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Short communication

Ethanol weakens cytochalasin B binding to the GLUT1 glucose transporter and drug partitioning into lipid bilayers

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Abstract

Ethanol weakens the specific interaction between the human red blood cell (RBC) glucose transporter GLUT1 and the inhibitor cytochalasin B (CB). The chromatographic retention volume of cytochalasin B on stationary phases consisting of GLUT1-containing membranes decreased with increasing ethanol concentration in the eluent. The apparent K_d values for the ethanol–GLUT1 interaction were 0.37, 0.45 and 0.64 M for red blood cells, red blood cell membrane vesicles and proteoliposomes, respectively, all much higher than the K_d values for D-glucose or cytochalasin B interaction with GLUT1. Ethanol also decreased the partitioning of cytochalasin B and drugs into phospholipid bilayers.
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Keywords: Immobilized biomembrane affinity chromatography; Cytochalasin B; Ethanol; Glucose; GLUT1

1. Introduction

Ethanol is known to inhibit the function of many membrane proteins, e.g. the nucleoside transporter [1] and several receptor-associated ion pumps [2,3], whereas the activity of some other proteins is enhanced [4–6]. Uptake of 2-deoxy-D-glucose into cultured human lymphocytes via the glucose transporter GLUT1 was suppressed to 50% by 150 mM ethanol, but no effect on GLUT3 and GLUT4 was observed [7]. The inhibition of the 2-deoxy-D-glucose uptake by propanol or butanol was stronger than that caused by ethanol [7]. Klepper et al. reported in Ref. [8] that 1% ethanol inhibited the 3-*O*-methyl-D-glucose uptake into red blood cells (RBCs) by 40%.

We studied the effects of ethanol on the binding of cytochalasin B (CB) to GLUT1 in RBCs, RBC membrane vesicles and GLUT1 proteoliposomes by using immobilized biomembrane affinity chromatography (IBAC) [9] and compared with the effects of other alcohols. We also studied the retention of CB and a set of drugs on egg yolk phospholipid liposomes by immobilized liposome chromatography (ILC) [10] in order to estimate whether ethanol has a non-specific effect on solute partitioning into membranes.

2. Experimental

2.1. Materials and buffers

We purchased cholic acid (>99%) from Fluka (Buchs, Switzerland), β-octyl glucoside (OG) from Dojindo (Kumamoto, Japan), CB from Sigma (St. Louis, MO, USA), D-glucose from BDH (Poole, UK), [^{3}H]L-glucose from NEN (Boston, MA), [4(*n*)-^{3}H]CB, Sephadex G-50 medium and Superdex 200 prep grade from Amersham Biosciences (Uppsala, Sweden), ethanol (99.5%) from Kemethyl (Haninge, Sweden), 1-butanol, methanol and 1-propanol from Merck (Darmstadt, Germany) and fresh human blood in citrate/phosphate/dextrose and RBC concentrate, stored for 6–7 weeks in saline/adenine/glucose/mannitol, from the Blood Bank of the University Hospital (Uppsala, Sweden). Hen's egg yolk phospholipids were prepared essentially as described in Ref. [11]. Previous analyses showed a content of 70% phosphatidylcholine, 21% phosphatidylethanolamine, 9% other phospholipids and lysophospholipids, and small amounts of cholesterol and other components [12]. Superporous agarose beads of particle diameter 106–180 μm with an average superpore size of 30 μm were prepared by P.-E.

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Gustavsson as described in [13], and wheat germ agglutinin (WGA) was purified by B. Ersson as described in [14] and was used and stored as freeze-dried material.

We used the following buffers set to pH 7.4 at 23 °C: buffer A (10 mM Tris–HCl, 150 mM NaCl, 1 mM EDTA) for preparation and IBAC analyses of columns containing membrane vesicles and proteoliposomes, as well as for preparation and ILC analyses of liposome-containing columns; buffer B (139 mM NaCl, 5 mM KCl, 50 mM mannitol, 3 mM NaN_3, 10 mM sodium phosphate) for RBC column preparation and analyses. When ethanol was included in the buffers the concentration is given in volume percent.

2.2. Immobilized biomembrane columns

Wheat germ lectin was coupled to superporous agarose beads and fresh RBCs were immobilized in these beads by alternating cell application and incubation, as described in [15]. The yields were 4.5 ml WGA/ml gel bed and 1.0×10^9 cells/ml gel bed, as determined by amino acid analysis of the amount of agglutinin.

