ACTIVATION OF SUCCINATE DEHYDROGENATION IN RAT LIVER BY NORADRENALIN, CYCLIC AMP, AND ACUTE COOLING

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Succinate dehydrogenase (SDH) is subject to multiple metabolic control [9, 14]. However, the influence of evolutionarily more progressive regulators has been very inadequately studied. Recently activation of liver mitochondrial respiration by catecholamines (CA) and cyclic AMP has been found during succinate utilization [4, 5], and succinate oxidase activity was found to be stimulated by cyclic AMP [2]. This suggested that CA and cyclic AMP can activate SDH [4]. According to one report, when incubated with hepatocytes dibutyryl-cyclic AMP stimulates SDH, but the authors cited used nonphysiological concentrations of the nucleotide [12]. The aim of the present investigation was to study this problem.

EXPERIMENTAL METHOD

Experiments were carried out on 94 Wistar rats of both sexes weighing 160-220 g. R(-) Noradrenalin hydrotartrate (NA) was injected subcutaneously in a dose of 11 μmoles/kg body weight 30 min before decapitation, and during this period stimulation of oyxgen utilization by the animal was maximal. In experiments *in vitro* homogenate or mitochondria were incubated with the regulators for 10 min at 28°C. Acute cooling was carried out at -17°C until the body temperature had fallen to 31°. Mitochondria were obtained from the anuclear fraction of the homogenate by centrifugation for 10 min at 10,000g in medium of the following composition: 0.25 M sucrose, 1 mM EDTA, 10 mM Tris-HCl buffer, pH 7.5. SDH activity was determined in a suspension of mitochondria by measuring dehydrogenation of succinate, using two electron acceptors: Phenazine methosulfate (PMS) [13] and the free radical N,N,N′,N′-tetramethyl-p-phenylenediamine (TMPD) [15] and the value of V_{max} were calculated by extrapolation to infinitely high concentrations of the acceptor [13, 15]. The reaction was triggered by the addition of mitochondria. In the control test succinate was replaced by malonate. Corresponding with [15], in the present experiments the basal value of V_{max} for succinate dehydrogenation was higher when the radical TMPD was used, but the degree of activation of the enzyme under the influence of all procedures used did not differ significantly ($P > 0.3$-1.0).

EXPERIMENTAL RESULTS

NA, whether administered *in vivo* or incubated with the homogenate, clearly activated succinate dehydrogenation in the liver (Table 1). This shows that the effect of CA is not mediated through other physiological systems but is the result of their direct influence on the liver.

Since most biochemical effects of CA are realized through the cyclic AMP system, the effects of exogenous cyclic AMP and of a nonhormonal activator of adenylate cyclase, namely NaF, were studied. The latter compound stimulates dehydrogenation of succinate during incubation with the homogenate to the same degree as NA. A similar effect also was found for cyclic AMP on incubation both with homogenate (Table 1) and with liver mitochondria (by 38.0 $\pm$ 5.0% with the TMPD radical, $P < 0.001$, and by 27.0 $\pm$ 9.5% with PMS, $P < 0.05$; $n = 15$).

The combined action of NA and cyclic AMP (Table 2) did not differ from their effect separately ($P > 0.5$). This shows that the mechanisms of SDH activation by CA and by cyclic AMP are identical, at least in their final stages. The equal stimulation of SDH by CA, NaF, and cyclic AMP and the absence of summation of the effects are evidence that CA act on succinate dehydrogenation through cyclic AMP.

All these rules have been established beforehand for stimulation of oxygen consumption by mitochondria when succinate was used as the substrate [3, 4]. This fact, and the about equal intensity of the two effects, suggest that stimulation of succinate dehydrogenation may be the cause of the activation of mitochondrial respiration on succinate.

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TABLE 1. Effect of NA, Cyclic AMP, and NaF on SDH Activity in Rat Liver (M ± m)

Series of experiments	Effector	Concentration	Injected (in vivo)	Incubation with homogenate (in vivo)	P
I	NA	11 μmoles/kg	30,0±5,1	—	<0,001
II	NA	10^{-6} M	—	21,0±3,3	<0,001
III	Cyclic AMP	10^{-6} M	—	30,0±4,6	<0,001
IV	F^-	10^{-2} M	—	24,0±5,2	<0,05

Legend. Results shown as increase in activity (in %) relative to control (with PMS 155 ± 12 nmoles/min/mg mitochondrial protein, with TMPD 363 + 28 nmoles/min/mg mitochondrial protein); PMS (series I-III) and TMPD radical (series IV) used as electron acceptor; 16-18 experiments in each series.

TABLE 2. Summation of Effects of NA and Cyclic AMP on Rat Liver SDH Activity (M ± m)

Index	NA	Cyclic AMP	NA + cyclic AMP
Activation, %	+21,0±6,1 (n=7)	+38,0±4,3 (n=5)	+27,0±4,5 (n=6)
P	<0,02	<0,001	<0,01

Legend. Results shown as increase in activity (in %) relative to control (332 ± 24 nmoles/min/mg mitochondrial protein), electron acceptor was TMPD radical; regulators (10^{-6} M) were incubated with rat liver homogenate for 10 min at 28°C.

