INCREASE IN HEPATIC NAD LEVEL – ITS EFFECT ON THE REDOX STATE AND ON ETHANOL AND ACETALDEHYDE METABOLISM

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1. Introduction

It is well-known that liver NAD can be increased many times with nicotinamide [1, 2]. This result has been used in two studies concerning regulatory effects of NAD on ethanol metabolism [3, 4]. These investigations showed that the rate of ethanol elimination does not change in animals treated with nicotinamide and the general conclusion was that the availability of NAD does not limit the ethanol oxidation. In one of these studies ethanol metabolism alone was measured, while in the other the total NAD and NADH in the liver were also determined before and during ethanol metabolism. Nothing was known about the cytosolic redox state of the livers under these conditions so that no information about the free NAD/free NADH ratio in this compartment was available and, without this, it is difficult to know anything certain about the availability of NAD for the alcohol dehydrogenase enzyme.

This study was undertaken to establish whether an increased total level of NAD in the liver would affect ethanol and acetaldehyde metabolism with respect to the relation between free and total nucleotides. No effects on ethanol metabolism were obtained, but the peripheral blood and the liver acetaldehyde levels were lower in nicotinamide treated rats. Total NAD/total NADH in the freeze-clamped rat livers showed no dependence on the respective ratio of the free cytosolic nucleotides. NAD deficiency may still limit ethanol oxidation in vivo because the increased NAD might not be available as free NAD in the cytosol.

2. Materials and methods

Male rats of Wistar origin, fed with standard laboratory diet and water ad lib to 4–6 months of age, were used. At 12 p.m. one group of rats received nicotinamide 500 mg/kg intraperitoneally. The experiments were started at 8 a.m. on the following day when the water and food were removed. The ethanol dose was 1.18 g/kg given intraperitoneally as a 10% (v/v) solution in saline. Control animals were given the same volume of saline.

In the first part of the investigations blood ethanol and acetaldehyde levels were measured at various times, and in the second part livers were freeze-clamped 2 hr after the ethanol administration. Both these techniques have been described previously [5]. For the calculation of the liver acetaldehyde an interpolation correction was made for the 'spontaneous' acetaldehyde formation from the ethanol present. Lactate, pyruvate and NAD were assayed enzymatically from the freeze-clamped livers by the methods described by Hohorst [6], Bücher et al. [7] and Klingenberg [8] respectively. NADH was assayed from the frozen liver power after treatment with hot alcoho!-KOH as described by Klingenberg [9]. Enzymes and coenzymes were supplied by C.F. Boehringer (Mannheim, West Germany).

3. Results and discussion

3.1. *Relation between total NADH/total NAD and cytosolic free NADH/free NAD ratios before and during ethanol metabolism*

Ethanol caused a decrease in total hepatic NAD

Table 1

Total NADH/total NAD and cytoplasmic free NADH/free NAD* ratios before and during ethanol metabolism in livers of nicotinamide-treated rats**.

Animals	NADH nmole/g	NAD nmole/g	$\frac{NADH}{NAD} \times 10^4$	Lactate nmole/g	Pyruvate nmole/g	$\frac{Lactate}{Pyruvate}$	$\frac{Free\ NADH}{Free\ NAD} \times 10^4$
Control	142 ± 25	822 ± 81	1750 ± 410	1730 ± 475	189 ± 113	12 ± 9	14 ± 10
Control + ethanol	220 ± 20	746 ± 65	2970 ± 450	1330 ± 450	29 ± 4	47 ± 11	52 ± 12
NA***	136 ± 33	3410 ± 951	444 ± 200	791 ± 234	66 ± 37	14 ± 5	15 ± 5
NA + ethanol	204 ± 63	3380 ± 357	616 ± 224	1250 ± 293	25 ± 7	55 ± 26	61 ± 29

* The cytoplasmic free NADH/free NAD ratio is calculated from the lactate/pyruvate ratio, with which it is in equilibrium, by using the value of 1.11×10^{-4} for the equilibrium coefficient of lactate dehydrogenase [10].

** Metabolite concentrations are expressed per liver wet wt. Averages ± S.D. of four animals are presented (except the control groups, where averages ± S.D. of eight animals were used for total NAD and NADH).

*** NA refers to nicotinamide-treated animals.

with a consequent rise in NADH in all rats (table 1). These effects in the total nucleotides are reflected in the ratio between the free nucleotides. The increase in both the total and free NADH/NAD ratios during ethanol metabolism is firmly supported by earlier investigations [11, 12].

The rats receiving nicotinamide showed a 5-fold increase in total liver NAD but no change in NADH (table 1). In contrast to the nicotinamide-induced increase in NAD, results of nicotinamide effects on the NADH level are quite few.

