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Triphasic effects of short chain *n*-alcohols on synaptic membrane transport of choline and of γ-aminobutyric acid

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n-Alcohols, when added in increasing concentrations, had an unusual triphasic effect on the uptake of choline and of γ-aminobutyric acid by isolated synaptosomes. There was slight inhibition of these uptakes at low *n*-alcohol concentrations, followed by a sharp peak of uptake enhancement, and then greater inhibition. The *n*-alcohol concentrations required for these effects were proportional to published *n*-alcohol membrane/buffer partition coefficients, with the peaks of uptake enhancement occurring at 60 mM *n*-propanol, 20 mM *n*-butanol and 7.5 mM *n*-pentanol. Synaptosomal membrane potential, as estimated from synaptosomal accumulation of the permeant cation [^{3}H]tetraphenylphosphonium, was not affected by *n*-alcohols in the concentrations used in this study, suggesting that neither the inhibitory or enhancing effects of these *n*-alcohols were attributable to changes in trans-synaptosomal membrane ion gradients. The inhibiting and enhancing effects of *n*-alcohols could be reproduced in determinations of γ-aminobutyric acid uptake by isolated synaptic plasma membranes, suggesting that the observed effects are due to a direct action of the *n*-alcohols on the synaptosomal plasma membrane. These effects may be attributable to a change in membrane binding of these alcohols from the membrane core to the membrane surface as alcohol concentration is increased.

Introduction

GABA and choline, a biosynthetic precursor of acetylcholine, are both taken up by isolated synaptic terminals (synaptosomes) via sodium gradient-dependent processes with characteristics suggesting active, carrier-mediated systems [1–4]. Such membrane-bound proteins are thought to be sensitive to the degree of disorder (or 'fluidity') of the surrounding lipid bilayer [5,6], which in turn is sensitive to alterations in membrane lipid composition [7,8] and to certain exogenous drugs [9].

Short chain *n*-alcohols have long been known for their anesthetic actions in vivo, for their lipid solubility in vitro, and for the close correlation between these activities (see Refs. 10 and 11). Recent evidence indicates that the actions of *n*-alcohols on biological membranes are selective for certain lipid domains. Ethanol, for instance, has been shown to selectively disorder the outer (exofacial) leaflet of synaptic plasma membranes [12], and to selectively disorder deeper portions of the SPM bilayer [13–16] while lesser changes or even an ordering effect is seen at the membrane surface [16]. Ethanol, *n*-propanol and *n*-butanol have been shown to partition preferentially into the interior of synthetic phospholipid bilayers at low alcohol concentrations and to preferentially bind to the bilayer surface at higher alcohol concentrations [17], an effect which may be due to cooperative binding of alcohol to the membrane surface [18]. These effects of short chain *n*-alcohols differ from the effects of some other 'membrane fluidizing' manipulations. Depletion of cholesterol from erythrocytes, for instance, appears to preferentially affect the inner (cytofacial) plasma membrane leaflet [19,20].

We have previously reported the effects of two different synaptic membrane perturbations on synaptosomal uptakes of GABA and of choline: depletion of synaptic membrane cholesterol using a lipid transfer protein [21], and partition of ethanol into the synaptic membrane [22]. The effects of these manipulations on synaptosomal uptake processes are quite different. Progressive cholesterol depletion is accompanied by a progressive

Abbreviations: GABA, γ-aminobutyric acid; SPM, synaptic plasma membrane; TPP^+, tetraphenylphosphonium ion.

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and reversible decrease in synaptosomal GABA uptake, while choline uptake is unaffected [21]. In contrast, increasing ethanol concentrations (50–400 mM) are accompanied by progressive and reversible decreases in synaptosomal choline uptake, while GABA uptake is unaffected [22]. In this paper we report the effects of *n*-propanol, *n*-butanol and *n*-pentanol on the uptakes of GABA and choline by synaptosomes, and on the uptake of GABA by SPM. These results are qualitatively different both from those obtained with ethanol and from those obtained with cholesterol depletion.

