CHANGES IN BICARBONATE STIMULATION OF RAT LIVER MITOCHONDRIAL ATPase UNDER DIFFERENT METABOLIC SITUATIONS

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1. Introduction

Reports from different laboratories have clearly established that mitochondrial ATPase preparations from ox heart [1], rat liver [2], rat liver submitochondrial particles [3] and purified mitochondrial ATPase (F_1), [3–5] are stimulated by bicarbonate. Ebel and Lardy [3] and Pedersen [6] have suggested the existence of an anion-binding site different from other substrate site or sites for Mg–ATP. Pedersen [6] has also shown that bicarbonate stimulation may be abolished by mercurial reagents, suggesting thus an implication of sulfhydryl groups in the bicarbonate binding. In a previous report from our laboratory [7] it was shown that physical training modified rat liver mitochondrial ATPase in such a way that bicarbonate stimulation of its activity was markedly decreased.

The present paper shows that with ATPase of isolated mitochondria from rats subjected to a variety of metabolic conditions, such as fasting, cold exposure, or diabetes, the bicarbonate activation effect may be either greatly decreased, or even completely abolished, without affecting the activity of the enzyme in the absence of this anion. The loss of bicarbonate sensitivity correlated well with an increase in plasma free fatty acids. It has also been observed that mitochondrial ATPase lacking bicarbonate sensitivity gradually recovered it, when mitochondrial suspensions were kept in 0.25 M sucrose, at 10°C, reaching the values of those of ATPase present in mitochondria obtained from control rats.

2. Materials and methods

Liver mitochondria were isolated from male Wistar rats weighing approximately 200 g following the method of Hogeboom [8].

Protein was determined by the method of Lowry et al. [9]. ATPase activity was determined essentially as described by Pullman et al. [10] in the absence of an ATP-generating system. Aliquots of the mitochondrial suspension (50–100 μg protein) were preincubated for 5 min at 30°C in 0.8 ml medium containing 50 μmol Tris-acetate, pH 7.4 and 3 μmol $MgCl_2$. When present, the amount of sodium bicarbonate was 10 μmol. The reaction was initiated by the addition of 6 μmol sodium ATP, pH 7.4, dissolved in 0.2 ml distilled water. The incubation was continued for 10 min and was stopped by the addition of 0.1 ml 50% trichloroacetic acid. Inorganic phosphorus was determined according to Fiske and Subbarow [11]. Reagent and enzyme blanks were determined in each experiment. It was always verified that under those conditions doubling the amount of mitochondrial protein the amount of P_i liberated also doubled and never exceeded 0.75 μmol in each determination. Plasma free fatty acids and glucose were determined respectively by techniques previously reported in the literature [12,13].

Rats were made diabetic by intraperitoneal injection of alloxan (0.2 mg/kg body wt). Animals were killed at the third day after injection. When indicated 10 U insulin were injected intraperitoneally 25 min before killing. Rats exposed to cold temperature were kept at 5°C under the same illumination conditions as those of the controls. No appreciable differences in food intake were observed when compared to controls.

3. Results and discussion

No appreciable changes in basal ATPase activity were observed in mitochondria isolated from animals subjected to different metabolic conditions. However, stimulation of ATPase activity by bicarbonate exhibited by mitochondria from control animals was affected to a greater or lesser extent depending on the metabolic situation of the animals (see table 1). Bicarbonate stimulation of mitochondrial ATPase in rats, fasted for periods of 14 h, 24 h, 48 h and 72 h, decreased from values of 110% to values as low as 20%, dependent on the time the animals were fasted. A similar effect was observed when rats having free access to food were exposed to cold temperature (5°C) for periods of 24 h or 48 h. Bicarbonate stimulation of ATPase in those cases was only 55% and 22%, respectively. Diabetic rats, after alloxan injection, showed also a lower stimulation of ATPase by bicarbonate. ATPase was stimulated by only 30%. Twenty minutes after an injection of insulin to diabetic rats, stimulation was partly recovered and values of 60% were obtained.

As shown in fig.1, there was an inverse relationship between the decrease in stimulation by bicarbonate of mitochondrial ATPase and the levels of plasma free fatty acids. Within the range of fatty acid concentrations of 0.2–0.8 mequiv./litre found in controls mitochondrial ATPase could be stimulated by bicarbonate up to 110%. Under conditions, such as fasting, diabetes, or cold exposure, an elevation of plasma free fatty acids took place together with a loss in the stimulation by bicarbonate.

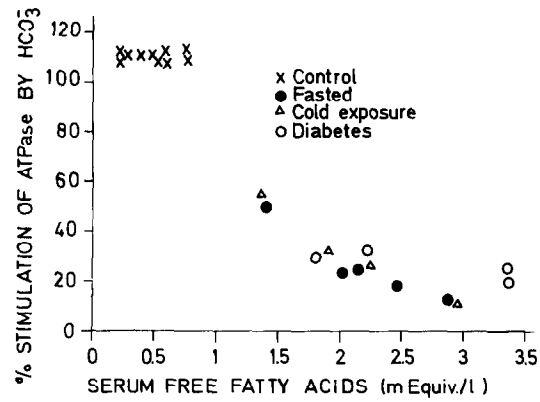

Fig.1. Relationship between serum free fatty acids and stimulation of liver mitochondrial ATPase from rats under a variety of metabolic situations.