From RBC concentrate, we prepared cytoskeleton-depleted membrane vesicles [16], from which we made GLUT1-proteoliposomes, essentially as in [17]. We prepared liposomes by rehydration of egg yolk phospholipid films as described in Ref. [10]. Membrane vesicles or (proteo)liposomes were immobilized by rehydration of dried Superdex 200 gel beads in a suspension of the membrane materials followed by freezing and thawing, as in [18], and the amount of immobilized phospholipids were determined by phosphorus analysis [19]. The GLUT1 in the entrapped material usually retains the CB-binding activity for months at room temperature [12,20–22].

2.3. IBAC and ILC

In frontal IBAC, a soluble ligand is retarded on a chromatographic column specifically in relation to the amount of immobilized binding sites, the ligand concentration and the affinity. The frontal elution volumes of [^{3}H]CB on GLUT1 containing columns were determined by fitting the data from an on-line flow-scintillation detector to Eq. (1) in [23]. Dissociation constants for CB-binding to GLUT1 were determined by non-linear regression analysis using Eq. (1) in [9], which is derived from [24]. The apparent glucose and ethanol affinities were determined by linear regression analysis [21] of the frontal elution volumes of 1 nM [^{3}H]CB inhibited by D-glucose (0–600 mM) or ethanol (0–6.8%; 0–1.2 M). Frontal runs of CB on GLUT1 were performed with immobilized RBCs (0.5 ml/min) and immobilized membrane vesicles and proteoliposomes (1.0 ml/min).

ILC reveals the partitioning of a solute into immobilized lipid bilayers, which can be expressed by the capacity factor, K_s, defined as:

$$K_S = \frac{V_R - V_0}{A} \quad (1)$$

where V_R is the retention volume for CB or a drug; V_0 is the retention volume for a non-interacting solute ([^{3}H]L-glucose or $Cr_2O_7^{2-}$) and A is the amount of phospholipids in the column. We analyzed the effect of ethanol on the ILC retention of CB (uncharged) and the pharmaceutical drugs alprenolol and metoprolol (positively charged) as well as ketoprofen and indomethacin (negatively charged) on lipid bilayers.

The IBAC elution volume of CB when the specific interaction with GLUT1 is suppressed (V_{min}) was determined by use of a high D-glucose concentration at 0% ethanol. The V_{min} values for the different ethanol concentrations were approximated to V_R calculated according to Eq. (1). The ILC CB data allowed correction of V_{min} on membrane vesicles and proteoliposomes, since the retention of drugs (and thus of CB) on egg yolk phospholipid bilayers is similar to their retention on RBC membrane vesicles (Fig. 2C in Ref. [10]). All IBAC and ILC analyses were done at 23 °C.

3. Results

3.1. Ethanol effects on CB binding to GLUT1 measured by IBAC

The specific retardation of CB on GLUT1 proteoliposomes or membrane vesicles with GLUT1 decreased at increasing concentrations of ethanol and was halved at approximately 2.0% (0.34 M) ethanol for both materials (Fig. 1A). At 5% ethanol the specific retardation of CB was only 12% of the retardation without ethanol. With immobilized RBCs, which contain an abundant amount of GLUT1 in their cell membranes, the elution volume was halved at $2.2 \pm 0.5\%$ (0.38 ± 0.09 M) ethanol ($n = 2$), not shown. All effects were completely reversed upon washing with a few column volumes of ethanol-free running buffer. The apparent K_d value for ethanol displacement of CB binding to GLUT1 decreased in the order proteoliposomes, vesicles and RBCs, similarly as reported for D-glucose (Table 1 and data in [23]). No high-affinity binding as preliminarily indicated in Ref. [17] was observed. Fig. 1B shows the increase in K_d, i.e. the decrease in the affinity, of CB or D-glucose binding to GLUT1 at increasing concentrations of ethanol. The ethanol effect on CB binding is much larger than that on D-glucose binding.