Materials and Methods

Materials

[^{3}H]GABA, [^{14}C]choline, [^{3}H]TPP$^+$, [^{14}C]methanol, [^{3}H]H_2O and [^{14}C]dextran were obtained from New England Nuclear (Boston, MA). Unlabelled GABA and choline were obtained from Sigma Chemical Co. (St. Louis, MO). 3,3′-Dipentyl-2-2′-oxacarbocyanine was obtained from Molecular Probes (Junction City, OR).

Preparation of synaptosomes and synaptic plasma membranes

Synaptosomes were isolated from the forebrains of Sprague-Dawley rats (Charles River Labs, Wilmington, MA) essentially by the method of Gray and Whittaker [23] as modified by Hajos [24]. Briefly, three to five unanesthetized rats were decapitated and their forebrains rapidly excised and placed in 0.32 M sucrose containing 2.5 mM Hepes (pH 7.4) ('sucrose-Hepes'), and homogenized at 0.2 g tissue/ml. Homogenization was performed in a Potter Elvehjem Teflon-glass homogenizer (1 inch inner diameter) using four to six strokes with a loose-fitting pestle (0.974 inch diameter) followed by three strokes with a tight-fitting pestle (0.991 inch diameter). The pestles were driven at 800–900 rpm by a Dyna-Mix overhead stirrer (Fisher Scientific, Pittsburgh, PA). An equal volume of sucrose-Hepes was added and the homogenate centrifuged at $1000 \times g$ for 10 min in a Sorvall SS-34 rotor. The resulting pellet was resuspended and recentrifuged and the supernatants from the two centrifugations were pooled. These were then centrifuged at $11\,000 \times g$ for 20 min in the SS-34 rotor and the resulting pellet was washed twice by resuspension and recentrifugation. The final pellet ('washed crude synaptosomal pellet') was resuspended in sucrose-Hepes (3–5 ml per g original brain tissue), layered in 10–15 ml aliquots over 30 ml of 0.8 M sucrose/2.5 mM Hepes (pH 7.4) and centrifuged for 25 min at $10\,000 \times g$ in a Beckman 25.2 rotor. The 0.8 M sucrose layer was diluted slowly (over an hour) with 3 vols of buffered Krebs medium (130 mM NaCl/5 mM KCl/1.3 mM $MgCl_2$/1.2 mM sodium phosphate/10 mM glucose/0.5 mM EGTA/10 mM Hepes (pH 7.4)) with periodic swirling. The diluted synaptosomes were pelleted ($10\,000 \times g$ for 20 min in a Beckman JA17 rotor) and resuspended in buffered Krebs medium containing 0.2 M sucrose ('buffered Krebs-sucrose') and used for functional studies immediately. For preservation of synaptosomal function, a sucrose concentration of 0.2 M was found to be optimal in the buffered Krebs medium (North, P., unpublished observations).

Preparation quality was assessed by thin-section electron microscopy and by qualitative assessment of synaptosomal membrane potential using the permeant fluorescent cation 3,3′-dipentyl-2-2′-oxacarbocyanine, the latter using a method based on that of Sims et al. [25] as applied to synaptosomes by Blaustein and Goldring [26]. By these criteria, isolated synaptosomes were similar in purity and integrity to previous preparations [21]. The enzymatic activity of choline acetyltransferase, determined by a radiochemical method [27], was enriched 4-fold (from 1.2 to 4.9 nmol/mg per min) in isolated synaptosomes in comparison with the crude homogenate.

