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Characterization of the Selective Inhibition of the *Delta* Subclass of Opioid Binding Sites by Alcohols

JACOB M. HILLER, LLOYD M. ANGEL, AND ERIC J. SIMON

Departments of Psychiatry and Pharmacology, New York University Medical Center, New York, New York 10016

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SUMMARY

We recently reported that ethanol and other aliphatic alcohols exert a selective inhibition on the binding of enkephalins to *delta* opioid binding sites. We report here a more detailed investigation of the characteristics of this inhibition. Opioid binding sites of the *kappa* subtype are similar to *mu* opioid binding sites in their relative insensitivity to inhibition by aliphatic alcohols. Scatchard analysis of saturation data of enkephalin binding showed that inhibition is the result of a decrease in affinity. Results of kinetic experiments demonstrated that the inhibition can be entirely accounted for by an increase in the dissociation rate of the ligand-receptor complex. The presence of sodium ions in the incubation medium and raising the temperature of incubation exacerbate the inhibitory effectiveness of alcohols. The order of potency among structural isomers of alcohols for inhibition of *delta* receptor binding is as follows: straight-chain primary > isoprimary > secondary > tertiary. The order of inhibitory potency of the aliphatic alcohols tested correlates well with their ability to disorder the cell membrane lipid bilayer. It is suggested that this is a probable mechanism by which alcohols inhibit binding to *delta* opioid binding sites.

INTRODUCTION

In the past few years considerable evidence has been accumulated in a number of laboratories which supports the existence of multiple opioid receptors. The earliest evidence for multiplicity of opioid receptors came from the laboratory of Martin and co-workers (1, 2). Based on differences in the pharmacological profile of morphine and several analogues in chronic spinal dogs, they proposed the existence of three classes of opioid receptors which they named for the prototypic ligands, *mu* for morphine, *kappa* for ketazocine, and *sigma* for SKF 10047 (*N*-allyl normetazocine). Data from *in vitro* bioassay systems and receptor binding studies produced evidence for both a morphine-preferring (*mu*) binding site, thought to be similar to or identical with the *mu* receptor of Martin *et al.*, and an enkephalin-preferring *delta* binding site (3–5).

The selective inhibition or inactivation of receptor subtypes by enzymes or chemical reagents other than receptor ligands initially met with little success. It was therefore necessary to resort instead to studies of selective protection against inactivation of receptor types by ligands having selectivity for a given type (6, 7).

We recently published a brief report which provided evidence for selective inhibition of the *delta* class of opioid binding sites by aliphatic alcohols (8). Considerable effort was expended to show that the effect of the alcohols is on the binding sites rather than on the peptide ligands. The inhibition of *delta* binding sites was found to be reversible, and the potency of the alcohols increased exponentially with their chain length. In the present paper we characterize further the interaction of alcohols with the three major types of opioid binding sites (*mu*, *delta*, and *kappa*).

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MATERIALS AND METHODS

Male Sprague-Dawley rats were purchased from Taconic Farms (Germantown, N. Y.), and male C57 black mice from Jackson Laboratories (Bar Harbor, Maine). Human brain tissue was obtained from Dr. E. Gross, Chief Medical Examiner of the City of New York. Crude brain membrane fractions were prepared by a method previously described by this laboratory (9). The membrane preparations were stored in 0.32 M sucrose at −70° until needed. For binding experiments, duplicate 2-ml samples (0.9–1.1 mg of protein per milliliter) in 0.05 M Tris-HCl (pH 7.4) containing 1 mM dipotassium EDTA were incubated with 1 nM tritiated ligand. To assess specific binding, these samples were incubated in the presence and absence of 1 μM unlabeled ligand. Incubation conditions are noted in the legends to the figures. After incubation, samples were filtered through Whatman GF/B filters. Filters were washed twice with 4 ml of buffer, and radioactivity remaining on dried filters was determined. Unless otherwise stated, all experiments were performed on rat brain crude membrane fractions.

[^{3}H]Naltrexone (specific activity 9.7 Ci/mmole) was kindly supplied by Dr. Richard Hawks, National Institute on Drug Abuse. [^{3}H]DHM[1]

[1] The abbreviations used are: DHM, dihydromorphine; DADL, D-Ala^2,D-Leu^5-enkephalin; DAGO, *H*-Tyr-D-Ala(Me)Phe-NH-CH$_2$-OH; DSTLE, *H*-Tyr-D-Ser-Gly-Phe-Leu-Thr.

