POOL SIZES AND METABOLISM OF CDPCHOLINE AND CDPETHANOLAMINE IN SKELETAL MUSCLE

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SUMMARY

1. The pool sizes of CDPcholine and CDPethanolamine in skeletal muscle were measured by isotopic dilution and found to be 0.7 and 1.5 μmole/100 g muscle respectively.

2. The specific radioactivities of phosphorylcholine derived from CDPcholine and of phosphorylethanolamine derived from CDPethanolamine determined 3 h after the administration of [^{32}P] orthophosphate were found to be 9.1% and 3.2% of the specific radioactivity of the serum inorganic phosphate respectively.

3. The specific radioactivity of muscle P_i after an injection of ortho [^{32}P] phosphate has also been determined in a time-course study during a 24 h period.

Measurement of the specific radioactivities of molecular species of phosphoglycerides has been carried out in liver [1,2] 1 h, and in muscle [3,4] 3 h after the injection of ortho [^{32}P] phosphate. The main difference in the incorporation pattern of ^{32}P between liver and muscle is in the ratio of specific radioactivity of phosphatidylcholine to that of phosphatidylethanolamine. Whilst in liver the average phosphatidylethanolamine has a specific radioactivity five times that of the average phosphatidylcholine, in muscle the ratio is 0.3.

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The aim of the present study was to measure the pool size and the specific radioactivity of CDPbases involved in the biosynthesis of phosphoglycerides in skeletal muscle. The specific radioactivity of the muscle P_i was determined during a 24 h period in order to establish whether a precursor-product relationship between serum P_i and the measured muscle P_i as well as between the muscle P_i and the phosphate moieties of phosphoglycerides could be demonstrated.

MATERIALS AND METHODS

Male Buffalo rats were placed on either a commercial cubes diet [3], a safflower seed oil containing diet [4], or a saturated fat diet [4], three weeks after birth and allowed continuous access to food and water. The animals were 6 months old when used. Five rats were used in each experiment and their tissues were pooled. The rats were injected with ortho [^{32}P] phosphate [3,4] and an incorporation time of 3 h was allowed.

CDPcholine (methyl-^{14}C), specific radioactivity 25 mCi/mmol, was purchased from New England Nuclear Co., 575 Albany Street, Boston, Mass., U.S.A. and CDP (2-^{14}C) ethan-1-ol-2-amine, specific radioactivity 28 mCi/mmol, from the Radiochemical Centre, Amersham, Bucks., England. The isotopes were each diluted with the respective CDPbase (Sigma Chemical Co., St. Louis, Mo., U.S.A.) to a specific radioactivity of 0.73 mCi/mmol for CDPcholine and 0.91 mCi/mmol for CDPethanolamine. Various amounts of either CDPbase were added to the aqueous extracts containing the phosphoglyceride precursors for pool size determination. The purity of the CDPbases used was checked by paper chromatography and found to be greater than 97%. Muscle and serum P_i were determined in pooled samples of five rats as described by Berenblum and Chain [5].

The pooled muscles were extracted with cold (-16°) 67% ethanol [6]and the residue subjected to lipid extraction. The 67% extract was purified [7] and chromatographed on Dowex AG1-X4 (200-400 mesh, chloride form, Bio Rad Laboratories, 32nd and Griffin, Richmond, Calif., U.S.A. [8].

A fraction collector was modified to collect eluates from the column into the scintillation vials, each vial containing 10 ml eluate.

All vials were examined for radioactivity and absorbance at 280 nm and the identification of CDPbases was facilitated by chromatographing a standard mixture of phosphorylcholine, phosphorylethanolamine, CDPcholine and CDPethanolamine under the same conditions (Fig. 1). The column chromatography method employed did not separate CDPbases from dCDPbases.

Fractions containing CDPbases were dried and pooled residues chromatographed on Whatman 3 MM paper (9) for 16 h. The purified bases

