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ATPase Activity, IF_1 Content, and Proton Conductivity of ESMP from Control and Ischemic Slow and Fast Heart-Rate Hearts[1]

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Earlier studies by Rouslin and coworkers showed that, during myocardial ischemia in slow heart-rate species which include rabbits and all larger mammals examined including humans, there is an IF_1-mediated inhibition of the mitochondrial ATPase due to an increase in the amount of IF_1 bound to the ATPase (Rouslin, W., and Pullman, M.E., *J. Mol. Cell. Cardiol.* **19,** 661–668, 1987). Earlier work by Guerrieri and colleagues demonstrated that IF_1 binding to bovine heart ESMP was accompanied by parallel decreases in ATPase activity and in passive proton conduction (Guerrieri, F., *et al., FEBS Lett.* **213,** 67–72, 1987). In the present study rabbit was used as the slow heart-rate species and rat as the fast heart-rate species. Rat is a fast heart-rate species that contains too little IF_1 to down regulate the ATPase activity present. Mitochondria were prepared from control and ischemic hearts and ESMP were made from aliquots by sonication at pH 8.0 with 2 mM EDTA. Oligomycin-sensitive ATPase activity and IF_1 content were measured in SMP prepared from the control and ischemic mitochondrial samples. After identical incubation procedures, oligomycin-sensitive ATPase activity, oligomycin-sensitive proton conductivity, and IF_1 content were also measured in ESMP samples. The study was undertaken to corroborate further what appear to be fundamental differences in ATPase regulation between slow and fast heart-rate mammalian hearts evident during total myocardial ischemia. Thus, passive proton conductivity was used as an independent measure of these regulatory differences. The results show that, consistent with the low IF_1 content of rat heart cardiac muscle mitochondria, control rat heart ESMP exhibit approximately twice as much passive proton conductivity as control rabbit heart ESMP regardless of the pH of the incubation and assay. Moreover, while total ischemia caused an increase in IF_1 binding and a commensurate decrease in passive proton conductivity in rabbit heart ESMP regardless of pH, neither IF_1 content nor proton conductivity changed significantly in rat heart ESMP as a result of ischemia.

KEY WORDS: Slow and fast heart-rate hearts; control and ischemic hearts; mitochondrial ATPase activity; mitochondrial ATPase inhibitor protein; IF_1; oligomycin-sensitive proton conductivity; rabbit and rat heart ESMP.

INTRODUCTION

The F_1-ATPase inhibitory subunit, IF_1, was first isolated from bovine cardiac muscle mitochondria 32 years ago (Pullman and Monroy, 1963). In so-called slow heart-rate mammalian species which include rabbits and all larger mammals thus far examined including the human (Rouslin, 1987a), the inhibitor binds to the ATPase in cardiac muscle mitochondria, one mole of inhibitor per mole of enzyme (Klein *et al.*, 1980),

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under nonenergizing conditions at low mitochondrial matrix pH such as occurs during myocardial ischemia (Rouslin, 1987a,b; Rouslin and Pullman, 1987). Subsequent mitochondrial energization causes the rapid release of much of the bound IF_1 (Schwertzmann and Pedersen, 1981; Husain and Harris, 1983; Klein and Vignais, 1983; Rouslin, 1987b; Rouslin and Pullman, 1987).

Glycolysis-driven cell acidification (Rouslin, 1983a,b, 1988; Rouslin and Broge, 1989, 1990; Rouslin *et al.*, 1986, 1990) characterizes most systems under ischemia. The lack of oxygen presumably also produces a gradual mitochondrial inner membrane deenergization resulting, in turn, in a relaxed, IF_1-receptive conformation of the F_0F_1-ATPase to which the inhibitor binds (Rouslin, 1987b; Rouslin and Pullman, 1987). The binding of the inhibitor during ischemia serves to diminish a wasteful hydrolysis of glycolytically produced ATP by the undriven mitochondrial ATP synthase (Rouslin, 1988, 1991; Rouslin *et al.*, 1986, 1990). Inhibitor binding thus slows net rates of tissue ATP depletion during ischemia thereby delaying cell injury and death (Rouslin, 1988; 1991; Rouslin *et al.*, 1986, 1990). Inhibitor binding also decreases proton conductivity of the F_0F_1-ATPase (Guerrieri *et al.*, 1987a).

