It should be noted in conclusion that the establishment of conditions for the preparation of liposomes with sufficiently high latent activity but close to zero free activity is the first step toward the development of transport measures capable of supplying enzymes to the tissues of patients with hereditary diseases.

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ACTIVATION OF RESPIRATION OF LIVER MITOCHONDRIA BY CATECHOLAMINES

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Catecholamines activate respiration (V_{O_2}) of rat liver mitochondria in vivo and in vitro on high concentrations of pyruvate, 2-oxoglutarate, and succinate. The effect is characteristic equally of free oxidation (state 4) and phosphorylating (state 3) or even dinitrophenol-uncoupled V_{O_2}. The addition of EDTA and bovine serum albumin did not abolish the effect. Activation of oxidoreductases by catecholamines is postulated.

KEY WORDS: catecholamines; tissue respiration; mitochondria.

Catecholamines (CA) increase the oxygen consumption (V_{O_2}) of adipose and muscular tissue [8-10]. The data for the liver are more contradictory [4, 8-10], which accords ill with the generally accepted universality of the metabolic effects of CA [10]. A major role in the mechanism of the calorigenic effect (CE) of CA is ascribed to the simple accumulation of oxidation substrates as a result of activation of glycogenolysis and lipolysis [7, 9] or to the uncoupling action of the accumulated fatty acids [7, 8, 10] or of calcium [7]. Moreover, these mechanisms are regarded as universal and unique, for the direct action of CA on terminal oxidation processes is denied [7]. This view contradicts the general principle that an important process must be controlled not only by unspecialized regulators such as substrates or allosteric effectors, but also by specialized regulators, more progressive from the evolutionary standpoint, such as hormones and cyclic nucleotides [2].

The object of this investigation was to reveal any possible effects of CA on V_{O_2} of the liver mitochondria and to investigate the mechanism of this effect. Preliminary reports were presented to the 10th and 11th All-Union Symposia on Biochemistry of the Mitochondria [3, 6].

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TABLE 1. Increase in Rates of Respiration (ΔV, in nanoatoms O_2 /min/mg protein) on Rat Liver Mitochondria after Addition of Noradrenalin (M ± m)

Substrate	V_0	V_3	V_4	V_{DNP}
Pyruvate + malate (n = 8)	8,1±0,89 ($P<0,001$)	5,5±3,3 ($0,1>P>0,05$)	7,3±1,2 ($P<0,001$)	1,5±2,67 ($0,1>P>0,05$)
2-Oxoglutarate (n = 9)	6,8±1,13 ($P<0,001$)	11,3±4,49 ($P<0,05$)	6,4±2,07 ($P<0,01$)	10,4±1.36 ($P<0,001$)
Succinate (n = 8)	9,2±1,77 ($P<0,001$)	10,4±4,77 ($0,1>P>0,05$)	8,7±3,1 ($P<0,05$)	9,7±4.14 ($P<0,05$)

TABLE 2. Increase in Rates of Respiration (ΔV, in nanoatoms O_2 /min/mg protein) on Succinate during Incubation of Rat Liver Homogenate with Noradrenalin (M ± m)

Conditions of incubation	V_0	V_3	V_4	V_{DNP}
Noradrenalin (n = 7)	19,4±5.62 ($P<0,001$)	28,0±5,34 ($P<0,001$)	29,5±6,36 ($P<0,01$)	26,6±6,62 ($P<0,001$)
Noradrenalin + albumin in incubation medium (n = 4)	14,5±3,9 ($P<0,01$)	27,1±9,86 ($P<0,05$)	9,7±4,19 ($0,1>P>0,05$)	26,1±10,17 ($0,1>P>0,05$)
Noradrenalin + 0.2 mM EDTA in respiration medium (n = 6)	27,2±9,24 ($P<0,05$)	32,3±10,68 ($P<0,05$)	26,9±9,68 ($P<0,05$)	—