Table 1
Dissociation constants, K_{dCB}, for CB interaction with GLUT1 in proteoliposomes, RBC membrane vesicles and RBCs and the apparent K_d values for D-glucose ($K_{d\,D\text{-}glc}$) and ethanol ($K_{d\,Ethanol}$) binding to GLUT1, determined by displacement of CB

	K_{dCB} (nM)	$K_{d\,D\text{-}glc}$ (mM)	$K_{d\,Ethanol}$ (M)
Proteoliposomes	49	63	0.64
RBC membrane vesicles	28	32	0.45
RBCs	70 ± 14[a]	12 ± 3[a]	0.37

[a] From Ref. [15]

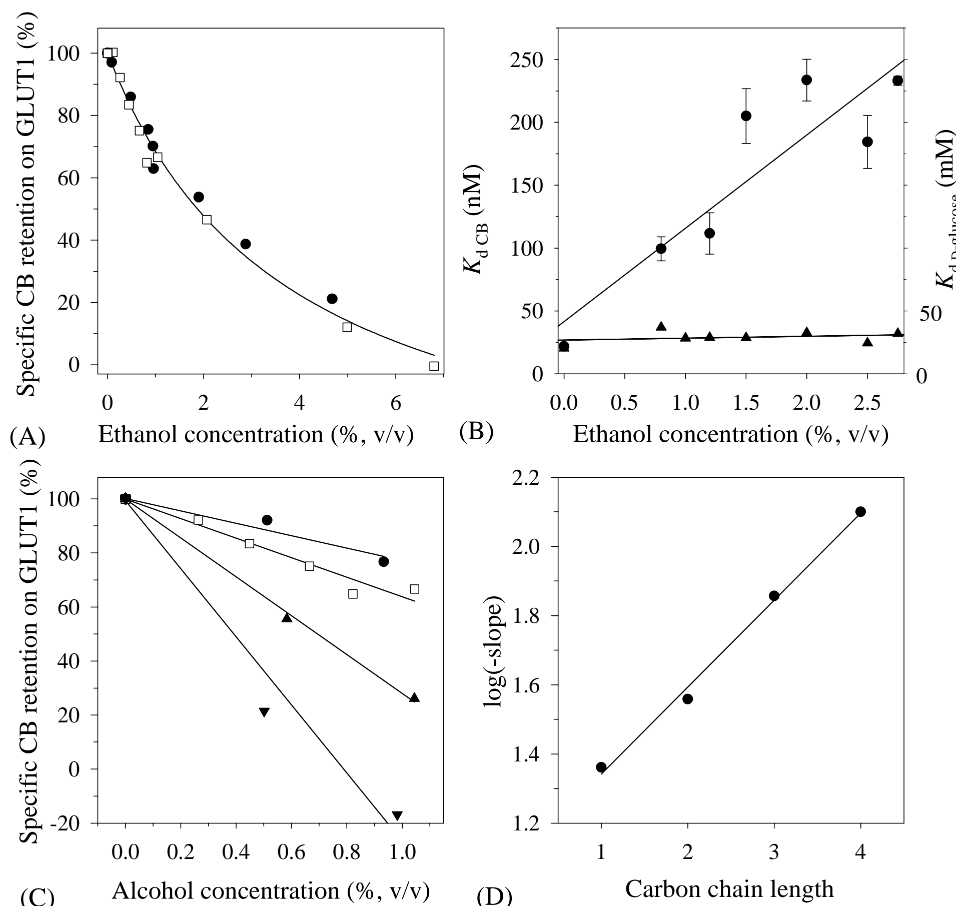

Fig. 1. (A) The percentual specific retention of CB on columns containing membrane vesicles (●) and GLUT1 proteoliposomes (□) vs. the ethanol concentration. The non-specific CB retention by interaction with the lipid bilayer was subtracted according to the measurements on the ILC column (see Fig. 2 (●)), whereby the lipid amounts in the different columns were taken into account. (B) K_d values for interaction between GLUT1 in membrane vesicles and CB ((●), left *y*-axis) and for displacement of CB by D-glucose ((▲), right *y*-axis) vs. the ethanol concentration. (C) The percentual CB retention on GLUT1 proteoliposomes vs. the concentration of methanol (●), ethanol (□), propanol (▲) or butanol (▼). Correction of V_{min} was made according to the ethanol effect on the ILC column, and may be underestimated for propanol and butanol and overestimated for methanol. This explains the negative value at high butanol concentrations. The ethanol data are taken from (A). (D) The slopes of the lines in (C) as a function of the carbon chain length.

