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Bicarbonate Stimulation of Mitochondrial ATPase. Effect of Physical Training *

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Bicarbonate stimulation of hepatic mitochondrial ATPase activity decreased in rats subjected to intense physical training and reached minimum values at the end of the third week. The stimulatory effect of bicarbonate on mitochondrial heart ATPase remained unaffected under equal conditions. ATPase stimulation by dinitrophenol and sensitivity to oligomycin, both in mitochondria from rat liver or heart, were not affected by physical training. Results suggest that stimulation by dinitrophenol and bicarbonate might be due to effects on separate sites of the enzyme.

Long ago RACKER (8) made the observation that HCO_3^- stimulated ATPase activity from ox heart mitochondria. Stimulatory effect of bicarbonate on ATPase activity has also been reported for rat liver mitochondria (2), rat liver submitochondrial particles (1) and isolated APTase from rat liver submitochondria (1, 4). EBEL and LARDY (1) have suggested the existence of an anion-binding site different from other substrate site or sites for Mg-ATP.

The effect of physical training on bicarbonate stimulation and on 2,4-dinitrophenol (DNP) stimulation of ATPase activity has been studied in isolated inner mitochondrial membranes both from rat liver and from rat heart. The stimulatory effect of bicarbonate on ATPase from liver decreased in rats subjected to an intense physical training reaching a minimum value at the end of the third week. The stimulatory effect on mitochondrial heart ATPase remained unaffected under the same conditions. The stimulation caused by DNP on mitochondrial ATPase remained unaffected by physical training both in liver and in heart. Oligomycin sensitivity was also unaffected by physical training in mitochondria from either tissue.

The results here reported support the hypothesis of a binding site for bicarbo-

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nate possibly located in a subunit different from that or those where the substrate site or sites are located.

Materials and Methods

Liver or heart mitochondria were isolated from male Wistar rats weighing approximately 200 g following the method of HOGEBOOM (3). Inner mitochondrial membranes were prepared according to the method of PARSONS *et al.* (6) with the modifications already described (9). When indicated, inner membranes (approximately 50 mg of protein) resuspended in 7.5 ml of 10 mM Tris-phosphate buffer, pH 7.5, and after 5 minutes 2.5 ml of a 1.8 M sucrose, 2 mM $MgSO_4$, 2 mM ATP solution added and kept on ice 5 minutes, were sonicated for 25 seconds on ice using an LSE sonifier at 7 amperes and centrifuged at 100,000 *g* for 90 minutes: a pellet and a supernatant were obtained.

Proteins were determined by the method of LOWRY *et al.* (5). ATPase activity was determined at pH 7.5 according to the technique of PULLMAN *et al.* (7).

Physical training was carried out letting the rats freely swim in water at 20° C until exhaustion, once per day. Exhaustion was reached after 30-40 minutes during the first week; after 1 or 2 hours during the second week; and after a maximum of 2 1/2 hours during the third week and thereafter.

Results and dicussion

Table I shows the values of DNP and bicarbonate stimulated ATPase activities in mitochondria and in inner mitochondrial membranes from rat liver and heart. It may be seen that liver ATPase was more sensitive to the stimulation by bicarbonate than heart ATPase, in agreement with previous findings of LAMBETH and LARDY (4).

Figure 1 shows the effect of increasing concentrations of bicarbonate on ATPase activity of inner mitochondrial membranes from rat heart and liver. In both cases a plateau was reached at approximately 25 mM HCO_3^-.

In fractions obtained by sonication of rat liver inner membranes, as described in Materials and Methods, ATPase activity and its sensitibity to bicarbonate were also determined. It may be seen in table II that ATPase activity present in the supernatant was not stimulated by bicarbonate, at difference with the ATPase activ-

Table I. *DNP and bicarbonate stimulated ATPase activities in inner membranes from liver and heart mitochondria.*

Final concentrations of the different compounds, added when indicated, were as follows: DNP, 0.5 mM; $NaHCO_3$, 10 mM; 5×10^{-3} M Mg^{++} was present in all cases. Specific activity (S.A.) of ATPase has been expressed as μmol ATP hydrolized $\times$ mg^{-1} protein $\times$ min^{-1}. Total activities (T.A.) in each fraction have been referred to 100 mg of original mitochondrial protein.

	ATPase activity					
			DNP		HCO_3^-	
	S.A.	T.A.	S.A.	T.A.	S.A	T.A.
Liver						
Mitochondria	0.62±0.11	62	0.75±0.12	75	1.3±0.19	130
Inner membrane	1.02±0.15	51	1.23±0.16	63	2.0±0.24	105
Heart						
Mitochondria	4.1±0.42	410	4.6 ±0.43	460	5.0±0.52	500
Inner membrane	6.6±0.70	330	7.5 ±0.71	380	8.1±0.76	409

Table II. *Rat liver mitochondrial ATPase specific activity and its sensitivity to bicarbonate and oligomycin in subfractions from inner mitochondrial membranes.*
Final concentrations of the different compounds, added when indicated, were as follows: 0.5 mM DNP; 10 mM $NaHCO_3$; 0.5 mM oligomycin. 5×10^{-3} M Mg^{++} was present in all cases. The activity ATPase has been expresed ad μmol ATP hydrolized $\times$ mg^{-1} protein $\times$ min^{-1}. Subfractions from inner membrane were obtained as indicated in Materials and Methods.

