

# Inhibitory effect of pyruvate on release of glutathione and swelling of rat heart mitochondria

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## Abstract

Pyruvate prevents the permeability transition of rat heart mitochondria induced by the system calcium ions + phosphate or by the dithiol reagent phenylarsenoxide and measured as swelling. Since swelling induced by the latter is relieved by the dithiol 2,3-dimercaptopropanol (BAL), it is inferred that the effect of pyruvate might be mediated by the reduction of lipoic acid. In isolated mitochondria, pyruvate also exerts a protective effect when calcium + phosphate-induced swelling is exacerbated by hypoxic conditions. These results agree with our previous observations that pyruvate markedly prevents the loss of cytosolic and mitochondrial glutathione after ischemia or ischemia followed by reperfusion.

**Keywords:** Myocardial ischemia; Reperfusion; Permeability transition; Pyruvate; Sulfhydryl groups

## 1. Introduction

Ischemia or hypoxia followed by reperfusion causes a large loss of both cytosolic and mitochondrial glutathione from isolated and perfused rat heart [1–8]. Pyruvate markedly prevents the cytosolic and mitochondrial loss either after ischemia or after ischemia followed by reperfusion [8] and, in addition, its presence instead of glucose determines a large recovery of contractile activity [3,8].

The intracellular concentration of calcium and phosphate increases in ischemic conditions [9,10] and both agents are well known to determine, in isolated mitochondria, a large amplitude swelling [11]. The latter depends on an increased permeability of the inner membrane also known as “permeability transition” and possibly dependent on the opening of an unselective pore stimulated by  $\text{Ca}^{2+}$  accumulation and several “inducing agents” [11]. It was also recently reported that the mitochondrial pore might be involved in reperfusion injury [12]. A mitochondria

dria permeability transition is also apparent in the presence of the dithiol reacting agent phenylarsenoxide [13]. The effect of pyruvate was tested on rat heart mitochondria subjected to permeability transition elicited by both  $\text{Ca}^{2+}/\text{Pi}$  and phenylarsenoxide, and in both cases it was observed that pyruvate exerts a protective action.

## 2. Materials and methods

For the preparation of mitochondria, rat hearts were homogenized with Ultra Turrax in 10 ml of 0.180 M KCl, 5 g/l BSA, 3 mM EDTA, 1 mM EGTA buffered with 5 mM Hepes/10 mM Tris (pH 7.4). Mitochondria were prepared according to Lindenmayer et al. [14]. Protein was determined with the biuret method [15]. Glutathione was essentially measured with the procedure of Tietze [16]. Mitochondrial swelling was estimated spectrophotometrically by following the decrease of absorbance at 540 nm [17]. The spectral data obtained from the various experiments and generated via the spectrophotometer software were stored and converted to the ASCII format. The data pairs format was used to transfer the data to a numerical analysis and graphics software. The data were therefore utilized for averaging the various curves of swelling which are the mean of 4 to 7 experiments obtained with 5 sampling points/min. The error bars, indicating the s.d., were taken every 2 min.

Abbreviations: BAL, British Anti-Lewisite (2,3-dimercaptopropanol); Cl-CCP, carbonyl cyanide-*m*-chlorophenylhydrazide; EGTA, ethyleneglycol bis( $\beta$ -aminoethylether) N,N'-tetraacetic acid; Hepes, N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid; Tris, tris (hydroxymethyl)-aminoethane; Pi, inorganic phosphate

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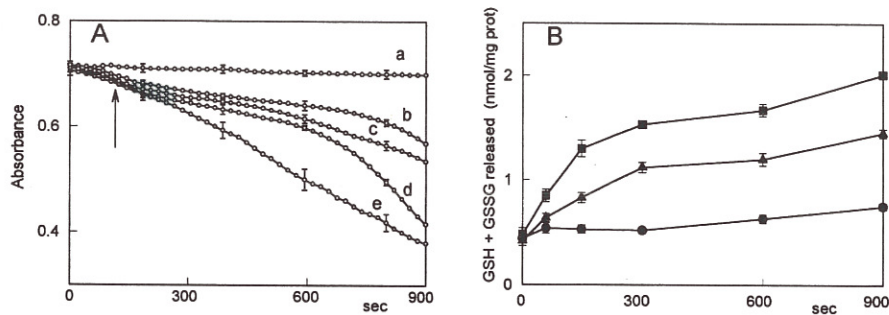


Fig. 1. Correlation between mitochondrial swelling and glutathione release induced by  $\text{Ca}^{2+}$ /Pi in rat heart mitochondria. Mitochondria (1 mg/ml) were incubated at 20°C in 215 mM mannitol, 71 mM sucrose, 3 mM Hepes (pH 7.4), 5 mM succinate, rotenone (0.25 μg/mg protein). Swelling (A) and glutathione release (B) were initiated by the addition of 50 μM  $\text{Ca}^{2+}$  and 2 mM phosphate. Other additions:  $\text{Ca}^{2+}$  and Pi omitted (a, ●); 5 mM pyruvate (b, ▲); 3 μM CI-CCP + 5 mM pyruvate (c); control (d, ■); 3 μM CI-CCP (e). CI-CCP was added at the arrow. The reported results are the average of 7 experiments.