Some years ago we demonstrated that the IF_1-mediated ATPase inhibition just described occurs only in the intact hearts or in the isolated cardiac muscle mitochondria of so-called slow heart-rate mammalian species (Rouslin, 1987a). This was not, however, evident in so-called fast heart-rate mammalian and in avian species that were studied (Rouslin, 1987a). Fast heart-rate mammalian species including the rat, hamster, and mouse contain only approximately 20–30% as much IF_1 per mg of cardiac muscle mitochondrial protein as slow heart-rate mammalian hearts (Rouslin, 1987a, 1988), an amount which is apparently too low to produce significant ATPase down regulation in intact mitochondria under nonenergizing conditions.

Earlier work by Guerrieri and coworkers demonstrated that IF_1 binding to bovine heart ESMP was accompanied by parallel decreases in ATPase activity and in oligomycin-sensitive proton conductivity (Guerrieri *et al.*, 1987a). The present study was undertaken to determine whether or not the changes in IF_1 binding that occur during ischemia in slow heart-rate hearts that underlie the ATPase inhibition observed might also result in changes in oligomycin-sensitive proton conductivity in ESMP prepared from control and ischemic hearts. The present study was also undertaken to determine whether or not ESMP from control mitochondria from different species that naturally possess different amounts of IF_1 in their cardiac muscle mitochondria also exhibit different proton conductivities.

In the present study rabbit was used as a slow heart-rate species that contains a full complement of IF_1, i.e., approximately one mole of IF_1 per mole of ATPase, and that exhibits marked IF_1,-mediated ATPase down regulation during ischemia. In contrast, rat was used as a fast heart-rate species that contains a low level of IF_1 in its cardiac muscle mitochondria, too low to significantly down regulate the ATPase activity present.

MATERIALS AND METHODS

Preparation of Control-Energized and Ischemic Rabbit and Rat Mitochondria; Rabbit and Rat Heart ESMP; Mitochondria and Mitochondrial IF_1-Containing Extracts from Hearts of Rabbit and Rat, and Pure Bovine IF_1

One kg male New Zealand White rabbits and 300 g male Sprague-Dawley rats were anesthetized with sodium pentobarbital (i.v. to effect for rabbits, i.p. to effect for rats) and killed by removal of the heart. The hearts were rapidly removed and placed either into ice-cold 180 mM KCl, 10 mM EGTA[4] (KE solution) (control samples) or incubated for 20 min in sealed Ziplok plastic bags immersed in a circulating water bath set at 37°C as described earlier (Rouslin, 1983a,b, 1987a,b) (ischemic samples).

The hearts were then minced finely in ice-cold 180 mM KCl, 10 mM EGTA, 0.5% bovine serum albumin, 10 mM MOPS-^{4}KOH, pH 7.2 (KEAM solution) and mitochondria were prepared from the cardiac muscle minces by Polytron homogenization as described earlier (Rouslin, 1983a,b, 1987a,b). For all experiments utilizing control-energized mitochondria, the mitochondria were energized by shaking them vigorously for 10 min at 37°C in 0.25 M sucrose, 1 mM EGTA, 20 mM MOPS-KOH, pH 7.2 (SEM solution), with 6.25 mM glutamate, 6.25 mM malate, and 2.5

[4] Abbreviations used: EGTA, [ethylenebis(oxyethylenenitrilo)]tetraacetic acid; MOPS, 3-(*N*-morpholino)propanesulfonic acid; MES, 2-(*N*-morpholino)ethanesulfonic acid; SMP, submitochondrial particles; ESMP, EDTA-treated submitochondrial particles; DCCD, dicyclohexylcarbodiimide.

Table I. ATPase Inhibitor Content, Maximal (Energized) Oligomycin-Sensitive ATPase Specific Activity, and Ratios of the Two in Rabbit and Rat Heart Mitochondria[a]

Species	IF_1 content (I.U.[b]/mg)	ATPase specific activity[c]	IF_1/F_1 ratio[d]
Rabbit	3.70 ± 0.17	3.32 ± 0.24	1.12 ± 0.04
Rat	0.56 ± 0.04	2.78 ± 0.48	0.20 ± 0.03

[a] All data are averages ± SEM of four separate determinations.
[b] One I.U. (inhibitory unit) is that amount of inhibitor which fully inhibits one international unit of enzyme activity by 100%, i.e., one μmol ATP hydrolyzed/min.
[c] Oligomycin-sensitive ATPase specific activity expressed as μmol/min/mg.
[d] The activities of the IF_1 and of ATPase are expressed in identical units.

mM Pi. This procedure served to maximally activate the mitochondrial ATPase at the beginning of the experiment before the start of subsequent *in vitro* incubations.