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(specific activity 71.0 Ci/mmole) and ^{3}H-labeled DADL (specific activity 31.0 Ci/mmole) were purchased from New England Nuclear Corporation (Boston, Mass.). [^{3}H]Bremazocine (specific activity 24.0 Ci/mmole) was a gift from Dr. D. Roemer, Sandoz, Ltd. (Basle, Switzerland). DADL was purchased from Peninsula Laboratories, Inc. (San Carlos, Calif.). DAGO, a peptide selective for *mu* binding sites, was a gift from Dr. M. J. Rance, Reckitt and Coleman Pharmaceuticals (Hull, United Kingdom). DSTLE, a peptide selective for *delta* binding sites, was a gift from Dr. B. P. Roques, School of Pharmacy, University of Paris (Paris, France). Alcohols and other solvents were of reagent grade and were purchased from Fisher Scientific Company (Pittsburgh, Pa.) and Aldrich Chemical Company (Milwaukee, Wisc.).

RESULTS

Effect of 1-butanol on binding to kappa sites. We have reported that opioid interaction with *mu* binding sites is considerably more resistant to alcohols than is binding to *delta* binding sites (8). Binding of ethylketocyclazocine, reported to have the pharmacological activity of a *kappa*-type opiate (10), was also found to be relatively resistant to alcohols (8). Since ethylketocyclazocine and other *kappa* ligands, such as bremazocine, have been found to bind with almost equal affinity to *mu*, *delta*, and *kappa* sites, no conclusion could be drawn from this result regarding inhibition of *kappa* sites by alcohols. The effect of 1-butanol on the binding of [^{3}H]bremazocine to *kappa* sites was therefore assessed by blocking *mu* and *delta* sites with saturating concentrations of appropriate ligands. This was accomplished by adding 100 nM each of DAGO and DADL, a technique developed by Gillian *et al.* (11) and previously used by our laboratory (12). Under these conditions, [^{3}H]bremazocine binding was very resistant to inhibition by 1-butanol. Exposure to 1% 1-butanol, which reduced the binding of [^{3}H]DADL by 70%, decreased [^{3}H]bremazocine binding by only 12%. This result indicates that *kappa* opiate binding sites are similar to *mu* sites in their resistance to 1-butanol.

Effect of 1-butanol on opioid binding after selective blocking of binding sites. To obtain further evidence for the selective effect of alcohols on *delta* sites, we performed experiments in which we attempted to block *delta* sites or non-*delta* sites (*mu* and *kappa*) selectively. When *delta* sites were blocked with the relatively selective *delta* ligand, DSTLE, the inhibition of [^{3}H]DADL was significantly less affected by 0.5% 1-butanol (81 ± 1% versus 57 ± 1% of control), whereas the binding of [^{3}H]naltrexone was essentially unaffected (94 ± 1.5% versus 98 ± 1% of control).

When an attempt was made to enrich the proportion of *delta* sites by blocking *mu* sites (with 10 nM DAGO or morphine) and *kappa* sites (with 10 nM dynorphin), the binding of [^{3}H]DADL was inhibited by 1-butanol to the same extent (57 ± 1% versus 58 ± 1.8%). There was significantly greater inhibition of [^{3}H]naltrexone binding in the blocked preparation (81 ± 1% versus 98 ± 1%). These results are consistent with our previous findings (8) that alcohol acts on the binding site rather than on the ligand.

Equilibrium and kinetic studies of the effect of 1-butanol on the binding of [^{3}H]DADL. In order to ascertain whether the inhibitory effect of alcohols on *delta* binding sites was due to a change in binding affinity or in the number of sites, saturation studies of the binding of [^{3}H]DADL in a concentration range of 0.5–10 nM were carried out in the presence and absence of 0.5% 1-butanol at 25°. Scatchard analysis of these saturation curves, presented in Fig. 1, yielded linear plots for this concentration range. There was no significant change in the maximal number of binding sites (0.12 pmole/ml for control preparations and 0.13 pmole/ml for preparations containing 0.5% 1-butanol). However, a 2-fold decrease in affinity was observed when 0.5% 1-butanol was added to the incubation mixture. K_d values for the binding of [^{3}H]DADL calculated from these equilibrium data were 1.66 nM for controls and 3.29 nM in the presence of 0.5% 1-butanol.