Additions	ATPase activity		
	I. Membrane	Pellet	Supernatant
None	1.02±0.15	1.10±0.15	0.40±0.04
DNP	1.23±0.16	1.35±0.17	0.51±0.05
DNP + Oligomycin	0.51±0.06	0.54±0.05	0.50±0.05
HCO_3^-	2.0 ±0.24	2.15±0.26	0.53±0.05
HCO_3^- + Oligomycin	0.72±0.07	0.81±0.09	0.53±0.05

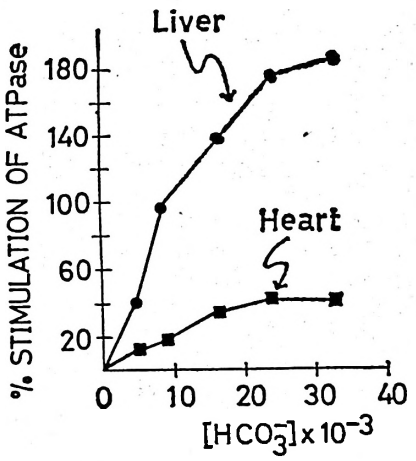

Fig. 1. *Effect of increasing concentration of HCO_3^- on the stimulation of mitochondrial ATPase activity.*
5×10^{-3} M Mg^{++} was present in all cases.

ity present in the pellet. The ATPase activity present in the pellet was oligomycin sensitive, whereas the ATPase activity in the supernatant was completely insensitive (table II). The fact that the ATPase, present in supernatants and pellets after sonication and centrifugation of mitochondrial membranes from control rats behaved differently towards HCO_3^- stimulation, is not completely understood. Since solubilization rendered an ATPase activity unaffected by bicarbonate a tentative interpretation migth be that a subunit with a specific site for bicarbonate has been lost. Other possibility could be that a change in composition could have taken place in the process of solubilization.

Table III shows that oligomycin sensitivity of ATPase from liver stimulated by DNP or bicarbonate was unaffected after three weeks of intense physical training.

Table III. *Physical training and oligomycin sensitivity of liver mitochondrial ATPase stimulated by DNP or bicarbonate.*
Final concentrations of the different compounds, added when indicated, were as follows: 0.5 mM DNP; 10 mM $NaHCO_3$; 5 mM oligomycin. 5×10^{-3} M Mg^{++} was present in all cases. The activity ATPase has been expresed as μmol ATP hydrolized $\times$ mg^{-1} protein $\times$ min^{-1}. Six animals were used for each determination. Physical training was carried out during three weeks as described in Materials and Methods.

Additions	ATPase activity	
	Control	After physical training
None	1.02±0.15	1.02±0.15
DNP	1.23±0.16	1.23±0.16
DNP+Oligomycin	0.51±0.06	0.51±0.06
HCO_3^-	2.0 ±0.19	1.39±0.14
HCO_3^-+Oligomycin	0.72±0.07	0.61±0.06

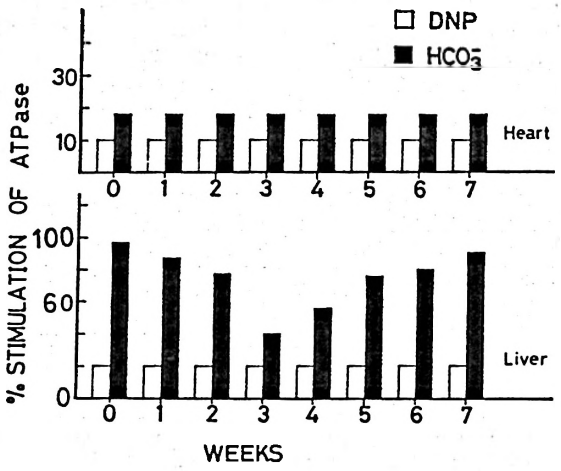

Fig. 2. *Effect of physical training on the stimulation of rat liver and heart mitochondrial ATPase by bicarbonate.*
Final concentrations of the different compounds, added when indicated, were as follows: DNP, 0.5 mM; $NaHCO_3$, 10 mM; 5×10^{-3} M Mg was present in all cases. Six animals were used for each determination. SEM was in all cases within ± 5 %.

Figure 2 shows the decrease in stimulatory effect of HCO_3^- on ATPase activity in isolated rat liver mitochondrial membranes along the period of several weeks of intense physical training. No decrease in this stimulatory effect was observed in ATPase from rat heart. On the other hand stimulation of mitochondrial ATPase activity from rat heart and liver by DNP remained unchanged along the time of the experiment. The values of the control rats remained unchanged during the time of the experiment.

The minimum values were reached at the third week; during the following weeks the stimulatory effect by bicarbonate started to increase approaching the values of the controls around the seventh week of training. This biphasic behavior may indicate some type of adaptation; at present we are unable to give a reasonable interpretation.

The results suggest that stimulations caused by DNP and by bicarbonate on ATPase activity from rat liver inner mitochondrial membrane would be due to effects on separate sites of the enzyme.

The decrease in response of mitochondrial liver ATPase to bicarbonate stimulation my reflect a modification of the enzyme induced during physical training. This modification might play some role in a mechanism of metabolic control. The possible molecular change of the enzyme would not affect neither the site responsible for DNP stimulation nor that conferring oligomycin sensitivity.

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Resumen

La estimulación por bicarbonato de la actividad ATPasa mitocondrial de hígado de rata decrece en ratas sometidas a entrenamiento obteniéndose el valor mínimo al final de la tercera semana. En las mismas condiciones el efecto estimulador de la ATPasa mitocondrial de corazón permanece inalterado. La estimulación de la ATPasa por dinitrofenol y la sensibilidad a la oligomicina de mitocondrias tanto de hígado como de corazón no se afecta por el entrenamiento. Los resultados sugieren que la estimulación por dinitrofenol y por bicarbonato se deben a efectos en lugares distintos del enzima.

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