Key Role of Succinate Dehydrogenase in Insulin-Induced Inactivation of Protein Tyrosine Phosphatases

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We studied the role of mitochondria in insulin-induced inactivation of protein tyrosine phosphatases in the liver. The mitochondrial respiratory chain is an insulin-sensitive source of H_2O_2 that acts as a physiological inhibitor of protein tyrosine phosphatases. Succinate dehydrogenase plays a key role in insulin-stimulated generation of H_2O_2 and inactivation of liver protein tyrosine phosphatases.

Key Words: *succinate dehydrogenase; protein tyrosine phosphatase; insulin; hydrogen peroxide; succinate*

Insulin receptor is regulated via reversible phosphorylation: receptor tyrosine kinase activates, while intracellular protein tyrosine phosphatases (PTP) dephosphorylate and inactivate insulin receptors [2]. Insulin inactivates PTP, enhances cascade transduction of receptor signals in cells, and stimulates the release of H_2O_2, which acts as a physiological inhibitor of PTP [6]. Previous studies showed that NADPH oxidase is an insulin-sensitive source of H_2O_2 in adipocytes [7]. The nature of other insulin-sensitive H_2O_2 sources involved in PTP inactivation remains unclear.

Mitochondria intensively generate H_2O_2 at high values of the membrane potential [4]. Succinate, the substrate for succinate dehydrogenase (SDH), provides most rapid generation of H_2O_2 compared to other respiratory substrates [1,3].

Here we studied the role of mitochondria in insulin-induced inactivation of liver SDH.

MATERIALS AND METHODS

Experiments with insulin were performed on liver slices from male Wistar rats. Liver slices (1 mm) were thoroughly washed with 0.15 M KCl. To estimate the dependence of PTP activity on the duration of incubation with insulin, the slices were incubated with 1 mU/ml insulin in a medium containing 30 mM Tris-HCl (pH 7.4) at 37°C. To evaluate the effects of additives on PTP activity, the slices were incubated with 1 mU/ml insulin, 1 mU/ml insulin+2 mM malonic acid, 2 mM succinate, or without additives (control) in a medium containing 30 mM Tris-HCl (pH 7.4) at 37°C for 5 min. After incubation these slices were thoroughly washed with 0.15 M KCl and homogenized for 1 min. Total PTP activity was measured.

For evaluation of the effects of additives on the rate of H_2O_2 release, the slices were incubated with 1 mU/ml insulin, 1 mU/ml insulin+2 mM malonic acid, 2 mM succinate, or without additives (control) in 30 mM Tris-HCl (pH 7.4) at 37°C for 10 min. H_2O_2 concentration in the incubation medium was measured.

Homogenates of rat liver were used in experiments with succinate. The liver was homogenized in a medium containing 100 mM Tris-HCl (pH 7.4) and 1.15% KCl. The homogenate was centrifuged at 200*g* for 10 min and supernatant was used in the experiment (35.6 mg protein/ml). The effects of catalase on the efficiency of succinate-induced inhibition of PTP were determined. The homogenate was incubated in a medium containing 30 mM Tris-HCl (pH 7.5) and 4 mM phosphotyrosine in the presence of 2 mM succinate,

