Primary Alcohols Modulate the Activation of the G Protein-coupled Receptor Rhodopsin by a Lipid-mediated Mechanism*

(Received for publication, May 10, 1996, and in revised form, June 14, 1996)

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The visual pigment rhodopsin is a prototypical member of the G protein-coupled receptor superfamily. In this study, we have investigated the effect of a series of n-alcohols on the formation of metarhodopsin II (MII), the photoactivated conformation of rhodopsin, which binds and activates transducin. When rhodopsin was photolyzed in the presence of several n-alcohols, increased MII formation was observed in the order ethanol > butanol > hexanol, whereas longer chain n-alcohols inhibited MII formation with decanol > octanol. The magnitude of the stimulatory effects was greater in a more highly unsaturated phospholipid. Alcohols, which enhanced MII formation also increased phospholipid acyl chain packing free volume, while those that decreased this bilayer property inhibited MII formation. An apparent discontinuity in the effect of these alcohols results when their potency is calculated in terms of the total aqueous alcohol concentration. In sharp contrast, a continuous variation in their behavior is observed, when their potency is calculated in terms of the amount of alcohol partitioned in the membrane. Our findings strongly support a lipid-mediated mechanism of action for alcohols on rhodopsin and, by analogy, for other G protein-coupled receptors.

The mechanism of action of alcohols and general anesthetics is generally discussed in terms of two opposing mechanisms. The first involves an alteration of phospholipid bilayer properties by these agents, resulting in a modulation of membrane protein function, while the second is based on the direct interaction with membrane proteins (1, 2). The lipid mechanism was based originally on the observation (3, 4) that anesthetic potency correlated directly with the solubility of an anesthetic in olive oil. Additional support for the lipid mechanism came from the observation that acute exposure of membranes to ethanol resulted in a disordering of the phospholipid acyl chain packing (5). Subsequent experiments demonstrating that the activity of a soluble enzyme, firefly luciferase, could be inhibited by a diverse group of alcohols and general anesthetics (6) have focused attention on the protein binding hypothesis.

Many of the recent studies aimed at elucidating the mechanism of action of alcohols and general anesthetics have dealt with the effect of these agents on ligand-gated channels, whose ligand binding sites is in a portion of the protein external to the bilayer (1, 7–10). The superfamily of G protein-coupled receptors has received relatively little study. In these receptors, the ligand binding sites are formed by their transmembrane helical segments and lie at the median point of the bilayer. One of the best characterized members of this superfamily is rhodopsin, which triggers the visual transduction pathway in rod cells. A metastable equilibrium between MI and MII (where $K_{eq} = [MII]/[MI]$) is established within milliseconds of photon absorption (11). MII binds and activates the visual G protein, transducin (12, 13). MII formation increases with higher levels of phospholipid acyl chain unsaturation (14–17) and decreasing levels of cholesterol (16, 18).

Differentiating between the two proposed mechanisms of action of alcohols and general anesthetics is difficult. However, studying the lipid dependence of the dose-response behavior of receptors in reconstituted systems is an explicit way of determining the lipid involvement in the action of these molecules. The membranes of postsynaptic neurons, retinal rod outer segments, and other excitable cells contain a preponderance of highly unsaturated phospholipids (for a review, see Ref. 19), suggesting a potential role for these phospholipids in mediating the effects of ethanol and other lipid-soluble agents. Recently, we published studies which support the involvement of a lipid mechanism in the enhancement of MII formation by ethanol (20). Variable chain length alcohols have been employed extensively as models for general anesthetics (1, 2). To further examine the involvement of lipids in the action of ethanol and general anesthetics, we have studied the effect of several n-alcohols on MII formation in ROS disks, PDPC, and POPC vesicles. In addition, the time-resolved anisotropy decay of the hydrophobic membrane probe, DPH, was used to characterize changes in phospholipid acyl chain packing properties in response to the addition of these alcohols.

EXPERIMENTAL PROCEDURES

Sample Preparation—Intact ROS disks were prepared from frozen bovine retinas as described previously (21). Samples for all studies were in a low salt buffer (10 mM HEPES, 50 μM diethyltriaminepentaacetic acid, pH 7.5; HNS buffer) and all measurements were made at 20°C. Suspensions of intact ROS disks (8 μM rhodopsin) were extruded 10x through a 0.2-μm pore filter to reduce light scattering. Rhodopsin was purified and reconstituted with defined phospholipids to yield rhodopsin-containing large unilamellar vesicles as described previously (22, 23). The phospholipids PDPC and POPC were purchased from Avanti Polar Lipids (Alabaster, AL) and used without further purification. The effect of the n-alcohols on ΔG for the MII → MII equilibrium at each concentration was determined according to $ΔG = ΔG_{alcohol} + ΔG_{alcohol}$, where $ΔG_{alcohol} = -RTln(K_{eq} = [MI]/[MII])$ is the gas constant, and $T$ is the absolute temperature. Bulk alcohol concentrations varied as follows: ethanol: 0.15–1.5 mM; butanol: 9–108 mM; hexanol: 1.16–7 mM; octanol: 0.25–0.98 mM; and decanol: 42–170 μM. Bilayer mole fractions, $x_{alcohol}$ of n-alcohols were calculated from

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3 The abbreviations used are: MI, metarhodopsin I; MII, metarhodopsin II; ROS, rod outer segment; POPC, 1-palmitoyl-2-oleoylphosphatidylcholine; PDPC, 1-palmitoyl-2-docosahexaenoylphosphatidylcholine; DPH, 1,6-diphenyl-1,3,5-hexatriene.
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RESULTS

Rhodopsin is uniquely well suited for studies of the mechanism of action of anesthetics, since the ligand, retinal, is covalently bound to the receptor and acts as a reporter of receptor conformation. In addition, the retinal absorbance is very sensitive to the position of the amino acids in the retinal binding site, and the presence of any of the alcohols in the retinal binding pocket would be readily detected as a shift in the retinal absorption spectrum. In our studies none of the alcohols altered the shape or location of the MI, MII, or rhodopsin absorption bands (Fig. 1), indicating that the alcohols did not partition into the retinal binding site.