EXPERIMENTAL METHOD

Experiments were carried out on 100 male albino rats weighing 150-220 g. DL-Noradrenalin hydrotartrate was injected subcutaneously in a dose of 11 μmoles/kg 30-40 min before decapitation or was incubated with liver homogenate at 28°C for 12 min in a concentration of $1.5 \cdot 10^{-6}$ M. Bovine serum albumin (fraction V) was added to the homogenates before incubation in a concentration of 5 mg/ml. The medium for homogenization, isolation, and incubation consisted of: 0.25 M sucrose, 1 mM EDTA, 10 mM Tris buffer, pH 7.5. Mitochondria were isolated by the usual method of differential centrifugation. The value of V_{O_2} was determined polarographically [1] in respiration medium: 0.29 M sucrose, 5.6 mM KCl, 3 mM KH_2PO_4, 10 mM Tris buffer, pH 7.5; the oxidation substrates were: 5 mM succinate, 2.5 mM 2-oxoglutarate, and 3 mM pyruvate + 1.5 mM malate. In the experiments in vitro 0.2 mM EDTA was added to the respiration medium. The following indices of mitochondrial respiration were measured: V_0) the rate of oxidation with the substrate, V_3) the rate of respiration on the addition of up to 100 μM ADP (state 3); V_4) the rate of respiration after all the ADP had been used up (state 4), V_{DNP}) the rate of respiration after addition of up to 30 μM dinitrophenol (DNP).

EXPERIMENTAL RESULTS

Noradrenalin increased the respiration of the mitochondria on all three substrates (Table 1). This is in agreement with the activation of respiration of liver slices on endogenous substrates by CA described by the writers previously [4].

If the hypothesis of the accumulation of oxidation substrates as the main cause of the increase in V_{O_2} were correct, this would be observed not only on endogenous substrates. Sufficiently high concentrations (of the order of $[S]_{0.5}$) of three different exogenous substrates were used, but nevertheless CE was very clearly manifested. The data in Table 1 are also evidence against another popular explanation of the CE of CA, namely uncoupling of respiration and phosphorylation. In fact, an increase in V_{O_2} was observed in all metabolic states of the mitochondria. Moreover, it is important, in principle, that the increase in V_3 and V_{DNP} was as a rule the same or even greater than the increase in V_0 and V_4, i.e., CA activates electron transport in both the phosphorylating (V_3) and the nonphosphorylating (V_0 and V_4) routes of oxidation, even when respiration and phosphorylation are completely uncoupled (V_{DNP}). Similar results also were obtained by another method of calculation: The increase in V_{O_2} under the influence of ADP ($V_3 - V_0$) and of DNP ($V_{DNP} - V_0$) and the decrease in V_{O_2} during accumulation of ADP ($V_3 - V_4$) after addition of noradrenalin were completely unchanged [3]. The equal stimulation of respiration of the mitochondria in the control and experimental animals under the influence of saturating concentrations of DNP and ADP indicates that the energetic control of respiration remained intact and confirmed the absence of an uncoupling effect of noradrenalin on the liver mitochondria.

To test this important conclusion experiments were carried out in which liver homogenates were incubated with noradrenalin (Table 2). Stimulation of mitochondrial respiration by noradrenalin ($1.5 \cdot 10^{-6}$ M) was preserved in the experiments in vitro also. Consequently, CE was not due to other physiological systems but was the result of the direct action of CA on the liver tissue. Later, just as in vivo, an increase in V_{O_2} was observed in all metabolic states of the mitochondria. This confirms the conclusion that CE of CA on the liver is due chiefly to the uncoupling of respiration and phosphorylation. In fact, neither fraction V of serum albumin, with high affinity for fatty acids, nor EDTA, binding metallic ions including Ca^{2+} (i.e., substances binding potential uncouplers) changed the activation of mitochondrial respiration produced by noradrenalin (the only exception was a smaller increase in V_4 in the experiments with albumin). Under these circumstances, the values of the Lardy and Chance respiratory controls increased when both substances (albumin and EDTA) were used, and especially when both were used together, but always equally in the control and in the presence of noradrenalin. These experiments are evidence that the accumulation of fatty acids and Ca^{2+} in the liver tissue cannot be regarded as the main cause of the CE of CA.

Stimulation of mitochondrial respiration in the liver by CA is thus not the result of the simple accumulation of oxidation substrates and (or) uncoupling of respiration and phosphorylation, but its mechanism is more complex. Presumably catecholamine stimulation of respiration in other tissues also cannot be reduced to the simple consequence of activation of biopolymer breakdown, but must also include the direct influence of these hormone mediators on the terminal oxidation processes. Previously, the writers found activation of mitochondrial NAD-isocitrate dehydrogenase under the influence of CA and cyclic AMP [5]. The presence of CE of CA on three different substrates may indicate that activation of mitochondrial enzymes is not confined to isocitrate dehydrogenase but is wider in character.

Taken as a whole, these results confirm the view that the routine control of mitochondrial respiration in mammals is not effected entirely by old intracellular mechanisms, but also by a direct neurohormonal mechanism.

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