Rabbit and rat heart ESMP[4] (EDTA-treated SMP[4]) were prepared from control and ischemic mitochondrial samples as described by Guerrieri *et al.* (1987b). Briefly, the mitochondria were sonicated at pH 8.0 in the presence of 2 mM EDTA and then centrifuged at 105,00 × *g* for 60 min.

IF_1-containing extracts used for the determination of IF_1 content, as in the experiments presented in Tables I, III, and IV, were prepared by alkaline extraction of intact mitochondria or of ESMP as described earlier (Pullman and Monroy, 1963; Frangione *et al.*, 1981; Rouslin, 1987b, 1988; Rouslin and Pullman, 1987). The pure bovine IF_1 used in the experiments presented in Fig. 3 was prepared according to Pullman (Pullman, 1986).

Table II. Control and 20-min Ischemic Mitochondrial ATPase Specific Activities and Ratios of the Two in Rabbit and Rat Heart SMP[a]

Species	Control ATPase specific activity[b]	20-min ischemic ATPase specific activity	Ischemic to control activity ratio
Rabbit	3.84 ± 0.20	1.32 ± 0.18	0.34 ± 0.03
Rat	3.45 ± 0.05	3.27 ± 0.11	0.95 ± 0.02

[a] All data are averages ± SEM of four separate determinations.
[b] ATPase specific activity is expressed as μmol/min/mg.

Assay Procedures

Mitochondrial ATPase activity was measured in sonicated mitochondria (Tables I and II) or in ESMP (Tables III and IV) using a modification of the method of Tzagoloff *et al.* (1968) as described by us previously (Rouslin, 1983a,b, 1987a,b). Briefly, the 1.0-ml reaction mixture contained approximately 45 μmol Tris-SO_4, pH 7.8, 10 μmol $MgCl_2$, and 50 μl of sonicated mitochondria at 0.5 mg/ml. The reaction was started by the addition of 10 μmol ATP-Tris, run for 5 min at 30°C, and stopped by the addition of 1 ml of 10% trichloroacetic acid followed by the assay of the Pi produced by the reaction. Specific activities were calculated as μmol Pi/mg/min.

The IF_1 content determinations presented in Tables I, III, and IV and in Fig. 1 were carried out as described previously using our rat heart SMP titration procedure (Rouslin, 1987a,b; Rouslin and Pullman, 1987). Briefly, IF_1-containing extracts were incubated with rat heart SMP for 20 min at 37°C at pH 6.2 in a final volume of 2.0 ml. The incubation medium contained 0.25 M sucrose, 0.1 mM MgATP, and 20 mM MES-KOH, pH 6.2. As demonstrated earlier (Rouslin and Pullman, 1987), the rat heart SMP titration procedure employed in the present study produced results when estimating the inhibitor from dog heart which were comparable to an RIA for the dog heart inhibitor. Moreover, a recent analysis of factors affecting functional assays for IF_1 supports the quantitative validity of the assay when applied to either a single species or to different species (Rouslin and Broge, 1994). Since units of ATPase inhibitory activity, i.e., of IF_1 activity, are the same as units of ATPase activity, i.e., μmol/min/mg, ratios of these two activities such as are presented in Table I were calculated directly from the activities of each.

The oligomycin-sensitive proton conductivity of ESMP in the experiments presented in Tables III and IV and in Figs. 2 and 3 was measured potentiometrically by monitoring the anaerobic release of the respiratory proton gradient as described earlier (Pansini *et al.*, 1978). In these experiments, the anaerobic release of the respiratory proton gradient was inhibited 70–80% by either oligomycin or DCCD[4]. It should be mentioned that it had been demonstrated earlier that it was primarily the IF_1-inhibitable portion of the passive proton conductivity of ESMP that is oligomycin sensitive (Guerrieri *et al.*, 1987b; Pansini *et al.*, 1978).