### 3. Results

In the presence of  $\text{Ca}^{2+}$  and Pi rat heart mitochondria undergo a large amplitude swelling (Fig. 1A) accompanied by a marked release of glutathione (Fig. 1B). The two events appear to be strictly correlated; in addition, all released glutathione is found as such in the medium, indicating that no oxidation occurred (not shown). In the presence of pyruvate, both swelling and glutathione release are partially prevented (Fig. 1). This effect of pyruvate does not appear to be energy-dependent since it still occurs in the presence of an uncoupling agent (Fig. 1A); the latter, on the other hand, stimulates the swelling induced by  $\text{Ca}^{2+}$ /Pi (Fig. 1A).

In hypoxic conditions mitochondrial functions appear to be markedly altered and characterized by a decline of the respiratory control ratio and ATP levels [18–20] together with a decrease of  $\beta$ -oxidative activity [19]. In the pres-

ence of relatively high concentrations of  $\text{Ca}^{2+}$  and phosphate, deprivation of oxygen is a further source of damage; therefore the experimental conditions utilized with isolated mitochondria are qualitatively similar to those induced in the isolated and perfused rat heart model [1–3]. As shown in Fig. 2, mitochondria, in the presence of  $\text{Ca}^{2+}$  and Pi, and incubated under nitrogen, undergo swelling more rapidly than normoxic mitochondria (compare b and d of Fig. 2); moreover, the presence of pyruvate reduces the swelling of both normoxic and hypoxic mitochondria (Fig. 2a and c).

Swelling can also be induced by the dithiol reagent phenylarsenoxide (Fig. 3). In this case, swelling occurs in the presence of EGTA and is therefore independent of calcium ions [13]. This type of swelling is almost completely inhibited by BAL (Fig. 3c), added a few minutes after the addition of phenylarsenoxide, again indicating the involvement of dithiol groups and the reversibility of this interaction. Also in this case the protection elicited by

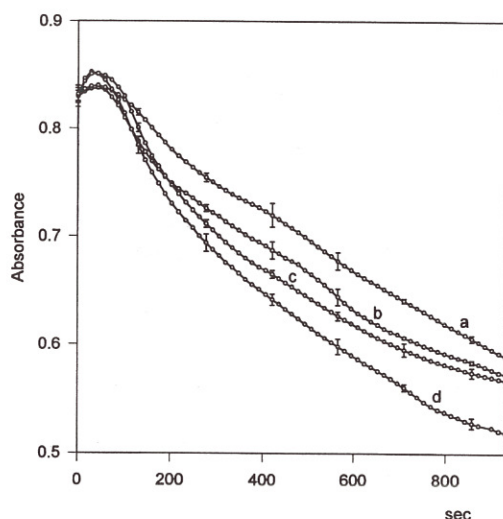


Fig. 2. Effect of pyruvate on hypoxic mitochondria. Rat heart mitochondria (0.25 mg/ml) were preincubated at 25°C with (a,c) and without (b,d) pyruvate (5 mM) in 213 mM mannitol/71 mM sucrose buffered with 5 mM Hepes/Tris (pH 7.4). Afterwards they were transferred (time 0) to spectrophotometric cuvettes containing 5 mM succinate, rotenone (10 μg/mg protein), oligomycin (12 μg/mg protein), 22 μM EGTA, 3 mM K-Pi and 50 μM  $\text{CaCl}_2$  and in the presence of 5 mM pyruvate (a,c) and under nitrogen (c,d). The reported results are the average of 4 experiments.

