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Short-chain alkanols and the functional efficiency of the Ca pump in the sarcoplasmic reticulum of rabbit skeletal muscles

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The effect of 10 low molecular mass alkanols on the activity of Ca-ATPase (EC 3.6.1.38), Ca uptake and Ca efflux as well as the functional efficiency of the Ca pump in the fragmented sarcoplasmic reticulum of rabbit skeletal muscles has been studied. Some alkanols, especially when taken at low concentration, have been found to stimulate the activity of the Ca pump and Ca-ATPase, namely *tert*-butanol, isopropanol and ethanol (from the group of hydrophilic alkanols), and pentanol, isopentanol and hexanol (from the more hydrophobic alkanols). Methanol (from the first group) and isobutanol, butanol and propanol (from the second) do not stimulate the Ca pump compared with the control. The specific effect of different alkanols cannot be explained in terms of a unitary mechanism based on 'fluidity' changes of the membrane. It is assumed that, at low concentrations, certain alkanols (or groups of related alkanols) are able to promote the specific transition of membrane proteins into the active state, whereas at higher concentrations all alkanols provide for the non-functional state of the proteins.

Short-chain alkanol; Ca^{2+} transport; Ca^{2+} pump; Ca^{2+}-ATPase; Sarcoplasmic reticulum

1. INTRODUCTION

Short-chain alkanols induce a variety of effects in the lipid bilayer of biomembranes [1-6]. In fragmented sarcoplasmic reticulum (FSR) vesicles, all alkanols were shown [5,6] to inhibit Ca uptake and to enhance the Ca-ATPase activity. But more recently a number of alkanols has been shown [3,4] to produce unexpected, drug-specific effects in some experimental systems. Therefore, we re-investigated how ethanol and other short-chain alkanols affect the functional activities of the Ca pump in FSR membranes of skeletal muscles. A preliminary communication was published earlier [7].

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2. EXPERIMENTAL

Rabbit FSR membranes were prepared as in [8]. To estimate the effect of alkanols, aliquots were taken, each containing 360 μg of membrane protein in 20 μl of the storage medium which consisted of 1 M sucrose, 25 mM imidazole, pH 7.0, at 4°C, 10^{-4} M EDTA and 10^{-4} M $CaCl_2$. The sample was mixed with an equal (20 μl) volume of an aqueous solution, containing the desired amount of the alkanol to be investigated. The mixture was incubated for 3 min at 4°C with continuous mixing on a 'Maxi-Mix' vortex-mixer (Thermolyne Corp.).

The standard assay of Ca-ATPase activity was carried out by monitoring the proton production due to ATP hydrolysis [9]; 20 μl aliquots were taken after preincubation and put into 4 ml of the reaction mixture containing 100 mM NaCl, 4 mM $MgCl_2$, 5 mM sodium oxalate and 2.5 mM imidazole, pH 7.0 at 37°C.

The Ca uptake and Ca release were registered with a Ca-selective F2110 electrode (Radiometer, Denmark). The initial concentration of the $CaCl_2$ solution was determined [10] by spectrophotometric titration with 0.3 mM murexide in 15 mM Tris-HCl buffer, pH 7.5, using a calibration curve obtained with a standard solution of $CaCl_2$. Possible errors (due to the drift of electrode potential) were minimized by calibrating the electrode before and after each assay.

Protein concentrations were determined as in [11].

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3. RESULTS AND DISCUSSION

The increase of alkanol concentration in the preincubation medium eventually lowers the efficiency of the Ca pump in alkanol-treated FSR vesicles (fig.1a). However, the curves depicted in fig.1a form two separate groups and not a smooth row of monotonically diminishing curves as in [5,6]. Both groups include alkanols that cause a distinct stimulation of the Ca pump. This is especially true when the alkanol concentration is relatively low. On the other hand, some alkanols, in the concentration range, do not stimulate the Ca pump compared with the control. Since the Ca/ATP values allow one to judge [12] both the efficiency of the pump operation and the intensity of Ca uptake, one may infer that certain alkanols stimulate Ca uptake, while others do not.

