Effect of Insulin on the Rate of Hydrogen Peroxide Generation in Mitochondria

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We studied the effect of insulin on H_2O_2 generation by mitochondria in rat liver and heart. Insulin markedly increased the rate of H_2O_2 generation, which was realized via short-term activation of mitochondrial succinate dehydrogenase. In terms of the Michaelis—Menten equation describing the dependence of H_2O_2 generation by mitochondria on succinate concentration (succinate dehydrogenase substrate), insulin decreased the Michaelis—Menten constant and increased the maximum rate of H_2O_2 generation compared to the control.

Key Words: *succinate dehydrogenase; mitochondrion; insulin; hydrogen peroxide; succinic acid*

Insulin receptors are regulated via reversible phosphorylation. Receptor tyrosine kinase activates, while intracellular protein tyrosine phosphatases (PTP) dephosphorylate and inactivate insulin receptors [2]. Insulin inactivates PTP and initiates cascade signal transduction in cells by stimulating the release of H_2O_2 that acts as a physiological inhibitor of PTP [4,7]. NADPH oxidase acts as an insulin-sensitive source of H_2O_2 in adipocytes [5]. Our previous experiments showed that the mitochondrial respiratory chain is an insulin-sensitive source of H_2O_2. Succinate dehydrogenase (SDH) plays a key role in insulin-induced generation of H_2O_2 and PTP inactivation in the liver [1].

Here we studied the effect of insulin on generation of H_2O_2, a physiological inhibitor of PTP, by heart and liver mitochondria.

MATERIALS AND METHODS

H_2O_2 concentration was determined by phenol red oxidation in the presence of horseradish peroxidase [6].

Experiments with insulin were performed on slices of the liver and heart from male Wistar rats. Slices (1 mm) were thoroughly washed with 0.15 M KCl. For evaluation of the effect of insulin on the rate of H_2O_2 generation by mitochondria the slices were incubated in a medium containing 30 mM Tris-HCl (pH 7.4) in the presence (experiment) or absence of 1 μU/ml insulin (control) at 37°C for 1 min. Mitochondria were isolated from slices by differential centrifugation in 0.25 M sucrose with 0.1 M EDTA. Nuclei were removed at 600*g*. Mitochondria were isolated at 10,000*g*. Isolated mitochondria were incubated in a medium containing 35 mM Tris-HCl (pH 7.6) and succinic acid (SA) in increasing concentrations at 37°C for 15 min. The concentration of H_2O_2 in the incubation medium was measured.

The significance of differences was evaluated by Student's *t* test. The maximum rate of H_2O_2 generation (Vm) and Michaelis constant (K_M) were calculated using regression analysis.

RESULTS

The dependence of the initial rate of H_2O_2 generation by isolated mitochondria on SA concentration (SDH substrate) was described by the Michaelis-Menten equation:

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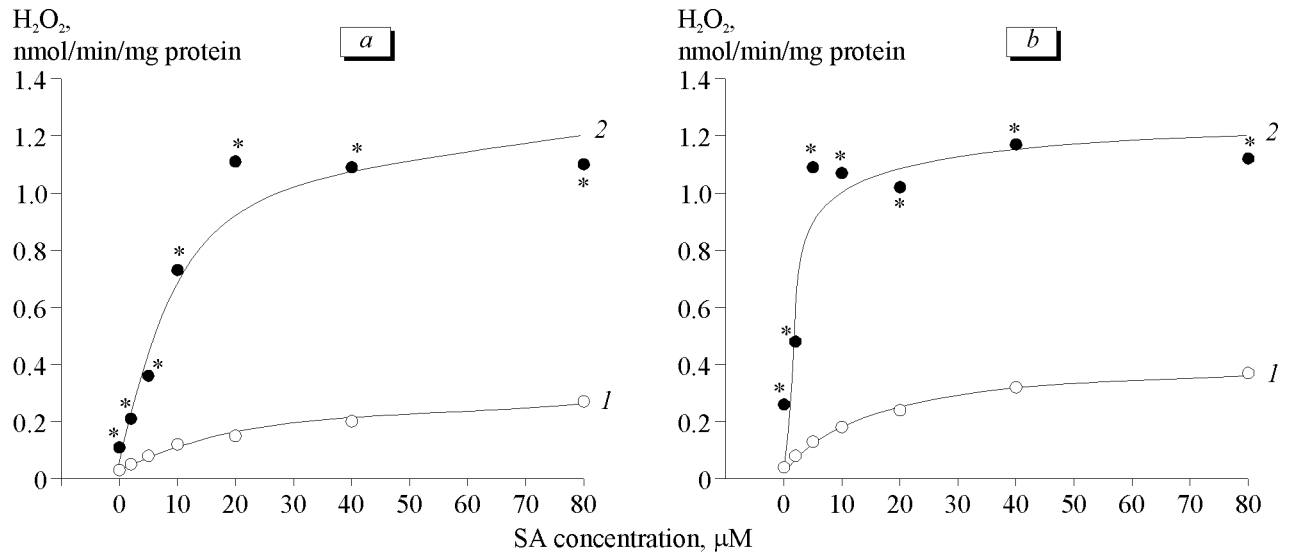

Fig. 1. Rate of H_2O_2 generation by heart (*a*) and liver (*b*) mitochondria as a function of succinic acid (SA) concentration: control (*1*) and pretreatment with insulin (*2*). Symbols: experimental data. Lines: estimated curves. *$p<0.01$ compared to the control ($n=3$).

$$v=VmS/(K_M+S),$$

where v is the initial rate of H_2O_2 generation, and S is SA concentration.

The rate of H_2O_2 generation in mitochondria isolated from insulin-pretreated tissues was higher than in the control (substrate SA, Fig. 1). Pretreatment with insulin modified parameters of the Michaelis—Menten equation describing the kinetics of H_2O_2 generation by mitochondria. Insulin increased Vm and decreased K_M compared to the control (Table 1).

These equations characterize the effect of insulin on the rate of H_2O_2 generation by mitochondria in the presence of SA in physiological concentrations (1-9 μmol/liter) [3]. In the presence of SA in these concentrations incubation of tissues with 1 μU/ml insulin for 1 min increased the rates of H_2O_2 generation by heart and liver mitochondria by 6-8 and 6-13 times, respectively.

It should be emphasized that incubation of tissues with insulin produced only a short-term increase in the rate of H_2O_2 generation by mitochondria. Increasing the time of treatment with insulin to 5 min was followed by a decrease in the rate of H_2O_2 generation to the control level.

Our results indicate that insulin increases the rate of H_2O_2 generation due to short-term activation of mitochondrial SDH. The molecular mechanism underlying insulin-induced activation of SDH remains unclear.

We showed for the first time that insulin increased the rate of H_2O_2 generation (physiological inhibitor of PTP) due to short-term activation of mitochondrial SDH.

TABLE 1. Parameters of Michaelis—Menten Equation Describing the Kinetics of H_2O_2 Generation by Mitochondria

Tissue pretreatment	Vm, nmol/mg protein/min	K_M, μmol/liter
Heart mitochondria		
Control	0.31	17.2
Insulin, 1 μU/ml	1.32	8.6
Liver mitochondria		
Control	0.42	12.4
Insulin, 1 μU/ml	1.20	1.8

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