THE REDOX STATE OF NAD^+/NADH SYSTEMS IN RAT LIVER DURING IN VIVO INHIBITION OF FATTY ACID OXIDATION BY ADENOSINE

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1. Introduction

The values of the NAD^+/NADH ratio in different cell compartments has been shown to be a key point in the control of metabolism [1,2]. Quite often, these NAD^+/NADH values are obtained by measuring the ratio of the concentration of the oxidized and reduced metabolites of suitable NAD^+-linked dehydrogenase systems according to the principles stated by Lynen [3,4] and elaborated by Bücher and Klingenberg [5].

Several investigators have reported the effect of fatty acid oxidation by rat liver in promoting a shift of the redox potential which increase the cytoplasmic reduced state [6–9]. In this work, the cytoplasmic and mitochondrial NAD^+/NADH systems from rat liver were studied under conditions in which an important inhibition of the fatty acid oxidation by the liver is produced in vivo by the administration of adenosine [10].

2. Materials and methods

2.1. *Animals*

Male Wistar albino rats, weighing between 120–150 g were used. They received a standard laboratory diet and were fasted for 16–20 h without restriction of water intake. Special care was taken to keep the animals in optimal conditions and with minimal handling. The animals received an intraperitoneal injection of either 0.9% NaCl (1 ml/100 g body wt) or adenosine (20 mg/ml in 0.9% NaCl) at a dose of 200 mg/kg body wt. The animals were sacrificed at different times after the injection. Control animals killed at 0 time were not injected.

2.2. *Preparation of liver extracts*

The animals were sacrificed by a blow on the head. The abdomen was immediately opened with a bistoury and 100–200 mg liver was homogenized at once in 2.0 ml 0.9 N $HClO_4$ at 0°C. Only those liver samples in which the homogenization was started within 5 s after the abdominal incision were used for the quantification of metabolites. The homogenate was centrifuged for 10 min at 3000 × *g*. The supernatant was saved and the precipitate was re-extracted with 0.66 ml 0.2 N $HClO_4$, and centrifuged again at 3000 × *g* for 10 min. The combined supernatants were brought to pH 7.0 by careful addition of 5 M K_2CO_3. The volume reached after pH adjustment was considered as the total volume of the extract. The wet weight of each sample was obtained from the dry weight of the perchloric acid precipitate, after washing once with water to eliminate the excess of perchloric acid, by multiplying by 3.27. This factor (3.27 ± 0.02 mean ± SE three determinations) was calculated by carefully weighing liver samples in an analytical balance and treating them in identical manner as described. The conversion factor is slightly modified by fasting conditions, sex and other environmental conditions.

2.3. *Quantification of metabolites*

The methods used in the determination of metabolites were the following: pyruvate by the method of Bucher, et al. [11]; lactate by the method of Hohorst [12]; 3-hydroxybutyrate by the method of

Williamson and Mellanby [13] and acetoacetate by the method of Mellanby and Williamson [14].

2.4. *Mitochondrial and cytoplasmic NAD$^+$/NADH ratio*

It was calculated from the ratio obtained in the quantification of the substrate pairs lactate/pyruvate and 3-hydroxybutyrate/acetoacetate, and with the equilibrium constants used by Krebs [2] for the lactate dehydrogenase and the 3-hydroxybutyrate dehydrogenase systems.

2.5. *Redox potential*

The mitochondrial redox potential (E_h) was calculated as described by Wilson et al. [15].

2.6. *Reagents*

The enzymes used in the quantification of metabolites were obtained from Boehringer Mannheim Corp. Coenzymes and substrates were obtained from Sigma Chemical Co. All other chemicals were of analytical grade.

3. Results

The levels of lactate, pyruvate, their ratio and the NAD$^+$/NADH ratio from the cytoplasmic liver cells of intact, saline and adenosine injected rats are shown in fig.1. The values obtained in non-injected animals are comparable to those reported by other investigators [1,2]. Adenosine did not produce any significant change in the parameters studied in fig.1. Some modifications observed after the injection of either saline or adenosine might be related to the stress response caused by the injection.