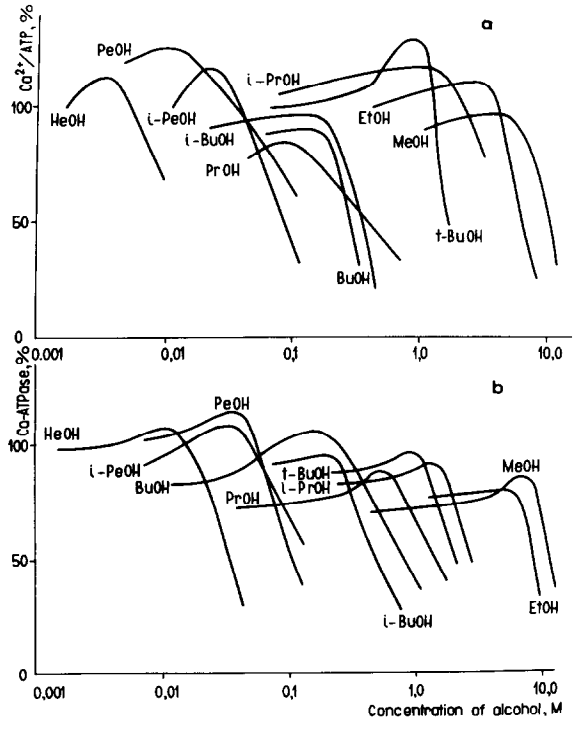

Fig.1. The functional efficiency of the Ca-pump (a) and the Ca-ATPase activity (b) of FSR vesicles in the presence of various alkanols. The functional efficiency of the Ca-pump was estimated by the Ca/ATP value [12]. Values obtained with the control FSR preparations were assumed as 100%, i.e. 4.8–6.4 μmol $P_i \cdot min^{-1} \cdot mg^{-1}$ of protein for Ca-ATPase activity and 0.9–1.4 for Ca/ATP. MeOH, methanol; EtOH, ethanol; PrOH, *n*-propanol; BuOH, *n*-butanol; PeOH, *n*-pentanol; HeOH, *n*-hexanol; i-PrOH, isopropanol; i-BuOH, isobutanol; i-PeOH, isopentanol; *t*-BuOH, *tert*-butanol.

Fig.1b shows the effect of alkanols on the Ca-ATPase activity. In this case, too, an increase in the concentration of any alkanol eventually results in a total disappearance of the enzyme activity. Yet, here as well, different alkanols have a different effect on the enzyme. Contrary to [5,6] an explicit stimulation of Ca-ATPase activity has been observed only in four cases: in the presence of pentanol, isopentanol, hexanol and butanol.

In order not to change our experimental conditions but to increase the period when the rate of Ca^{2+} is linear, Ca uptake was registered with a Ca-selective electrode in the same media in which the activity of Ca-ATPase had previously been measured. The response of the electrode was linear within 10^{-6} to 10^{-3} M. In the presence of 4 mM Mg^{2+} the Nernstian slope factor varies from 26 to 29 mV, while the response linearity at a concentration of free Ca^{2+} far below 10 μM significantly deviates from the straight line. The relatively slow response is due to the design features of the selectrode.

Fig.2 shows that, in the absence of alkanols (curve 9), the FSR vesicles normally absorb 4 additions of Ca^{2+}, 125 nmol each, without any noticeable release of the ion. Of particular interest is the fact that the Ca uptake, close to the control (curves 3, 6 and 8), is observed in those preparations in which a decrease in Ca/ATP (fig.1a) and in the Ca-ATPase activity (fig.1b) is detectable. This is in good agreement with the data of Ohnishi et al. [13] who demonstrated that one of the alkanols studied by us, ethanol, causes only an insignificant release of Ca^{2+} from a 'light' FSR provided it is added to the medium prior to ATP. A further increase in the concentration of this or that alkanol leads to a distinct change of the properties of the membrane itself. In this case, Ca^{2+} leakage from vesicles (curves 2, 5 and 7) is suddenly induced, or conditions are created when it is impossible to monitor Ca uptake by vesicles (curves 1 and 4). From time to time such dramatic changes in the membrane properties are not detectable or disguised after a single addition of Ca^{2+}; they become apparent only after another addition of Ca^{2+} or even after several additions of the ion. A possible reason may be that higher alkanol concentrations act upon Ca release and not on the Ca pump which