ESMP prepared as described above were incubated at a concentration of 3 mg/ml for 30 min under

Table III. IF_1 Content, Oligomycin-Sensitive Proton Conductivity, and Oligomycin-Sensitive ATPase Activity of ESMP from Control and Ischemic Rabbit (Slow Heart-Rate) and Rat (Fast Heart-Rate) Hearts at pH 8.0[a]

Rabbit			Rat		
IF_1 content (IU/mg)			IF_1 content (IU/mg)		
Control	Ischemic	Increase in IU	Control	Ischemic	Increase in IU
0.48 ± 0.02	1.97[b] ± 0.16	1.50	0.19 ± 0.01	0.21[b] ± 0.02	0.02
Rabbit			Rat		
Proton conductivity ($1/t_{1/2}$)			Proton conductivity ($1/t_{1/2}$)		
Control	Ischemic	% Inhibition	Control	Ischemic	% Inhibition
1.06 ± 0.01	0.82 ± 0.02	22.64	1.82 ± 0.03	1.96 ± 0.06	−0.07
Rabbit			Rat		
ATPase activity (μmoles/min/mg)			ATPase activity (μmoles/min/mg)		
Control	Ischemic	% Inhibition	Control	Ischemic	% Inhibition
5.05 ± 0.09	3.70 ± 0.11	26.73[b]	4.53 ± 0.06	3.83 ± 0.04	15.37[c]

[a] All data in this table are averages ± SEM of four separate determinations.

[b] The procedure used to prepare ESMP (sonication at pH 8.0 in the presence of 2 mM EDTA) as well as the 30-min incubation with succinate employed for the proton conductivity measurements significantly decreased the IF_1 contents of ESMP from ischemic rabbit and rat hearts relative to the IF_1 contents of mitochondria from these tissues (cf. IF_1 content data in Table I). Also, the degree of ATPase inhibition observed in ESMP from ischemic rabbit heart was significantly lower than that in SMP from ischemic rabbit heart (cf. rabbit heart ATPase data in Table II).

Much of the ATPase inhibition (loss) observed in ischemic rat hearts in these experiments was not pH dependent (cf. rat heart ATPase data in Table IV) and appeared to be linked to factors other than IF_1 binding. SMP prepared from ischemic rat hearts exhibited only approximately 5% ATPase inhibition relative to controls (cf. rat heart ATPase data in Table II).

N_2 flux at 25°C in a mixture containing 200 mM sucrose, 10 mM KCl, and 20 mM potassium succinate. After this incubation, proton cycles were induced by the addition of H_2O_2 in the presence of catalase and the rate of passive proton conduction was estimated by determining the $t_{1/2}$ of the anaerobic release of the proton gradient induced by H_2O_2 additions (Pansini *et al.*, 1978). In the experiments presented in Table IV and Fig. 2, the pH of the 30-min incubations was varied as indicated. Protein was estimated by the Lowry procedure (Lowry *et al.*, 1951).

RESULTS

Table I presents the IF_1 contents, ATPase specific activities, and the activity ratios of the two in rabbit and rat heart mitochondria. The data are averages ± SEM of four separate experiments. As reported earlier (Rouslin, 1987a; Rouslin and Broge, 1990, 1993, 1995), rat heart mitochondria contain only approximately 15% as much IF_1 per mg mitochondrial protein as rabbit heart mitochondria. Thus, while the IF_1-to-ATPase activity ratio in rabbit heart is close to unity, that of rat heart is only 0.2. Table II shows the degrees of IF_1-mediated ATPase inhibition produced by 20 min of total ischemia. The data are averages ± SEM of four separate experiments. Again, as reported earlier (Rouslin, 1987a; Rouslin and Broge, 1995), and consistent with the IF_1 content data presented in Table I, rabbit heart exhibited a marked IF_1-mediated ATPase inhibition to 34% of controls whereas rat heart showed only approximately 5% ATPase inhibition.

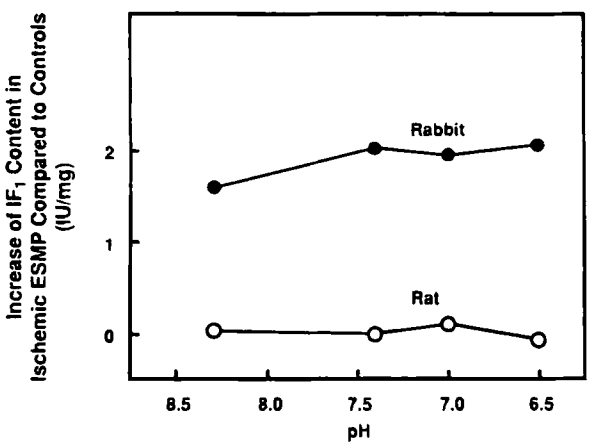

Fig. 1. Effects of 20 min of total ischemia on the IF_1 content of ESMP from rabbit and rat hearts as a function of the pH of incubation. ESMP were prepared by sonication at pH 8.0 in the presence of 2.0 mM EDTA. They were then incubated with succinate for 30 min at 25°C before being recentrifuged and assayed for IF_1 content.