We then studied the kinetics of the binding of [^{3}H]DADL in the presence and absence of 1-butanol (0.5%) at 25°. The rates of binding of [^{3}H]DADL under the two conditions are shown in Fig. 2. Second-order constants calculated for association rates were 4.5×10^{-7} M^{-1} min^{-1} for control samples and 3.8×10^{-7} M^{-1} min^{-1} for binding in the presence of 1-butanol. The difference between these rate constants is not statistically significant. The first-order rate constants for dissociation of [^{3}H]DADL under control and experimental conditions were 4.6×10^{-2} min^{-1} ($t_{1/2}$ = 16 ± 1.4 min) and 9.9×10^{-2} min^{-1} ($t_{1/2}$ = 7.0 ± 0.3 min), respectively (Fig. 3). It is evident from these data that the inhibition of DADL binding by 0.5% 1-butanol can be totally accounted for by the increase in the rate of dissociation of the receptor-ligand complex.

When the dissociation constants were calculated from the kinetic data ($k_d/k_a = K_d$), values of 1.02 nM for binding in control samples and 2.60 nM for 1-butanol-treated samples were obtained. These values are in satisfactory agreement with the results obtained from the binding experiments.

Saturation studies of the binding of [^{3}H]DADL were also performed in the presence of 10 nM morphine and 10 nM dynorphin. This protocol should, presumably, enrich the proportion of *delta* sites. Scatchard analysis of the saturation curves yielded linear plots for the

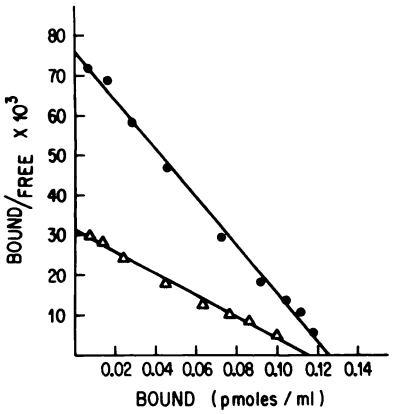

FIG. 1. *Scatchard analysis of saturation curves for the specific binding of [^{3}H]DADL to rat brain membrane preparations in the presence (△) and absence (●) of 0.5% 1-butanol.*

Binding of tritiated ligand (0.5–10 nM) was carried out at 25° for 45 min as described under Materials and Methods. Lines of best fit were determined by regression analysis.

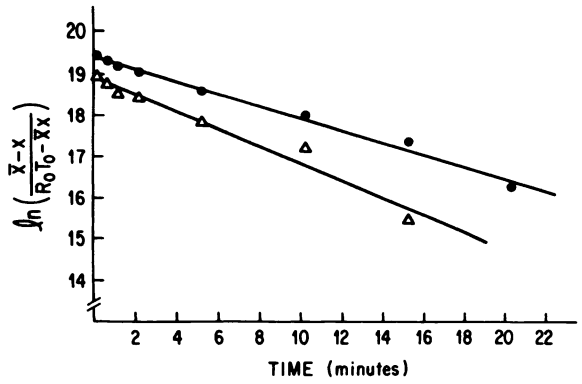

FIG. 2. *Kinetics of specific binding of [^{3}H]DADL to opioid binding sites in rat brain membrane preparations at 25° in the presence (△) and absence (●) of 0.5% 1-butanol.*

The concentration of [^{3}H]DADL binding sites (R_0) equaled 0.1 pmole/ml, and the tritiated ligand concentration (T_0) was 1 nM. The equilibrium concentration of receptor-ligand complex ($\bar{x}$) in controls was 2.8×10^{-11} M; in the presence of 0.5% 1-butanol, 1.7×10^{-11} M. The applied rate law (13)

$$K_2 = \frac{1}{t}\,\frac{\bar{x}}{x^{-2} - R_0 \cdot T_0}\left(ln\,\frac{\bar{x} - x}{R_0 \cdot T_0 - \bar{x}x} + ln\,\frac{R_0 \cdot T_0}{\bar{x}}\right)$$

was then used to determine association rate constants.

