THE POSSIBLE ROLE OF CARNITINE AND CARNITINE ACETYL-TRANSFERASE IN THE CONTRACTING FROG SKELETAL MUSCLE

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1. Introduction

By promoting the translocation of the activated acyl-groups across the mitochondrial acyl-CoA barrier carnitine and the long-chain acyl-CoA carnitine-acyl-transferase (CPT) (EC 2. 3. 1. 23) play an important role in the oxydation of fatty acids [1,2]. This function of CPT is emphasized by its intracellular localization on the inner and outer surface of the mitochondrial inner membrane [3–6]. The function of the short chain acyl-CoA carnitine-acyltransferase (CAT) (EC 2. 3. 1. 7.) is not clear. The enzyme is supposed to play a role in the transport of the intramitochondrially formed acetyl groups into the cytoplasm where are used for fatty acid synthesis and for other processes consuming acetyl groups [7–10]. According to Fritz [1] the enzyme facilitates the intramitochondrial acetyltranslocation within the different acetyl-CoA pools. There are also suggestions for an acetyl group buffering system consisting of carnitine and CAT. By this system CoASH regeneration would be sufficient even in the case of increased fatty acid and carbohydrate degradation [11–14], and at the same time acetyl groups would be preserved in an active form (high-energy acetate reservoir) [14].

The activity of carnitine acyltransferases and the concentration of carnitine and carnitine esters in the muscle are high and intensive carbohydrate and fat oxidation can be produced by stimulating the muscle. Therefore the effect of stimulation on the carnitine and carnitine-ester content of the contracting muscle may be informative for a better understanding of carnitine-metabolism.

Different types of muscles metabolise nutrients at different proportions, which suggests an unequal importance of carnitine and the transferase enzyme in the various types of muscles. The gastrocnemius muscle of the frog used in the present study is a typical 'white' muscle and its main source of energy is carbohydrate-catabolism. Therefore our experiments could be expected to provide information chiefly on the interrelationships between carnitine and carbohydrate metabolism.

2. Materials and methods

Adult frogs, *Rana esculenta*, freshly collected in June and July were used as experimental animals. One hr prior to the stimulation of the gastrocnemius muscle the animals were immobilized by keeping them in a small box or by the intraperitoneal injection of 0.5 mg of syncurarin. By using the latter method the concentration of acetylcarnitine could be maintained at a lower level and scattering could also be significantly reduced.

In the 'in situ' experiments skin covering the gastrocnemius muscle was opened and the muscle was directly stimulated for various times (frequency of the stimulation: 2/sec; duration: 6 msec; voltage: 4–8 V). The gastrocnemius muscle of the other hind leg served as control.

In the in vitro experiments the effect of isometric and isotonic contraction was studied. The gastrocnemii were dissected out and mounted in a muscle holder immersed in non-oxygenated frog-Ringer solution.

The stimulation was performed under the same conditions as described above. Paired muscles served as controls and were kept in separate chambers. The longest period of stimulation was two min this time, resulting in total exhaustion of the muscle, that is it became refractory to further stimulation.

After the concentration had ceased, the muscle was removed within a few seconds and frozen in acetone containing solid CO_2. The further preparation and extraction of muscle for the estimation of carnitine and carnitine ester content was made as described by Pearson et al. [15]. Acetyl-carnitine was measured by a combined optical method [15] and free carnitine was estimated by Ellmans-reagent (5,5'-dithiobis-2-nitrobenzoic acid) [15]. The analysis of total acid-soluble and long chain acyl-carnitine, respectively, was performed after alkaline hydrolysis of the samples and was expressed as free carnitine [15]. Pyruvate and lactate concentrations were measured by lactate dehydrogenase [16,17]. The tissue concentrations of each metabolites were expressed as nmol/g muscle. The preparation of the muscle extract and the analysis of each metabolite were performed on the same day. The recovery data of the respective compounds added to the muscle varied between 95.6% and 104.9%. CPT was extracted and measured by the hydroxamic acid method as described [18]. CAT extraction and estimation by a combined optical test was performed according to [19]. The activities of both enzymes were expressed as U/g muscle 1 U corresponding to the formation of 1 μmol $NADH_2$ or palmitoyl-hydroxamate/min at 25°C. *P* values were determined with Student's test of the means. Carnitine acetyltransferase, citrate-synthase, malate dehydrogenase and CoASH were the products of Boehringer, Mannheim, DL-carnitine-hydrochloride was produced by Schuhardt, München, acetyl-chloride and palmitoyl-chloride by The British Drug Houses Ltd., England NAD, $NADH_2$, LDH and all the other reagents were manufactured by Reanal, Budapest. DL-acetylcarnitine, DL-palmitoylcarnitine and acetyl-CoA were prepared after standard procedures [8,20,21].

3. Results and discussion

To our knowledge no data concerning the carnitine metabolism of frog muscle have yet been published. Our results are shown in table 1.

