exception of the nonvolatile anesthetic propofol).

other along at least one-quarter of their cell bodies (19). The percentage of cells in clusters was calculated by dividing the number of cells present in clusters by the total number of cells (150–200) in each field. Values obtained for each field of a treatment group were then averaged.

Cell Adhesion Assay. Cell–cell adhesion was measured by using a modified short-term aggregation assay of subconfluent cells (12). Cells were detached by gentle agitation, mechanically dissociated to obtain a single-cell suspension, and diluted in PBS supplemented with 0.1 mg/ml DNase to 350,000 cells per ml for the NIH 3T3 cells and 250,000 cells per ml for the NG108–15 cells. One milliliter of the cell suspension was added per well (4.5 cm^2) to a 12-well plate. Concentrations of the various alcohols were chosen by using published or calculated membrane–buffer partition coefficients (15) to provide the same molar membrane alcohol concentration as 50–100 mM ethanol (Table 1). After addition of the alcohols, the cells were gently mixed and mechanically shaken for 30 min at room temperature on an orbital shaker set at 60–80 rpm. Cells were viewed at a final magnification of ×200, and each well was scored for single and adherent cells in five or six microscopic fields of view. We counted approximately 100 cells per field of view and 600 cells per well. The percentage of adherent cells was calculated for each microscopic field of view and averaged. To calculate the magnitude of ethanol inhibition, we subtracted the values for cell adhesion with the Vec-1A5 cells from those of the 2B2-L1 or 2A2-L1 cell lines. Similarly, the values for cell–cell adhesion in NG108–15 cells cultured in serum-free medium were subtracted from those of NG108–15 cells treated with BMP-7.

Results

Effects of 1-Alcohols on Cell–Cell Adhesion in Two Model Systems. We tested the effects of a series of alcohols on cell–cell adhesion in two well-characterized model systems. NG108–15 cells were incubated for 48 h in serum-free medium supplemented with 10–20 ng/ml BMP-7 to induce L1 and N-CAM gene expression (10). Parallel experiments were performed by using two ethanol-sensitive NIH 3T3 cell lines transfected with human L1 (2B2-L1 and 2A2-L1) (12) and a NIH 3T3 cell line transfected with the empty expression vector (1A5-V). Fig. 1 depicts the structures of the alcohols used in this study and categorizes them as active (inhibits cell–cell adhesion) or inactive (no effect on cell–cell adhesion). For each alcohol studied, similar results were obtained in BMP-7-treated NG108–15 cells and in L1-expressing NIH 3T3 cells (Table 1).

Treatment with BMP-7 greatly increased the percentage of adherent NG108–15 cells (control, 17.0 ± 1.1%; BMP-7, 50.0 ± 1.4%). Similarly, L1-transfected NIH 3T3 cells exhibited higher levels of cell–cell adhesion than vector-transfected cells (1A5-V, 19.4 ± 1.1%; 2B2-L1, 46.1 ± 1.0%; 2A2-L1, 55.9 ± 2.1%). Consistent with previous reports (10, 12), 100 mM ethanol or 2 mM 1-butanol maximally inhibited cell–cell adhesion in both cellular systems. In contrast, cell–cell adhesion was not inhibited by concentrations of 1-pentanol (5 mM) and 1-octanol (0.15 mM) that disorder cell membranes to the same extent as 200–300 mM ethanol (15). We also observed a cutoff for cyclic alcohols; cyclobutanol inhibited cell–cell adhesion, whereas cyclopentanol and benzyl alcohol did not. The inactivity of several alcohols (1-pentanol, 1-octanol, cyclopropylethanol, 4-methyl-1-pentanol, 3-buten-1-ol, and cyclopentanol) was confirmed by using concentrations 5- to 10-fold higher than those reported in Table 1 (data not shown). An anesthetic concentration of the nonvolatile anesthetic propofol did not inhibit cell–cell adhesion; hence, it is unlikely that alcohol or anesthetic effects on cell–cell adhesion play a role in intoxication.

