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Effect of amrinone on myocardial mitochondria function

Valentina Bobyleva-Guarriero, Cristina Mussini, U. Muscatello, C. Cappello* and G. Mattioli*

Institute of General Pathology and **Chair of Cardiology, University of Modena, Italy*

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The effect of amrinone on cardiac mitochondria of guinea pig was studied. It was found that amrinone does not change the respiratory function of cardiac mitochondria in the presence of α-ketoglutarate, whereas it inhibits glutamate oxidation. It was also found that amrinone strongly inhibits the activity of glutamic dehydrogenase of both crude extract from sonicated heart mitochondria and of purified preparation from bovine liver. This inhibition may explain the effect of amrinone on the oxidation of glutamate in mitochondria. These results are discussed in view of the contradictory effects of amrinone on cardiac and other tissues.

Amrinone; Cardiac mitochondria; Glutamate dehydrogenase

1. INTRODUCTION

Amrinone (5-amino[3,4'-bipirydin]-6(1H)-one; WIN 40680) is a nonglycosilic, noncatecholamine-dependent, inotropic vasodilator agent which has been proposed for the treatment of patients with severe heart failure [1]. However, some criticism has been raised as to its efficacy as a harmless drug since its positive effects are paralleled by significant toxic effects on cardiac and other tissues [2].

Apart from the problem as to its suitability in medical practice, amrinone has been shown to possess a positive inotropic effect both in vivo and in vitro [1]. However, the site of its effect on cardiac muscle contractility remains unknown. It therefore seems of some interest to investigate whether amrinone has any effect on the mechanism of energy supply. In the present work the effect of amrinone on the respiratory activity and the phosphorylative efficiency of mitochondria isolated from guinea pig heart were studied.

2. MATERIALS AND METHODS

Male albino guinea pigs of Morini strain weighing 400–500 g were used. The animals were maintained on the standard diet and water ad libitum. Mitochondria were prepared following the method of Palmer et al. [3]. Only intermyofibrillar mitochondria were used. The respiratory states studied were those defined in [4]. The respiratory rates and the phorphorylative efficiency were measured as described by Guarriero-Bobyleva et al. [5]. The activity of glutamic dehydrogenase (EC 1.4.1.2) was determined as in [6]. Aspartate aminotransferase (EC 2.6.1.1) was determined as indicated by Schwartz and Bodansky [7]. Protein concentration was measured as in [8]. Purified preparation of glutamate dehydrogenase of bovine liver was purchased from Boehringer Mannheim GmbH, that of aspartate aminotransferase was from Sigma. Amrinone was from Winthrop-Breon (USA).

Correspondence address: V. Bobyleva-Guarriero, Institute of General Pathology, via Campi, 287, 41100 Modena, Italy

3. RESULTS

It appears from table 1 that the oxidation of glutamate is strongly inhibited upon addition of amrinone in isolated cardiac mitochondria. Both the respiratory rate in state 3, that is in the presence of all limiting factors [4], and the respiratory control are inhibited. The phosphorylative capacity is almost lost. These effects also persist when the mitochondria, preincubated with amrinone, are then washed in saline (results not given) indicating that amrinone is irreversibly bound to mitochondrial structure. Table 1 also shows that amrinone has little if any effect on the respiratory control and ADP/O ratio when α-ketoglutarate is used as the substrate. The respiratory rate is slightly increased in the presence of this drug.

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Table 1

Effect of amrinone on respiration rate, phosphorylative efficiency and respiratory control of isolated guinea pig heart mitochondria

Substrate (2 mM)	Respiration rate in state 3		ADP/O		Respiratory control	
	Control	+ amrinone	Control	+ amrinone	Control	+ amrinone
Glutamate	132 ± 25.0	31.1 ± 6.4*	2.82 ± 0.5	1.20 ± 0.2	17.6 ± 7.9	3.8 ± 0.8
α-KG	88.7 ± 2.6	107 ± 7.6**	2.80 ± 0.2	2.78 ± 0.4	11.4 ± 6.4	9.8 ± 3.4

The respiration rate in state 3 [4] is expressed as ng atoms of oxygen per mg of protein/min. The respiratory control is the ratio of respiration rate in state 3 to respiration rate in state 4 [4]. Mitochondria were prepared from guinea pig heart. Mitochondria were incubated under the condition indicated in section 2. Protein concentration varied from 0.7 to 0.8 mg per ml of incubation medium. When added, the concentration of amrinone was 0.08 mg per ml of incubation medium. Values represent the mean ± SD of 3-4 experiments. * $p<0.01$ with respect to control; ** $p<0.02$ with respect to control