Table III shows the IF_1 content, proton conductivity, and ATPase activity of ESMP prepared from con-

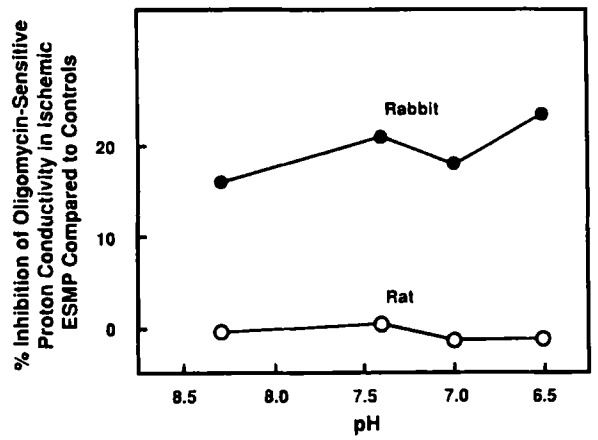

Fig. 2. Effects of 20 min of total ischemia on the oligomycin-sensitive proton conductivity of ESMP from rabbit and rat hearts as a function of the pH of incubation and assay. ESMP were prepared by sonication at pH 8.0 in the presence of 2.0 mM EDTA. They were then incubated with succinate for 30 min at 25°C before being assayed for oligomycin-sensitive proton conductivity.

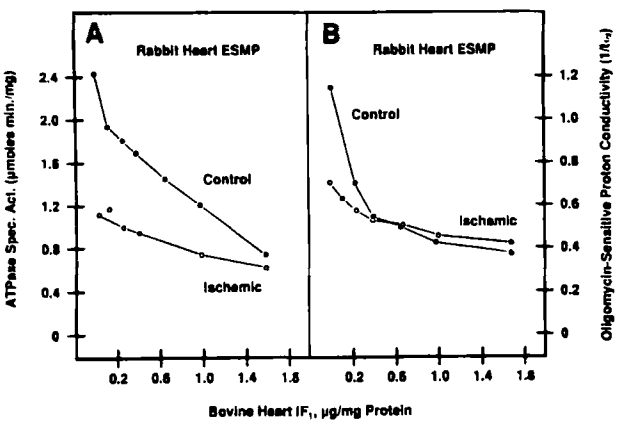

Fig. 3. A. Titration of pure bovine heart IF_1 on control and ischemic rabbit heart ESMP. Pure bovine heart IF_1 was incubated with control and 20-min ischemic rabbit heart ESMP prepared as described in the legends to Figs. 1 and 2 for 20 min at 37°C at pH 6.5. Aliquots of the mixtures were then assayed for ATPase activity (panel A) and oligomycin-sensitive proton conductivity (panel B).

trol and ischemic rabbit and rat heart mitochondria. In these experiments, the ESMP were prepared by sonication at pH 8.0 in the presence of 2 mM EDTA and subsequently incubated with 20 mM succinate at pH 8.0. The data are averages ± SEM of four separate experiments. While 20 min of total ischemia produced a large increase in the IF_1 content of rabbit heart ESMP, it produced essentially no increase in the IF_1 content of rat heart ESMP. Consistent with the changes in IF_1 content, ischemic rabbit heart ESMP showed a significant decrease in oligomycin-sensitive proton conductivity relative to controls, whereas the proton conductivity of ischemic rat heart was essentially unchanged relative to controls. For the proton conductivity determinations presented in Table III, the K_I for oligomycin was 0.10 ± 0.02 and 0.09 ± 0.01 μg oligomycin/mg protein for control and ischemic rabbit heart ESMP, respectively, and 0.07 ± 0.01 and 0.07 ± 0.01 μg/mg protein for control and ischemic rat heart ESMP, respectively (n = 4 for all data).

