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Ethanol stimulates the plasma membrane calcium pump from human erythrocytes

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Abstract

The plasma membrane Ca^{2+}-ATPase from human erythrocytes can be stimulated by different treatments such as addition of calmodulin or acidic phospholipids and controlled proteolysis. In this report we show that short chain alkyl alcohols also stimulated this enzyme. At 5% (v/v) ethanol, the maximal velocity of the enzyme was about 2.4-fold higher than in the control, and thus, was also higher than the maximal velocity obtained in the presence of calmodulin (about 2-fold). When ethanol and calmodulin were present simultaneously, the stimulatory effect was additive (3.4-fold stimulation). On the other hand, the stimulatory effect of ethanol was preserved after treatment of the enzyme with trypsin to stimulate the Ca^{2+}-ATPase and render it independent of calmodulin, thus suggesting that the interaction of ethanol and calmodulin with the Ca^{2+}-ATPase occurred through a different mechanism. Other short chain alkyl alcohols (methanol, n-propanol and n-butanol) stimulated the Ca^{2+}-ATPase activity to the same extent than ethanol but with different efficacy. Thus, the larger the carbon number, the lower the concentration needed to get the same maximal stimulation. Ethanol also increased the affinity of the enzyme for ATP to a larger extent and additively, when compared to calmodulin. All the effects of ethanol mentioned above were identically observed on the membrane-bound enzyme (i.e., erythrocyte ghosts) ruling out any effect of the alcohols attributable to the solubilized purified enzyme. Furthermore, Ca^{2+} transport by inside-out vesicles was also stimulated by ethanol, showing both the same concentration-dependence as the Ca^{2+}-ATPase activity and the additive effect observed when calmodulin was also present. The stimulatory effect of ethanol was significant at pharmacological concentrations, thus suggesting potential implications of toxicological relevance.

Keywords: ATPase, Ca^{2+}-; Calcium ion transport; Ethanol; Alcohol; Calmodulin; Erythrocyte; (Human)

1. Introduction

The plasma membrane Ca^{2+}-ATPase is responsible for the maintenance of the intracellular Ca^{2+} concentration at the resting level in eukaryotic cells [1,2]. It belongs to the so-called P-type ion-motive ATPases [3]. The activity of this enzyme is highly regulated. Thus, it can be stimulated by calmodulin [4,5], acidic phospholipids and polyunsaturated fatty acids [6]. Phosphorylation of the enzyme by cAMP-dependent protein-kinase [7] and by protein-kinase C [8] also stimulates the ATPase activity. Beside, controlled proteolysis with trypsin [9,10] and other proteolytic enzymes [11] stimulates the Ca^{2+}-ATPase to the same extent to that attained with calmodulin. Auto-aggregation of the enzyme is also translated in an increase of the ATPase activity [12]. Modification of the water structure surrounding the enzyme medium by the addition of organic solvents also mimics calmodulin [13,14]. In this regard, we have shown that poly-alcohols (i.e., ethylene glycol, glycerol, etc.) are more effective than dimethyl sulfoxide, with respect to their stimulatory effect on the Ca^{2+}-ATPase activity [13].

It has been shown that alcohols produce a profound effect in many membrane enzymes, either through a direct interaction with the protein moiety [15,16], or indirectly, by interacting with the phospholipid bilayer [17]. Recently, it has been reported that intracellular calcium homeostasis could be affected by ethanol [18]. The studies on the effect of alcohol on the plasma membrane Ca^{2+}-ATPase are reduced to few contradic-

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tory reports. Yamamoto and Harris [19] reported a low stimulatory effect of ethanol using membrane preparations (from erythrocytes and synaptosomes), mainly on the affinity of the enzyme for Ca^{2+}, while Kosk-Kosicka and co-workers reported an inhibition of the stimulatory effect of calmodulin or auto-aggregation of the enzyme by short chain alkyl alcohols, using a solubilized purified enzyme from human erythrocytes [20].

In the present study, using purified enzyme as well as membrane preparations, we show that the activity of the Ca^{2+}-ATPase was stimulated by short-chain alkyl alcohols. The stimulatory effect observed was additive to that obtained when the enzyme was stimulated by calmodulin or by trypsin proteolysis. Ethanol also stimulated Ca^{2+} transport by the pump with the same concentration-dependence observed for the ATP hydrolytic activity.

2. Experimental procedures

2.1. Chemicals

ATP, EGTA, arsenazo III, phosphatidylcholine, trypsin, soy bean trypsin inhibitor, were from Sigma. All other reagents were analytical grade.