concentration range of [^{3}H]DADL used (0.5–5.0 nM). No significant changes were observed in the maximal number of binding sites (55 ± 8 fmoles/ml in the absence of 1-butanol and 50 ± 3 fmoles/ml in the presence of 1-butanol). The K_d value for [^{3}H]DADL binding in the absence of 1-butanol was 1.17 ± 0.17 nM; in the presence of 1-butanol, 2.04 ± 0.02 nM. Thus the ratios of K_d values obtained when *mu* and *kappa* sites were blocked (0.57) and in the absence of *mu* and *kappa* blocking agents (0.51) were not significantly different.

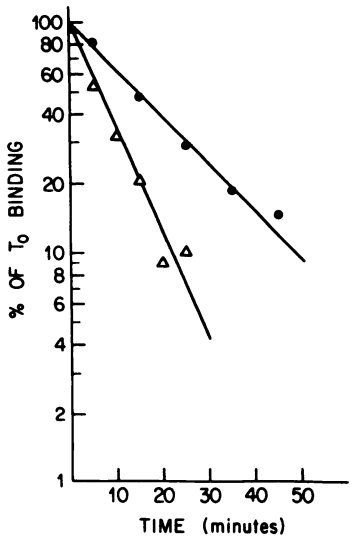

FIG. 3. *Dissociation of [^{3}H]DADL specific binding from rat brain membrane preparations in the presence (△) and absence (●) of 0.5% 1-butanol.*

Binding of tritiated ligand (1 nM) was carried out at 25° for 45 min. At time 0 (T_0), a 1000-fold excess of unlabeled drug was added to the homogenates, and specific binding remaining in aliquots, at indicated time intervals, was determined and plotted as percentage of T_0 binding.

Effect of temperature on inhibition of DADL binding by 1-butanol. The influence of incubation temperatures on the effect of 1-butanol on receptor binding was investigated. Length of incubation was selected so as to permit binding to reach steady state at each temperature. As seen from Table 1, it was found that 1-butanol was significantly more effective at 37° than at either 24° or 0° in inhibiting DADL binding. This was apparent at both concentrations of 1-butanol tested. Although the inhibition of [^{3}H]DHM and [^{3}H]naltrexone binding by 1-butanol at 37° was also significantly greater than that seen at 24°, the inhibition was still well below that for [^{3}H]DADL binding.

Effect of sodium on alcohol inhibition of DADL binding. The effect of 1-butanol on opioid binding in the presence of sodium salts was investigated (Table 2). Sodium did not confer any degree of protection from the effects of 1-butanol on [^{3}H]DADL binding. On the contrary, sodium seemed to exacerbate somewhat the inhibitory effect of this alcohol on [^{3}H]DADL binding. In the case of [^{3}H] naltrexone binding, there was a small inhibitory effect of 1-butanol on the increased binding observed in the presence of sodium.

The binding of [^{3}H]DHM, which was depressed in the presence of NaCl, showed some additional inhibition by 1-butanol (0.5%) which was not seen at this concentration in the absence of NaCl.

Effect of chain branching and position of hydroxyl group on the inhibitory potency of alcohols. The effects of various primary, secondary, tertiary, and branched-chain alcohols on the binding of [^{3}H]DADL and [^{3}H]DHM to rat brain membrane preparations are presented in Table 3. With all alcohols tested, *delta* binding was depressed and *mu* binding was either increased or depressed to a much lesser extent than *delta* binding. These data also show that secondary alcohols are less inhibitory to *delta* binding than the respective primary alcohols with the same carbon chain length. For example, 2-pentanol, 2-butanol, and 2-propanol are weaker inhibitors than 1-pentanol, 1-butanol, and 1-propanol, respectively. Tertiary butyl alcohol, the only tertiary alcohol tested, was found to be even less inhibitory than 2-butanol. Furthermore, the inhibitory effect of straight-chain primary alcohols on *delta* binding was somewhat greater than that observed with branched primary alcohols having equal numbers of carbon atoms; e.g., 1-butanol and 1-pentanol were comparatively more effective inhibitors than isobutyl alcohol and isopentyl alcohol. Benzyl alcohol, the only aliphatic alcohol tested containing an aromatic ring, had a potency equivalent to that of a five-carbon, primary straight-chain alcohol.