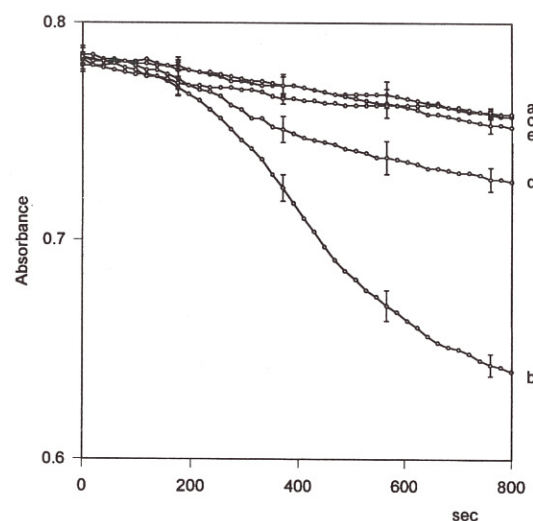


Fig. 3. Effect of pyruvate on phenylarsenoxide-induced swelling. Mitochondria (0.25 mg/ml) were incubated at 20°C in 215 mM mannitol, 71 mM sucrose, 3 mM Hepes (pH 7.4), rotenone (1 μg/mg protein). Swelling was initiated by the addition of 10 μM phenylarsenoxide. Other additions: (a) phenylarsenoxide omitted; (b) control; (c) 0.17 mM BAL; (d) 5 mM pyruvate; (e) 5 mM pyruvate + 0.17 mM BAL. The reported results are the average of 7 experiments.



pyruvate is observed (Fig. 3d) and is stronger than that occurring with  $\text{Ca}^{2+}$  and phosphate.

#### 4. Discussion

Mitochondria are involved in the ultrastructural changes appearing in myocardial cells as a consequence of oxygen deprivation; they, in fact, undergo swelling and loss of matrix density [21] together with the appearance of intramitochondrial granules of calcium phosphate [22]. As previously reported, in isolated mitochondria,  $\text{Ca}^{2+}$  and phosphate determine a large amplitude swelling [11]. As a consequence, matrix content is lost and essential metabolites such as pyridine nucleotides or Coenzyme A may leak out of the matrix [23]. Similarly, glutathione leaks out of the mitochondrion leaving the system unprotected against oxidative injury since mitochondrial glutathione appears to act mostly against oxidative stress [24] and cellular glutathione deficiency is associated with major alterations of mitochondria. From the results reported in this paper it appears that pyruvate protects from both  $\text{Ca}^{2+}$ /Pi-induced swelling and glutathione loss (Fig. 1A and B).

Previous research has demonstrated that, similarly to the organ on the whole, also isolated mitochondria undergo damage in hypoxic conditions [18–20]. As reported in Fig. 2, mitochondria incubated with  $\text{Ca}^{2+}$ /Pi and in hypoxic conditions undergo swelling more rapidly than normoxic mitochondria. The presence of pyruvate slightly reduces the amplitude of swelling of not only normoxic but also hypoxic mitochondria. This result is in agreement with our previous observation that pyruvate strongly protects the isolated/perfused rat heart during ischemia/reperfusion [8].

In order to provide an insight into the mechanism of action of pyruvate, mitochondrial swelling was also induced with phenylarsenoxide that stimulates a mitochondrial swelling independent of the presence of  $\text{Ca}^{2+}$  ion [13]. Also in this case pyruvate is strongly protective (Fig. 3). Several other thiol groups reagents are known to induce mitochondrial swelling, while low molecular weight thiols appear to act as preventive agents [11]. The phenylarsenoxide-induced swelling is relieved by the dithiol agent BAL, indicating that BAL and pyruvate might act through a common mechanism. In fact pyruvate, through the pyruvate dehydrogenase complex, reduces lipoic acid to dihydrolipoic acid which might be involved, more or less directly, in the protective action.

The prevention of swelling elicited by pyruvate is more difficult to explain in the case of  $\text{Ca}^{2+}$ /Pi, since a direct involvement of thiol groups is not apparent. Nevertheless, according to Le Quôc [25], after treatment with acetoacetate that causes a massive oxidation of pyridine nucleotides, a modification of a small amount of mitochondrial protein sulfhydryl groups is apparent.

The protective effect of pyruvate on isolated and perfused rat heart is multifactorial, and different modes of action appear to be operative, i.e., direct scavenging of hydrogen peroxide [26,27], preservation of pyruvate dehydrogenase from the action of free radicals (derived from hydrogen peroxide) [28] or prevention of inhibition of the

phosphorylated form [29] and decrease of intracellular phosphate [30]. In fact, in isolated-perfused heart phosphocreatine increases when pyruvate replaces glucose as perfusing substrate. Finally, pyruvate, through aminotransferase reaction, can provide malate which is transformed to fumarate and then to succinate therefore giving rise to an anaerobic mitochondrial production of ATP that might have an important role in maintaining the viability of tissues exposed to anoxia [31].

In conclusion, the preventive action of pyruvate against  $\text{Ca}^{2+}$ /Pi or phenylarsenoxide-induced swelling observed in the isolated mitochondria could be included among the different protective mechanisms observed in ischemia/reperfusion; the potential reducing capacity of pyruvate towards protein disulfides should also be taken into account.

From preliminary experiments it has been observed that the effect elicited by pyruvate is shared by other pyridine nucleotide-dependent substrates and particularly by glutamate and isocitrate.

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