ACTIVATION OF RESPIRATION OF LIVER MITOCHONDRIA IN VARIOUS METABOLIC STATES BY CYCLIC 3',5'-AMP

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Cyclic 3',5'-AMP (10^{-6} M) activates respiration of the liver mitochondria in all metabolic states and neither changes nor increases the rate of phosphorylation during oxidation of saturating concentrations of isocitrate and succinate. For the effect to be manifested, preincubation of the mitochondria or liver homogenate with cyclic AMP is necessary. The fifth fraction of serum albumin and EDTA do not abolish the effect. Noradrenalin (NA) increases mitochondrial respiration only on incubation with the homogenate. Effects of NA and cyclic AMP do not undergo summation, and the effect of the former is probably mediated by cyclic AMP. The results do not confirm the decisive role of uncoupling of respiration and phosphorylation or accumulation of the oxidation substrate, but instead they suggest activation of mitochondrial enzymes.

KEY WORDS: cyclic 3',5'-AMP; catecholamines; respiration of mitochondria; rat liver.

Recent research has demonstrated the universal control of many types of metabolism by cyclic nucleotides. Against this background, one of the few exceptions is the regulation of oxygen consumption (V_{O_2}). The existing data are very contradictory both for whole cells [6, 10] and for mitochondria [5, 7]; in most cases, moreover, experiments have been carried out with nonphysiological concentrations of cyclic 3',5'-AMP (10^{-2}-10^{-4} M). Meanwhile, administration of cyclic AMP and dibutyryl cyclic AMP in vivo increases the O_2 consumption of the body and reduces pO_2 in the tissues [2]. Incubation of catecholamines and cyclic AMP with homogenates and cyclic AMP with mitochondria activates NAD-isocitrate dehydrogenase of several tissues [3, 8].

Since this enzyme is important for the regulation of the Krebs cycle it was natural to suggest that cyclic AMP may affect the oxygen consumption of the mitochondria. The object of the present investigations was to test this hypothesis and to choose optimal conditions for demonstration of the effect.

EXPERIMENTAL METHOD

Experiments were carried out on 80 noninbred male rats aged 3-4 months. In some cases Wistar rats also were used. Mitochondria from 2 or 3 rats were pooled in each experiment in the series (6-15 experiments in each series). The liver was homogenized in 8 volumes of 0.25 M sucrose, made up in 1 mM EDTA and 10 mM Tris-HCl, pH 7.5. Cyclic AMP and noradrenalin (NA) were incubated with the homogenate or with the isolated mitochondria for 12 min at 28°C, or the substances were added to the polarographic cell. The O_2 consumption was determined polarographically [1] in medium consisting of 0.29 M sucrose, 5.6 mM KCl, 3 mM KH_2PO_4, 10 mM Tris-HCl, pH 7.5. The following respiration rates of the mitochondria were measured: (initial velocity V_0) – in medium with the oxidation substrate; the rates of respiration in Chance's state 3 (V_3) and 4 (V_4): V_3 on the addition of 100 μM ADP and V_4 after utilization of all the ADP; and also V_{DNP} – after addition of 45 μM 2,4-dinitrophenol (DNP). The rate of phosphorylation [ADP]/t also was calculated. The optical density of the suspension of mitochondria was measured at 520 nm on the SF-4A spectrophotometer, [9].

EXPERIMENTAL RESULTS

NA and cyclic AMP in physiological concentration, on incubation with the liver homogenates (Table 1), and cyclic AMP, on incubation with mitochondria (Table 2), increased the O_2 consumption of the mitochondria in all metabolic states. The stimulation not only of free (V_0 and V_4), but also of phosphorylating (V_3) and even of uncoupled (V_{DNP}) respiration shows clearly the hypothesis that uncoupling is the basic and only cause of the calorigenic effect of the catecholamines and cyclic AMP. A decrease in the rate of phosphorylation was

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TABLE 1. Increase in Respiration Rate of Liver Mitochondria of Wistar Rats after Incubation of Homogenates with NA and Cyclic AMP (M ± m). Substrate: 5 mM Succinate

Regulator	Concentration, μM	Increase in respiration rate				Change in [ADP]/t
		ΔV_0	ΔV_3	ΔV_4	ΔV_{DNP}	
NA	1,5	10,5±3,1*	14,1±4,5*	7,7±2,1*	21,5±7,1*	—4,2±3,3
Cyclic AMP	1,0	18,3±7,1*	18,3±7,2*	17,2±8,0	22,2±9,6	+8,6±7,7
NA + cyclic AMP	1,5+1,0	16,7±5,3*	20,9±6,8*	16,3±6,4*	28,2±8,1*	—2,4±7,1

Legend to Tables 1-3: 1) V represents difference between VO_2 in experiment and control. Absolute values of VO_2 in control with 5 mM succinate: $V_0 = 29-50$, $V_3 = 40-68$ nanoatoms O_2/min/mg mitochondrial protein; with 5 mM isocitrate: $V_0 = 11.2$ $V_3 = 22.2$; [ADP]/t expressed in nanomoles ADP/min/mg protein. 2) Values for which $P < 0.05$ compared with control marked by asterisk.