Structure Activity Analysis of the Alcohol Target Site. We first examined alcohols related to 1-butanol, the 1-alcohol that most

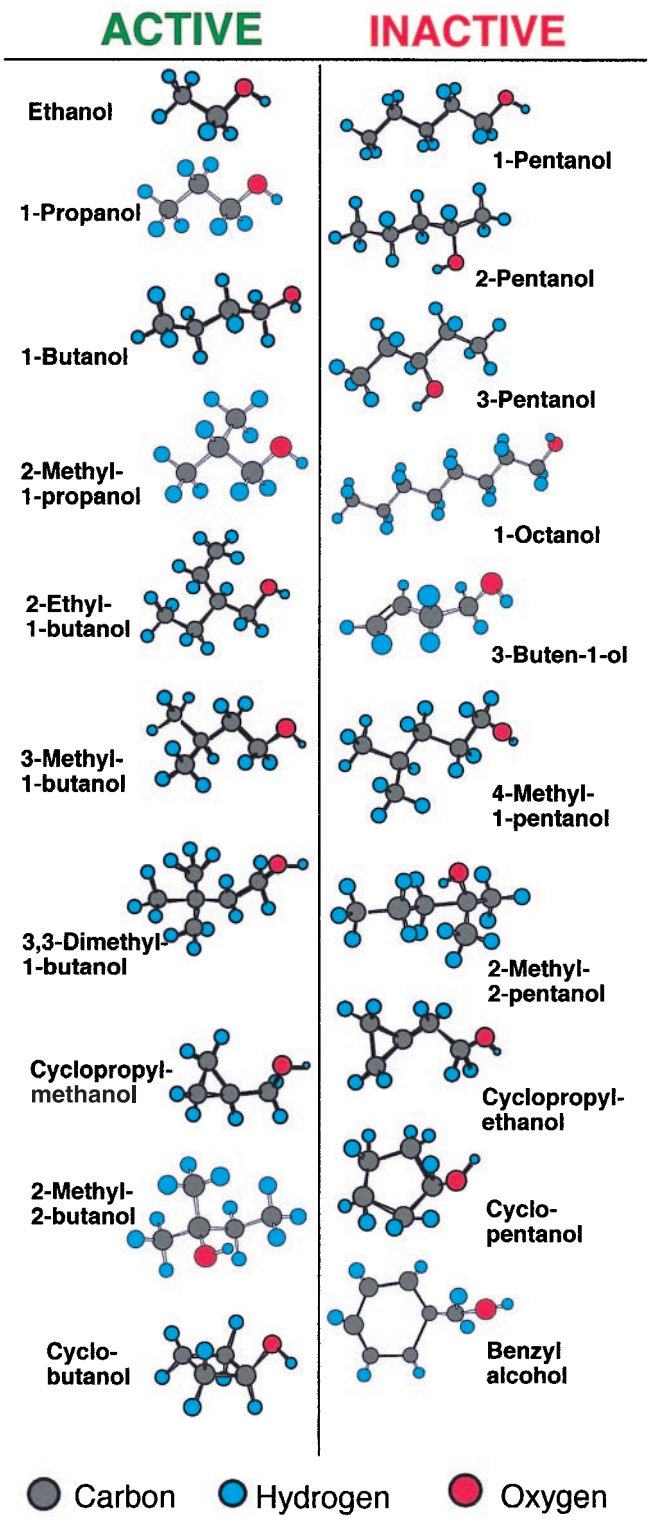

Fig. 1. Structure–activity relation of alcohols. Alcohols are categorized as active (inhibit cell–cell adhesion) or inactive (do not inhibit cell–cell adhesion). Ball and stick figures of the alcohols were produced with CHEMOFFICE (CambridgeSoft, Cambridge MA).

potently inhibited cell–cell adhesion. 2-Pentanol differs from 1-butanol through the addition of a methyl group at the 1-carbon position. This slight modification abolished activity (Table 1). In contrast, the addition of up to two methyl groups at the 2-carbon and 3-carbon positions of 1-butanol (2-ethyl-1-butanol; 3-methyl-1-butanol; and 3,3-dimethyl-1-butanol) did not reduce activity. Thus, alcohol activity is not a simple function of molecular volume or the total number of carbons.