Selectivity of other organic solvents in inhibiting opioid binding. Other organic solvents were also tested for their ability to inhibit selectively *delta* binding. Results seen in Table 4 show that the three solvents tested, acetone, acetonitrile, and ethyl acetate, inhibited the binding of DADL to a greater extent than the binding of DHM. Although the differential effects were less dramatic than those seen with alcohols, they are nevertheless still apparent, suggesting that a hydroxyl group is not absolutely indispensable for this differential inhibition. The dihydroxy compound, ethylene glycol, and the trihydroxy

TABLE 1

Effect of 1-butanol on [^{3}H]DADL and [^{3}H]naltrexone binding: influence of temperature of incubation

Binding of [^{3}H]DADL, [^{3}H]naltrexone, and [^{3}H]DHM, each at a concentration of 1 nM, to rat brain homogenate was carried out at three temperatures, as described under Materials and Methods, in the presence of two concentrations of 1-butanol. Values presented are the means ± standard error of the mean of at least three experiments.

Incubation temperature	Length of incubation	% of control binding[a]					
		[^{3}H]DADL		[^{3}H]naltrexone		[^{3}H]DHM	
		0.25% 1-Butanol	0.5% 1-Butanol	0.25% 1-Butanol	0.5% 1-Butanol	0.25% 1-Butanol	0.5% 1-Butanol
	min						
37°	25	66 ± 2.0**	40 ± 3.1***	84 ± 2.8*	63 ± 1.3***	89 ± 2.9*	83 ± 1.5**
24	60	79 ± 0.3	54 ± 1.3	98 ± 2.4	90 ± 2.1	109 ± 4.6	106 ± 5.0
0	120	72 ± 0.9	54 ± 2.1	97 ± 5.2	90 ± 6.4	110 ± 6.9	110 ± 9.9

[a] Significance: ** $p < 0.02$, ** $p < 0.01$, *** $p < 0.001$, significantly lower than 24° values containing the same concentrations of alcohol.

derivative, glycerol, were also tested. They showed inhibition of opiate binding only at very high concentrations and with little or no selectivity. This is probably due to the high degree of polarity of these polyalcohols.

DISCUSSION

Our previous results, indicating that aliphatic alcohols inhibit *delta* binding sites at concentrations at which *mu* sites are affected little or not at all (8), have been extended to *kappa* sites. When the binding of bremazocine was studied under conditions in which *mu* and *delta* sites were saturated, it was found that the remaining bremazocine sites, presumed to be largely of the *kappa* type, were quite resistant to inhibition by 1-butanol. It thus appears that inhibition by aliphatic alcohols at relatively low concentrations is selective for *delta* sites. At higher concentrations, both *mu* and *kappa* sites began to be inhibited as well.

Selective blocking of *delta* sites significantly reduced the inhibition of [^{3}H]DADL binding by 1-butanol, whereas the blocking of *mu* and *kappa* sites increased the inhibition of [^{3}H]naltrexone binding. These results confirm that the action of alcohol is exerted on the binding sites.

The inhibition by 1-butanol of [^{3}H]naltrexone binding in the presence of *mu* and *kappa* blockers was not as striking as that observed by us previously (8) in NG108-15 cells, a source of essentially pure *delta* sites. This is undoubtedly due to the incomplete saturation of *mu* and *kappa* sites at the low concentrations of blockers used to avoid blocking *delta* sites. The lack of increased alcohol sensitivity of [^{3}H]DADL binding when non-*delta* sites were blocked suggests that DADL, at the concentrations used, binds predominantly to *delta* sites. This was confirmed by the linear Scatchard plot for DADL binding over the concentration range used in our experiments.

Data from saturation binding experiments strongly suggest that 1-butanol decreases the affinity for opioid peptides without significantly affecting receptor number. Kinetic studies generated a K_d which was in good agreement with that obtained from equilibrium experiments and showed that the change in affinity was caused by an increase in the dissociation rate of DADL in the presence of alcohol.