TABLE 2. Increase in Respiration Rate of Liver Mitochondria on Their Incubation with Cyclic AMP (10^{-6}M) (M ± m)

Series	Increase in respiration rate				Change in [ADP]/t
	ΔV_0	ΔV_3	ΔV_4	ΔV_{DNP}	
A					
Isocitrate 5 mM	3,9±1,3*	4,5±1,2*	5,5±0,94*	—	+12,4±6,0
Succinate 5 mM	7,5±1,7*	7,4±3,3*	6,4±1,9δ	10,9±3,7*	+20,4±9,2*
B					
Succinate 5 mM	10,0±3,2*	15,3±5,6*	10,5±2,7*	18,5±3,8*	+20,4±9,0
Succinate 5 mM + BSA 5 mg/ml	9,1±2,4*	27,4±5,2*	12,7±3,1*	—	+11,4±6,9
Succinate 5 mM + 0,4 mm EDTA	15,0±0,9*	17,9±4,5*	12,7±3,9*	23,3±3,3*	—0,8±5,5
Succinate + BSA + EDTA	4,9±1,4*	10,2±2,4*	12,0±3,0*	—	+28,1±9,2*
Succinate 10 mM	11,8±2,1*	19,2±6,6*	22,0±5,2*	10,9±4,5	+1,13±8,7

Legend. A) Noninbred rats, B) Wistar rats, BSA) Bovine serum albumin.

TABLE 3. Respiration of Liver Mitochondria of Noninbred Rats on Addition of Cyclic AMP directly to Polarographic Cell (M ± m). Substrate: 5 mM succinate

Duration of preincubation of mitochondria	V_0 in control	V_0 in various concentrations of cyclic AMP		
		10^{-7} M	10^{-6} M	10^{-5} M
Not incubated	29,5±2,4	26,8±2,5	27,1±3,4	29,7±3,6
5 min	30,1±0,7	30,0±0,9	28,7±0,9	29,6±0,6
12 min	31,4±1,4	31,4±1,8	31,4±1,8	30,0±2,0

never observed, and indeed it never changed or increased (Tables 1 and 2). These facts are in agreement with observations showing an increase (of 21-24%) in the optical density of the suspension of mitochondria isolated from homogenate incubated with NA and (or) cyclic AMP, compared with the incubated control. ATP is known to increase the density of mitochondria [9].

The experiments with binding of potential uncouplers by the mitochondria also provided evidence against the hypothesis of uncoupling of respiration and phosphorylation by cyclic AMP. Neither the fifth fraction of bovine serum albumin, with high affinity for fatty acids, nor EDTA, which binds bivalent ions, nor a combination of both caused any change, as a rule, in the basic effects of cyclic AMP. The values of the respiratory control after Lardy and Chance under these conditions increased both in the control and on incubation with cyclic AMP.

The results likewise do not confirm the other hypothesis of the mechanism of the effect of cyclic AMP on VO_2, namely the accumulation of oxidation substrate. In fact, an effect was observed in the presence of high concentrations of exogenous substrates, corresponding to saturation levels. For instance, with an increase in the succinate concentration to 10 mM the respiration rates showed no significant change in the presence and absence of cyclic AMP (Table 2).

The coincidence of the effects of NA and cyclic AMP and the total absence of summation of the effects of these two regulators (Table 1) are evidence in support of a single mechanism of action, at least in the final

stages. However, there was a significant difference in the action of NA and cyclic AMP: the former was completely inactive on mitochondria (not shown in the Table 1), whereas cyclic AMP clearly activated the O_2 consumption when two different substrates – isocitrate and succinate – were used (Table 2). Cyclic AMP can evidently act directly on these subcellular particles, whereas NA realizes its effect through an extramitochondrial factor, most probably through the adrenergic receptor–adenylate cyclase system, located in the plasma membrane. This agrees fully with the mechanism of activation of isocitrate dehydrogenase under the influence of catecholamines and cyclic AMP [3, 8].

Activation of respiration of the mitochondria by the use not only of isocitrate, but also of succinate, confirms the writers' hypothesis [8] that catecholamines and cyclic AMP can activate not only isocitrate dehydrogenase, but also other mitochondrial enzymes, in this case succinate dehydrogenase.

Unlike in the experiments with preincubation (Table 2), no effect of cyclic AMP was found even in high concentrations (Table 3). The need for preincubation of the mitochondria with cyclic AMP, which was not taken into account in earlier investigations [5], shows that the effect of cyclic AMP takes time for its realization. This is evidence in support of the indirect action of cyclic AMP.

The natural hypothesis that the intermediate factor is swelling of the mitochondria during incubation is opposed by the absence of effect of cyclic AMP when added to the polarographic cell after preincubation of the mitochondria under the same conditions (Table 3).

Cyclic AMP may exert its effect on the mitochondria in two ways: 1) by its action on the outer membrane of the mitochondria through certain sensitive centers (receptor proteins), 2) penetration directly into the mitochondrion and its influence on proteins of the matrix and (or) the inner membrane. Although the permeability of the mitochondria to cyclic AMP is unknown, the second hypothesis appears more likely, for cyclic AMP activates isocitrate dehydrogenase, the enzyme of the matrix. This last fact has also been found to apply also to a solubilizing enzyme isolated from mitochondrial "ghosts" [3].

It is natural to suggest that cyclic AMP exerts its influence on the mitochondria through protein kinase: 1) the enzyme present in mitochondria [11], 2) the specific protein inhibitor of protein kinase abolishes the action of cyclic AMP on isocitrate dehydrogenase [8]. Confirmation of this hypothesis would be very important, for phosphorylation of mitochondrial proteins may not only change the activity of the enzymes, but may also substantially affect the level of the membrane potential, oxidative phosphorylation, and transport processes in the mitochondria.

Activation by cyclic AMP of both NAD-isocitrate dehydrogenase [3, 8] and mitochondrial respiration on various substrates and in all metabolic states (this investigation) shows that the cyclic nucleotides must attract just as much attention in mitochondriology as in other fields of modern biochemistry.

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