3.2. Effects of different alcohols on the CB binding to GLUT1

In addition to the series of runs with increasing concentration of ethanol, CB was run on GLUT1 proteoliposomes in the presence of two concentrations of methanol, propanol and butanol. The CB interaction with the membranes clearly decreased with the length of the carbon chain of the alcohol (Fig. 1C and D). The effects were completely reversed by washing with a few column volumes of buffer A.

3.3. Interaction of CB and drugs with liposomes

The K_s values for CB and all model drugs run on protein-free phospholipid columns decreased markedly at increasing ethanol concentrations (Fig. 2), independently of the drug charge. Washing with a few column volumes of buffer A could reverse this effect. The presence of 5% ethanol decreased the K_s values by 36% for indomethacin, 22% for alprenolol, 39% for CB, 29% for ketoprofen and 30% for metoprolol (Fig. 2). The K_s values for methanol, ethanol, propanol and butanol were close to 0.

4. Discussion

Ethanol inhibited CB binding to GLUT1 in RBCs (Table 1), membrane vesicles and proteoliposomes (Fig. 1A and B) and decreased the non-specific interaction between CB and the lipid bilayers, similarly as for all other tested drugs (Fig. 2). This is apparently due to suppression of hydrophobic interactions since the inhibition of specific CB-binding to membrane vesicles was enhanced with the length of the alcohol carbon chain (Fig. 1C and D). Furthermore, 2-deoxy-D-glucose transport by GLUT1 is also inhibited by ethanol [7], perhaps indicating that

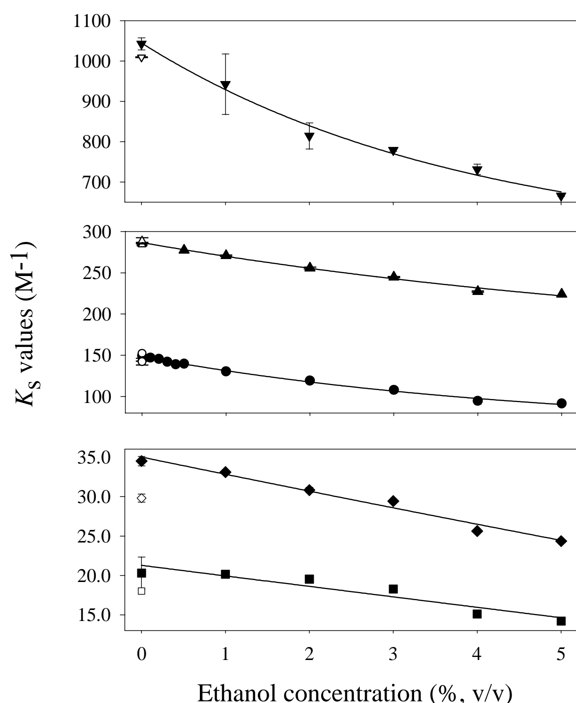

Fig. 2. The K_s values of the drugs indomethacin ((▼), negatively charged), alprenolol ((▲), positively charged), CB ((●), neutral) ketoprofen ((◆), negatively charged) and metoprolol ((■), positively charged) vs. the ethanol concentration in the eluent. Open symbols show the retention volumes for the drugs without ethanol in the eluent measured after the experimental series in order to demonstrate the reversibility of the ethanol effect.

hydrophobic interactions are involved in the GLUT1 conformational changes in the transport process. The inhibition of CB binding to GLUT1 by ethanol was much weaker than that caused by the GLUT1 substrate D-glucose. The decreased partitioning of drugs into lipid bilayers at increasing ethanol concentration (Fig. 2) was probably caused by the decreased polarity of the mobile phase.

Dissolving amphipahtic molecules such as CB at high concentrations often requires the presence of an organic solvent. The application of ethanol at concentrations higher than 0.1% to GLUT1 systems apparently requires control experiments in order to determine the additional effects of ethanol. An effect of a supplementary organic solvent may also explain the inhibition of aromatic amino acid transport attributed to CB in Ref. [25], where CB concentrations above the solubility limit were used, which probably was accomplished by the inclusion of ethanol or dimethylsulfoxide. Other solvents like β-mercaptoethanol or acetonitrile at the relatively high concentrations used in immobilized artificial membrane chromatography [26] and in HPLC similarly affect the outcome of a separation process. However, the ethanol effect on CB-binding to GLUT1 that we observed in the physiological tolerance interval of ethanol in humans (up to approximately 0.5% (v/v) or 0.4% (w/w)) was small.

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