REGULATION OF OXIDATIVE PHOSPHORYLATION BY ADRENALIN AND INSULIN: ROLE OF THE INSULIN-DEPENDENT CYTOPLASMIC REGULATOR

Ya. Kh. Turakulov,* M. Kh. Gainutdinov, I. I. Lavina, and M. S. Akhmatov

UDC 612.26-06:[612.452+612.349].018

The insulin-dependent regulator (IDR) was shown to inhibit oxidation of pyruvate and succinate in rat liver mitochondria. Since insulin increases but adrenalin reduces IDR activity in the liver cytoplasm, it is suggested that a change in the concentration of IDR in the cytoplasm is one mechanism of regulation of substrate oxidation in mitochondria in vivo. Activation of oxidation by adrenalin was shown to be inducible not only by an increase in IDR activity in the cytoplasm, but also by another mechanism independent of the IDR concentration.

KEY WORDS: liver mitochondria; adrenalin; insulin; oxidative phosphorylation.

The cellular mechanisms of the action of adrenalin and insulin on mitochondria in vivo have not yet been discovered. The existence of such mechanisms is postulated on the basis of experimental observations showing the existence of hormonal regulation of mitochondrial function in vivo [2, 4-6] and the absence of any action of adrenalin and insulin in physiological concentrations directly on mitochondria in experiments in vitro. Most attention has been paid to the role of cyclic nucleotides in this regulation [2, 5]. Meanwhile, the present writers have shown that a factor of glycoprotein nature is present in the cytoplasm which, in low concentrations, changes the functional state of liver mitochondria; the activity of this factor increases after injection of insulin into rats [1, 3]. It is suggested that an insulin-dependent cytoplasmic regulator (IDR) is indirectly responsible for the action of insulin on liver mitochondria [3].

In connection with antagonism in the action of adrenalin and insulin on liver metabolism it is interesting to study the role of IDR in the cell mechanisms responsible for this antagonism at the mitochondrial level. The investigation described below was devoted to the study of this problem.

EXPERIMENTAL METHOD

The techniques used were fully described previously [3]. Adrenalin was injected subcutaneously into rats. Mitochondria were isolated from rat liver in 0.45M sucrose, made up in 10 mM Tris-HCl, pH 7.4. The rate of oxygen utilization by the mitochondrial suspension was measured polarographically. After centrifugation of the liver homogenate in 0.3M sucrose, 10 mM Tris-HCl, pH 7.4 (1 mg/g) at 30,000g the cytoplasmic fraction was obtained.

EXPERIMENTAL RESULTS

The addition of small quantities of cytoplasm from the liver of a satiated rat to the liver mitochondrial suspension led to an increase in the ability of the mitochondria to accumulate Ca^{++}. It was shown previously [1, 3] that injection of insulin under these conditions into rats in vivo potentiates the activating action of the cytoplasm, and subsequent separation of the cytoplasmic fractions showed that this effect is due to IDR [3]. After injection of adrenalin the activating effect of the cytoplasm was sharply reduced (Fig. 1). Dependence of the action of control cytoplasm and cytoplasm from the liver of rats receiving adrenalin on concentration is shown in Fig. 2. In the case of control cytoplasm, saturation was reached at lower concentrations (of

*Academician of the Academy of Sciences of the Uzbek SSR.

Institute of Territorial Medicine, Ministry of Health of the Uzbek SSR. Institute of Biochemistry, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 88, No. 9, pp. 301-304, September, 1979. Original article submitted August 17, 1978.