It is evident that the degree of inhibition is quite dependent on the concentration of ligand. It is therefore critical to use appropriate concentrations when comparisons are made of different ligands at a single concentration. In all of these experiments, 1 nM concentrations of the various ligands were used. Since the K_d for high-affinity binding of bremazocine, naltrexone, DADL, and DHM varies at most by a factor of 2 from 1 nM, we felt this to be a suitable concentration. It is assumed that an approximately equal fraction of the type of opioid binding

TABLE 2

Effect of 1-butanol on opioid binding in the presence of sodium ions

Binding of tritiated ligands to brain membrane preparations in the presence and absence of sodium chloride and 1-butanol was carried out at 25° for 45 min, as described under Materials and Methods. Figures in parentheses represent percentages of control binding. Values are means ± standard error of the mean of at least three experiments.

^{3}H-Labeled ligand	Specific binding		
	0 Na^+	10 mM NaCl	25 mM NaCl
		fmoles/ml	
DADL			
Control	45 ± 2.2	27 ± 3.3	21 ± 2.7
0.5% 1-Butanol	24 ± 1.4 (54 ± 1.3)	10 ± 1.2 (40 ± 2.5)	7 ± 0.6 (33 ± 2.5)
Naltrexone			
Control	119 ± 5.1	158 ± 7.9	171 ± 1.7
0.5% 1-Butanol	107 ± 6.5 (90 ± 2.1)	135 ± 7.1 (86 ± 0.3)	135 ± 6.1 (79 ± 3.0)
DHM			
Control	41 ± 1.5	31 ± 2.7	21 ± 2.6
0.5% 1-Butanol	43 ± 2.1 (106 ± 5.0)	26 ± 1.8 (86 ± 3.5)	17 ± 2.9 (82 ± 11.7)

TABLE 3

Effect of alcohols on the binding of mu and delta ligands: influence of position of the hydroxyl group and chain branching on alcohol potency

Rat brain membrane preparations were specifically bound with ^{3}H-labeled ligand, as described under Materials and Methods, in the presence of the indicated concentrations of alcohol (v/v) for 45 min at 25°. Where standard errors are given, the value represents the mean of at least three experiments. Where no standard errors are shown, the two individual values are given.

Alcohol and concentration	% of control binding	
	[^{3}H]DADL (1 nM)	[^{3}H]DHM (1 nM)
%		
1-Propanol		
0.5	79 ± 2	123, 129
1.0	59 ± 3	138, 145
2.0	30, 33	78, 92
5.0	1, 4	12, 25
Isopropanol		
2.0	68, 64	121, 118
5.0	24, 19	89, 89
1-Butanol		
0.25	79 ± 0.3	98 ± 2
0.5	57 ± 2	104 ± 1
1.0	20 ± 1	87 ± 7
2-Butanol		
0.25	77, 100	113, 118
0.5	73 ± 4	112 ± 8
1.0	44 ± 4	112 ± 7
Isobutanol		
0.5	64 ± 2	102 ± 2
1.0	33 ± 1	84 ± 8
t-Butanol		
0.5	96 ± 7	119, 128
1.0	87 ± 5	121, 122
1-Pentanol		
0.25	51, 49	78, 75
0.5	16, 14	45, 44
Isopentanol		
0.25	52, 56	73, 67
0.5	23, 26	47, 43
2-Pentanol		
0.25	66, 74	118, 98
0.5	41 ± 6	90, 93
1.0	11, 13	82, 64

TABLE 4

Effect of other organic solvents on the binding of various opioid ligands to rat brain membranes

Rat brain membranes were specifically bound, as described under Materials and Methods, for 45 min at 25° with tritiated ligand in the presence of indicated concentrations of the various solvents (v/v). Values are means ± standard error of the mean for at least three experiments.

Solvent and concentration	% of control binding	
	[^{3}H]DADL (1 nM)	[^{3}H]DHM (1 nM)
%		
Acetone, 2.0	69 ± 4.0	97 ± 3.7
Acetonitrile, 2.0	64 ± 1.4	81 ± 0.9
Ethyl acetate, 1.0	58 ± 2.1	90 ± 0.9
Glycerol		
2.5	92 ± 1	98 ± 2
5.0	84 ± 1	87 ± 3
10.0	68 ± 3	68 ± 1
Ethylene glycol		
2.5	85 ± 1	99 ± 2
5.0	76 ± 2	87 ± 3
10.0	50 ± 2	72 ± 2

site to which each binds with highest affinity was occupied by the ligands.