2.2. Purification of the erythrocyte Ca^{2+}-ATPase

Erythrocyte ghosts deficient in calmodulin were prepared as described in Ref. [21]. from recently outdated human blood. Purified Ca-ATPase was obtained using a calmodulin affinity column as described before [21]. Routinely, 0.5–0.6 mg of ATPase was obtained from 500–600 mg of ghost protein. The purified ATPase was stored under N_2 at −70°C at a concentration of 100–200 μg/ml, in a buffer containing 0.05% Triton X-100, 130 mM KCl, 20 mM Hepes-KOH (pH 7.2), 2 mM EDTA, 2 mM $MgCl_2$, 50 μM $CaCl_2$, 2 mM dithiothreitol, 0.5 mg/ml phosphatidylcholine and 5% glycerol (v/v). Bovine brain calmodulin was obtained as described by Guerini et al. [22].

2.3. Determination of ATPase activity

Aliquots of purified Ca^{2+}-ATPase (about 1–2 μg protein/ml) were incubated in a medium containing 130 mM KCl, 20 mM Hepes-KOH (pH 7.2), 1 mM ATP, 1 mM $MgCl_2$, 1 mM EGTA, and the appropriate concentrations of $CaCl_2$ to obtain the desired free calcium concentration. The final concentration of calcium ions was calculated employing an iterative computer program as described before [23]. Since the rates of ATPase activity were linear over 45 min incubation at 37°C, the reaction was arrested at 45 min by the addition of 8% (final concentration) cold trichloroacetic acid. When membrane preparation were used, the mixture was centrifuged and the supernatant was kept for inorganic phosphate determination. The latter was carried out according the colorimetric method of Fiske and Subbarrow [24], modified by the use of ferrous sulfate as reducing agent. When necessary, appropriate blanks were made to correct the slight interference of high concentration of alcohols with the colorimetric method. Alternatively, a coupled-enzyme assay system was used to measure the Ca^{2+}-ATPase activity of the purified enzyme as described previously [21]. Ethanol at 5% concentration (v/v) had no interference with the coupled enzyme assay. In order to determine if the effect of ethanol was reversible, crude plasma membrane preparations (i.e., erythrocyte ghosts) were incubated with 5% concentration (v/v) of the alcohol for 30 min at 37°C. Then the membranes were centrifuged, the supernatant removed and the pellet was resuspended and washed twice. After this procedure, the measured Ca^{2+}-ATPase activity was identical to that obtained with control preparation where addition of ethanol was omitted. After washing, the ethanol treated membranes were still able to be stimulated by ethanol (results not shown). The figures show the mean value of at least five independent experiments.

2.4. Limited proteolysis of the purified Ca^{2+}-ATPase

Controlled trypsin proteolysis of the purified ATPase was performed as previously described [14,21]. The digestion was carried out at 4°C for 30 min. Trypsin (50 μg/ml) was added to aliquots containing 100–200 μg/ml of purified Ca^{2+}ATPase in the same solution in which the enzyme was stored. The digestion was arrested by addition of 10-fold excess soybean trypsin inhibitor.

2.5. Preparation of erythrocytes inside-out plasma membrane vesicles and determination of Ca^{2+} transport

Inside-out plasma membrane vesicles from human erythrocytes were obtained by the method of Sarkadi et al. [25], with the modifications introduced in Ref. [26]. Ca^{2+} transport were followed by measuring the changes in the absorbance spectrum of arsenazo III, using a SLM Aminco DW2a spectrophotometer at the wavelength pair 675–685 to avoid interference with magnesium, as described before [29]. Ethanol up to 10% concentration (v/v) had no interference with the determination of Ca^{2+} movements by the above method. Concentrations of ethanol up to 5% had no effect on the Ca^{2+} permeability of the vesicles. After 10% ethanol, the vesicles became gradually 'leaky' to Ca^{2+} (not shown).

2.6. Determination of the protein concentration

The protein concentration was determined by the method of Lowry et al. [27]. To avoid interferences with Triton X-100, the protein was precipitated by trichloroacetic acid in the presence of deoxycholate [28].

