EFFECT OF DL-CARNITINE ON TISSUE CONTENT OF ADENOSINE TRIPHOSPHATE AND CREATINE PHOSPHATE IN INSCHAEMIC MYOCARDIAL TISSUE

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ABSTRACT

For rhythmic myocardial contraction, high energy phosphates are supplied via efficient biochemical pathways of oxidative metabolism of substrates including carbohydrates, proteins and fats. In this study induction of ischaemia (which causes a reduction in oxygen supply and /or substrate delivery) was shown to ultimately reduce the formation of the high energy phosphates (adenosine triphosphate – ATP – and creatine phosphate- CP-). The ATP values were reduced to 7.2 ± 2.7μmols/g dry weight and and CP values to 6.9 ± 1.5 μmols/g dry weight for in ischaemic myocardial tissue. This contrasts with control values of 16.2 ± 5.2μmols /g dry weight for ATP and 11.7± 2.5 μmols/g dry weight for CP in normal (non ischaemic) myocardial tissue. Continuous perfusion of the hearts with perfusate containing 4.9μmols/ml DL-Carnitine appeared to prevent the lowering of ATP and CP levels as a result of induction of ischaemia. The values (14.4S±2.5μmols/g dry weight for ATP and 13.1±4.0μmols/g dry weight for CP) are significantly ($P<0.05$) higher than corresponding values for ischaemic myocardial tissue not per fused with DL-Carnitine (ATP 7.2±2.7μ mols/g dry weight and CP 6.9±1.5μ mols/g dry weight). These observations indicate that DL-Carnitine prevents the depletion (and conserves to near normal values) the content of ATP and CP in ischaemic myocardial tissue. This finding is of potential value in the management of ischaemic heart diseases.

Key words: DL- carnitine, ATP, CP myocardial inschaemia

INTRODUCTION

The heart is relatively poorly per fused considering the percentage of cardiac output (4%) it receives in the form of coronary blood flow and its overall work load. The myocardium is said to be ischaemic when its oxygen demand is not correspondingly met by coronary supply of oxygen (coronary blood supply). This often results in metabolic disorders in which essentially there is a change from aerobic to anaerobic metabolism leading the accumulation of lactic acid which together with other by-products of metabolism account for the chest pain in ischaemic heart disease – IHD – (Katz, 1967; Wolffendbuttel et al, 1993).

Myocardial oxygen extraction or removal from coronary blood is nearly maximal at rest, therefore there is little reserve even at rest to meet any increased oxygen demand.

Furthermore, the oxygen content of blood cannot be significantly increased under normal atmospheric conditions (Katzung and Chatterjee, 1998). Thus increased myocardial demands for oxygen in the normal heart are usually met by augmenting coronary blood flow –CBF- (Tomlinson et al, 1992; Katzung and Chatterjee 1998).

Carnitine (formally called vitamin B_1) is a natural constituent of striated muscles, liver and in relatively small quantities in the heart (Gibbs, 1978).
Its role as a facilitator of the transfer of fatty acids from cytosol into mitochondrial matrix (i.e. across the two mitochondrial membranes) is today a well established fact (Bremer, 1968; Gieville and Tubbs, 1968; and Bressler, 1970).

The objective of this work is to investigate the possible effect of exogenously supplied DL-Carnitine on the content of the energy-rich molecules ATP and CP in ischaemic myocardial tissue.

METHOD

Male adult rabbits (585-613g) obtained from the animal house of the Department of Pharmacology, De Mont fort University, England; were used in this study. The animals were administered 500 I.U. heparin prior to sacrifice to prevent blood coagulation. Immediately after sacrifice, the thorax was quickly opened to expose the heart, which was then removed by excising just below the point of bifurcation of the carotid artery and the aorta. The heart was immediately immersed in pre-cooled (3-4^{0}C) Ringer–Langendorf heart solution, squeezing gently to rid the chambers of any blood.

The hearts were then canulated and fastened carefully to avoid any damage to the aortic value. A constant pre-determined perfusion pressure height of 93 cm was maintained throughout experimentation.