The presence of the long chain acylcarnitine esters and CPT indicates that fatty acid oxydation in the frog skeletal muscle is carnitine dependent. Since the levels of the long chain esters remained unchanged under stimulation and the amounts of the short chain esters were practically identical with that of acetyl-carnitine (see table 1; the sum of free plus acetyl-carnitine nearly corresponds to total acid-soluble carnitine in each case) only the concentrations of acetylcarnitine and free carnitine were measured in the stimulation experiments. Lengthening the time of stimulation resulted in an increase of the acetyl

Table 1
Concentration of carnitine and carnitine esters and the activity of CAT and CPT in resting gastrocnemius muscle of the frog

	nmol/g muscle				U × 10^3/g muscle	
Group of animals	Acetyl-carnitine	Free-carnitine	Total acid soluble carnitine	Long chain acyl-carnitine	CAT	CPT
Untreated	26 ± 1.9	167 ± 9.7	183 ± 10.3	28 ± 2.6	588 ± 59	62 ± 5
Syncurarin treated	10 ± 0.7	178 ± 8.3	176 ± 9.4	21 ± 2.1	–	–

Ten animals in each group. Means ± ESM

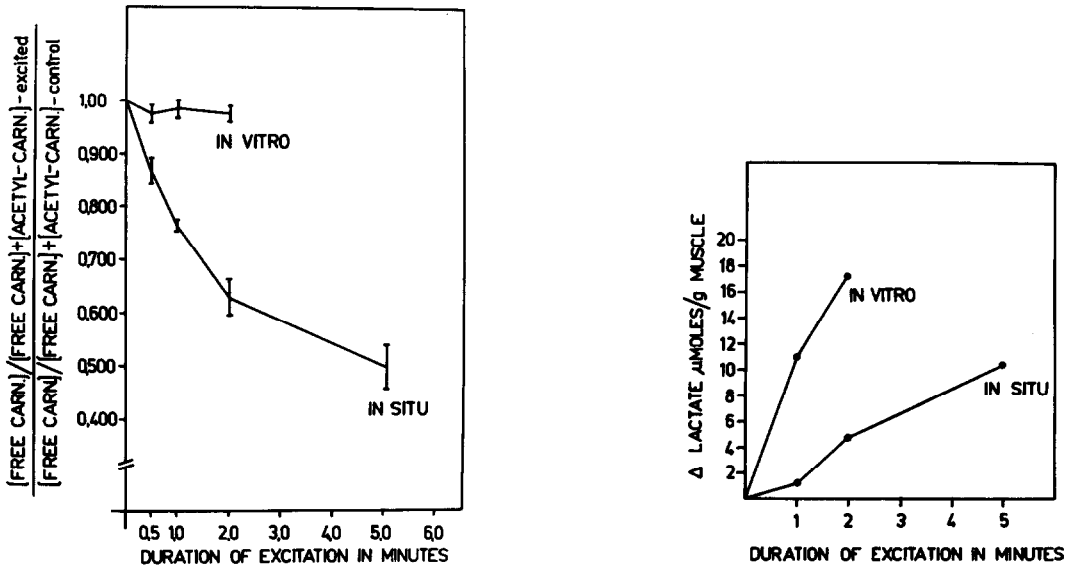

Fig.1. A) The effect of contraction on the acetyl-content of the carnitine-pool in frog gastrocnemius muscle. The contralateral gastrocnemius muscle served as control in each case. Five animals were stimulated. Means ± SEM. B) Lactate formed upon stimulation of the muscle. Data are differences between stimulated and control gastrocnemius. The figures are means of three separate experiments.

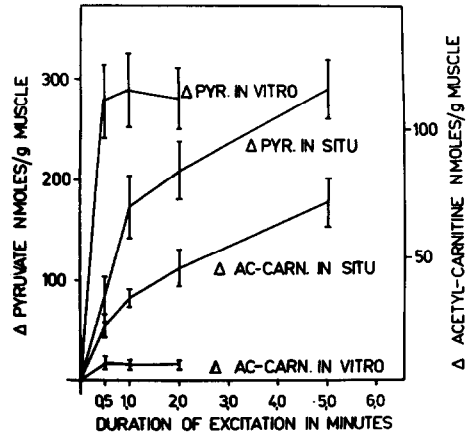

Fig.2. Pyruvate and acetylcarnitine formed during the 'in situ' and 'in vitro' stimulation of gastrocnemius muscle. Results are expressed as concentration in the stimulated muscle minus concentration in the control muscle. There was a significant difference between in vitro and in situ concentrations of both substrates at each time. ($P < 0.05$). Five animals were studied. Means ± SEM.

content of the carnitine pool (in situ experiments, fig.1A). This was the same in both the curare-treated and the untreated animals. Only the data of treated animals are shown for the reasons given in Materials and methods. No such effect could be observed in vitro. Neither the isometric nor the isotonic contractions resulted in changes of the respective metabolites which was probably due to the unefficient oxygen supply. Our data concerning lactate estimations seem to confirm this assumption (fig.1B).

The origin of acetylcarnitine formed under stimulation in the 'in situ' experiments is not clear. Under 'in vitro' stimulation more pyruvate is formed than 'in situ' (fig.2).

The patterns of the pyruvate and acetyl-carnitine curves observed 'in situ' suggest that the acetylcarnitine may have been formed from pyruvate under these conditions and therefore may be of carbohydrate origin.

Our in situ experiments show that the acetyl-content of the carnitine pool in the frog gastrocnemius muscle increases during contraction suggesting an 'acetyl-buffering' role for carnitine and CAT in this

carbohydrate oxydizing organ. This observation is in accordance with the data of Childress et al. [14] who studied the role of carnitine in the flight-muscle of the β-oxydation-deficient *Phormia regina*.

It would be interesting to compare the effect of contraction on the carnitine metabolism in different types of ('white' and 'red') skeletal muscle.

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