Table 1
ATPase activity and bicarbonate stimulation in liver mitochondria from rats subjected to different metabolic situations

Treatment	No. expts	ATPase activity (μmol ATP hydrolyzed/mg protein . min)	
		Mg^{2+}	$Mg^{2+} + HCO_3^-$
Controls	40	0.62 ± 0.05	1.34 ± 0.11
14 h Fasted	10	0.62 ± 0.06	1.30 ± 0.09
24 h Fasted	5	0.60 ± 0.05	0.99 ± 0.06
48 h Fasted	7	0.63 ± 0.05	0.93 ± 0.07
72 h Fasted	8	0.64 ± 0.06	0.74 ± 0.05
24 h Cold exposure	4	0.62 ± 0.05	0.95 ± 0.07
48 h Cold exposure	5	0.63 ± 0.05	0.76 ± 0.06
Diabetic rats	5	0.60 ± 0.05	0.78 ± 0.05
Diabetic rats plus insulin	4	0.61 ± 0.06	0.96 ± 0.06

Final concentration of Na HCO_3 was 10 mM. 5×10^{-3} M Mg^{2+} concentration was present in all cases. Blood glucose in controls, 90 ± 10 mg%. Diabetic rats 550 ± 50 mg%

Table 2
Effect of oleic acid infusion on the stimulation of mitochondrial ATPase activity by bicarbonate

Infusion	No. expts	Plasma free fatty acids (mequiv./litre)	% Stimulation ATPase by HCO_3^-
None	3	0.5 ± 0.3	115 ± 5
Albumin	4	1.8 ± 0.8	85 ± 9
Albumin + 2 μl oleic acid	2	3.0 ± 1.0	50 ± 8
Albumin + 5 μl oleic acid	2	4.5 ± 1.0	10 ± 5
Albumin + 10 μl oleic acid	3	5	0
Albumin + 15 μl oleic acid	2	5	0

Rats were anesthesized by a subcutaneous injection of 12.5% urethane (1 ml/100 g wt). Jugular vein was canulated and 1 ml 10% albumin containing oleic acid in the indicated amounts was injected for 20 min. Emulsion of oleic acid in albumin were prepared by 10 s sonication immediately before use. Animals were killed by decapitation and blood samples collected for fatty acid determinations

Oleic acid was also infused in groups of rats, and the levels of serum free fatty acids determined as well as the percent stimulation of liver mitochondrial ATPase by bicarbonate. As shown in table 2 a rise in the circulating fatty acids was accompanied by a decrease in stimulation of ATPase activity by bicarbonate.

In preliminary experiments carried out 'in vitro' a large decrease in bicarbonate sensitivity of ATPase was also observed in mitochondria isolated from a total rat liver homogenate previously incubated in the presence of albumin-bound oleate.

It was also observed that mitochondria with ATPase having a low sensitivity to bicarbonate stimulation gradually recovered that sensitivity, and values close to those of the mitochondria from control rats could be reached. Figure 2 shows the percent stimulation of ATPase of mitochondria kept at 10°C after different intervals of time. Although a slight decrease in stimulation was found in the controls, an increase in stimulation of ATPase activity by bicarbonate took place in mitochondria with low sensitivity, obtained from animals subjected to 72 h fast, or 48 h cold exposure, or with alloxan diabetes.

The fact that the sensitivity to bicarbonate stimulation of mitochondrial ATPase was affected by a variety of metabolic situations, all of them having in common changes in plasma free fatty acid levels, may very well be the expression of a possible regulatory control.

Pedersen [14] has recently shown that bicarbonate affects the kinetics of ATP hydrolysis catalyzed by

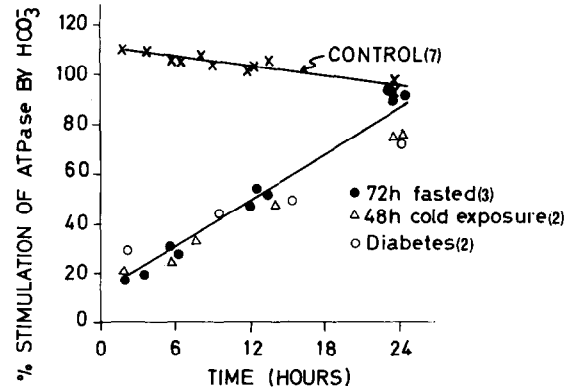

Fig.2. Recovery of sensitivity to bicarbonate stimulation of ATPase in mitochondria isolated from livers of rats subjected to various metabolic situations. Mitochondria from livers of rats maintained under the specified conditions were isolated [8] and kept at 10°C in 0.25 M sucrose, without any added substrates. Percent stimulation of ATPase activity by bicarbonate was determined independently for each mitochondrial suspension in aliquots taken after different intervals of time. The number of separate mitochondrial suspensions studied has been indicated in parentheses.

mitochondrial ATPase, whereas other reactions, such as ATP-dependent transhydrogenase and ATP–P_i exchange, which are believed to be catalyzed by the same enzyme complex remain unaffected in the presence of this anion. These findings suggested that the bicarbonate effect would be rather specific for ATP hydrolysis. If this reaction actually takes place

within the cell it might play some role in the regulation of intramitochondrial ATP levels.

In metabolic situations such as fasting or diabetes a release of fatty acids from fat depots takes place together with an elevated production of ketone bodies by the liver. During prolonged cold exposure an elevation of plasma free fatty acid levels also occurs probably due to an increased excretion of catecholamines. In all these cases we have found a decrease or even loss in the sensitivity of mitochondrial ATPase to bicarbonate stimulation. This could be interpreted as a way to minimize ATP hydrolysis in the mitochondria. In agreement with this suggestion would be the recent finding of Siess and Wieland [15] who have demonstrated an increase of the ATP/ADP ratio in experiments on isolated hepatocytes incubated in the presence of albumin-bound oleate. Under these conditions the flux of the Krebs cycle would slow down through an inhibition of citrate synthetase and of isocitrate dehydrogenase. This situation would favor the utilization of oxaloacetate for gluconeogenesis, and of acetyl CoA for the synthesis of ketone bodies.

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