Table IV shows the effects of the pH of the 30-min ESMP incubation with 20 mM succinate at 25°C on the IF_1 content, proton conductivity, and ATPase activity of ESMP prepared from control and ischemic rabbit and rat heart mitochondria. The increases in IF_1 content and percent inhibition of oligomycin-sensitive proton conductivity due to ischemia in rabbit and rat heart ESMP shown in Table IV are presented graphically in Figs. 1 and 2. It is apparent from these figures that only rabbit heart ESMP exhibited increases in both IF_1 content and proton conductivity inhibition due to ischemia and that both increases were relatively independent of the pH of the ESMP incubation prior to the measurement of proton conductivity.

The experiments presented in Fig. 3 were done to verify that the changes observed in ATPase activity and proton conductivity in the rest of the study were in fact attributable to variations in the IF_1 content of ESMP. In these experiments, pure bovine IF_1 was added in varying amounts to either control or ischemic rabbit heart ESMP. Thus, ESMP at 5 mg/ml were incubated with the amounts of bovine IF_1 indicated in Fig. 3 for 20 min at 25°C at pH 6.5 in a mixture containing 200 mM sucrose, 20 mM KCl, and 20 mM potassium succinate. An aliquot of the mixture was removed for the assay of ATPase activity and the remainder was used for the measurement of passive proton conduction.

As may be seen, control rabbit heart ESMP were able to bind considerably more bovine IF_1 than ischemic particles. This was due to the fact that there were fewer unbound IF_1 binding sites available on the ischemic particles due to the presence of greater amounts of endogenous rabbit heart IF_1 on them (see the IF_1 content data in Tables III and IV). Thus, the addition of increasing amounts of bovine IF_1 inhibited both the ATPase activity and the proton conductivity of control ESMP to a greater extent than of ischemic particles. However, the total inhibition of ATPase activity and proton conductivity due to the presence

Table IV. IF_1 Content, Oligomycin-Sensitive Proton Conductivity and Oligomycin-Sensitive ATPase Activity of ESMP from Control and Ischemic Rabbit (Slow Heart-Rate) and Rat (Fast Heart-Rate) Hearts as a Function of pH

	Rabbit IF_1 content (IU/mg)			Rat IF_1 content (IU/mg)		
pH	Control	Ischemic	Increase	Control	Ischemic	Increase
8.3	0.51	2.09	1.58	0.21	0.25	0.04
7.4	0.57	2.61	2.04	0.19	0.20	0.01
7.0	0.59	2.61	2.02	0.16	0.27	0.11
6.5	0.76	2.85	2.09	0.22	0.18	−0.04
	Rabbit Proton conductivity ($1/t_{1/2}$)			Rat Proton conductivity ($1/t_{1/2}$)		
pH	Control	Ischemic	% Inhibition	Control	Ischemic	% Inhibition
8.3	0.83	0.70	15.7	1.82	1.96	−0.077
7.4	1.39	1.10	20.9	2.86	2.70	+0.056
7.0	0.95	0.78	17.9	2.50	2.86	−0.144
6.5	0.76	0.58	23.7	1.20	1.37	−0.142
	Rabbit ATPase activity (μmoles/min/mg)			Rat ATPase activity (μmoles/min/mg)		
pH	Control	Ischemic	% Inhibition	Control	Ischemic	% Inhibition
8.3	5.34	4.29	19.66[a]	4.38	3.64	16.89[b]
7.4	5.28	3.72	29.54[a]	4.33	3.73	13.86[b]
7.0	5.11	3.61	29.35[a]	4.41	3.69	16.33[b]
6.5	5.03	3.46	31.21[a]	4.27	3.59	15.92[b]

[a] The procedure used to prepare ESMP (sonication at pH 8.0 in the presence of 2 mM EDTA) as well as the 30-min incubation with succinate employed for the proton conductivity measurements significantly decreased the level of ATPase inhibition relative to that observed in SMP from ischemic rabbit heart (cf. rabbit heart ATPase data in Table II).

[b] Much of the ATPase inhibition (loss) observed in ESMP from ischemic rat hearts in these experiments was not pH dependent and appeared to be linked to factors other than IF_1 binding. SMP prepared from ischemic rat hearts exhibited only approximately 5% ATPase inhibition relative to controls (cf. rat heart ATPase data in Table II).

of both added bovine and endogenous rabbit IF_1 was approximately the same.