Modifications to the 4-carbon position of 1-butanol were also informative. 1-Cyclopropylethanol differs from 1-butanol through the addition of a methyl group that bonds to both the 3-carbon and 4-carbon atoms to form a cyclopropyl moiety (Fig. 1). This modification abolished activity. To determine whether the inactivity of 1-cyclopropylethanol is related to its bulky cyclopropyl group, we examined several additional cyclic alcohols. Cyclopropylmethanol, a cyclic derivative of 1-propanol, was active, as were the related alcohols 1-propanol (10, 11) and 2-methyl-1-propanol (Table 1). Another effect of the cyclopropyl modification in 1-cyclopropylethanol is to constrain rotation about the 4-carbon position of 1-butanol. To investigate whether rotation about this axis is necessary for activity, we studied 3-buten-1-ol, a molecule that differs from 1-butanol only in the presence of a double bond between the 3- and 4-carbons. This small modification abolished activity. Thus, the target site appears to discriminate among structurally related alcohols.

Alcohols as Multivalent Ligands. If alcohol action has strict structural requirements, then it is likely that potent alcohols, such as 1-butanol, must align with the target site in a specific orientation to inhibit cell–cell adhesion. Alcohols that present multiple 1-butanol moieties have a higher probability of aligning correctly with a 1-butanol recognition site and should therefore be more potent than 1-butanol. Fig. 2 shows the structure of several multivalent alcohols related to 1-butanol. 3-Methyl-1-butanol and 2-ethyl-1-butanol each present a butanol moiety from two separate alignments, whereas 3-dimethyl-1-butanol does so from three. Dose–response curves for inhibition of cell–cell adhesion were analyzed in L1-expressing NIH 3T3 cells. Maximal inhibition of cell–cell adhesion was comparable for all four alcohols (Table 1); however, potency differed severalfold (Fig. 2), increasing as a function of the number of 1-butanol moieties rather than the molecular volume or the membrane/buffer partition coefficient.

Long-Chain Alcohols Antagonize the Effects of Short-Chain Alcohols on Cell–Cell Adhesion. Although 1-alcohols longer than 1-butanol have no intrinsic activity, they might still antagonize the effects of shorter 1-alcohols by competing for binding at a putative hydrophobic target site. We used BMP-7-treated NG108–15 cells to test whether 1-pentanol or 1-octanol could antagonize the inhibition of cell–cell adhesion by a maximally effective concentration of 1-butanol (2 mM). Both 1-pentanol and 1-octanol abolished the effects of 1-butanol (Figure 3*A*). Antagonism was dose-dependent, and 1-octanol (IC_{50} = 3.6 μM) was approximately 200 times more potent than 1-pentanol (IC_{50} = 715 μM). Both 1-pentanol and 1-octanol also abolished the effects of 100 mM ethanol and 3 mM 3-methyl-1-butanol (data not shown). Similar results were obtained in L1-expressing NIH 3T3 cells (2B2-L1) (not shown).

To explore the mechanism of antagonism, we performed dose-response curves of 1-butanol in the absence and presence of two concentrations of 1-octanol (5 and 50 μM). 1-Octanol reduced the maximal effectiveness of 1-butanol in 2B2-L1 cells (Fig. 3*B*), consistent with a noncompetitive mechanism of inhibition. To determine whether 1-octanol was a reversible antagonist, 2B2-L1 cells growing in T75 tissue culture flasks were incubated for 30 min at 37°C in the absence and presence of 50 μM 1-octanol, washed three times with 10 ml of medium, and harvested for adhesion assays. Pretreatment with 1-octanol followed by washing did not reduce 1-butanol inhibition of cell–cell adhesion (1-butanol inhibition: control, 43.6 ± 3.0%; 1-octanol pretreatment, 55.7% ± 11%).

