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Energy metabolism of cardiac cell cultures during oxygen deprivation: effects of creatine and arachidonic acid

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In recent years, many studies have shown that oxygen deficiency in heart results in a decline of ATP production and cessation of contractile activity [1, 2].

Myocardial cell cultures are a useful model for studying myocardial nucleotide metabolism during oxygen deprivation. In the myocyte, such deprivation induces modifications of cell membranes, cytoplasmic enzyme leakage and lysosomal damage [3–5]; ATP, derived from glycolytic pathway, might play an important role in membrane maintenance [6]. The aim of the present study was to determine whether creatine or arachidonic acid could reduce the energy deficiency due to oxygen and glucose deprivation in cardiac cell cultures.

As regards creatine, it is now well documented that the creatine–PCr–mitochondrial creatine kinase (CK, EC 2.7.3.2) system, in heart cells, serves for regulatory function in the coupling of energy production (mitochondrial site) to energy utilization (myofibrillar site) [7, 8]. Creatine, added to the culture medium, was already shown to induce, in normoxic conditions, an increase in phosphocreatine (PCr) [9] and in mitochondrial CK [10] in myocardial cells. Thereby, it was of interest to investigate if creatine might induce, in normoxic conditions, an increase in contractile activity simultaneous with an increase in PCr synthesis in myocardial cells and, in oxygen deprived conditions, might protect cells against the fall of PCr synthesis already observed in ischemic whole heart [2].

On the other hand, arachidonic acid, as a component of membrane phospholipids, has been suggested to be implicated in membrane integrity and fluidity [1]. Moreover, we showed previously, that non esterified arachidonic acid disappeared from the myocyte when oxygen and glucose deprived cells stopped beating [11]. Consequently, it was suggested that arachidonic acid could protect cellular membrane, thereby reduce enzyme leakage and maintain intracellular energy content.

Materials and methods

Cultures of heart cells. Cultures of cardiac cells were prepared from 3 day-old rats (Sprague–Dawley), according to the method of Harary and Farley [12] with some modifications [13]. Cells were grown in Eagle's minimum essential medium with 10% calf serum and antibiotics.

Experimental procedure. On day 8, just before the beginning of the experiment, glucose concentration in the culture medium was controlled to be near 0 by glucose-oxydase method (Biochemica-Test Combination, Boehringer, Mannheim). Thereafter, half of the flasks were supplemented with glucose to obtain final concentration of 10^{-3} M. Each of the two groups, with or without glucose, was divided into two parts, one of which was gassed with air 95% + CO_2 5%, the other with nitrogen 95% + CO_2 5%, for 55 min at 37°. Air-gassed cultures had average pO_2 values of 117 ± 6 mm Hg and oxygen deprived cultures had pO_2 values of 25 ± 2 mm Hg; pO_2 remained at this level for the 3 h experiment. In air- as well as in nitrogen(N_2)-gassed cultures, we obtained thereby four groups: air or N_2 without glucose, air or N_2 with glucose, air or N_2 with treatment, air or N_2 with glucose and treatment. Creatine (5.10^{-3} M) was added to the treated cultures once a day for 8 days, the latter addition being made just before gassing. Arachidonic acid was dissolved, under N_2, in Na_2CO_3 solution (10^{-1} M); 12.5 μl of the solution 10^{-3} M (i.e. 1‰ of the volume of medium) was added to each culture flask to give a final concentration of 10^{-6} M. Treatment was applied once in control and oxygen deprived cultures just after gassing.

Beating rate was measured at 37° under an inverted microscope at a magnification of 300 in 5 randomly selected microscopic fields for each culture flask, at least 3 times before starting the experiment, then every half hour. The results were expressed as percentages of the mean pre-hypoxic values.

Biochemical assays were performed in control and oxygen deprived cultures 3 hr after the beginning of the gassing. Proteins were determined by Lowry's method [14]. ATP and PCr were measured in acid soluble extracts of the cells as described by Seraydarian *et al.* [15].

As regards statistics, the data were submitted to variance analysis according to Snedecor [16]. The results were expressed as M ± S.E.M.

Results

Beating activity (Fig. 1). In normoxic cells, the beating activity was maintained at 100% throughout the experiment. Oxygen deprivation induced a decrease of beating frequencies, this decrease being different according to glucose concentration. In 8-day-old cultures, the glucose of medium being nearly exhausted, the cells stopped beating within 1 hr of oxygen deprivation. If the medium was supplemented with 10^{-3} M glucose just before gassing, the beating activity of the oxygen deprived cells exhibited a retarded decline, the beating frequencies slowing down within 3 hr, from 38 to 72% of prehypoxic values. The cells grown in 5.10^{-3} M creatine (Fig. 1A) did not exhibit higher beating frequencies than did controls, throughout 8 days in culture. Arachidonic acid (Fig. 1B) did not modify beating frequencies of normoxic cultures. In oxygen-deprived cultures, creatine (5.10^{-3} M) or arachidonic acid (10^{-6} M) did not prevent the beating from stopping or, in the presence of glucose, from slowing down.