The experiments reported here in which binding of naltrexone and DHM was compared as to inhibition by 1-butanol under various conditions of temperature and sodium concentration confirm our previous finding that a *mu*-selective agonist and a *mu*-preferring antagonist behave relatively similarly.

An apparent discrepancy between this paper and our earlier report should be pointed out and discussed. In our earlier paper (8) we reported the K_d for the binding of DADL to be 4.9 nM in control samples and 11.9 nM in the presence of 0.5% 1-butanol. In this paper we report K_d values that are approximately 3-fold lower. This difference is the result of the temperature at which these experiments were performed. The earlier saturation studies were done at 37° and the present ones at 24°. We have consistently found that the affinity of DADL binding is significantly lower at 37° than at room temperature. This has also been reported by others (14). In view of these findings, the data presented in Table 1 showing increased potency of 1-butanol at 37° may be explained by the decreased affinity and therefore the lower binding site occupancy of [^{3}H]DADL at this temperature.

Our studies of solvents other than monohydroxyl aliphatic alcohols have shown that compounds that do not contain a free alcohol hydroxyl group can nonetheless inhibit opioid binding. There is some selectivity for inhibition of *delta* binding sites, but this is far less clear-cut than that observed with alcohols. Moreover, polyalcohols are very poor inhibitors, probably because of their low lipid solubility.

Biophysical techniques have been employed by others to demonstrate that alcohols cause a structural disordering of natural membranes (15–18). Lyon *et al.* (19) have observed that the membrane-disordering potency of straight-chain alcohols, as measured by an electron paramagnetic resonance technique, increases logarithmically with the increase in the number of methylene groups. We have previously shown that the potency of straight-chain alcohols in inhibiting enkephalin binding to the *delta* opioid binding sites is increased exponentially with the length of the carbon chain (8). Furthermore, Lyon *et al.* (19) reported that the membrane-disordering potencies by groups of structural isomers were as follows: straight-chain primary > isoprimary > secondary > tertiary. We have observed the same order of potency among structural isomers of alcohols in inhibiting *delta* binding (Table 3). Lyon *et al.* (19) have also suggested that the membrane-disordering potency of an alcohol reflects the ability of that alcohol to partition into a membrane bilayer. They have, in fact, shown that the logarithm of the membrane-disordering potency is linearly related to the logarithm of the membrane/buffer partition coefficient with a slope of about 1. In Fig. 4 we present correlations between the relative inhibitory potencies (based on IC_{50} determinations) of alcohols on the