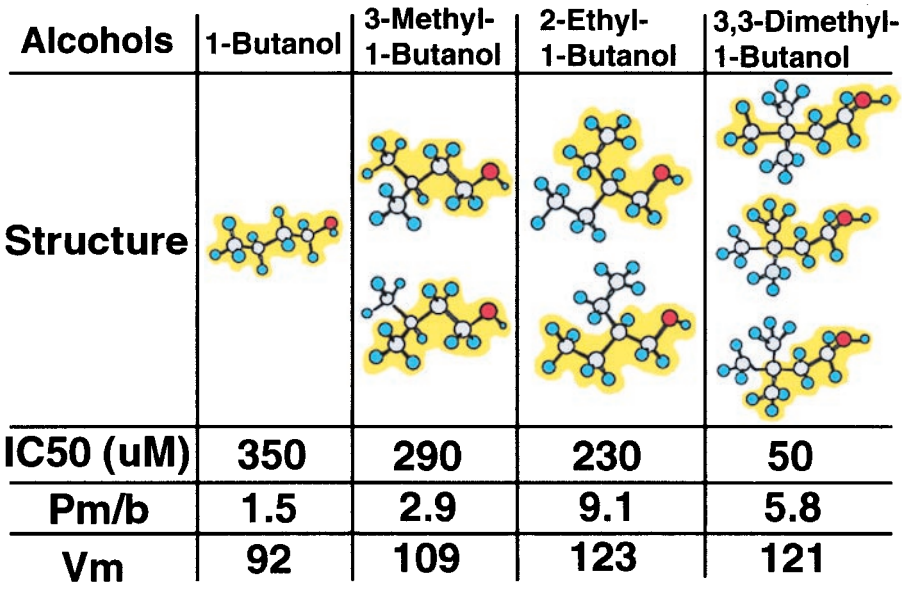

Fig. 2. Relation between the number of 1-butanol moieties and the potency for alcohol inhibition of cell–cell adhesion. Dose–response curves for the mean percent inhibition of cell–cell adhesion in BMP-7-treated NG108–15 cells were calculated from three to six independent experiments. The IC_{50} was determined by log-logit analysis of the mean data (not shown). The number of alignments that can present a 1-butanol moiety (highlighted in yellow) to a target are depicted for each alcohol. Also shown is the membrane/buffer partition coefficient ($P_{m/b}$) and molar volume (V_m) of each alcohol. Note that 3,3-dimethyl-1-butanol, which can present a 1-butanol moiety from three possible alignments, is less lipid soluble than 2-ethyl-1-butanol, but more than 4 times as potent.

Ethanol Inhibition of BMP-7 Morphogenesis Is Also Antagonized by 1-Octanol. Treatment with BMP-7 for 48 h causes NG108–15 cells to grow in clusters of adherent cells (BMP-7 morphogenesis), rather than as predominantly single cells. We showed previously that low concentrations of ethanol inhibit BMP-7 morphogenesis (10). We therefore asked whether 1-octanol could antagonize ethanol inhibition of BMP-7 morphogenesis. NG108–15 cells were cultured for 3 days in the presence of 0.1–40 ng/ml BMP-7; parallel BMP-7-treated cultures were treated with 50 mM ethanol or 50 mM ethanol and 50 μM 1-octanol. BMP-7 caused a dose-dependent increase in the percentage of cell clusters, and this effect was inhibited significantly by 50 mM ethanol (Fig. 4). Octanol abolished ethanol inhibition of BMP-7 morphogenesis.