SPMs were prepared from synaptosomes, following hypo-osmotic shock, in a manner similar to that of Jones and Matus [28]. The washed crude synaptosomal pellet was incubated in 5 mM Tris (pH 8.1) for 1 h at 4°C, and then diluted with 48% (wt/wt) sucrose (585 g/l)/5 mM Tris (pH 8.1) to a final sucrose concentration of 34% wt/wt (390 g/l). 20 ml aliquots of this were overlaid with 18 ml aliquots of 28.5% (wt/wt) sucrose (319 g/l)/5 mM Tris (pH 8.1) and centrifuged overnight at $49\,000 \times g$ (Beckman SW 27 rotor, 19 000 rpm). The plasma membrane band was collected from the 28.5–34% sucrose interface, diluted 2-fold with cold water and then recovered by centrifugation at $87\,000 \times g$ for 2 h (SW 27, 25 500 rpm), forming a pellet with a white outer rim and a slightly darker center. The outer rim, consisting of SPMs of greater purity, and the inner rim, consisting of less pure SPMs, were separated with a spatula and resuspended in sucrose-Hepes medium at 10–20 mg SPM protein per ml. The purer outer rim SPMs were used for these studies. If the membranes were not used immediately, they were stored in liquid nitrogen.

The protein content of the preparations was assayed using a modification [29] of a Folin technique [30].

Measurement of synaptosomal uptake of choline and GABA

Synaptosomal uptakes of choline and of GABA were measured by means of an isotopic technique [21] based on a centrifugation method described by Simon and Kuhar [31] for [^{3}H]choline. Synaptosomes (0.5 mg synaptosomal protein) were preincubated at 37°C for 10 min in 1 ml of the buffered Krebs-sucrose solution described above, with or without *n*-alcohol. Uptake was started by the addition of [^{3}H]GABA (0.08 μCi) and [^{14}C]choline (0.05 μCi) to give final GABA and choline

concentrations of 1 μM. Uptake was terminated after 2 min by addition of 4 vols of ice-cold incubation medium containing unlabelled GABA and choline (1 μM each). Zero-time blanks were obtained by simultaneous addition of labelled GABA/choline mixture and of cold, unlabeleld GABA/choline mixture. Synaptosomes were collected by centrifugation (20000 × g × 15 min in a Beckman JA17 rotor); the pellets were twice surface-rinsed with 5 ml ice-cold NaCl (0.15 M) and then dissolved overnight with Protosol (New England Nuclear, Boston, MA) prior to liquid scintillation counting. Values for total uptake of choline and GABA were obtained by subtracting the zero-time blanks from the timed measurements made in buffered Krebs-sucrose. Sodium-independent choline uptake represented about 20% of total choline uptake, and sodium-independent GABA uptake represented less than 0.5% of the total GABA uptake [22].

Measurement of GABA uptake by synaptic plasma membrane vesicles

Sodium gradient-dependent uptake of [^{3}H]GABA by SPM vesicles was determined at 25°C by the filtration method of Kanner [32]. The vesicles were incubated for 15 min at 37°C in 0.1 M potassium phosphate/1 mM $MgCl_2$ (pH 7.0) to load the vesicles with potassium phosphate. This loading is virtually complete within 10 min, as determined by following passive ^{86}Rb uptake [21]. The vesicles were then cooled on ice for 10 min, centrifuged at 80000 × g for 20 min, and resuspended in 0.1 M potassium phosphate, 1 mM $MgCl_2$ (pH 7.0) at 2–3 mg SPM protein/mg. These potassium phosphate-loaded vesicles were incubated for 1 min at 25°C in 0.1 M NaCl/1 mM $MgCl_2$/0.15 μM [^{3}H]GABA (10–15 Ci/mmol). The membranes were collected on Millipore HAWP 0.45 μm pore-size filters and washed twice with 4 ml of ice-cold 0.15 M NaCl/0.15 μM unlabelled GABA, prior to drying and preparation for liquid scintillation counting. A zero-time blank was subtracted from the 1 min values.