Acute cooling is known to cause mobilization of endogenous CA. In the present experiments it considerably activated succinate dehydrogenation in the liver (by 21.0 ± 2.2%, n = 13; $P < 0.001$), but administration of the classical β-adrenolytic Inderal, in a dose of 34 μmoles/kg, completely abolished the effect of cooling (by 3.2 ± 4.2%, n = 16; $P > 0.5$). Consequently, activation of succinate dehydrogenation during acute cooling is brought about through endogenous CA and β-adrenoreceptors.

This important result and the fact that the acting concentrations of CA and cyclic AMP were within the physiological range are clear evidence in support of the real biological significance of activation of succinate dehydrogenation by these regulators. Although SDH is not the only enzyme regulator of the tricarboxylic acid cycle, control of its activity is fully justified. First, the preceding organic acids of the Krebs' cycle are not the only source of succinate, among which may also be mentioned methionine, valine, and isoleucine, which account for a considerable part of the amino acids found in the composition of proteins [8], and also fatty acids with an odd number of carbon atoms. Activation of SDH probably will facilitate energy production on account of these additional substrates. Second, there is Kondrashova's idea [3] of a switch to predominant utilization of succinate under hypoxic conditions and in other types of stress. Activation of succinate dehydrogenation by CA and cyclic AMP may be one of the concrete mechanisms of this switch.

The results of the present experiments are in good agreement with activation of liver mitochondrial respiration by glucagon during utilization of succinate and SDH activity [12], for glucagon exerts its effects on the liver through cyclic AMP.

Activation of SDH makes a significant addition to previous data on stimulation by CA and cyclic AMP of mitochondrial functions such as oxygen consumption [4, 5], NAD-isocitrate dehydrogenase activity [5, 6], and citrate synthetase activity [7]. The important difference between SDH and the last two enzymes is that it is a membrane and not a matrix enzyme, transferring electrons directly to the respiratory chain. This underlines the breadth of cyclic nucleotide control over mitochondrial functions.

The mechanism of SDH activation requires special investigation. It has recently been found that on incubation with mitochondria labeled cyclic AMP penetrates into all compartments of the mitochondria [1], and that the mitochondria contain a cyclic AMP-dependent protein kinase [10, 11]. Phosphorylation of the membrane proteins is already a known fact, so that it can be tentatively suggested that phosphorylation of SDH increases its activity.

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EFFECT OF *Bryonia alba* ROOT EXTRACT ON LIPID PEROXIDATION IN THE LIVER OF RATS WITH ALLOXAN DIABETES

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In previous investigations [14] the writers showed that in experimental alloxan diabetes (AD) an extract of *Bryonia alba* roots has a hypoglycemic action, due to the fraction of trihydroxyoctadecadienoic acids, which exhibit prostaglandin-like activity.

In diabetes depression of insulin secretion is accompanied by specific disturbances in fatty acid and prostaglandin metabolism [8, 9], and the role of special factor regulating the secretion of the hormone is ascribed to phospholipase A_2 [12]. The polyenic fatty acids set free are not only transformed into prostaglandins, but also are oxidized into monohydroperoxides, which subsequently break down to form hydroxy and epoxy acids [7]. The trihydroxyoctadecadienoic acids are C-18 homologs of the end products of the lipoxygenase pathway of oxidation of arachidonic and icosa-8, 11, 14-trienoic acids. Their precursors – the corresponding monohydroperoxides – can inhibit prostacyclin biosynthesis in blood vessel walls [6], and this is regarded as one possible cause of the development of atherosclerotic complications that are characteristic of diabetes [10], accompanied by a simultaneous rise in the blood lipid peroxide (LP) level [15].

The object of the present investigation was to study the effect of *Bryonia alba* root extract (BAE) on the formation of lipid peroxidation products in homogenates and the microsomal fraction of the liver (MFL) of albino rats with AD, and also on the fatty acid composition of individual phospholipids (PL).

EXPERIMENTAL METHOD

Diabetes was induced by the method described previously [3]. From the 7th day of the disease the animals were given a daily intramuscular injection of an aqueous solution of BAE in a dose of 5 mg/kg body weight. The MFL was isolated by differential centrifugation. The malonic dialdehyde (MDA) content was determined by the method in [2]. Total and individual PL and free fatty acids were isolated by the standard methods [5]. Gas–liquid chromatography (Pye Unicam) was done on 1200 × 3 mm, 180C columns, with 8% PEGA and 3% E-30 on the Gas Chrom Q instrument.

EXPERIMENTAL RESULTS

The results indicate an increase in MDA formation both in homogenates and in MFL of rats with AD (Table 1), in agreement with data on the increase in the LP level in various pathological states [2]. These changes were accompanied

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