Figure 4 shows the effect on the proton conductivity of control and ischemic rat ESMP of the addition of pure bovine IF_1 to the particles. These experiments were conducted under the same conditions used in the experiment presented in Fig. 3. The primary difference between the results obtained with rabbit and rat heart ESMP is that, while ischemic rabbit heart ESMP bound considerably less bovine IF_1 than control rabbit particles due to the higher endogenous IF_1 content of the ischemic rabbit heart ESMP (Tables III and IV), ischemic rat heart ESMP bound only slightly less bovine IF_1 than control rat heart particles. The similar results observed with control and ischemic rat heart ESMP with respect to the effect of exogenous IF_1 binding on the proton conductivity of the particles are consistent with the similar and low endogenous IF_1 contents of control and ischemic rat heart ESMP (Tables III and IV).

DISCUSSION

The synthesis of ATP by the mitochondrial ATP synthase is accompanied by the movement of protons from the cytoplasmic side of the inner membrane to the matrix space. Consistent with this, ATP hydrolysis by the reversal of the mitochondrial ATP synthase is accompanied by the movement of protons back through the enzyme in the opposite direction. Thus, during mitochondrial ATP hydrolysis *in situ* such as occurs during myocardial ischemia, protons move through the F_0F_1-ATPase complex from the matrix space into the cytoplasm. A number of agents that inhibit ATP hydrolysis by the mitochondrial ATPase also block proton movement through the enzyme. Thus, while oligomycin and the natural inhibitor subunit, IF_1, each bind to the F_0F_1-ATPase complex at different sites, the binding of each has the dual effect of inhibiting both ATP hydrolysis and proton movement.

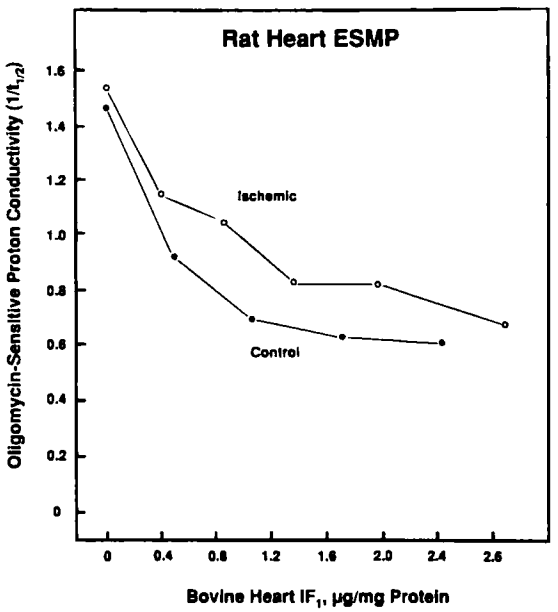

Fig. 4. Titration of pure bovine heart IF_1 on control and ischemic rat heart ESMP. Pure bovine heart IF_1 was incubated with control and 20-min ischemic rat heart ESMP prepared as described in the legends to Figs. 1 and 2 for 20 min at 37°C at pH 6.5. Aliquots of the mixtures were then assayed for oligomycin-sensitive proton conductivity.

Some years ago we demonstrated that the ATPase inhibition which occurs during myocardial ischemia *in situ* or in isolated cardiac muscle mitochondria made acidotic *in vitro* involves the reversible binding of IF_1 to the enzyme (Rouslin, 1987b; Rouslin and Pullman, 1987). This IF_1-mediated ATPase inhibition occurred only in so-called slow heart-rate hearts, but was not evident in so-called fast heart-rate mammalian and avian species that were studied (Rouslin, 1987a). Fast heart-rate mammalian species including the rat, hamster, and mouse contain only approximately 20–30% as much IF_1 per mg of cardiac muscle mitochondrial protein as slow heart-rate mammalian hearts (Rouslin, 1987a, 1988), an amount which is apparently too low to produce significant ATPase down regulation in intact mitochondria under nonenergizing conditions.

Earlier work by Guerrieri and coworkers demonstrated that IF_1 binding to bovine heart ESMP was accompanied by parallel decreases in ATPase activity and in oligomycin-sensitive proton conductivity (Guerrieri *et al.*, 1987a). It thus seemed likely that the changes in IF_1 binding we observed during ischemia in slow heart-rate hearts or the differences we observed in IF_1 content between slow and fast heart-rate cardiac muscle mitochondria would be accompanied by parallel changes or differences in oligomycin-sensitive proton conductivity like those observed earlier by Guerrieri and coworkers. The present study was thus undertaken to determine whether or not the changes in IF_1 binding that occur during ischemia in slow heart-rate hearts that underlie the ATPase inhibition observed might also result in changes in oligomycin-sensitive proton conductivity in ESMP prepared from control and ischemic hearts. The present study was also undertaken to determine whether or not ESMP from control mitochondria from different species that naturally possess different amounts of IF_1 in their cardiac muscle mitochondria might also exhibit different proton conductivities.