Measurement of synaptosomal membrane potential

Synaptosomal membrane potential was measured by means of the permeant cation [^{3}H]TPP^+ using a technique described by North and Fleischer [21]. Synaptosomes were preincubated 10 min in a buffered Krebs-sucrose solution with or without alcohol, as described above for measurements of choline and GABA uptake. Then TPP^+ (0.21 μM) was introduced along with a trace quantity (0.04%) of [^{14}C]methanol (used as an index of the pellet volume). Following equilibration (10 min at 37°C), the synaptosomes were pelleted and both the supernatants and pellets were counted, allowing calculation of the relative TPP^+ concentrations in the intrasynaptosomal and extrasynaptosomal spaces. A parallel incubation with [^{3}H]H_2O and [^{14}C]dextran enabled us to calculate the synaptosomal internal volume. The membrane potential was then calculated using the Nernst equation. Nonspecific binding of TPP^+ was determined by parallel assay using depolarizing media (130 mM KCl substituted for 130 mM NaCl), and was subtracted (on a per mg protein basis) prior to calculation of the membrane potential.

Statistical analysis

Differences between values in the presence and absence of alcohols were compared using Student's *t*-test for paired data.

Results

Short-chain *n*-alcohols (*n*-propanol, *n*-butanol and *n*-pentanol), when added at increasing concentrations, showed a curious triphasic effect on synaptosomal uptake of choline (Fig. 1). These alcohols produce a 10–15%, concentration-dependent inhibition of choline uptake at low alcohol concentrations. The concentration range in which this is seen decreases with increasing alcohol chain-length, ranging from 20–40 mM *n*-propanol to 3–6 mM *n*-pentanol. At slightly higher alcohol concentrations (60 mM *n*-propanol, 20 mM *n*-butanol, 7.5 mM *n*-pentanol) there is an abrupt peak of uptake activity, at which choline uptake may equal or exceed the control values. At still higher alcohol concentrations, there is greater (30–40%) inhibition of uptake. The heights of the peaks increase with increasing alcohol chain-length; they are greatest with *n*-pentanol and smallest with *n*-propanol, and the concentrations at which this uptake-enhancing effect is seen are proportional to published membrane/buffer partition coefficients for these alcohols (Fig. 2). This suggests that the effect occurs at a certain, constant intramembranous concentration of the alcohols, and is amplified by longer alcohol chain-lengths.

A similar triphasic effect of short-chain *n*-alcohols is seen in determinations of synaptosomal GABA uptake (Fig. 1). This uptake is also inhibited by these higher-order *n*-alcohols, but the degree of inhibition is less than that seen for choline uptake, and is generally not statistically significant in our experiments until rather high concentrations of these alcohols are reached. Peaks of enhanced GABA uptake are seen, however, and at the same alcohol concentrations that produce peaks of enhanced choline uptake.

This uptake-enhancing effect of certain *n*-alcohol concentrations was also demonstrable in determinations of GABA uptake by isolated SPMs, following imposition of a trans-membrane sodium gradient (Fig. 3). No inhibition, or only slight, of SPM GABA uptake is seen at low concentrations of *n*-propanol and *n*-butanol, followed by a peak of activity similar to that seen in determinations of syaptosomal GABA uptake (Fig. 1),