3. Results

As can be observed in Fig. 1, short-chain alkyl alcohols stimulated the Ca^{2+}-ATPase activity to a larger extent to that attained with calmodulin (i.e., 2.4-fold instead of 2-fold). The maximal velocity obtained was similar, independently of the n-alcohol used (Fig. 1A, B, C and D). However, as the number of carbon atoms increased the concentration of alcohol needed to achieve the half maximal response diminished (Fig. 2), independently of the presence of calmodulin. It can also be observed in Fig. 1 that, independently of the length of the carbon chain, the stimulatory effect of calmodulin was additive to that of the n-alcohol, reaching a maximal velocity of more than 3-fold with respect to the basal activity when optimal concentration of the respective alcohol was used and calmodulin was present. Also, under these conditions, the same reciprocal correlation with the carbon number of the n-alcohol

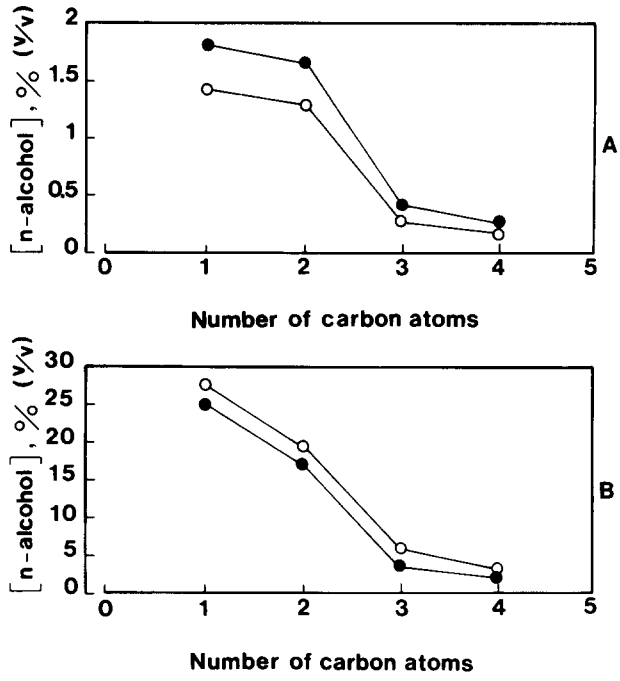

Fig. 2. Effect of the length of short chain alkyl alcohols on the Ca^{2+}-ATPase activity. (A) The concentration of the different n-alcohols needed to stimulate the Ca^{2+}-ATPase activity to 50% of its maximal stimulation are plotted against the number of carbon atoms of the different alcohols. (B) The concentrations of different alcohols needed to inhibit the Ca^{2+}-ATPase activity to the half of its maximal value are plotted against the number of the carbon atoms.

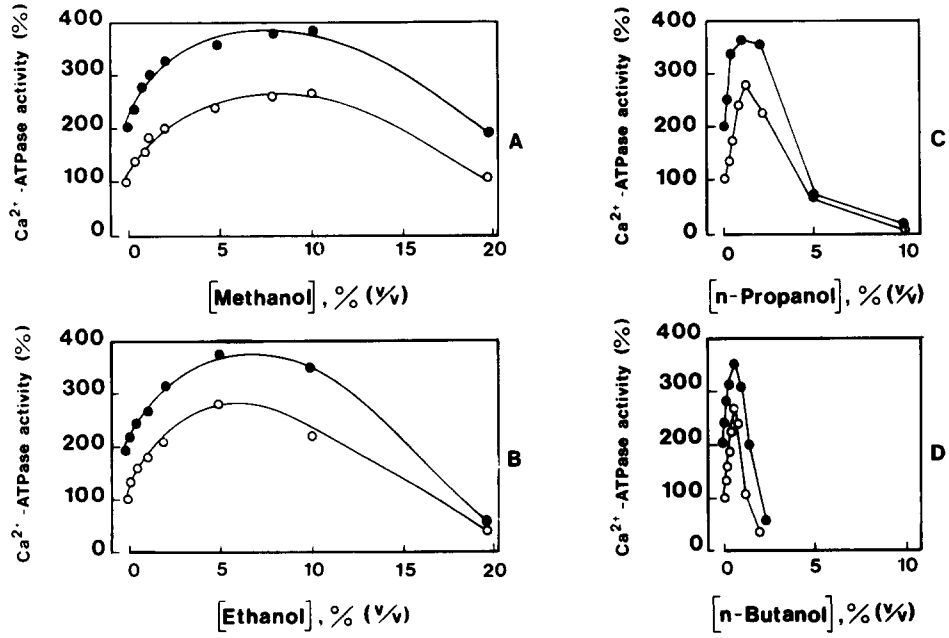

Fig. 1. Effect of ethanol and others short chain alkyl n-alcohols on the purified Ca^{2+}-ATPase activity. The reaction medium (0.5 ml, 37°C) contained 130 mM KCl, 20 mM Hepes-KOH (pH 7.2), 1 mM ATP, 1 mM $MgCl_2$ 1 mM EGTA and the amount of $CaCl_2$ to give a final Ca^{2+} concentration of 10 μM, and the indicated concentration (v/v) of the respective n-alcohol. (○) no additions; (●) 5 μg/ml calmodulin. The reaction was incubated for 45 min and arrested by the addition of cold trichloroacetic acid (8% final concentration), and organic P_i was determined as explained in Experimental procedures.