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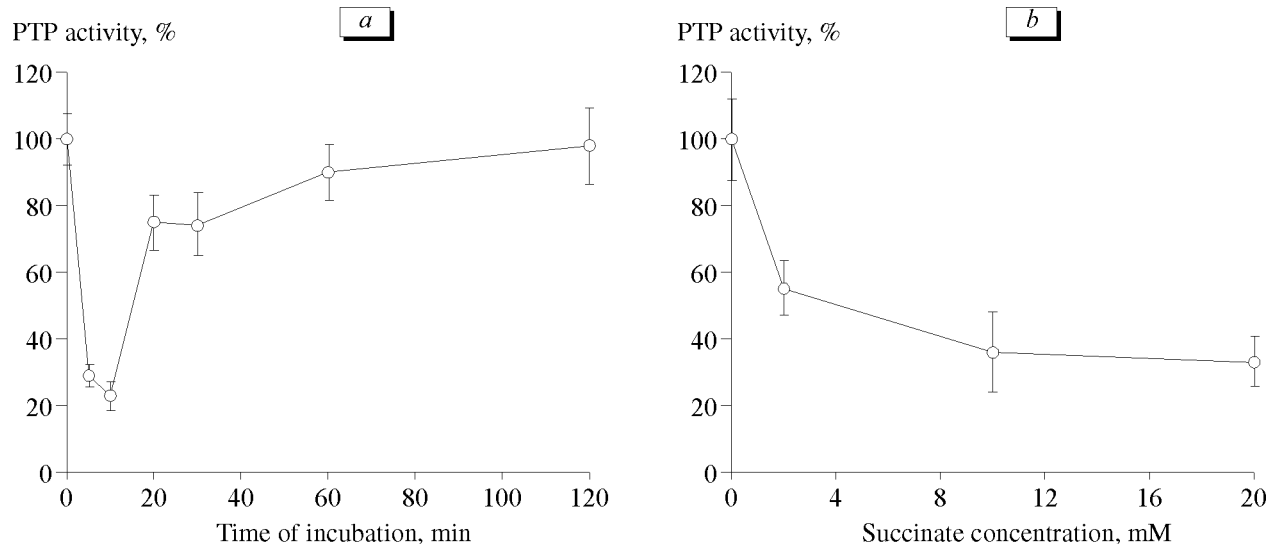

Fig. 1. Activity of protein tyrosine phosphatase (PTP) as a function of the duration of incubation with insulin (*a*) and succinate concentration (*b*).

2 mM succinate+7.6 U catalase, or without additives (control) at 37°C for 10 min. PTP activity was measured. The dependence of PTP activity on succinate concentration was evaluated. The cell fraction was incubated in a medium containing 30 mM Tris-HCl (pH 7.5), 4 mM phosphotyrosine, and sodium succinate in increasing concentrations at 37°C for 10 min. PTP activity was measured.

PTP activity was estimated by the release of inorganic phosphate from phosphotyrosine [5]. H_2O_2 concentration was determined by oxidation of phenol red in the presence of horseradish peroxidase [8].

The data are presented as means±standard deviations. The results were analyzed by Student's *t* test.

RESULTS

Insulin rapidly and reversibly decreased PTP activity (Fig. 1, *a*) and increased the rate of H_2O_2 release in liver samples (Table 1). SDH inhibitor malonic acid attenuated the effect of insulin. Therefore, the mitochondrial respiratory chain is an insulin-sensitive source of H_2O_2. SDH plays a role in insulin-stimulated generation of H_2O_2.

PTP activity was measured in the presence of various additives for evaluation of the role of SDH in insulin-induced inactivation of the enzyme (Table 1). Insulin significantly decreased PTP activity. Malonic acid had no effect on enzyme activity, but attenuated the inhibitory effect of insulin on PTP. Succinate decreased PTP activity (similarly to insulin). The degree of PTP inactivation depended on succinate concentration (Fig. 1, *b*). These data indicate that SDH plays a key role in insulin-induced inactivation of PTP.

For evaluation of the role of H_2O_2 in PTP inactivation, liver samples were incubated with catalase.

TABLE 1. Effects of Additives on H_2O_2 Release and PTP Activity (*M*±*m*, *n*=10)

Experimental conditions	Rate of H_2O_2 release, arb. U	PTP activity, %
Control	1.0±0.1	100±4
Insulin, 1 mU/ml	10.8±3.4*	27±4*
+malonic acid, 2 mM	3.9±1.5$^+$	65±3$^+$
Succinate, 2 mM	7.6±0.5*	28±3*

Note. $p<0.001$: *compared to the control; $^+$compared to insulin.

PTP activity in the presence of 2 mM succinate was 55±8% of the control ($p<0.001$). In the presence of 2 mM succinate and 7.6 U catalase PTP activity was 92±15% of the control ($p<0.001$ compared to succinate). Thus, catalase completely blocked succinate-induced inactivation of PTP, which indicates that H_2O_2 is involved in the inhibition of this enzyme.

Our results indicate that H_2O_2 generation by mitochondria contributes to transduction of insulin signals in cells. Therefore, mitochondria and factors modulating H_2O_2 production in these organelles play a role in the development of insulin resistance.

Thus, we showed for the first time that the mitochondrial respiratory chain is an insulin-sensitive source of H_2O_2. SDH plays a key role in insulin-induced generation of H_2O_2 and inactivation of PTP in the liver.

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