ATP and CP levels (Fig. 2 and 3). Under normoxic conditions, glucose (10^{-3} M) had no effect on the levels of ATP and PCr. In oxygen deprived cultures, ATP and PCr levels were depressed when compared with controls ($P < 0.001$); glucose maintained ATP and PCr levels as the controls.

In control cultures, the intracellular concentration of PCr increased in response to 5.10^{-3} M creatine ($P < 0.001$) (Fig. 2); no change in ATP concentration was observed. Glucose

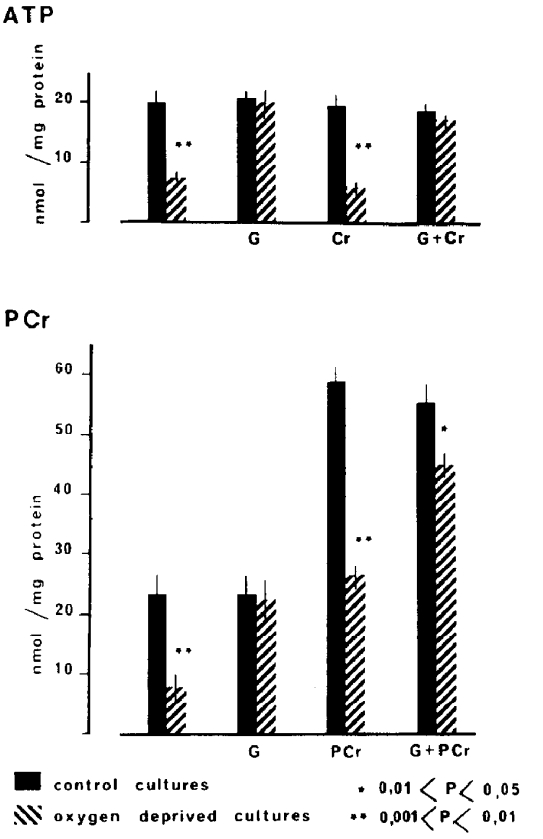

Fig. 2. Effect of creatine (Cr, 5.10^{-3} M), in the presence of glucose (G, 10^{-3} M) or not, on ATP and PCr levels in control and oxygen deprived cultures.

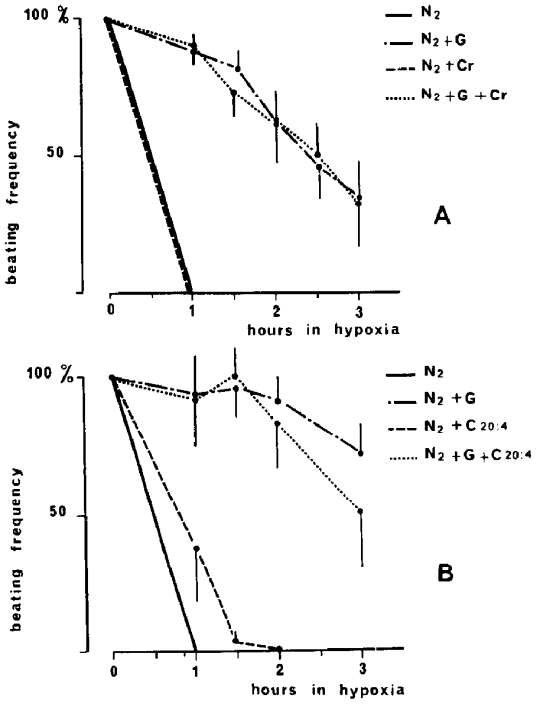

Fig. 1. Beating frequencies of oxygen deprived heart cells in culture, in the presence of glucose (G, 10^{-3} M) or not: (A) effect of creatine (Cr, 5.10^{-3} M); (B) effect of arachidonic acid (C20: 4, 10^{-6} M).

(10^{-3} M) did not modify these results. In creatine treated cultures, oxygen deprivation induced a significant fall of PCr ($P < 0.001$); however, the values obtained were of the same order as control values without creatine. In the presence of glucose and creatine, oxygen deprivation induced but a slight fall of PCr values ($0.01 < P < 0.05$).