was obtained (Fig. 2A). Concomitantly, the concentration of n-alcohol needed to inhibit the Ca^{2+}-ATPase activity to a 50% of its maximal value diminished as the length of the carbon chain increase, independently of the presence of calmodulin (Fig. 2B).

In order to study its effect on the affinity of the enzyme for Ca^{2+}, ethanol was used at a concentration which produced the maximal stimulation of the Ca^{2+}-ATPase activity (i.e., 5%, v/v). Fig. 3 shows that this concentration of ethanol increased the affinity of the enzyme for Ca^{2+} to the same level to that observed in the presence of calmodulin. Thus, Lineweaver-Burk and Eadie-Hofstee plots shown in Fig. 3B indicated that the K_m of the control was 1.15 ± 0.14, while in the presence of calmodulin or ethanol the K_m decreased to 0.52 ± 0.07 and 0.45 ± 0.05, respectively. When both effectors were present (Fig. 3A and B) an additive effect on the Ca^{2+} affinity was observed (K_m 0.28 ± 0.02). All the K_m values were statistically significant ($P < 0.001$, Student's t-test) with respect to the control. The additivity of the stimulatory effect of ethanol and calmodulin on the affinity of the enzyme for Ca^{2+} and on the maximal velocity of ATP hydrolysis suggests that the mechanism of interaction of both effectors is different. Since the effect of calmodulin can be mimicked by controlled trypsin proteolysis of the Ca^{2+}-ATPase, which in turn originates a calmodulin-independent form of the enzyme [9,10], this treatment represents an easy form to study if the stimulatory effects of ethanol and calmodulin occur through different mechanisms. Thus, the purified enzyme was digested with trypsin under conditions which result in a Ca^{2+}-ATPase activity similar to that achieved in the presence of calmodulin but independent of the protein modulator ([10,14] and Fig. 3). Under these conditions, we found (Fig. 3) that ethanol was able to further increase the rate of ATP hydrolysis and the affinity for Ca^{2+} of the proteolysed enzyme to the same extent achieved when both calmodulin and ethanol were present. The above results strongly suggest that the mechanism of interaction of ethanol with the calcium pump is different to that of calmodulin.

Operationally, all P-type ionic pumps are characterized by the presence of a high affinity (catalytic) and a low affinity (regulatory) ATP binding sites [30]. In the case of the plasma membrane calcium pump the expression of the second binding site is reported to be dependent on the presence of calmodulin [31,32]. We have recently shown, using the purified enzyme, that addition of organic solvents mimics calmodulin, increasing the affinity of the Ca^{2+}-ATPase for ATP at the low affinity nucleotide binding site [14]. Now we show (Fig. 4) that ethanol (at 5%, v/v) also increased the affinity of the enzyme for ATP at the low affinity ATP binding site. Similarly as it was observed on the affinity of the enzyme for Ca^{2+}, an additive effect was obtained upon addition of calmodulin when ethanol was already present. The maximal velocity of the enzyme was also additively increased when both effector were present (Fig. 4). Trypsin proteolysis of the purified Ca^{2+}-ATPase under the same conditions described in Fig. 3 also mimicked calmodulin with respect to the effect on the affinity of the enzyme for ATP (Fig. 5). We also show here that addition of ethanol

