THE TERMINAL STEP OF THE DE NOVO SYNTHESIS OF DIACYL AND ALKYLACYL PHOSPHOLIPIDS IN SUBFRACTIONS OF RABBIT SARCOPLASMIC RETICULUM ONTOGENESIS

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1. Introduction

The sarcoplasmic reticulum represents a specialized form of endoplasmic reticulum present in the muscle cell which regulates the contraction-relaxation cycle by modulating Ca^{2+} concentration in the sarcoplasm (review in [1]). The membrane, fragmented during homogenization of muscle, yields a microsomal fraction which in the previous work from this laboratory was resolved into a light and a heavy subfraction. Both subfractions isolated from mature and undeveloped muscle were recently characterized with respect to phospholipid content, composition and localization [2,3]. Although phospholipids are absolutely essential for the main activities of the sarcoplasmic reticulum [4,5], their metabolism in this membrane has not been sufficiently studied. Especially, there is no information about the de novo synthesis of ether-linked phospholipids, despite the high content of this type of phospholipid in sarcoplasmic reticulum [6]. The only reports to date concern the acylation of ether-linked phospholipids in adult muscle [7,8].

The presence of enzymes involved in diacyl phospholipid biosynthesis was already demonstrated in the total microsomal fraction isolated at various stages of muscle development [9]. However, in view of the differences in phospholipid content in the light and the heavy fraction [2,3], detailed studies on phospholipid-synthesizing enzymes in both subfractions appeared to be especially interesting. The present paper describes the last step of the de novo synthesis of choline and ethanolamine phospholipids in both microsomal subfractions isolated from muscle at various stages of development. The synthesis of diacyl and alkylacyl types of phospholipids was investigated.

2. Materials and methods

Rabbit skeletal muscle microsomes were obtained by the method described previously [2,3] for various stages of development of the animals, including adult rabbits. Briefly, the method consisted of separation of a muscle homogenate fraction sedimenting between 8000 and 50 000 × *g*/h on a sucrose density gradient into two subfractions (called 'light' and 'heavy microsomal fraction' later on).

The preparation of labeled [*Me*-^{14}C]CDP-choline and [*Me*-^{14}C]CDP-ethanolamine, 1,2-diacyl-*sn*-glycerols and 1-alkyl-2-acyl-*sn*-glycerols was described previously [10]. CDP-ethanolamine: 1,2-diradyl*-glycerol phosphoethanolaminetransferase (EC 2.7.8.1) and CDP-choline: 1,2-diradylglycerol phosphocholinetransferase (EC 2.7.8.2) were assayed for 15 min in a shaking water bath at 37°C in a final volume of 0.15 ml containing 50 mM Tris–HCl, pH 8.0, 13 mM $MnCl_2$, 0.88 mM [*Me*-^{14}C]CDP-ethanolamine (0.5–1.0 Ci/mol), 1.8 mM diradylglycerols, and 50 μg microsomal protein for the former, and 50 mM Tris–HCl, pH 8.0, 13 mM $MgCl_2$, 1 mM dithiothreitol, 1 mM ethyleneglycol-bis (2-aminoethyl)-*N,N'*-tetraacetic acid, 0.4 mM [*Me*-^{14}C]CDP-choline (0.5–1.0 Ci/mol), 1.8 mM diradylglycerols, and 50 μg microsomal protein for the latter enzyme. Diradylglycerols were added as sonicated emulsions in 0.1% Tween 20 [11]. The reactions were stopped and lipid-associated radioactivity determined according to [12].

* Radyl denotes alkyl or acyl

3. Results and discussion

The dependence of the specific activities of microsomal phosphocholine- and phosphoethanolamine-transferase on the age of the animal (or embryo) is shown in figs.1 and 2, respectively. The most striking feature is the drop of specific activity from the high values in embryonic muscle to a minimum occurring around birth in the light fraction and few days later in the heavy one. The drop of enzymatic activity is subsequently followed – especially for the diacylglycerol-dependent activities (figs.1A and 2A) – by an increase in the mature muscle. This rather unusual shape of the curve is, however, in agreement with our previous results obtained on unfractinated microsomes with diacylglycerols as a substrate [9]. The drop in