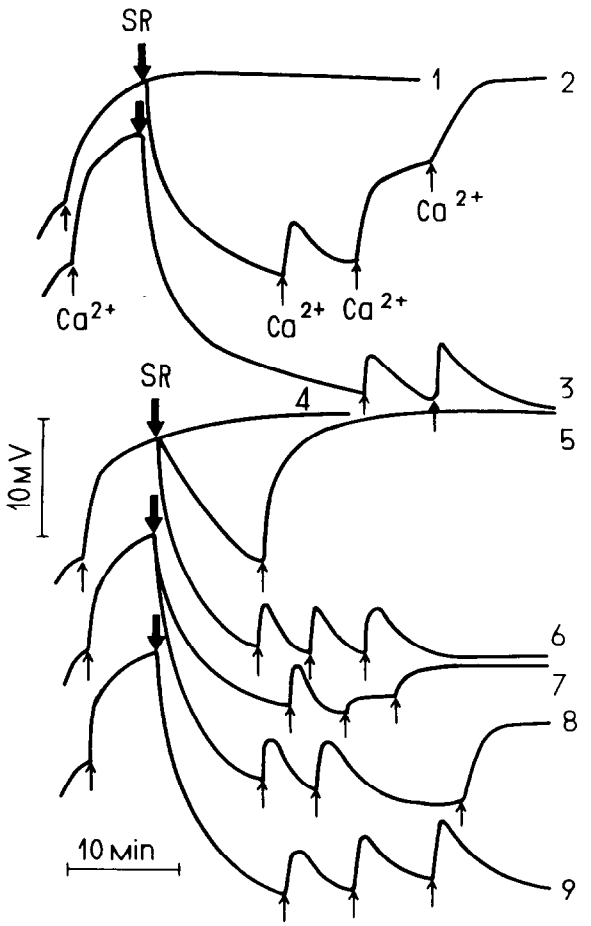

Fig.2. The effects of various alkanols on the Ca^{2+} transport in FSR vesicles. Curves: 1, 0.55 M BuOH; 2, 0.37 M BuOH; 3, 0.04 M HeOH; 4, 2.61 M i-PrOH; 5, 1.96 M i-PrOH; 6, 1.84 M i-PrOH; 7, 1.07 M PrOH; 8, 0.8 M PrOH; 9, control. The incubation medium is supplemented with FSR vesicles (180 μg protein); arrows indicate the addition of 125 nmol Ca^{2+}.

continues to operate and, for a period of time, is capable of compensating for Ca^{2+} leakage.

The correlation analysis [14] revealed that the concentration (C) of alkanol at which the maximal value of Ca/ATP is attained, with one exception, i.e. propanol, correlates well with the value of the partition coefficient (P) in the two-phase system. The pattern observed (fig.3) is in good agreement with the semi-empiric data [2] on the dependence of the distance between the hydrophobic and hydrophilic gravity centers in an alkanol molecule on the hydrocarbon chain-length. The dependence is changed sharply after the transition through the

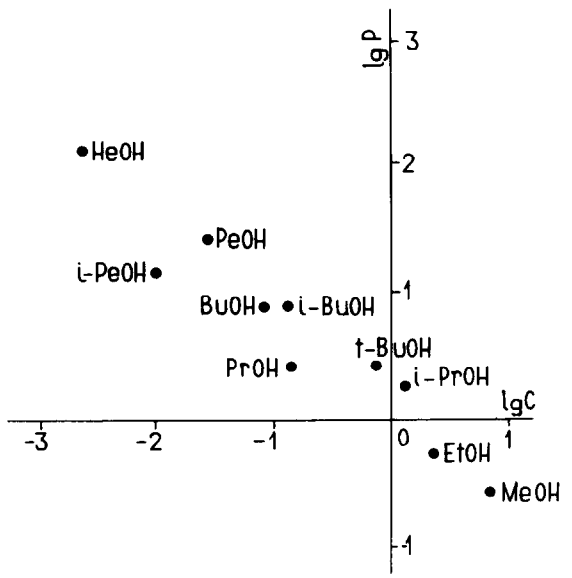

Fig.3. Partition coefficient (P) dependence of alkanol concentration (C) at which the maximal value of Ca/ATP is achieved. The correlation coefficients (r) for small samples were calculated as in [14]. The values of P in the water/octanol system have been taken from [15].

$3<n<4$ point (where n is the number of carbon atoms) and thus is described by two branches. In [2] a hyperbolic curve was also represented which reflects the hydrophile-lipophile balance as a function of n, the transition from the descending branch of the curve to the ascending one being located in about the same region as $3<n<4$. A similar transition point was observed when estimating the changes of the anesthetic activity of alkanols as the function of the length of the hydrocarbon chain [16].

So, the different effect of different alkanols cannot be explained in terms of a unitary mechanism based on the change in membrane fluidity. In all likelihood, the effects observed are conditioned by the direct interaction of alkanols with the Ca-ATPase protein itself. Such interaction must be specific for each alkanol or a group of related alkanols that affect in a different way the rate of conformational changes and, at low concentrations, by acting on definite domains in the membrane, are able to promote the specific transition of membrane proteins into the active state, whereas at higher concentrations all alkanols provide for the non-functional state of the proteins.

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