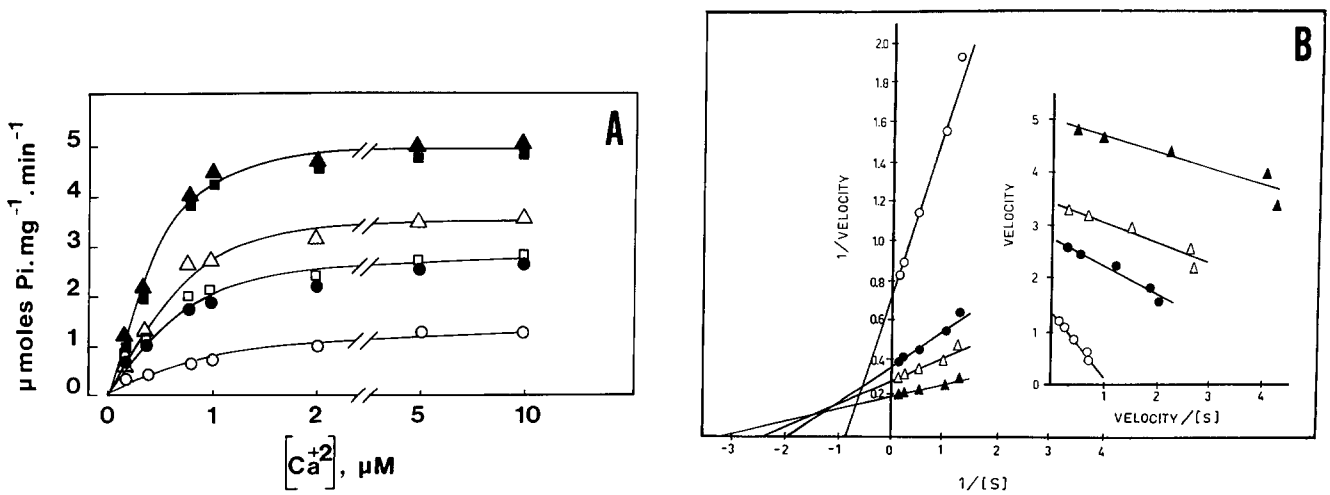

Fig. 3. Effect of ethanol, calmodulin and trypsin proteolysis on the Ca^{2+}-ATPase affinity for Ca^{2+}. Experimental conditions were as in Fig. 1. The enzyme (0.1–0.2 mg/ml) was proteolysed at 4°C for 30 min in the presence of trypsin (50 μg/ml). The digestion was arrested with a 10-fold excess of soybean trypsin inhibitor. Other condition were as indicated in Experimental procedures. (A) Control (○); 5 μg/ml calmodulin (●); proteolysed Ca^{2+}-ATPase (□); ethanol 5% (v/v) (△); proteolysed Ca^{2+}-ATPase plus ethanol 5% (v/v) (■); ethanol 5% (v/v) plus 5 μg/ml calmodulin (▲). Data points represents the mean value of at least nine independent experiments. (B) Lineweaver-Burk and Eadie-Hoftee (inset) plots of data from (A). The K_m values were 1.15 ± 0.14 (control); 0.52 ± 0.07. (calmodulin); 0.45 ± 0.05 (ethanol); 0.28 ± 0.02 (ethanol plus calmodulin). Control (○); Ethanol 5% (v/v) (△); 5 μg/ml calmodulin (●); ethanol 5% (v/v) plus 5 μg/ml calmodulin (▲).

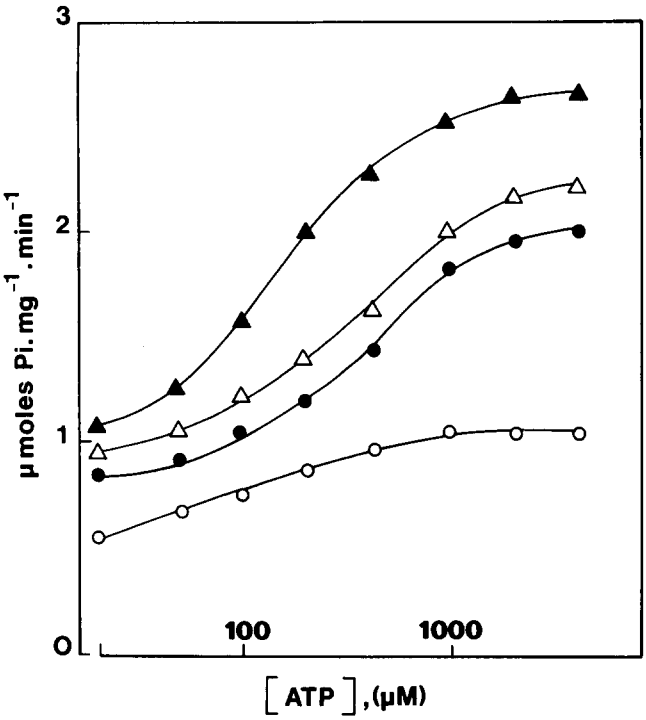

Fig. 4. Effect of ATP on the stimulation of the Ca^{2+}-ATPase by ethanol and calmodulin. Experimental conditions were as in Fig. 1. Final free Ca^{2+} concentration were 10 μM. ATP concentrations were maintained constant by the use of 1 mM phospho*enol*pyruvate and 1 IU piruvate kinase. Control (○); 5 μg/ml calmodulin (●); 5% ethanol (v/v) (△); 5% ethanol plus 5 μg/ml calmodulin (▲).