Discussion

These experiments provide evidence for a highly specific interaction between alcohols and a target that regulates cell–cell adhesion. Alcohols of specific size and shape selectively inhibit cell–cell adhesion in L1-transfected NIH 3T3 cells and in BMP-7-treated NG108–15 cells. The pharmacological profile of 20 different alcohols is identical in the two cellular systems, consistent with the existence of a common molecular target. Previous data suggest that this target is L1 or an L1-associated protein (11–13).

Our structure activity analysis indicates that the alcohol target discriminates among alcohols of equivalent molecular volume and is exquisitely sensitive to molecular shape. Cell–cell adhesion is inhibited with increasing potency by methanol, ethanol, 1-propanol, and 1-butanol (10, 11). This suggests that short-chain 1-alcohols interact with a hydrophobic recognition site. The existence of a cutoff above 1-butanol and cyclobutanol implies that this site has limited dimensions. Membrane lipid solubility is not a critical determinant of alcohol action, because membrane/buffer partition coefficient increases sharply as a function of carbon chain length for alcohols across the range of active and inactive 1-alcohols (15). The total number of carbons is also not a critical determinant of activity. The most potent 1-butanol derivatives, 2-ethyl-butanol and 3-dimethyl-1-butanol, have more carbons (six) and larger molecular volumes than the inactive alcohol, 1-pentanol.

1-Butanol is the most potent 1-alcohol, and molecules related to 1-butanol were the most informative about the structural requirements for alcohol activity. The addition of methyl groups to the 2- and 3-carbon positions increases potency; in fact, molecules that comprise multiple 1-butanol moieties appear to act as multivalent ligands. Derivatives of 1-butanol become inactive if there is restricted rotation between the 3 and 4 carbons (3-buten-1-ol and cyclopropylethanol) or interference with the hydroxyl group (2-pentanol). These data indicate that the target site is optimally engaged by molecules related to 1-butanol, but also imposes structural constraints on the presentation of the 1-butanol molecule. In this respect, alcohols appear to act like classical receptor ligands in inhibiting cell–cell adhesion.

Dwyer and Bradley (8) have derived a loose consensus sequence of structural features in alcohol-sensitive proteins. In their model, the methyl groups of the alcohol lie within a hydrophobic cavity or groove, whereas the hydroxyl group participates as a hydrogen bond donor. The hydrophobic groove and the hydrogen acceptor site are formed from several structural elements, such as loops and turns, often near an α-helix. Our structure activity analysis is consistent with the presence of both a discrete hydrophobic binding groove and a nearby hydrophilic allosteric regulatory site. For 1-butanol, alignment of the hydroxyl group with the allosteric site must require some flexibility within the hydrophobic groove, because restriction of rotation about the 4-carbon abolishes activity. 1-Alcohols longer than 1-butanol may be inactive because they are too large to fit within the hydrophobic groove. Alternatively, long-chain 1-alcohols may fit within the hydrophobic groove, but project their hydroxyl groups too far from the allosteric site.

The presence of a highly selective target site predicts the existence of antagonists. A major finding of this study is that 1-pentanol and 1-octanol, although inactive alone, completely abolish the effects of 1-butanol or ethanol on cell–cell adhesion.