0007-4888/79/8809-1008$07.50

TABLE 1. Effect of Adrenalin and IDR on Rate of Oxygen Consumption by Suspension of Liver Mitochondria*

Index	Substrate: 1 mM pyruvate				Substrate: 1 mM succinate + rotenone, 1 μg/ml			
	V_0	V_3	V_4	V_{DNP}	V_0	V_3	V_4	V_{DNP}
Control	6,4±0,8	14,2±1,7	5,1±0,4	13,7±1,5	18,4±2,0	58,1±5,6	19,2±2,3	80,2±7,8
Adrenalin (1.5 mg/kg)	8,1±0,5	22,3±2,6	8,8±0,9	20,4±2,7	21,2±18	71,2±6,5	23,6±4,2	109,1±11,5
Adrenalin (1.5 mg/kg) + IDR (10^{-5} μg protein/ml)	4,9±0,4	14,0±1,3	3,0±0,4	12,0±1,4	15,9±1,3	48,1±3,7	14,1±1,2	63,2±7,6

*0.12M KCl, 0.1M sucrose, 1 mM KH_2PO_4, 10 mM Tris-HCl, pH 7.4; rate of oxygen consumption measured in nanoatoms O_2/min/mg mitochondrial protein; M ± m.

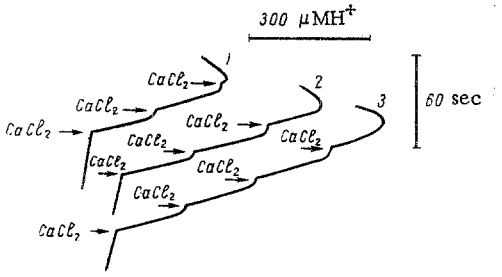

Fig. 1

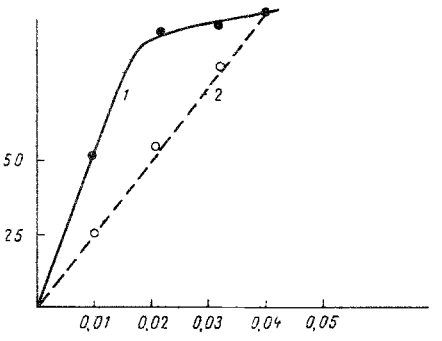

Fig. 2

Fig. 1. Action of liver cytoplasm from rats receiving adrenalin on Ca^{++} transport in mitochondria. Control cytoplasm isolated from liver of satiated rats. Adrenalin (1.5 mg/kg) injected subcutaneously 20 min before sacrifice. Incubation medium (3 ml) contained 0.1M KCl, 5 mM succinate, rotenone 1 μg/ml, 1.0 mM KH_2PO_4, 10 mM Tris-HCl, pH 7.15, mitochondria, 1 mg protein/ml, 0.15 mM $CaCl_2$, 0.01 ml cytoplasm. 1) Control; 2) cytoplasm (control); 3) cytoplasm (adrenalin).

Fig. 2. Action of cytoplasm from liver of rats receiving adrenalin and from control rats as a function of concentration. Abscissa, cytoplasm (in ml); ordinate, activation of Ca^{++} transport (in %). 1) Control; 2) adrenalin.

protein) of cytoplasm than in the case of cytoplasm from the liver of rats receiving adrenalin. Adrenalin thus has the opposite action to adrenalin on IDR activity in the cytoplasm, which is in good agreement with the antagonism between the action of these two hormones on metabolism.

Since oxidation of exogenous substrates in the mitochondria was activated by adrenalin (Table 1), in good agreement with data in the literature [2, 7], it was suggested that IDR has the opposite action to adrenalin on respiration. Experiments to study the action of IDR on respiration were carried out on liver mitochondria from rats receiving adrenalin (1.5 mg/kg 20 min before sacrifice). Purified preparations of IDR in experiments with isolated liver mitochondria induced inhibition of oxidation of pyruvate and succinate in all metabolic states (Table 1); in the case of pyruvate, moreover, the respiratory control of the mitochondria was increased. An increase in respiratory control during oxidation of succinate on the addition of IDR was observed in experiments with preparations of mitochondria which had aged for a few hours at 0-2°C. Inhibition of oxidation of pyruvate and succinate by IDR in the presence of 2,4-dinitrophenol (DNP) indicates that this inhibition cannot be explained by inhibition of the adenine-nucleotide translocase of the liver mitochondria. The inhibitory action of IDR on succinate oxidation, incidentally, was exhibited under conditions when succinate oxidation could be limited by succinate transport in the mitochondria (the mitochondria were isolated in hypertonic isolation medium; the tonicity of the incubation medium was increased, but the succinate concentration was relatively low). Consequently, one possible explanation of the inhibition of oxidation is inhibition of substrate transport in the mitochondria. This explanation is most likely with respect to inhibition of oxidation of pyruvate, because of the very low activity of the monocarboxylate carrier in liver mitochondria [4, 7].