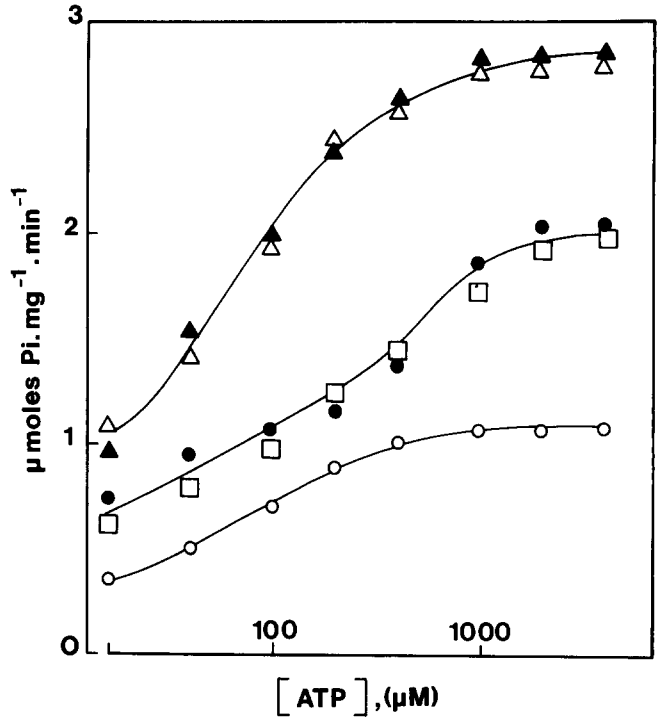

Fig. 5. Effect of ATP on the stimulation of the Ca^{2+}-ATPase by ethanol and trypsin proteolysis. Trypsin proteolysis was carried out as indicated in Fig. 3. Other experimental conditions were as in Fig. 4. Control (○); 5 μg/ml calmodulin (●); proteolysed enzyme (□); proteolysed enzyme plus 5% ethanol (v/v) (△); proteolysed enzyme plus 5% ethanol (v/v) plus 5 μg/ml calmodulin (▲).

(5%, v/v) to the proteolysed Ca^{2+}-ATPase had an additive effect on the maximal velocity and on the affinity of the enzyme for ATP, further supporting that the mechanism of stimulation of ethanol and calmodulin are different in nature.

In order to investigate if the stimulatory effect of ethanol was limited to the purified solubilized enzyme, or if it could be reproduced on the enzyme in situ, we studied the effect of ethanol on the same parameters mentioned above, using plasma membrane preparations from erythrocytes (as described under Experimental procedures) as a source of Ca^{2+}-ATPase. All the effects of ethanol mentioned above were identically obtained when plasma membrane preparations were used instead of purified enzyme (not shown).

In order to investigate if the effect of ethanol on the ATP hydrolytic activity can be reproduced on the Ca^{2+} transporting activity, we studied the effect of this alcohol on Ca^{2+} transport, by the use of calmodulin-depleted inside-out vesicles (IOVs). Fig. 6 shows that ethanol, at the same concentration range used in the experiments shown in Fig. 1, was able to stimulate Ca^{2+} transport with the same concentration-dependence to that observed on the ATP hydrolytic activity. Fig. 6 also shows an additive effect of ethanol and calmodulin on Ca^{2+} transport, similarly to that obtained on the ATP-hydrolyzing activity. It is worth noting that, as in the case on the ATP hydrolytic activity, the effect of ethanol on the basal and on the calmodulin-stimulated Ca^{2+} transport is significant at