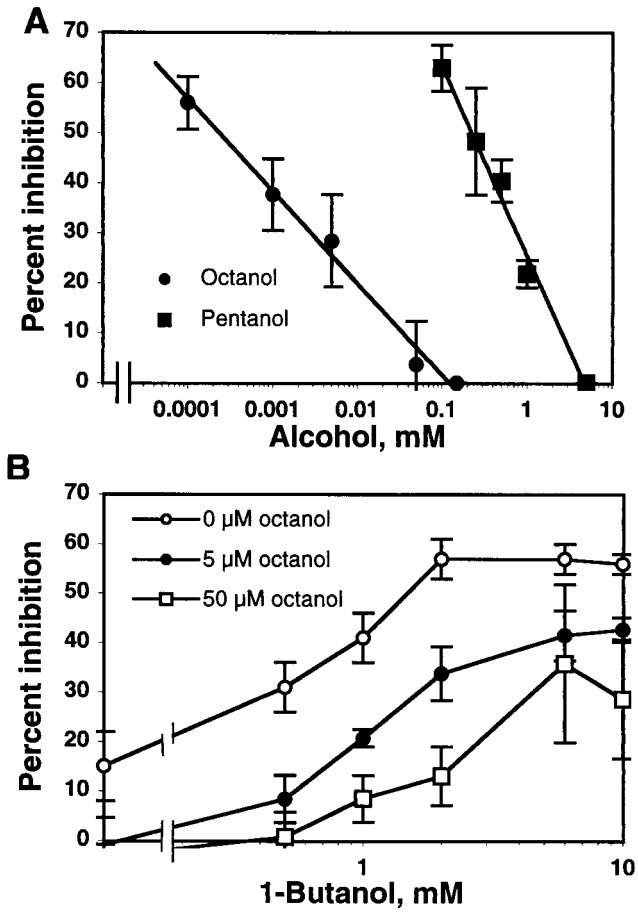

Fig. 3. Antagonism of 1-butanol inhibition of cell–cell adhesion by 1-octanol and 1-pentanol. (*A*) Cell adhesion assays were performed with BMP-7-treated NG108–15 cells in the presence of 2 mM 1-butanol and the indicated concentrations of 1-octanol or 1-pentanol. Shown are means for the percent inhibition of cell–cell adhesion by 1-butanol ($n = 3–5$). Similar results were obtained by using L1-expressing NIH 3T3 cells expressing human L1 (not shown). (*B*) Dose–response curves for 1-butanol inhibition of cell–cell adhesion in NIH 3T3-L1 (2B2-L1) cells were determined in the presence of the indicated concentrations of 1-octanol. Shown are the mean ± SEM percent inhibition of cell–cell adhesion for three to five experiments.