After stabilization of heart contractions, the hearts were made ischaemic by introduction into coronary vasculatures sephadex micro spheres (4mg in 0.5ml of Ringer – Langendrof perfusate). Contractions were recorded for 30 minutes or more after which the hearts were quickly de-mounted into liquid nitrogen using metal tongues (pre –cooled for 20 minutes in liquid nitrogen).

The procedure was repeated but with the Ringer – Langendrof perfusion fluid containing 4.9 mg/ml DL-Carnitine.

All hearts were saved in a biofreezer for ATP and CP extraction and subsequent quantitative analysis.

ATP And CP Extraction.

This essentially involved homogenization of a sample of frozen left ventricular tissue and subsequent extraction of ATP and CP contained in the myocardium using excess 0.6N perchloric acid (HC104).

For each heart, a set of nine test tubes divided into three series A, B and C and labelled A1, A2, A3, B1, B2, B3 and C1, C2, C3, were used. To test tubes in series A 3 mls of 0.6N perchloric acid was added. Each test tube plus content was then weighed and immersed in ice at least one hour before the start of homogenization.

The heart to be analyzed for ATP and CP was quickly transferred into the metal crusher piece of equipment, also kept cold by addition of a volume of liquid nitrogen. Using a hammer several blows were employed to homogenize the heart tissue which was placed in between the two halves of the metal crusher. With the crushing metal back in liquid nitrogen a spatula (pre-cooled in liquid nitrogen) was used to properly mix the homogenized tissue and a sample removed and added to the $HC10_4$ in test tubes A1,A2, and A3. The contents were than thoroughly mixed and re-weighed. A sample of the heart was also transferred into pre-dried (to constant weight) crucibles for tissue dry weight determination.

The test tubes A1, A2, and A3 immersed in ice were transferred to a cold room where calculated amounts of 0.6N $HC10_4$ were further added to give a $HC10_4$: tissue weight ratio of 4:1 and allowed 20 minutes extraction time. Contents of each test tube were then centrifuged at 3000 revolutions per minute (rpm) for 15 minutes and then re-mixed and re-centrifuged (3000 rpm) for a further 15 minutes.

2 mls of the supernatant were transferred to corresponding test tubes in the B series. To each supernatant in B series (A1 intoB1; A2 intoB2; and A3 intoB3). 10ml of methyl orange indicator was added and the excess per chlorate neutralized with a measured volume of potassium hydroxide. The precipitate was sedimented by centrifuging for 15 minutes at 3000rpm.

Again the yellowish supernatant from B series test tubes were transferred to the corresponding test tubes in the C series (B1 into C1; B2 into C2; B3 into C3). Contents of these test tubes were frozen and saved for ATP and CP quantitative analysis.

B. Assay for ATP and CP

This was done using the Perkin – Elmer UV-VIS spectrophotometer (No 550-5) at wavelength 340nm with a tungsten lamp light source.

Into each cuvettes 1ml of double distilled water was added, followed by 1ml of 0.2M TRIS buffer (pH 7.5).

100 micro-liters of 1M magnesium chloride solution were then added followed by 100 micro-liters of 1.0M (in TRIS buffer) nicotinamide adenine dinucleotide phosphate (NADP).

To test cuvettes, 100 micro-liters of appropriate extract (previously allowed to melt immersed in ice) was added while 100µL of double distilled water was added to the blank cuvettes. 10micro-litres of 0.5M glucose was added to all cuvettes and volumes made up to 3mls by addition of calculated volumes of 0.2m TRIS buffer, and contents thoroughly mixed by disposable cuvette stirrer.

For each of these cuvettes, four (4) spectrophotometric readings were obtained as follows:

Reading I: For this reading, 10micro-litres of glucose-6- phosphate dehydrogenase (G6PDH) in 3.2M ammonium sulphate solution were added to blank, standard and test cuvettes and contents stirred immediately. Reaction was

allows to proceed to completion (7 minutes) and contents were stirred once more before reading 1 was taken.