On the basis of the results described above the activation of oxygen consumption by the perfused liver in the presence of adrenalin and inhibition of oxidation by insulin can be explained by a change in the IDR concentration in the cytoplasm; the most likely mechanism of action of IDR, moreover, is restriction of the supply of oxidation substrate from the cytoplasm to the mitochondria.

Is this mechanism of stimulation of oxidation by adrenalin the principal or indeed the only one? In mitochondria isolated from the liver of rats receiving adrenalin, activation of substrate oxidation persists for a few hours after removal. Can this effect be due to a decrease in the IDR concentration in the mitochondrial suspension? The concentrations of IDR in mitochondrial preparations can be estimated on the basis of the action of exogenous IDR on them. Inhibition of oxidation of pyruvate and succinate by IDR is observed in the presence of IDR in concentrations two orders of magnitude higher thanthose required to increase the calcium capacity of the mitochondria; moreover the graph of the relationship between the action of IDR on Ca^{++} transport and its concentration is bell-shaped and activation of Ca^{++} transport by IDR levels out completely at IDR concentrations required to inhibit respiration [3].

These two actions of IDR on mitochondria are independent of one another. It was shown previously that low concentrations of IDR increase the calcium capacity of recently isolated mitochondria [3]. Consequently, IDR is present in a mitochondrial suspension in trace amounts, within the range of concentrations from 10^{-14} to 10^{-15}M and it cannot have any significant inhibitory action on substrate oxidation. During isolation of mitochondria the components of the cytoplasm are rinsed out, with the result that the IDR concentration in the mitochondrial suspension is evidently reduced. The existence of an enzyme system inactivating IDR in the mitochondria is proved by the strong inactivation of exogenous IDR arising during incubation with mitochondria at 37°C for 10 min.

The fact that stimulation of respiration in the mitochondria continues after isolation of the mitochondria from the liver of rats receiving adrenalin cannot be explained by a decrease in IDR activity in the cytoplasm after injection of adrenalin and it points to the presence of a mechanism of activation of oxidation that is independent of IDR. The action of adrenalin on respiration of liver mitochondria in vivo is thus due to a decrease in IDR activity in the cytoplasm and the consequent abolition of the inhibitory action of IDR, and to stimulation of oxidation that is independent of IDR and persists in preparations of mitochondria after their isolation.

LITERATURE CITED

1. M. Kh. Gainutdinov, Ya. Kh. Turakulov, M. S. Akhmatov, et al., Khim. Prir. Soedin., No. 1, 141 (1978).
2. V. I. Kulinskii, L. M. Vorob'eva, and L. V. Trufanova, in: Mitochondria [in Russian], Moscow (1977), pp. 12-14.
3. Ya. Kh. Turakulov, M. Kh. Gainutdinov, I. I. Lavina, et al., Dokl. Akad. Nauk SSSR, 234, 1471 (1977).
4. A. P. Halestrap, Biochem. J., 148, 85 (1975).
5. R. S. Haynes, in: Energy Metabolism and the Regulation of Metabolic Processes in Mitochondria, New York (1972), pp. 239-245.
6. R. K. Yamazaki, J. Biol. Chem., 250, 7924 (1975).
7. M. A. Titheradge and H. G. Coore, FEBS Lett., 71, 73 (1976).