The effect of various alcohols was characterized by the incremental change in Gibbs free energy, \( \Delta(\Delta G) \) (see "Experimental Procedures"), for the MI-MII equilibrium that resulted from the addition of the alcohol. All five alcohols altered the MI-MII equilibrium in a dose-dependent manner, producing unique linear correlations between \( \Delta(\Delta G) \) and the mole fraction of alcohol in the membrane, \( \chi_{\text{alcohol}} \).

Fig. 1. Difference spectra, corrected for the presence of unbleached rhodopsin, of the MI-MII equilibrium for ROS disks (C) and ROS disks plus 40 mM butanol (\( \bullet \)), in pH 7.5 HNS buffer at 20 °C. The solid curves are the deconvoluted MI and MII bands for ROS disks plus 40 mM butanol.

Fig. 2. Dose-response curves for the effect of ethanol (\( \gamma \)), butanol (\( \Delta \)), hexanol (\( \odot \)), and decanol (\( \bullet \)) on the MI-MII equilibrium of rhodopsin in ROS disks (A), PDPC vesicles (B), and POPC vesicles (C). \( \Delta G \) has the dimensions of cal/mol.
manner that depends upon acyl chain composition. Ethanol is reported to bind in the interfacial region of the bilayer, presumably hydrogen-bonded to the carbonyl oxygen of the glycerol backbone (31). The localization of ethanol in the interfacial region and the positive correlation between MII production and acyl chain packing free volume (16, 18, 30) provide a context for interpreting the disparate effects of the various alcohols studied here. Ethanol binding in the interfacial region is reported to increase the average head group spacing. This should increase the bilayer acyl chain packing free volume, and MII formation, which is consistent with our observations shown in Figs. 4 and 2, respectively. As the alcohol chain length increases, it progressively occupies more space behind the head group, reducing the gain in bilayer free volume associated with the increased head group spacing. For chain lengths greater than hexanol, the result is a net loss in bilayer free volume, Fig. 4, resulting in an inhibition of MII formation, Fig. 2. The effect of free volume on MII formation is consistent with the observation that the formation of MII is associated with an increase in volume of about 100 ml/mol, suggesting that MII has an expanded molecular volume relative to MI (32). Our data indicate that alcohols that promote MII formation disorder acyl chain packing, as reflected by increasing $f_v$, whereas those alcohols that inhibit MII formation order acyl chain packing and decrease $f_v$. Similar results were observed to differentiate anesthetic and nonanesthetic alcohols when the lipid acyl chain order parameter was plotted against $\gamma_{\text{alcohol}}$ in nicotinic acetylcholine receptor-rich membranes (33). All anesthetic alcohols decreased acyl chain order, whereas nonanesthetic alcohols increased the acyl chain order parameter. The exact mechanism whereby higher levels of phospholipid acyl chain unsaturation amplifies the effect of the $n$-alcohols relative to MII formation, as shown in Fig. 3C, is not yet determined. However, possible explanations are either that the partition coefficients for the alcohols change with the acyl chain composition or that at equivalent $\gamma_{\text{alcohol}}$, the acyl chain packing of polyunsaturated phosphatidylcholine is more readily perturbed than that of the monounsaturated phosphatidylcholine.

Upon going from hexanol to octanol, the receptor response changes from excitatory to inhibitory. When potency is given in...
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terms of $M_{alcohol}$, this transition appears as a sharp discontinuity, Fig. 3A. However, when potency is stated in terms of $X_{alcohol}$, this transition is seen to be part of a continuous decline in potency of the alcohols to promote MII formation. Thus the apparent discontinuity in behavior between hexanol and octanol is an artifact of the manner in which the data is expressed, i.e. correlating the response with $M_{alcohol}$ rather than $X_{alcohol}$ and is not an intrinsic system property. Although cutoff points have been described in certain systems, the class of receptors represented by rhodopsin does not exhibit this mode of behavior. These findings demonstrate that the manner in which the alcohol concentration is expressed can produce an apparent discontinuity in behavior between hexanol and octanol, the transition is seen to be part of a continuous decline in potency of the alcohols to promote MII formation. Thus the cutoff effect and reverse the order of potency of the alcohols.

While there is evidence to support the action of alcohols directly on ligand-gated ion channels (2, 7) and some peripheral membrane proteins (34), no such mechanism has been established for liganded receptors of the type studied here. Our results provide strong evidence that alcohols can modulate the formation of the G protein-activating conformation of rhodopsin, MII, by a lipid-mediated mechanism. By analogy, one would expect other G protein-coupled receptors, such as those that mediate the action of several neurotransmitters, to be sensitive to the presence of alcohols in a manner similar to rhodopsin. Neuronal and retinal tissue are rich in highly polyunsaturated phospholipid acyl chains, such as 22:6n-3 (19), which are more sensitive to the effects of alcohols. Thus, our findings suggest that the receptors in the membranes of neurons and photoreceptors will be particularly sensitive to the effects of alcohols and other lipid soluble agents.