In a paper by Gibb and Brody [13], female rats of Sprague-Dawley or Holtzmann strain treated with nicotinamide, which starts 24 hr before sacrifice, exhibit an increase in hepatic NADH. Such an increase in NADH is also reported by Lagunas et al. [14] in rats injected 4 and 6 hr before sacrifice with the same dose of nicotinamide as was used here. There was also an increase in the cytoplasmic free NAD/free NADH ratio in freeze-clamped livers but not in liver slices. In our work, however, the cytoplasmic free NADH/free NAD ratio calculated from the lactate/pyruvate ratio showed no response to the increased total NAD either before or during ethanol metabolism. In fact the ethanol-induced increased cytosolic free NADH/free NAD ratio was slightly higher in the nicotinamide rats than in the controls. The discrepancy between the results reported here and those of Lagunas et al. could be due to several factors, including different strains, sexes, nutritional states and intervals between nicotinamide injection and sacrifice. Changes in the redox state of the liver are surely sensitive to differences in nutritional states caused by different feeding habits during the experimental period. The timing of the experiments of Lagunas et al. in relation to the feeding/sleeping cycle was not reported, which makes it difficult to compare their results to those reported here.

The results from these experiments can be interpreted in two ways. Either NAD accumulated in some form in a compartment other than the cytosol (presumably in the nucleus were the final step of the biosynthesis of NAD takes place [15], or the increased NAD liberated bound NADH in the cytosol, which would keep the ratio between the free nucleotides unchanged.

3.2. *Regulation of ethanol and acetaldehyde metabolism*

The rate of ethanol oxidation in the liver cytosol can be modified primarily by the enzyme activity, acetaldehyde/ethanol ratio and free NADH/free NAD ratio in this cell compartment. The dominating role of the nucleotide ratio is supported by findings in which the oxidation of NADH, generated during ethanol metabolism, has been proposed as the main regulator of the ethanol oxidation rate [16, 17]. More directly, Hillbom, in changing the ethanol oxidation rate by different drugs, showed that the oxidation rate negatively correlated with the cytosolic free NADH/free NAD ratio and was independent of the alcohol dehydrogenase activity [18].

The mechanism by which the free nucleotide ratio modifies the rate may be a result of NAD deficiency. The regulation should then take place in the first step

of the ethanol oxidation sequence, when NAD bonds to the alcohol dehydrogenase. No effect on ethanol elimination was obtained by increasing the hepatic NAD with nicotinamide (fig. 1). These results agree with earlier reports, in which it was concluded that NAD deficiency does not limit ethanol oxidation [3, 4]. However, from these results showing no relation between total and free cytosolic nucleotides, it cannot be excluded that the nicotinamide-induced NAD increase is not available for the cytosolic alcohol dehydrogenase reaction. On the other hand, it is difficult to understand that the freshly synthesized NAD is stored in some other compartment without equilibrating throughout the cell.

Blood acetaldehyde in the nicotinamide-treated rats was significantly lowered during ethanol metabolism (fig. 1). The freeze-clamped livers showed the same significant differences; 130 ± 51 nmoles/g (n = 8) for the control rats and 71 ± 19 nmoles/g of wet wt liver (n = 4) for the nicotinamide-treated rats. Previous studies with 'drinking' and 'non-drinking' rat strains have shown that at least enzyme activity may regulate the hepatic acetaldehyde oxidation during ethanol metabolism [5]. Other recent investigations also indicate the importance of the intra-mitochondrial redox state [19] and NADH re-oxidation [20]. Assuming that the cell compartment where acetaldehyde is oxidized (mainly the mitochondria [17, 21] is supplied with some of the increased NAD, then the lowered acetaldehyde level in the liver and blood of rats treated with nicotinamide suggests that NAD deficiency is one factor limiting the rate of hepatic acetaldehyde oxidation. Alternatively, instead of a difference in the rate of acetaldehyde oxidation the lower acetaldehyde levels in the nicotinamide-treated rats could be due to the slightly decreased ethanol oxidation rate (= acetaldehyde formation rate) which was found in these animals (fig. 1).

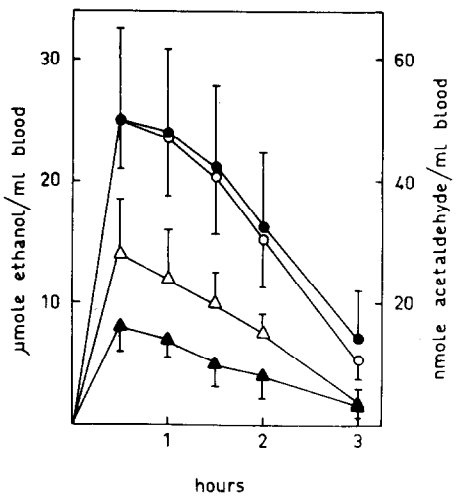

Fig. 1. Ethanol (○, ●) and acetaldehyde (△,▲) in blood after i.p. ethanol injection of 1.18 g/kg. Nicotinamide-treated rats (●,▲) and control rats (○, △).

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