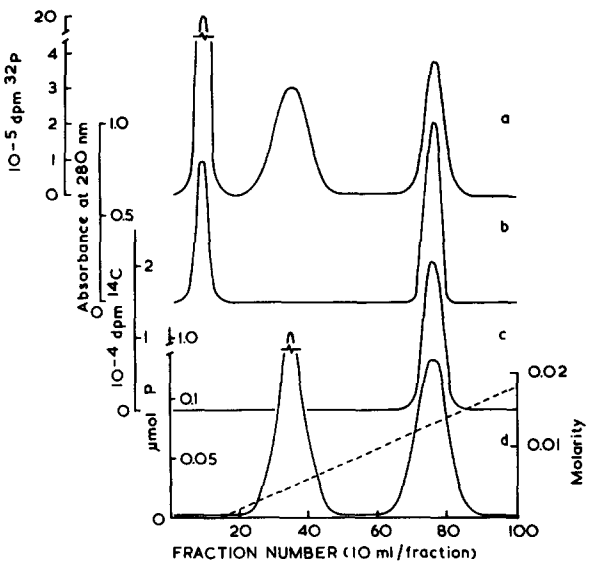

Fig. 1. Column chromatography of a 67% ethanol extract of leg muscles of five rats which had been injected with [^{32}P] orthophosphate 3 h before death. CDPcholine (methyl-^{14}C) was added as an internal standard. a) distribution of [^{32}P]. (b) absorbance at 280 nm. c) distribution of [^{14}C] radioactivity. d) distribution of phosphate of a standard mixture of 0.5 mg phosphorylcholine, 0.5 mg phosphorylethanolamine, 0.25 mg CDPcholine (methyl-^{14}C) containing approx. 100,000 dpm) and 0.25 mg CDPethanolamine chromatographed under the same conditions. The broken line shows the increase in the concentration of formic acid during the elution, which began from fraction 15.

were eluted with 0.01 M formic acid by centrifugation [10] and hydrolyzed with 1 M HCl for 1 h at 100°. After evaporation of HCl, the residues were chromatographed for 16 h for separation of phosphorylcholine [9] and for 46 h for separation of phosphorylethanolamine (11).

Cytidine-containing compounds were quantified by their absorbance at 280 nm (E_{max} = 13000 $l.mol^{-1}.cm^{-1}$ at pH 2) whilst phosphate esters were detected under uv light after spraying with acid molybdate spray [11] and quantified as described by Itaya and Ui [12]. Using these methods the following molar cytidine/phosphorus were obtained: CDPcholine 1:1.92, CDPethanolamine 1:1.94 and CMP 1:0.94 and 1:0.96 respectively.

Radioactivity was measured as previously described [3,4].

RESULTS

CDPbases

The pool size of CDPcholine in skeletal muscle was determined in control rats and in rats deficient in essential fatty acids [4],

Table 1. Pool sizes of CDPbases and relative specific radioactivities of phosphorylbase moieties derived from CDPbases. CDPbases were extracted with 67% ethanol from pooled leg muscles or five rats injected with ortho [^{32}P] phosphate 3 h before sacrifice. The extract was chromatographed on Dowex AG1-X4 and fractions containing CDPbases were purified by paper chromatography. The pools of CDPbases obtained may be slightly smaller because they may have included small amounts of dCDPbases. Figures in brackets are results of an experiment with rats deficient in essential fatty acids.

Parameter	Unit	CDPcholine	CDPethanolamine
Added CDPbase	µmol	0.782	1.1
Specific radioactivities (^{14}C) of added CDP base	dpm/µmol x 10^{-3}	1619	2018
Isolated CDPbase	µmol	0.448 (0.352)	0.24
Specific radioactivity (^{14}C) of isolated CDPbase	dpm/µmol x 10^{-3}	623 (655)	820
Muscle weight	g	181 (102)	106
Pool size/100g muscle	µmol	0.69 (1.13)	1.5
Specific radioactivity (^{32}P) of isolated CDPbase	dpm/µmol x 10^{-3}	91.2 (82.8)	62.2
Initial specific radioactivity (^{32}P) of CDPbase	dpm/µmol x 10^{-3}	148 (1391)	105
Relative specific radioactivity of CDPbase	percentage of the specific radioactivity of serum P_i*	25.1 (78.5)	12.8
Relative specific radioactivity of phosphorylbase derived from CDPbase		9.1 (13.2)	3.2
Highest relative specific radioactivity of any phosphoglyceride species		1.94 (4.2)**	0.62

* 592,000 dpm/µmol for control rats and 1,770,000 dpm/µmol for rats deficient in essential fatty acids

** 1-oleoyl-2-linoleoyl phosphatidylcholine (1-oleoyl-2-palmitoleoyl phosphatidylcholine [4]is the probable product of phosphorylcholine moiety of CDPcholine as 1-palmitoyl/oleoyl-2-docosahexaenoyl phosphatidylethanolamine [3] is the product of the phosphorylethanolamine moiety of CDPethanolamine.

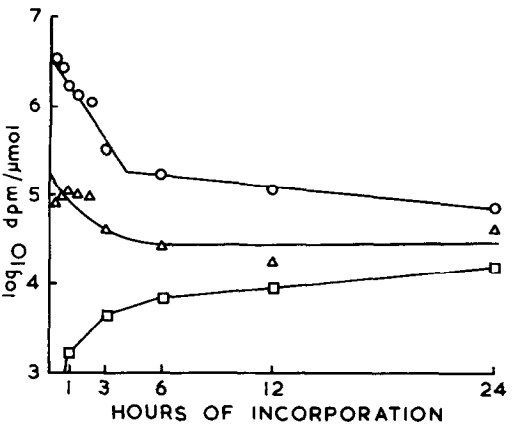

Fig. 2. Specific radioactivities of serum (O——O) and muscle (Δ——Δ)P_i as a function of time. Similar measurements for the phosphatidylcholine fraction (□——□) possessing the highest specific radioactivity [3] are shown for comparison. The sera and tissues of five rats were pooled for samples obtained after 1, 3, 6, 12 and 24 h of incorporation. Other samples originated from a single rat.

and it was found to be 0.7 µmol and 1.1 µmol per 100 g muscle respectively. The relative specific radioactivity of the phosphorylcholine moiety was 8.1% for control and 13.2% for essential fatty acid deficient rats.