Reading II: For this reading 10 micro liters of Hexose Kinase (HK) in 3.2M ammonium sulphate was added to blank standard and test. Contents was stirred and reaction allows to proceed to completion (7 minutes) and stirred again before reading II was taken.

Reading III: For this reading 50 micro-litres of 10nM adenosine diphosphate (ADP) in TRIS buffer was added to blank, standard and test cuvettes. Contents were stirred before reading III was taken.

Reading IV: For this reading 10 micro-litres of creatine kinase –CK- (6 mg CK/0.3 mls of TRIS buffer solution) were added to test, standard CP and blank cuvettes. Contents were mixed and reaction allows to proceed to completion (25 minutes). Contents were again stirred just before reading IV was taken.

The differences between readings II and I for standard ATPcuvettes were plotted against the amount of ATPpreviously added to obtain a standard ATP curve. Similarly the difference between readings IV and III were plotted against initial amount of CP added to obtain the standard CP curve. The tissue concentration of ATP and CP in test cuvettes are obtained by reading-off from standard ATP and CP curves, the values that correspond to the differences between readings II and I ; and IV and III. Since the volume of the extract used and the dry weight of the heart tissue from which ATP and CP extraction was done, the tissue concentration of ATP and CP can be calculated (expressed in micro-mols/g tissue dry weight.

RESULTS

The results below show tissue content of ATP and CP expressed per unit dry weight of myocardinal tissue.

	ATP µmols/gm dry weight	CP µmols/gm dry weight
i. Control hearts (n = 5)	16.2 ± 5.2	11.7 ± 2.5
ii. Control Ischaemic hearts (n = 5)	7.2 ± 2.7	6.9 ± 1.5
iii. Ischaemic hearts per fused with DL-Carnitine (n = 5)	14.4 ± 2.5	13.1 ± 4.0

DISCUSSION

The principle of cellular metabolism leading to the generation and utilization of energy is similar in all mammalian tissues the myocardium inclusive. However under resting conditions, the heart muscle uses mainly (70%) fatty acids for energy generation during rhythmic contractions in preference to carbohydrates. On the other hand just as it is the case with other tissues, in anaerobic or ischaemic conditions, myocardial metabolism employs anaerobic glycolytic mechanisms for energy production. The energy so generated is in the form if molecules containing high energy bonds for example ATP and CP.

In this work, the normal control hearts (non ischaemic) could be perceived to be operating under "non-stressful" or resting conditions. Introduction of ischaemia using sephadex micro spheres had the effect of reducing coronary flow to the myocardium resulting in decreased supply of oxygen and substrates necessary for energy generation. (Katz, 1976; Gibbs, 1978; Gotto and Farmer, 1988; Chatterjee, 1990;). As mentioned earlier, carbohydrates become a major substrate for the production or generation of energy rich molecules in ischaemic conditions (Rozanski and Berman, 1987), reduction in carbohydrate nutrient supply to myocardium (in this work by experimental ischaemia) expectedly resulted in a substantial decrease in ATP (from 16.2$\pm$5.2 to7.2$\pm$2.7μ mols/g dry weight) and CP (from 11.7$\pm$2.5 to 6.9$\pm$1.5μ mols/g dry weight).

Carnitine has been shown to facilitate entry of substrates particularly fatty acids into mitochondrial matrix where most oxidative metabolic reactions take place. These reactions result in the generation of ATP and CP (Fritz et al, 1962; Pearson and Tubbs 1967; and Brasman and Fritz 1971). In this work the perfusion of ischaemized hearts with DL-Carnitine either restored to near normal or prevented the depletion of ATP and CP (ATP and CP values for DL-Carnitine per fused ischaemic hearts are almost equal values for non-ischaemic hearts in the table above). This observation is in agreement with that of earlier authors Fritz et al, 1962; Pearson and Tubbs, 1967).

It can therefore be concluded the DL-Cardnitine by yet unknown mechanism/s prevent the depletion of myocardial tissue content of ATP and CP by experimental ischaemia.

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