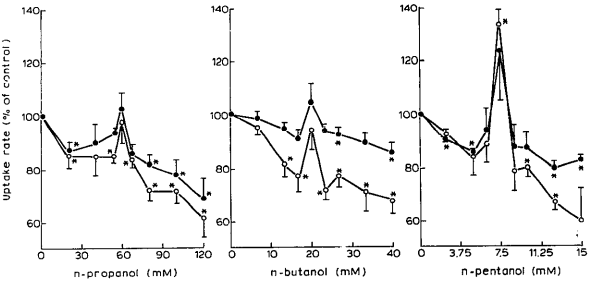

Fig. 1. Effect of short-chain *n*-alcohols on synaptosomal uptakes of choline and of GABA. Isolated synaptosomes were incubated at 37 °C in the presence of [^{3}H]GABA and [^{14}C]choline, 1 μM each, following a 10 min equilibration period. Uptake was terminated after 2 min by dilution with a cold, unlabelled GABA/choline mixture, followed by centrifugation to collect the synaptosomes. The data are shown as percent of control (zero alcohol) values, and as means ± S.E. for five determinations (*n*-propanol), seven determinations (*n*-butanol) or three determinations (*n*-pentanol). Control (zero alcohol) uptake values for the 2 min incubations were 19 ± 4 pmol choline/mg synaptosomal protein and 136 ± 24 pmol GABA/mg synaptosomal protein for *n*-propanol experiments, 15 ± 2 pmol choline/mg synaptosomal protein and 135 ± 25 pmol GABA/mg synaptosomal protein for *n*-butanol experiments, and 15 ± 1 pmol choline/mg synaptosomal protein and 124 ± 29 pmol GABA/mg synaptosomal protein for *n*-pentanol experiments (means ± S.E. for five, seven, and three preparations, respectively). (○) Choline uptake, (●) GABA uptake. (*) Difference from control (zero alcohol) value is statistically significant ($P < 0.05$ or better).

and then greater inhibition of GABA uptake at higher alcohol concentrations. This result suggests that the observed effects of *n*-alcohols on synaptosomal choline and GABA uptake are attributable to a direct effect on the synaptic plasma membrane, rather than to a secondary effect resulting from *n*-alcohol actions elsewhere within the synaptosome.

Synaptosomal membrane potential, and the effect of *n*-alcohols on this potential, was estimated from synaptosomal accumulation of the membrane-permeable TPP^+. These determinations showed only small and statistically nonsignificant changes in membrane potential over the *n*-alcohol concentration ranges used in this study (Fig. 4). These results suggest that the effects of *n*-alcohols on synaptosomal uptakes of choline and

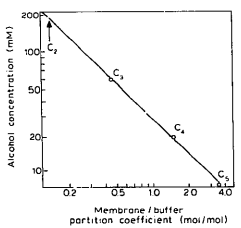

Fig. 2. Double-logarithmic plot of *n*-alcohol membrane/buffer partition coefficients versus the alcohol concentrations producing enhancement of synaptosomal choline and GABA uptake. Partition coefficients are from Seeman [47] and the uptake-enhancing alcohol concentrations are from Fig. 1. C_3 = *n*-propanol, C_4 = *n*-butanol, C_5 = *n*-pentanol. Extrapolation of these data predicts enhancement of synaptosomal choline and GABA uptake at 200 mM ethanol (C_2, arrow).

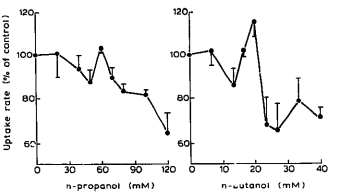

Fig. 3. Effect of short-chain *n*-alcohols on uptake of GABA by isolated SPMs. SPMs were prepared from isolated synaptosomes following hypo-osmotic shock. Uptake of GABA was determined at 25 °C following imposition of a trans-membrane sodium gradient. The SPMs were incubated for 1 min in the presence of [^{3}H]GABA (0.15 μM) and then the solution was filtered. The data are shown as percent of control (zero alcohol) values, and as means ± S.E. for three determinations (*n*-propanol) or five determinations (*n*-butanol). Control (zero alcohol) uptake values were 23.7 ± 0.9 pmol GABA/(mg protein) per min for *n*-propanol experiments and 37 ± 5 pmol GABA/(mg protein) per min for *n*-butanol experiments (means ± S.E. for three and five determinations, respectively).

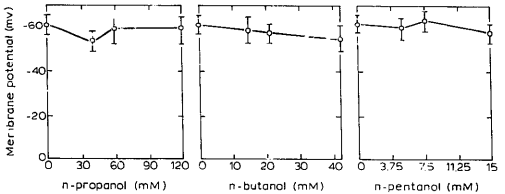

Fig. 4. Effect of short-chain *n*-alcohols on synaptosomal membrane potential. Membrane potential was estimated, using the Nernst equation, from synaptosmal accumulation of the permeant cation, [^{3}H]tetraphenylphosphonium. The values are shown as means ± S.E. for seven determinations.

GABA are not attributable to *n*-alcohol effects on synaptosomal ionic gradients (see Discussion).