In the saline treated animals the levels of 3-hydroxybutyrate and acetoacetate were studied. The amount of both mitochondrial substrates remained nearly constant through the 2 h experimental period (fig.2); therefore their ratio and the NAD$^+$/NADH ratio from the mitochondrial compartment remained also constant (fig.2). Besides, these ratios are in agreement with those reported by Wilson et al. [15] in isolated hepatocytes and by Parrilla et al. [16] in perfused rat liver, but are lower than those reported by other

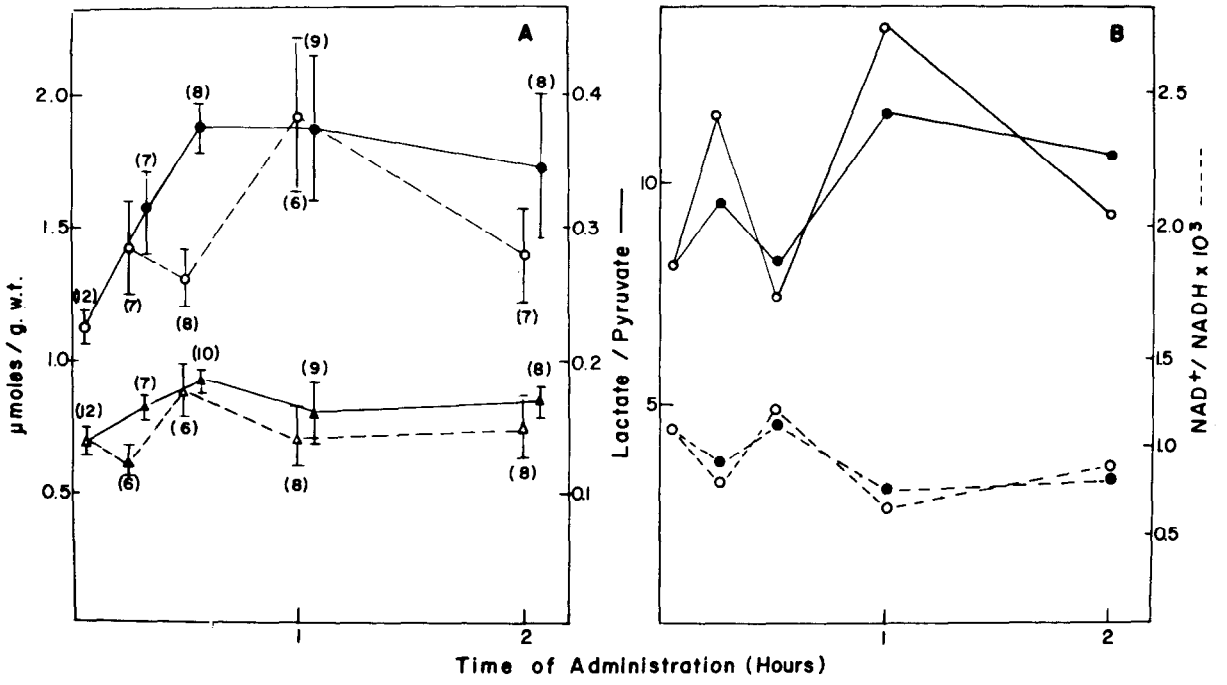

Fig.1. Time course of the effect of adenosine in cytoplasmic NAD$^+$/NADH ratio in the liver. A The values are the mean ± SE of the number of animals in parenthesis. Lactate: (○- - -○) saline and (●——●) adenosine; pyruvate: (△- - -△) saline and (▲——▲) adenosine. B Lactate/pyruvate ratio and NAD$^+$/NADH (○) saline, (●) adenosine.