The pool size of CDPethanolamine was determined in rats fed a cubes diet [3]. The pool size was found to be 1.5 µmol/100 g muscle and the relative specific radioactivity of the phosphorylethanolamine moiety derived from CDPethanolamine was 3.2%. The results are listed in Table 1.

Muscle P_i

The semilogarithmic plot of the specific radioactivity of serum and muscle P_i as well as that of a phosphatidylcholine fraction (possessing the highest specific radioactivity) as a function of incorporation time is shown in Fig. 2. An extrapolation of the P_i curves results in an intersection time of approx. 70 h.

DISCUSSION

The incorporation of orthophosphate into muscle phosphoglycerides involves the following intermediates: P_i → serum P_i → muscle P_i → phosphorylbases → phosphorylbase moieties of CDPbases → phosphoglyceride molecules directly synthesized via CDPbases. We have found that the relative specific radioactivity of phosphorylcholine derived from CDPcholine in control rats was 8.1% and in rats deficient in essential fatty acids 13.2%. The relative specific radioactivity of the muscle P_i in both control rats and those deficient in essential fatty acids (unpublished results) was 10% after 3 h of incorporation. The specific radioactivity of phosphorylcholine derived from CDPcholine was lower than that of muscle P_i in control rats and therefore a precursor-product relationship is possible. However in rats deficient in essential fatty acids the specific radioactivity of phosphorylcholine derived from CDPcholine was higher than that of muscle P, and a precursor-product relationship could no longer exist. Any scheme of phosphate incorporation into muscle includes muscle P_i as a mandatory intermediate. Hence, a possible reason of our findings is that the measured pool of P_i is heterogeneous and only a small compartment of th this pool is involved in phosphoglyceride biosynthesis. It has been demonstrated that a considerable fraction of intracellular P_i in muscle is bound to myofibrollar proteins [13] as an intermediate in myosin-ATPase activity [14,15] and therefore is not available as a phosphate precursor in phosphoglyceride biosynthesis. Moreover, extracellular P seems to penetrate slowly into the intracellular space of muscle cells [16]. Both findings support the concept of heterogeneity of muscle P_i.

The pool size of CDPcholine in skeletal muscle is approx. 1/10 of that of liver. Some of the discrepancy in data regarding the amount of CDPcholine in liver (Table 2) can be accounted for by methodology

Table 2

Pool size of CDPcholine and CDPethanolamine in rat liver μmol/ 100g liver).

Source	CDPcholine	CDPethanolamine
Sundler [17]		2.4*
Sundler et al [18]	0.87	
Shamgar and Collins (unpublished results)	4.8	
Trewhella and Collins (unpublished results)	10.4	
Wilgram et al [7]	5.0	
Kennedy and Weiss [19]	10.4	5.3
Schneider [20]	3.4	4.3

* Based on 10 g wet weight of liver.

[18]. However, in relation to the amounts of CDPbases in muscle, we used cold [-16°] 67% ethanol for the homogenization and extraction of muscle and hence the likelihood of enzymic changes in the amount of CDPbases was reduced.

The pool size of CDPethanolamine in muscle is approx. 1/3-1/2 of the corresponding pool in liver.

The actual pools of CDPbases in muscle could be slightly smaller because they may have included dCDPbases. The liver dCDPbases amount to less than 1/20 of the corresponding CDPbases [20].

The specific radioactivities of muscle phosphatidylcholines are considerably higher than those of phosphatidylethanolamines [3,4] whereas the reverse is true for liver [19,20]. In muscle, the specific radioactivity of the phosphorylcholine moiety of CDPcholine is approx. 3 times that of the phosphorylethanolamine moiety of CDPethanolamine.

A possible cause for the higher specific radioactivities of muscle phosphatidylcholines compared with those of phosphatidylethanolamines could be the relatively high specific radioactivity of their precursor, i.e. the phosphorylcholine moiety of CDPcholine.

The recent demonstrations of the back reactions [17,21] could explain the fact that the specific radioactivities of liver phosphatidylcholines are much lower than those of their precursors. Although the presence of the back reaction has not been demonstrated in muscle, the possibility of some CDPcholine formation from phosphatidylcholine cannot be excluded.

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