Discussion

We describe an unusual triphasic effect of short-chain *n*-alcohols, added at increasing concentrations, on synaptosomal uptakes of choline and of GABA, and on SPM uptake of GABA (Figs. 1 and 3). Uptake inhibition at low and high *n*-alcohol concentrations is interrupted by sharp peaks of uptake enhancement at intermediate *n*-alcohol concentrations. We are not aware of any previously described triphasic effects of alcohols on membrane transport processes, although an analogous pattern has been described for the effects of charged anesthetics on Na^+/K^+-ATPase activity in plasma membranes of cultured, transformed mouse fibroblasts [33].

The uptake-enhancing *n*-alcohol concentrations are proportional to published membrane/buffer partition coefficients for these *n*-alcohols (Fig. 2). Extrapolation of these data to ethanol predicts an uptake-enhancing effect of 200 mM ethanol (Fig. 2). Since the uptake-enhancing effect is smaller with shorter chain-length (Fig. 1), one might expect this predicted uptake-enhancing effect of 200 mM ethanol to be small. In fact, determinations of ethanol effects on synaptosomal uptakes of choline and of GABA do show a slight (but statistically nonsignificant) enhancing effect on GABA uptake at this concentration, and show a plateau of inhibition of choline uptake between 50 and 200 mM ethanol [22].

One possible mechanism for these results is an alcohol effect on trans-membrane ionic gradients, with consequent secondary effects on the sodium gradient-dependent uptakes of GABA and of choline. Synaptosomal uptake of GABA is dependent on the presence of a trans-membrane sodium gradient [34], with a negligible sodium-independent component [4,22]. In contrast, uptake of choline by synaptosomal preparations consists of two components which differ in sodium-dependence and in affinity for choline [35,36]. Under the experimental conditions used here, approx. 80% of the measured choline uptake activity is attributable to a high-affinity, sodium-dependent component, while the remaining 20% is attributable to a low-affinity, sodium-independent component [22]. Synaptosomal uptake of GABA, in addition to its dependence on the presence of a trans-membrane sodium gradient, is known to decrease with KCl-induced depolarization of synaptosomes [37,38], as is synaptosomal high-affinity choline uptake [31,39].

To assess the possibility of an *n*-alcohol effect on trans-membrane ion gradients, we estimated synaptosomal membrane potential from synaptosomal accumulation of the membrane-permeable cation, TPP^+. This was not significantly affected by *n*-alcohols over the concentration ranges used in these studies (Fig. 4). Synaptosomal membrane potential and synaptosomal uptakes of choline and of GABA can be related quantitatively using literature values. Simon and Kuhar [31], for instance, have reported that raising the extrasynaptosomal potassium chloride concentration from 10 to 20 mM results in a 33% drop in sodium-dependent, high-affinity choline uptake by rat hippocampal synaptosomes. The extrasynaptosomal sodium concentration was constant at 126 mM in these experiments. Assuming intrasynaptosomal potassium and sodium concentrations of 140 mM and 10 mM, respectively [40], one can use the Goldring equation [41] to calculate synaptosomal membrane potentials of −65 and −49 mV for 10 and 20 mM extrasynaptosomal potassium, respectively, from the data of Simon and Kuhar [31]. Thus the 33% decrease in choline uptake they have reported corresponds to an approx. 16 mV decrease in synaptosomal membrane potential. Similar calculations can be performed for synaptosomal GABA uptake. These calculations suggest that the effects of *n*-alcohols on synaptosomal choline and GABA uptake which we observe here cannot be attributed to *n*-alcohol effects on synaptosomal membrane potential or on its constituent ionic gradients. The insensitivity of the syn-

aptosomal membrane potential to *n*-alcohols which we describe (Fig. 4) is consistent with the observations of Houck [42] that *n*-alcohols, in the concentrations used here, have no effect on the resting membrane potentials of lobster axons.