In normoxic cultures, arachidonic acid (10^{-6} M) did not affect ATP and PCr levels (Fig. 3). In oxygen deprived conditions, arachidonic acid tended to reduce ATP decrease and maintained PCr at its normoxic value; glucose did not modify the effects of arachidonic acid.

Discussion

Most of the studies concerned with ischemia are focused on the metabolic changes in the whole heart; the deleterious effects of ischemia are characterized by an early fall of high energy phosphates content. Only few studies [6, 17, 18] refer to energy metabolism of myocardial cell cultures in oxygen deprived conditions.

Our data confirm that energy synthesis, in oxygen deprived cultures, is mainly dependent on extracellular glucose; in this regard glycolytic activity and intracellular ATP were shown, in anoxic heart cell cultures, inversely correlated with CK release [18]. In our experimental conditions, ATP, generated in the glycolytic pathway, was sufficient to support partially the beating activity for 3 hr; the remanent level of β-oxidation, present in oxygen deprived cultures, supplied only the minimal energy required for the survival of the cells [11].

In normoxic muscle cells, it was already demonstrated that creatine stimulates oxidative phosphorylation [19]; its subsequent phosphorylation to PCr is catalyzed by mitochondrial CK coupled to adenine nucleotide translocase

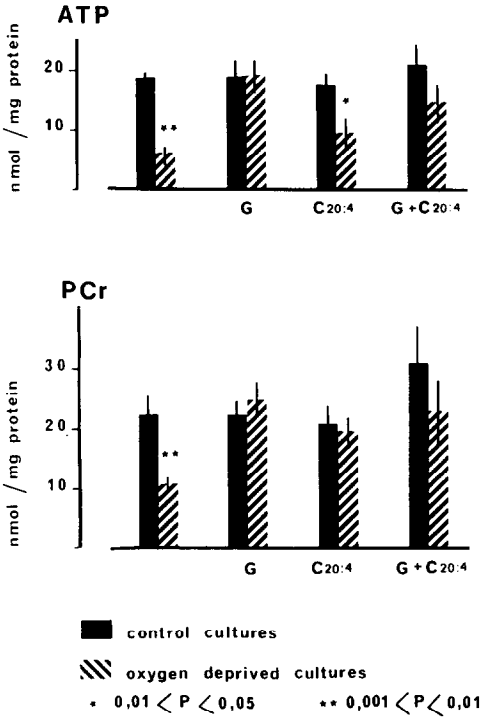

Fig. 3. Effect of arachidonic acid (C20: 4, 10^{-6} M), in the presence of glucose (G, 10^{-3} M) or not, on ATP and PCr levels in control and oxygen deprived cultures.

[8]. Consequently, creatine, when added to the culture medium, induced a new steady state of cellular energy owing to increase in PCr synthesis. These results are in agreement with Seraydarian's data in cardiac and skeletal muscle cells of the rat [7].

In oxygen-deprived conditions, it appeared that creatine partially preserved mitochondrial CK activity, maintaining a high level of PCr. However, low ATP content and arrest of beating activity suggested a possible impairment of myofibrillar CK activity in spite of creatine addition. According to observations of CK release by heart cell in ischemia-simulating conditions [17], a preferential leakage of myofibrillar CK isozyme has also to be considered.

Unlike creatine, arachidonic acid did not modify energy metabolism of heart cell in normoxic conditions. Nevertheless, arachidonic acid, preventing the PCr decrease in oxygen deprived cultures, had an effect comparable with creatine. It also reduced ATP decrease; however, ATP content remained too low to support the beating activity. In a previous work [11], a fall of non-esterified arachidonic acid was shown concomitant with the loss of beating function. These results support the hypothesis that this unsaturated fatty acid, owing to its insertion in membrane phospholipids (work in hand), would preserve membrane integrity and reduce enzyme leakage (as CK isozymes). According to Katz *et al.* [20], low concentrations of fatty acids stabilize cell membrane and increase its resistance against deleterious factors. Another hypothesis could be envisaged: the mechanism responsible for arachidonic protective effect could be related to the prostaglandin synthesis. It is now clear that prostaglandin synthesis is not limited to the vascular elements of the heart. Cultured myocardial cells synthesize various prostaglandins [21, 22]; this synthesis is reduced from 50% in oxygen deprived cultures [22]. As for creatine, additional investigations have to be performed for a better comprehension of arachidonic acid effect in oxygen deprived cardiac cells.

In summary, creatine and arachidonic acid protected partially against the fall of high energy phosphate content in oxygen deprived heart cells: yet this protective effect did not seem sufficient to maintain the beating activity of the cells as glucose did.

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