Since the oxidation of glutamate requires the conversion of this substrate to α-ketoglutarate via the glutamate dehydrogenase pathway or via aspartate aminotransferase activity, the effect of amrinone on both enzyme activities was tested. Fig.1 shows that amrinone strongly inhibits the glutamic dehydrogenase activity both in crude extract from guinea pig heart mitochondria (fig.1A) and in purified enzyme preparation from bovine liver (fig.1B). The inhibition by amrinone is much higher in liver preparation than in heart preparation (cf. fig.1A and B). Amrinone also has an inhibitory effect on aspartate aminotransferase activity. However, the quantitative evaluation of this effect requires further investigation.

4. DISCUSSION

The results reported in the present paper show that one of the possible sites of action of amrinone is at the level of the enzyme glutamic dehydrogenase. In fact, the activity of this enzyme is strongly inhibited both in crude extract from isolated cardiac mitochondria and in purified bovine liver preparation by the addition of amrinone. This may provide an explanation for the drastic reduction of glutamate oxidation observed in isolated guinea pig heart mitochondria upon addition of amrinone. In fact, glutamic dehydrogenase has been shown to be active in mitochondria isolated from guinea pig heart [9],

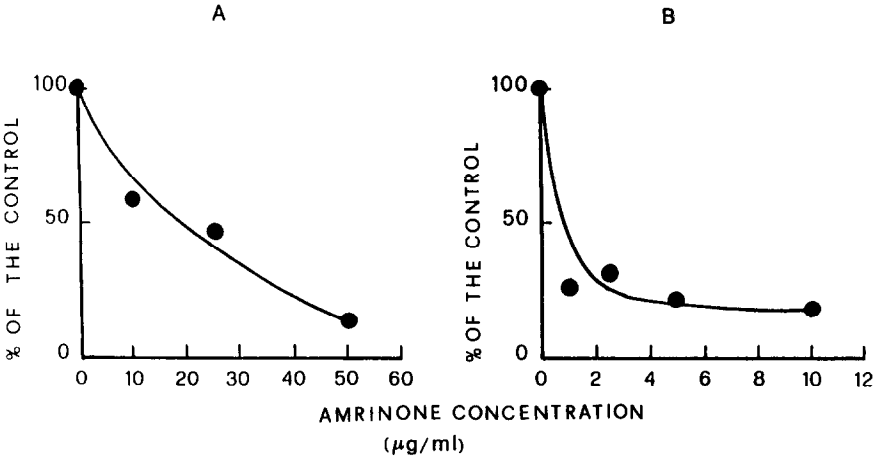

Fig.1. Effect of amrinone on the activity of glutamate dehydrogenase. The enzyme activity was measured as indicated in [6], except that NADH was substituted by NADPH. Crude extract of enzyme was prepared by sonication and centrifugation of suspension of intermyofibrillar mitochondria. The purified bovine liver enzyme was from Boehringer Mannheim (127701) and was diluted 20 times before use. (A) crude extract of myocardial enzyme; (B) purified bovine liver enzyme. Note that the scales of amrinone concentration are different for A and B.

although at a much lower rate as compared with that seen in liver mitochondria [10]. The consequence of this inhibitory effect on the energy metabolism at the cell level, may well be contradictory. In fact, the inhibition of glutamic dehydrogenase reduces the transformation of α-ketoglutarate into glutamate, thus increasing the supply of α-ketoglutarate as an oxidable substrate for the Krebs cycle. The slight increase of respiration seen with α-ketoglutarate in the presence of amrinone adds support to this conclusion. On the other hand, the inhibition of this enzyme may result in negative effects on the overall activity of the energy metabolism. In fact, the activity of the glutamic dehydrogenase has been suggested to play a role for catalytic regulation of Krebs cycle intermediates and thus of the rate of aerobic energy production [9]. Furthermore, the activity of this enzyme is essential for the removal of ammonia in conditions such as ischemia, characterized by increased concentration of this substance [11,12]. An inhibition of this enzyme may be deleterious under this condition: in fact, a high level of ammonia may have negative effects on activities of some enzymes of Krebs cycle and on the intramitochondrial concentration of pyridine nucleotides [13]. Therefore, it is possible that many of the negative collateral effects, observed during therapy with amrinone [2] are due to its inhibitory effect on glutamate dehydrogenase of tissues other than cardiac tissue.

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