The data reported here suggest that *n*-alcohols have at least two independent effects on synaptosomal membrane transport of choline and GABA. (1) An inhibitory effect, with inhibition of choline uptake somewhat greater than inhibition of GABA uptake, which is manifested similarly by homologous alcohols at similar intramembranous alcohol concentrations, and (2) a stimulatory effect, seen only within a narrow intermediate range of intramembranous alcohol concentrations, which is greater with longer *n*-alcohol chainlengths. This second effect suggests some change in the nature of the alcohol–membrane interaction as the alcohol concentration is increased.

Evidence for such a qualitative change in alcohol–membrane interaction as alcohol concentrations are increased is provided by studies of synaptic membrane lipid order and by studies of thermotropic behavior of synthetic phosphatidylcholine bilayers. In SPMs, low concentrations of ethanol have greater disordering ('fluidizing') effects in the membrane core than near the membrane surface, as measured by electron-spin resonance probes [13] and fluorescent probes [14,15]. At ethanol concentrations above 200 mM, in contrast, these probes report effects at the membrane surface of synaptic and erythrocyte plasma membranes [14,43]. Nuclear magnetic resonance spectroscopy has also been used to measure synaptic plasma membrane order in the presence and absence of ethanol. These studies show a disordering effect of ethanol on terminal acyl methyl group protons in the membrane interior, and an ordering effect on choline headgroup protons at the membrane surface [16]. Thermotropic studies of ethanol, *n*-propanol and *n*-butanol effects on synthetic bilayers suggest that these alcohols, at low concentrations, partition into fluid-phase lipid, and that they bind specifically to sites on the bilayer surface at high alcohol concentrations [17,44–46]. This is supported by nuclear magnetic resonance studies of deuterated ethanol binding to synthetic bilayers. These studies show the partition coefficient for ethanol binding to the membrane interior to be constant between 35 mM and 3.5 M ethanol, while binding to the membrane surface is highly cooperative [18], indicating that surface binding becomes proportionately greater with higher ethanol concentrations.

Selective effects of *n*-alcohols on different membrane domains within the SPM may also be important in the effects of these agents on membrane transport functions. For instance, ethanol has been shown to selectively disorder the outer (exofacial) leaflet of SPMs [12]. This is in contrast to membrane cholesterol depletion, which appears to selectively decrease the lipid order of the inner (cytofacial) leaflet of plasma membranes, at least in erythrocytes [19,20]. Indeed, the biochemical effects of synaptic membrane cholesterol depletion on synaptosomal uptake of choline and of GABA are qualitatively different from the effects of short-chain *n*-alcohols on these uptake processes. North and Fleischer [21] have found that decreasing the synaptosomal cholesterol/phospholipid ratio results in decreased synaptosomal uptake of GABA, while the uptake of choline is unaffected, in contrast to the effects of ethanol [22] and short chain *n*-alcohols. These qualitatively different biochemical results suggest that selective 'fluidization' of different membrane lipid domains may be reflected in different effects on membrane transport functions.