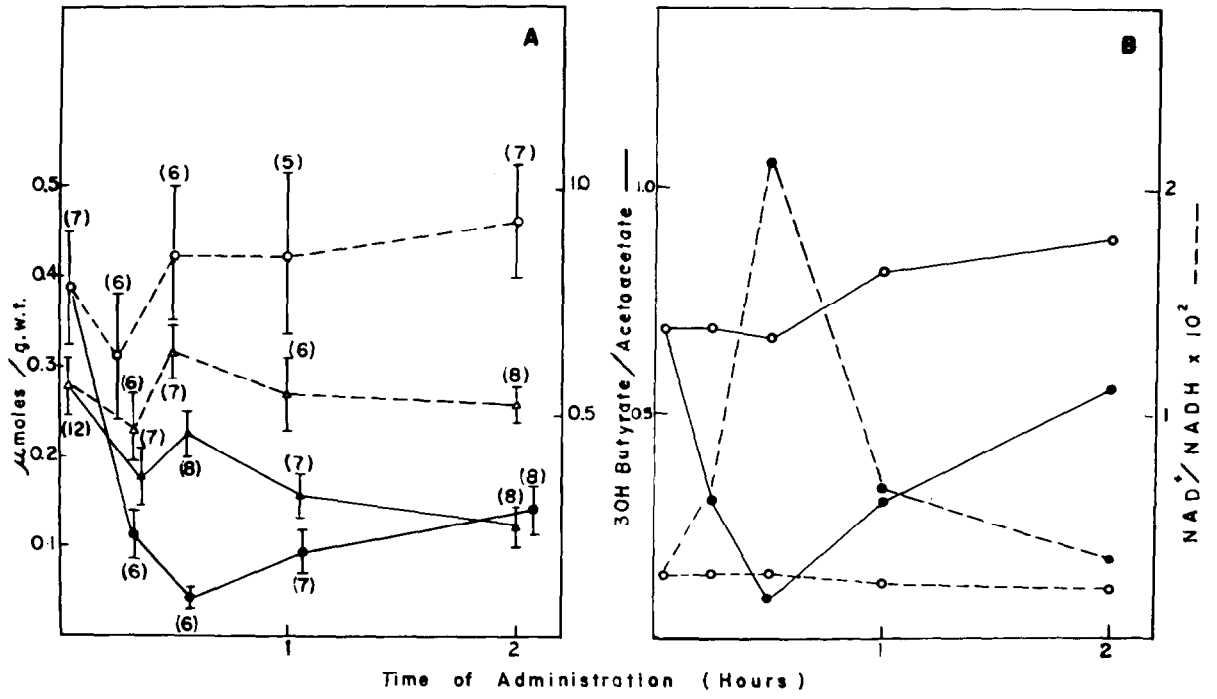

Fig.2. Time course of the effect of adenosine in the mitochondrial $NAD^+/NADH$ ratio in the liver. A The values are the mean ± SE of the number of animals in parenthesis. 3-Hydroxybutyrate: (○- - -○) and (●——●) adenosine; acetoacetate: (△- - -△) saline, and (▲——▲) adenosine. B 3-hydroxybutyrate/acetoacetate ratio and $NAD^+/NADH$ (○) saline, (●) adenosine.

authors [2,17,18]. After the injection of adenosine a decrease in acetoacetate and 3-hydroxybutyrate was evident (fig.2). However, the decrease was more pronounced in the level of 3-hydroxybutyrate; the ratio of these substrates and therefore, that of the mitochondrial $NAD^+/NADH$ was clearly modified. This latter ratio reached a 7-fold increase 30 min after the administration of the nucleoside (fig.2).

4. Discussion

In the experiments here described and in some reported by Krebs [2], the changes in the mitochondrial dehydrogenase system do not always follow those of the cytoplasmic system. The transport of reducing power from mitochondria to cytoplasm and vice-versa, has been studied by numerous investigators [5,6,19] and will not be discussed here. In any event, in some metabolic conditions of the hepatocyte, as in alloxan diabetes [2] or adenosine treatment, the equilibrium in the redox potential between cytoplasm and mitochondria can not always insure parallel changes in their respective $NAD^+/NADH$ ratios.

The substrate pairs more suitable for the assessment of the $NAD^+/NADH$ ratios in the cytoplasm and mitochondria of rat liver were employed [2]. Although the value of these ratios is limited by the assumptions in which they are based [16,20], they are useful in demonstrating that the E_h of the mitochondrial NAD^+ system in control rats (from −261 mV to −265 mV) increases (becomes less negative) due to adenosine treatment (−251 mV at 15 min and 60 min after the injection and −235 mV at 30 min after the injection). The data agree with those of Löffler et al. [21], who observed that the absence of caprylate in perfused rat liver resulted in an increase of the mitochondrial redox potential. Our results are also in complete agreement with the adenosine mediated inhibition in the oxidation of fatty acids by intact rat liver and by liver homogenates [10]. They are also in accordance with

an important decrease in the level of ketone bodies in blood produced by adenosine [10].

Furthermore, they represent just the antagonistic conditions observed when the oxidation of fatty acids in the liver cells is promoted [6,7,9].

In isolated hepatocytes incubated with adenosine the cytosolic $NAD^+/NADH$ ratio remains unchanged [22] as in the present work. However, in the same cells the mitochondrial $NAD^+/NADH$ ratio and the E_h, as well as the utilization of oleic acid and the production of ketone bodies [22] were only slightly modified, but in an opposite direction by incubation with the nucleoside, if compared with intact liver (fig.2) [10]. These differences might result from the trauma associated with the isolation of hepatocytes [23] and/or the experimental conditions for the incubation of the liver cells. Nevertheless, the role of some extra-hepatic tissues conditioning the response to adenosine by the liver in the whole animal can not be discarded.

The changes in the mitochondrial dehydrogenase system produced by adenosine (fig.2) were quite probably a consequence of an inhibition in the activation of free fatty acids [10] since both effects were detected simultaneously.

Therefore, it is suggested that in liver mitochondria of the intact rat the fatty acids are the main oxidizable substrate and they play a fundamental role in the maintenance of the mitochondrial redox state. Both suggestions have been reached independently in more simplified systems: Bryla et al. [24] postulated the long-chain fatty acids as the main endogenous respiratory substrates in isolated liver mitochondria and Löffler et al. [21] suggested that the availability of free fatty acids is important in the maintenance of the mitochondrial redox state in perfused rat liver.

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