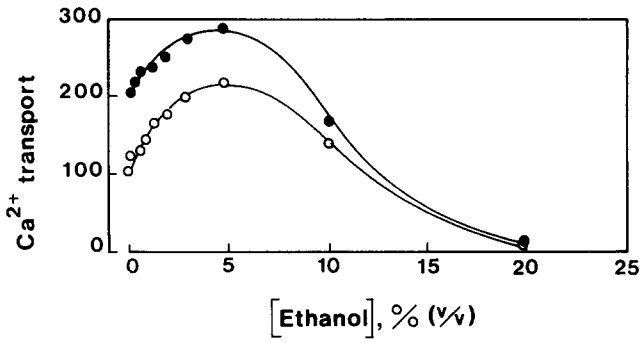

Fig. 6. Stimulation by ethanol of the Ca^{2+} transport by inside-out plasma membrane vesicles from erythrocytes. The reaction medium (1 ml, 37°C) contained, 130 mM KCl, 30 mM Hepes-KOH (pH 7.2), 0.5 mM $MgCl_2$, 1 mM Na-ATP, 50 μM arsenazo III and 10 μM free Ca^{2+}. The reaction was started by the addition of IOVs (0.5 mg/ml final). The initial velocity of the Ca^{2+} transport was plotted against each concentration of ethanol as indicated. The basal specific activity (corresponding to 100%) of the Ca^{2+} transport was 21 ± 1.9 nmol Ca^{2+}/min/mg protein. Other experimental conditions as described under Experimental procedures. The figure shows the mean value of at least five independent experiments. Control (○); 5 μg/ml calmodulin (●).

concentrations of ethanol achieved after ethanol intoxication in humans (0.25–0.5%, v/v). By the use of plasma membrane preparations, under conditions described under Experimental procedures, it was observed that all the effects of ethanol mentioned above were fully reversible (not shown).

4. Discussion

The Ca^{2+}-ATPase from plasma membrane has been submitted to extensive studies in order to clarify its regulatory mechanisms. Several effectors besides calmodulin have been reported which are able to stimulate its hydrolytic activity as well as its associated Ca^{2+} transport. Most of them, however, only increase the affinity of the enzyme for Ca^{2+} [33] and/or increase its maximal velocity to the same extent obtained in the presence of calmodulin [2,30,33]. In the present work we demonstrate that ethanol stimulated the Ca^{2+}-ATPase to a larger extent to that obtained when this enzyme was activated by calmodulin. The increase in the degree of activation was observed on the affinity of the enzyme for Ca^{2+} and for ATP as well on its maximal velocity. Albeit the effect of ethanol on the maximal velocity of the enzyme is unambiguous, the effect on the affinity of the enzyme for Ca^{2+} should be taken more carefully. It is possible that ethanol, being an organic solvent, may affect the activity of the dissolved calcium. Thus, In principle, the presence of ethanol could affect the enzyme structure but also the apparent affinity of the enzyme and the Ca^{2+}-buffering system.

Interestingly, the effect of ethanol was additive to that of calmodulin. This additive response was observed on the affinity of the Ca^{2+}-ATPase for its substrates, and also on the maximal velocity of the enzyme, thus suggesting that both effectors interact with the Ca^{2+}-ATPase through different mechanisms This was confirmed by the experiments on the trypsin-proteolyzed enzyme. It was shown that when the Ca^{2+}-ATPase was made calmodulin-independent by digestion with trypsin, the effect of ethanol was essentially the same to that observed when the alcohol was added simultaneously with calmodulin.

We have previously shown, in experiments where different fragments of the proteolyzed Ca^{2+}-ATPase were reconstituted in liposomes, that this enzyme can be reduced to 60% of its molecular mass and still be able to transport Ca^{2+} with high efficacy [29]. This suggests that about 40% of the molecular mass of the Ca^{2+}-ATPase could be involved on its regulation. It has also been demonstrated that part of this 40% is involved in the regulation of the pump by calmodulin and by acidic phospholipids [33]. Acidic phospholipids increase the affinity of the enzyme for Ca^{2+} to a larger extent to that achieved with calmodulin, but the maximal velocity attained is similar for both effectors [34,35]. Since ethanol is able to increase the maximal velocity of the enzyme to a larger extent than calmodulin (or acidic phospholipids), this fact suggests that the Ca^{2+}-ATPase can be further regulated by other unknown effectors, which would interact with the enzyme through different(s) domain(s).

Hydrophobic interactions are known to affect the plasma membrane Ca^{2+}-ATPase activity [13,36]. The fact that the optimal concentration of n-alcohol which gives the maximal stimulatory effect strongly depended of the length of the carbon chain could be easily interpreted as an hydrophobic effect related to the partition coefficient of the different alcohols between water and the lipid bilayer. Therefore, the stimulatory effect observed could be due to an increase in the fluidity of the membrane. However, if this could be an easy explanation on the experiments where membrane preparations were used, the same can not be directly extrapolated to the experiments with the purified solubilized enzyme. In this case, the protein was in mixed micelles of detergent and phospholipids where they can not be considered as an organized structure. However, the detergent/phospholipid mixture could create a hydrophobic environment mimicking the situation in the membrane. Alcohols might cause local changes in this environment in a similar fashion to the situation in intact membranes, leading to alterations in parameters such as fluidity. On the other hand, a direct effect on the protein moiety can not be ruled out.