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References

1 Kuhar, M.J. and Murrin, L.C. (1978) J. Neurochem. 30, 15–21.
2 Krnjevic, K. (1974) Physiol. Rev. 54, 418–540.
3 Wheeler, D.D. (1979) J. Neurochem. 32, 1197–1213.
4 Wheeler, D.D. and Hollingsworth, R.G. (1979) J. Neurosci. Res. 4, 265–289.
5 Shinitzky, M. and Henkart, P. (1979) Int. Rev. Cytol. 60, 121–147.
6 Shinitzky, M. (1984) in Physiology of Membrane Fluidity, Vol. I (Shinitzky, M., ed.), pp. 1–51, CRC Press, Boca Raton, FL.
7 Quinn, P.J. (1981) Prog. Biophys. Mol. Biol. 38, 1–104.
8 Stubbs, C.D. and Smith, A.D. (1984) Biochim. Biophys. Acta 779, 89–137.
9 Goldstein, D.B. (1984) Annu. Rev. Pharmacol. Toxicol. 24, 43–64.
10 Brink, F. and Posternak, J.M. (1948) J. Cell. Comp. Physiol. 32, 211–233.
11 McCreery, M.J. and Hunt, W.A. (1978) Neuropharmacology 17, 451–461.
12 Schroeder, F., Morrison, W.J., Gorka, C. and Wood, W.G. (1988) Biochim. Biophys. Acta 946 85–94.
13 Chin, J.H. and Goldstein, D.B. (1981) Mol. Pharmacol. 19, 425–431.
14 Harris, R.A. and Schroeder, F. (1981) Mol. Pharmacol. 20, 128–137.
15 Harris, R.A. and Schroeder, F. (1982) J. Pharmacol. Exp. Ther. 223, 424–431.
16 Hitzemann, R.J., Schueler, H.E., Graham-Brittain, C. and Kreishman, G.P. (1986) Biochim. Biophys. Acta 859, 189–197.
17 Rowe, E.S. (1983) Biochemistry 22, 3299–3305.
18 Kreishman, G.P., Graham-Brittain, C. and Hitzemann, R.J. (1985) Biochem. Biophys. Res. Commun. 130, 301–305.
19 Flamm, M. and Schachter, D. (1982) Nature 298, 290–292.
20 Schachter, D., Abbot, R.E., Cogan, U. and Flamm, M. (1983) Ann. N.Y. Acad. Sci. 414, 19–28.
21 North, P. and Fleischer, S. (1983) J. Biol. Chem. 258, 1242–1253.
22 Mrak, R.E. and North, P.E. (1988) Eur. J. Pharmacol. 151, 51–58.
23 Gray, E.G. and Whittaker, V.P. (1962) J. Anat. (Lond.) 96, 79–88.
24 Hajos, F. (1975) Brain Res. 93, 485–489.

25 Sims, P.J., Waggoner, A.S., Wang, C.-H. and Hoffman, J.F. (1974) Biochemistry 13, 3315–3330.
26 Blaustein, M.P. and Goldring, J.M. (1975) J. Physiol. 247, 589–615.
27 Fonnum, F. (1975) J. Neurochem. 24, 407–409.
28 Jones, D.H. and Matus, A.I. (1974) Biochim. Biophys. Acta 356, 276–287.
29 Schacterle, G.R. and Pollack, R.L. (1973) Anal. Biochem. 51, 654–655.
30 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
31 Simon, J.R. and Kuhar, M.J. (1976) J. Neurochem. 27, 93–99.
32 Kanner, B.I. (1978) Biochemistry 17, 1207–1211.
33 Sweet, W.D. and Schroeder, F. (1986) Biochem. J. 239, 301–310.
34 Martin, D.L. and Smith, A.A., III (1972) J. Neurochem. 19, 841–855.
35 Whittaker, V.P., Dowdall, M.J. and Boyne, A.F. (1972) Biochem. Soc. Symp. 36, 49–68.
36 Yamamura, H.I. and Snyder, S.H. (1972) Science 178, 626–628.
37 Sellstrom, A. and Hamberger, A. (1975) J. Neurochem. 24, 847–852.
38 Blaustein, M.P. and King, A.C. (1976) J. Membr. Biol. 30, 153–173.
39 Vaca, K. and Pilar, G. (1979) J. Gen. Physiol. 73, 605–628.
40 Guyton, A.C. (1971) Textbook of Medical Physiology, 4th Edn., p. 39, W.B. Saunders, Philadelphia, PA.
41 Hodgkin, A.L. (1958) Proc. R. Soc. (Lond.) Ser. B 148, 1–37.
42 Houck, D.J. (1969) Am. J. Physiol. 216, 364–367.
43 Logan, B.J., Laverty, R. and Peake, B.M. (1983) Pharmacol. Biochem. Behav. 18, Suppl. 1, 31–35.
44 Jain, M.K. and Wu, N.M. (1977) J. Membr. Biol. 34, 157–201.
45 Rowe, E.S. (1985) Biochim. Biophys. Acta 813, 321–330.
46 Rowe, E.S. (1985) Alcohol 2, 173–176.
47 Seeman, P. (1972) Pharmacol. Rev. 24, 583–655.