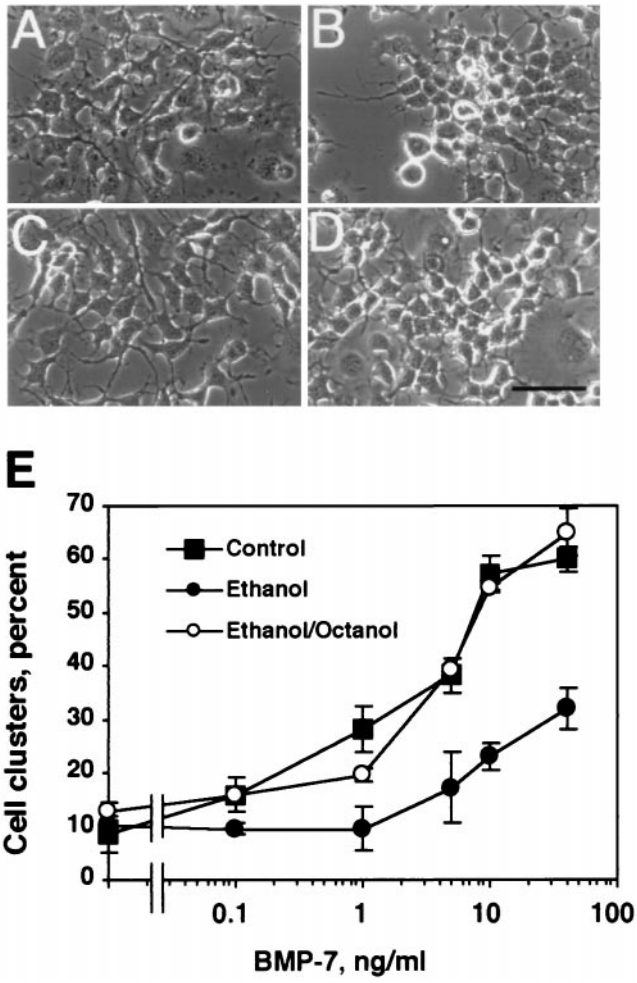

Fig. 4. 1-Octanol antagonizes ethanol inhibition of BMP-7 morphogenesis. NG108–15 cells were cultured for 3 days in serum-free medium supplemented with the indicated concentrations of BMP-7, ethanol, and 1-octanol. (*A–D*) Photomicrographs were obtained under phase-contrast microscopy (×200 magnification) from cells treated as follows: (*A*) no additions (control); (*B*) 10 ng/ml BMP-7; (*C*) 10 ng/ml BMP-7 and 50 mM ethanol; (*D*) 10 ng/ml BMP-7, 50 mM ethanol, and 0.05 mM 1-octanol. (*E*) NG108–15 cells were cultured for 3 days in the presence of 0.1–40 ng/ml BMP-7. Parallel cultures were treated with 50 mM ethanol or 50 mM ethanol plus 0.05 mM 1-octanol. The percentage of cells in clusters of three or more cells was scored from two separate fields viewed under ×200 magnification. Shown are the mean ± SEM for the percentage of cell clusters from three to four independent experiments. (Scale bar = 100 μm.)

1-Octanol was more potent than 1-pentanol, suggesting that antagonism also requires interaction with a hydrophobic site. This demonstrates that 1-alcohols above a cutoff can antagonize the actions of 1-alcohols below the cutoff. These data also provide a description of a specific, nonenzymatic antagonist of ethanol in neural cells. The mechanism of antagonism remains unclear. The antagonists may compete with active alcohols for access to the hydrophobic target site. Alternatively, binding of the antagonists may induce a conformational change that moves an allosteric site out of range of the active alcohols, without disrupting cell–cell adhesion. The noncompetitive nature of antagonism by 1-octanol is more consistent with this latter possibility. Antagonism by long-chain alcohols is not a universal property of alcohol targets that exhibit a cutoff. 1-Octanol did not antagonize ethanol enhancement of GIRK1/4 potassium channel activity (20), which exhibits a cutoff effect between 1-propanol and 1-butanol.

To learn whether 1-octanol antagonism modifies the effects of ethanol on a cellular response that depends on cell adhesion, we used a cellular model in which the induction of L1 and N-CAM alters the morphology of proliferating neural cells. We showed previously that ethanol inhibits the morphological changes induced by BMP-7 in NG108–15 cells (10). Here, we demonstrate that 1-octanol potently antagonizes ethanol inhibition of BMP-7 morphogenesis. Thus, 1-octanol antagonizes both the short-term effects of ethanol on cell–cell adhesion and the long-term effects of ethanol in a morphogenetic assay of proliferating neural cells.

Among different clonal, L1-transfected NIH 3T3 cell lines, only a subset were ethanol-sensitive, suggesting that host-cell factors modify the effects of ethanol on L1-mediated cell–cell adhesion (12). Myeloma cells and insect S2 cells transfected with L1 were also insensitive to ethanol (14, 21). In effect, 1-octanol converts L1-expressing NIH 3T3 cells from an ethanol-sensitive to an ethanol-insensitive phenotype. If long-chain alcohols can prevent ethanol interaction with L1, then other cell-specific, posttranslational modifications of the molecule might do likewise.

L1 plays a critical role in neural development (22) and has also been implicated in processes related to learning and memory (23). We have speculated that effects of ethanol on L1 and cell adhesion could contribute to the development of fetal alcohol syndrome and to the cognitive impairment of alcoholics (11). 1-Octanol and related compounds may prove useful in dissecting the role of L1 and cell adhesion in both of these adverse effects of ethanol on the nervous system; L1 would be a candidate target for actions of ethanol that are blocked by low concentrations of 1-octanol. Compounds that block ethanol effects on L1 might also reduce ethanol teratogenesis.

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