Another possibility is that the stimulatory effect of ethanol on the calcium pump would occur through a modulation of the intersubunit interactions which are known to exist in this enzyme [12,37]. The oligomerization of the pump has been reported to stimulate its activity [12]. However, this effect is though to occur through an interaction with the calmodulin-binding domain of the enzyme [38]. Since the effect of ethanol appears to be independent of calmodulin, the above explanation for its stimulatory effect does not seem to be adequate. Other experiments should be done in order to clarify the mechanism of stimulation of the Ca^{2+}-ATPase activity by n-alcohols.

Other P-type ATPases such as the Ca^{2+}-ATPase from sarcoplasmic reticulum are also affected by ethanol. Thus, a biphasic effect of short-chain alkyl alcohols has been reported on this Ca^{2+} pump [39], but the degree of stimulation is relatively low, and higher concentration inhibit the enzyme. Other groups has reported inhibition of the mentioned Ca^{2+} pump by ethanol and other anesthetics [40]. In the case of the plasma membrane Ca^{2+}-ATPase, as mentioned previously, there are contradictory reports. In red blood cells as well as in synaptosomes a small but significant increase in the rate of Ca^{2+}-ATPase activity upon

addition of ethanol has been reported [19], which was more pronounced on the affinity of the enzyme for Ca^{2+}. However, in this report no attempt to characterize the behavior of the Ca^{2+}-ATPase activity in the presence of ethanol and calmodulin was made. Beside, these authors used only membrane preparations [19]. On the other hand, Kosk-Kosicka an co-workers recently showed in a short report an inhibition of the stimulatory effect of calmodulin or auto-aggregation of the enzyme by short chain alkyl alcohols, using a solubilized purified enzyme from human erythrocytes [20]. However, no control with alcohol alone was reported and the assays were performed in the presence of a 150 μM of the detergent $C_{12}E_8$. The detergent could have masked the stimulatory effect of ethanol in those preparations. Another difference between the methodology used by the mentioned authors and this work concerns the isolation of the Ca^{2+}-ATPase. Kosk-Kosicka et al. added 20% glycerol (v/v) in the storage solution of the calmodulin-affinity eluted Ca^{2+}-ATPase, in order to preserve the activity of the enzyme after freezing [12], while we use only 5% glycerol (v/v) for the same purpose. This might be important since it has been demonstrated that glycerol has an stimulatory effect on the purified Ca-ATPase [14]. At present we can not ascertain the reason for this discrepancy.

It is worth while to mention here that other plasma membrane Ca^{2+}-ATPases from different origins are also stimulated by ethanol. By the use of plasma membrane vesicles from the human parasite *Leishmania braziliensis* [41], we observed the same overall stimulatory effect of ethanol on the Ca^{2+}-ATPase of the mentioned organism (Benaim, G. and Cervino, V., unpublished observations). These results strongly suggest that this phenomena is not an attribute solely of the human erythrocyte enzyme, but can be extended to other plasma membrane Ca^{2+}-ATPases.

Ethanol was able to significantly stimulate the Ca^{2+} transport at concentration [18,42] which are easily achieved in the blood after its human consumption (0.25–0.5%, v/v). At this concentration range, the stimulation observed, though relatively small (30–40%), was additive to that of calmodulin. It has been reported [43] that addition of ethanol at this concentration range in skeletal muscle produce a decrease in the intracellular Ca^{2+} concentration, as measured with the intracellular Ca^{2+} indicator fura-2. On the other hand, after the addition of ethanol at the same concentration range to hepatocytes [18,44], an increase in the intracellular Ca^{2+} concentration is observed. However in this case, the change is transitory and parallel to the stimulation of the inositol lipid-specific phospholipase C, and the concomitant increase in the levels of inositol 1,4,5-trisphosphate [44]. Other effects of ethanol have been related to the alteration of the intracellular Ca^{2+} homeostasis [18]. A possible direct pharmacological role of ethanol through an interaction with the plasma membrane Ca^{2+}-ATPase remains to be elucidated.

Acknowledgments

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