In the present study rabbit was used as a slow heart-rate species that contains a full complement of IF_1, i.e., approximately one mole of IF_1 per mole of ATPase (see Table I) and that exhibits marked IF_1-mediated ATPase down regulation during ischemia (see Table II). In contrast, rat was used as a fast heart-rate species that contains a low level of IF_1 in its cardiac muscle mitochondria (Table I), too low to significantly down regulate the ATPase activity present (Table II). As expected, when we compared ESMP from control rabbit and rat hearts, we observed that control rat heart ESMP contained only a fraction of the IF_1 per mg protein present in control rabbit heart ESMP and, consistent with this, they exhibited nearly double the proton conductivity of rabbit heart ESMP (Tables III and IV).

When ESMP from control and ischemic hearts were compared, there were again marked differences observed between the rabbit and rat which were consistent with our earlier observations on differences between the two species with respect to both mitochondrial ATPase inhibition and changes in IF_1 content. Thus, as shown in Table IV and in Figs. 1 and 2, 20 min of total ischemia caused a marked increase in both the IF_1 content and proton conductivity inhibition of rabbit heart ESMP, but produced essentially no change in either parameter in rat heart particles. Thus, as expected, the differences in IF_1-mediated ATPase regulation observed earlier between rabbit and rat hearts were again reflected in differences between these two species with respect to proton conductivity in ESMP.

Within the context of ischemic cardiac muscle, a low pH develops rapidly in the cytosol due largely to an increased rate of glycolytic ATP production and subsequent hydrolysis. This cytosolic acidosis is quickly transmitted to the mitochondrial matrix compartment through the action of the Pi/H^+ symport activ-

ity of the Pi carrier (Rouslin and Broge, 1989). However, the IF_1-mediated blockage of H^+ efflux through the F_0F_1-ATPase complex that takes place in slow heart-rate hearts probably contributes very little to the overall changes in either matrix pH or in delta-pH that occur in the ischemic cells.

Within the context of inherent species differences in mitochondrial inner membrane passive proton conductivity, Brand and coworkers have reported a body mass dependence of proton leak in liver mitochondria in which smaller mammals such as rat and mouse exhibited markedly higher proton leak rates than larger mammals such as rabbit and horse (Porter and Brand, 1993). While other studies by Brand suggest that an animal's thyroid status is an important determinant of mitochondrial proton leak rate and thus of basal metabolic rate (Nobes *et al.*, 1990), the present study suggests that, in fast heart-rate species, low IF_1 content may contribute to mitochondrial passive proton conductivity and thus to basal metabolic rate.

IF_1 presumably binds directly only to the β subunits of the F_0F_1-ATPase complex (Klein *et al.*, 1980). Together with the α subunits, the β subunits make up the bulk of the catalytic headpiece of the complex, most of which lies a rather large distance from the main part of the proton channel, i.e., that residing in the basepiece of the complex (Capaldi *et al.*, 1992, 1994). It may thus be difficult to visualize how a small inhibitor peptide that binds to a catalytic headpiece component can also act as a proton channel gate. The dual effects of IF_1 on both ATPase catalysis and proton conductivity may, however, be understood in terms of a topological model of the F_0F_1-ATPase complex in which F_1 binds to a lower extremity of a β subunit that lies relatively close to the top of the stalk region of the complex. Binding at such a site very low on the headpiece could explain the dual effects of IF_1 binding on both ATPase catalytic and proton conductivity. Alternatively, the binding of IF_1 to any part of a β subunit, even at a site relatively distant from the junction of the headpiece and stalk region, could cause a conformational alteration in the β subunit which, in turn, could alter F_1–F_0 interaction resulting in an inhibition of proton conduction through the enzyme (Guerrieri *et al.*, 1987b). Indeed, this latter alternative is perhaps the more plausible one in view of the long-distance interaction between the head- and basepieces of the ATPase complex suggested by, among other things, the effects of oligomycin and DCCD on both ATPase activity and proton conductivity.

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