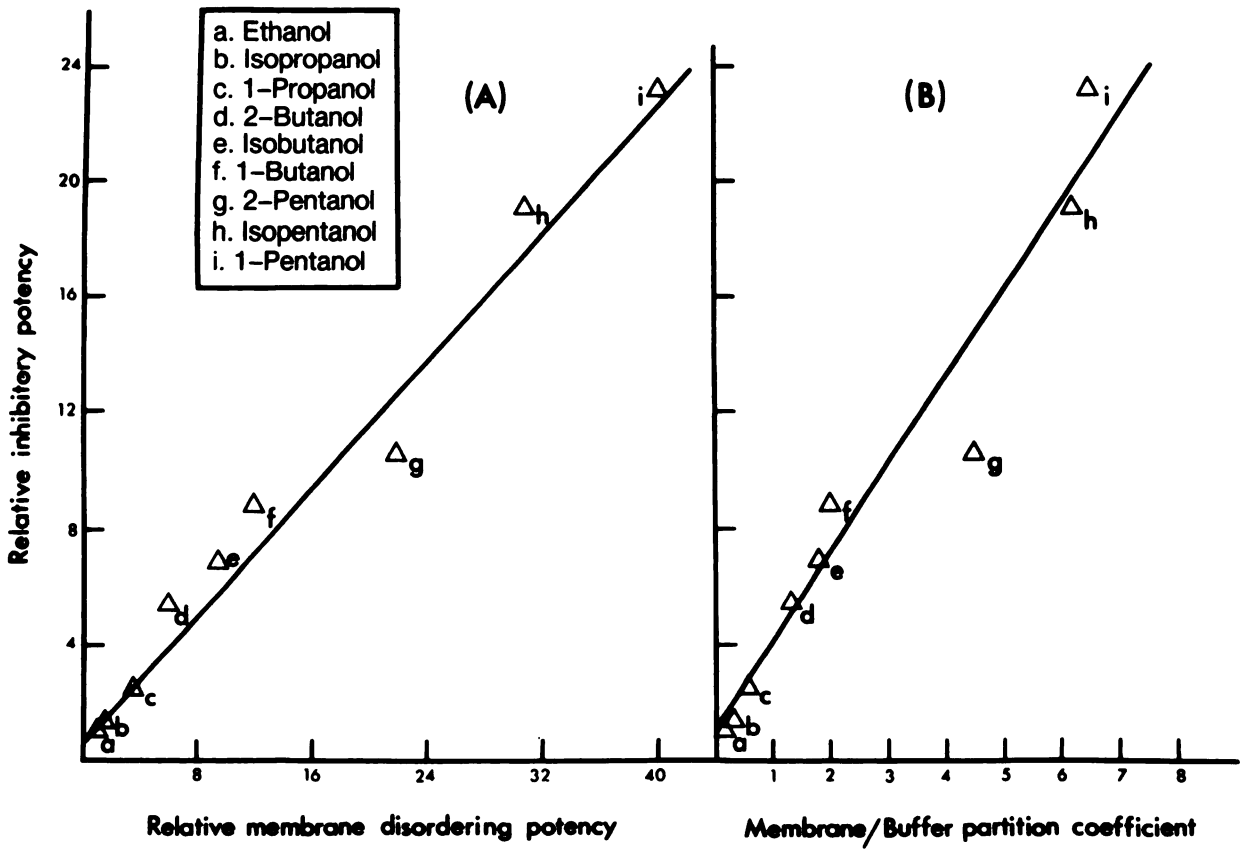

FIG. 4. *Correlation between the relative potency of alcohols to inhibit [^{3}H]DADL specific binding and (A) their relative membrane-disordering potencies and (B) their membrane/buffer partition coefficients.*

The relative inhibitory potency (ethanol = 1) for each alcohol was determined from their IC_{50} values for the inhibition of [^{3}H]DADL binding. Values for relative membrane-disordering potency and membrane/buffer partition coefficient (moles per kilogram of membrane per mole per liter of water) are from Lyon *et al.* (19). Lines of best fit were determined by regression analysis.

binding of [^{3}H]DADL and (a) the relative membrane-disordering effect and (b) the membrane/buffer partition coefficients of the same alcohols. The correlation coefficient (r) for each of these two plots was 0.99. Although we recognize that these data are merely correlational, they do support the idea that the mechanism of the inhibition of *delta* binding sites by alcohols may be via the disordering of the membrane lipid bilayer, which in turn may be determined by the ability of the alcohol to dissolve in the membrane lipids.

In recent experiments on the binding of [^{3}H]DADL at various temperatures, we have observed that binding reaches a peak at 25°. The decrease in DADL binding at higher temperatures begins at about 31–33°, the temperature range at which membrane lipid bilayers are known to have a phase transition point (15). This finding is consistent with our working hypothesis that alcohols act by disordering membrane lipid bilayers, an effect similar to that of temperature.

An interesting hypothesis advanced by Bowen *et al.* (20) suggests that the apparent multiple opioid sites may represent different conformational states of a single binding site. This group envisions a three-state allosteric model consisting of *mu* agonist-, *mu* antagonist-, and adenylate cyclase-coupled *delta* agonist-preferring states. The findings of this paper, when viewed in the light of this hypothesis, may be interpreted to indicate that the disordering of the membrane lipids by alcohols alters in some way the adenylate cyclase-receptor coupling and thus selectively affects the *delta*-preferring state of the Type 1 opiate receptor.

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Send reprint requests to: Dr. Jacob M. Hiller, Department of Psychiatry, New York University Medical Center, 550 First Avenue, New York, N. Y. 10016.