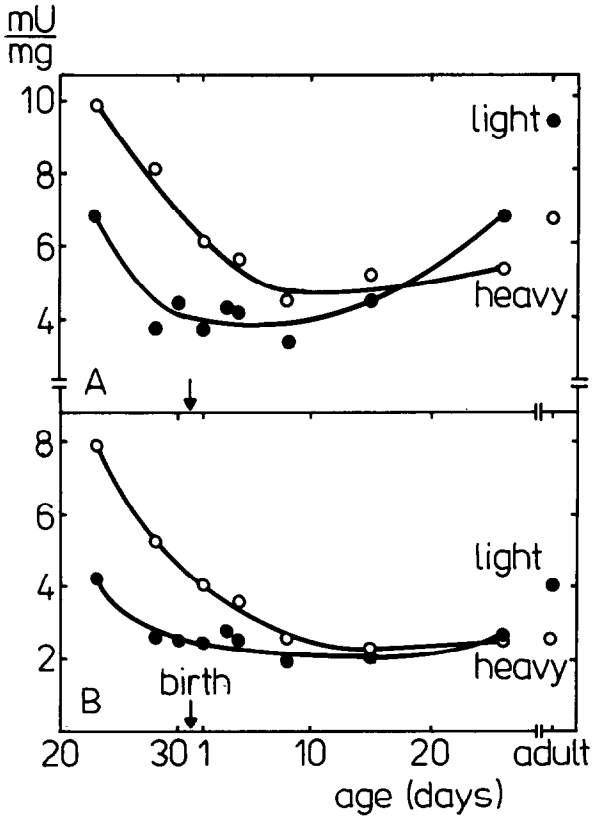

Fig.1. The dependence of microsomal phosphocholine-transferase activity on the age of the animal. (A) diacylglycerols; (B) alkylacylglycerols as a substrate. The presence of endogenous diradylglycerols was neglected because of the low amount of protein used for the assays. Closed and open symbols indicate the light and the heavy microsomal fraction, respectively.

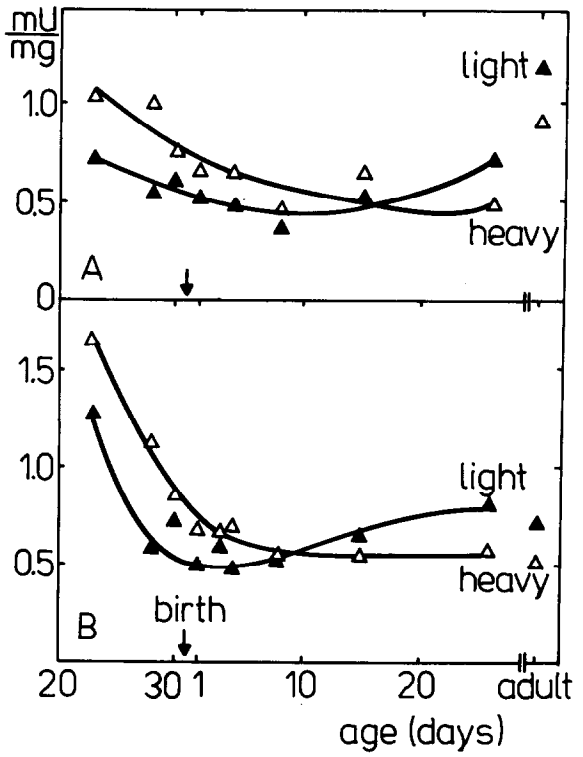

Fig.2. The dependence of microsomal phosphoethanolaminetransferase activity on the age of the animal. See legend to fig.1 for details.

the enzymatic activities cannot be explained by proteolysis artifact, ([3], and unpublished results). In addition, several other microsomal enzymes show a maximum of activity during the early postnatal period [9,13], also arguing against enhanced unspecific proteolysis.

The alkylacyl-glycerol-dependent phosphocholine- and phosphoethanolaminetransferase activities are shown in figs.1B and 2B, respectively. The high values of enzymatic activities observed in the embryonic muscle decrease during development in a similar way as diacylglycerol-dependent activity except that the increase in the activities during postnatal period is less pronounced, especially for the heavy fraction.

Fig.3, showing the ratio of synthesis rates of diacyl phospholipids to their ether-linked analogues, allows us to draw additional conclusions. This ratio is lower for ethanolamine phospholipids than for choline phospholipids, thus correlating well in the adult muscle sarcoplasmic reticulum [6] with the lower content of ether-linked phospholipids in the latter than in the former class. It is remarkable that the ratio of both

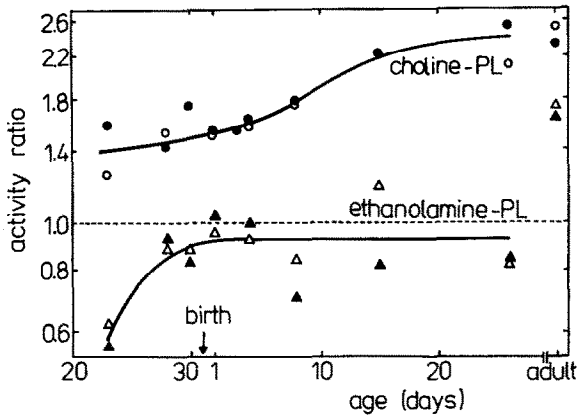

Fig.3. The ratio of diacylglycerol-dependent to alkylacylglycerol-dependent phosphocholinetransferase (○,●) and phosphoethanolaminetransferase (△,▲) activity in the course of muscle development. Open symbols represent the heavy and closed symbols the light microsomal fraction.

activities changes during development. This could mean a shift in the specificity of the transferases in the direction favouring diacylglycerols in later stages of development. More likely, it indicates the existence of separate enzymes for both lipid substrates, the relative amounts of which could change during ontogenesis. This conclusion would be in line with our previous findings on the solubilization of phosphoethanolaminetransferase in liver endoplasmic reticulum [10].

Figs.1 and 2 show that in the embryonic and early postnatal period all transferases activities are higher in the heavy fraction than in the light one. At the age of ~10–20 days the activities in both fractions become equal. Later the activity of light fraction exceeds that of the heavy one. The heavy and the light microsome fractions of mature muscle consist of vesicles derived most probably from different regions of the native sarcoplasmic reticulum membrane [2,14, 15]. If the phospholipid-synthesizing enzymes are concentrated in certain membrane regions, they would be enriched in one of the microsomal fractions. The presence of 'inside-out' vesicles in the light microsomal fraction of adult muscle [2] might cause a latency of the transferases activity, which – at least in endoplasmic reticulum of liver – is located on the cytoplasmic side of the membrane [16,17]. This fact, however, does not interfere with the results of present study, since the vesicles of the light fraction tend to be more permeable to nucleotides [2], especially in the presence of detergents introduced into the assay with the diradylglycerol emulsion.

In undeveloped muscle, the transferase activities are higher in the heavy microsomal fraction, which already has some features of the mature sarcoplasmic reticulum [3]. In the case of the light fraction the situation is more complicated because it also contains membraneous elements of non-reticular origin [3]. Despite that, it seems to be clear that precursors of sarcoplasmic reticulum containing the transferase activities are present in the light fraction from the earliest stage studied, and that these activities change in an even fashion in later development up to the age of an adult animal.

The absolute specific activities of the transferases found in muscle microsomes are remarkably high. For instance, in the sarcoplasmic reticulum of adult muscle they are, depending on the fraction, only three to four times lower than in rat liver endoplasmic reticulum, when measured under identical conditions. Such high activities of enzymes catalyzing the biosynthesis of the two major membrane phospholipids make it imperative to reevaluate the common view of the sarcoplasmic reticulum as a membrane specialized only to carry out Ca^{2+} transport.

Summarizing, we conclude that a massive de novo synthesis of phosphatidylcholine and phosphatidylethanolamine in their diacyl and alkylacyl variety is an intrinsic property of the sarcoplasmic reticulum. Taking into account the relatively low turnover rate of phospholipids constituting the sarcoplasmic reticulum membrane (roughly one fifth that of liver endoplasmic reticulum [18,19]), the transferase activities, which are only 3–4 times lower than in liver, may suffice to synthesize in situ all phospholipids required for this membrane structure and function.

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