EFFECT OF A TRANSAMINASE INHIBITOR ON THE TRANSPORT OF CYTOSOLIC REDUCING EQUIVALENTS INTO MITOCHONDRIA

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SUMMARY

The effects of a transaminase inhibitor, cycloserine (CS), on extra- and intramitochondrial redox state were studied in perfused rat liver. The addition of CS induced a rapid increase in perfusate lactate/pyruvate (L/P) ratio indicating a shift towards reduction in the cytosolic NAD(H) pool. The flavoprotein absorbance, measured from a lobe of liver, increased simultaneously indicating an oxidation of the intramitochondrial redox state. CS potentiated the effects of ethanol on the cytosolic redox state (L/P ratio), but decreased the ethanol-induced reduction of intramitochondrial flavoproteins. The results can be interpreted to mean that transamination is of importance in the transport of reducing equivalents across the mitochondrial membrane.

There are several metabolic processes in the liver which require transport of reducing equivalents into and out of the mitochondria. However, the mitochondrial membrane is relatively impermeable to NADH (1); so alternative shuttle mechanisms have been proposed which transfer reducing equivalents through the mitochondrial membrane. According to present knowledge, the most important of these mechanisms is the malate-oxaloacetate (mal-OAA) shuttle (2-7). Because of the low permeability of the mitochondrial membrane to OAA (8), transamination of OAA to aspartate is necessary for the functioning of the shuttle (2,3,7). There are numerous studies on isolated mitochondria, indicating that transamination is important in transfer of hydrogen (3,6,7). However, the evidence about the role of transamination in intact tissue is still scanty (5). In addition, an argument has been presented against the operation of the mal-OAA shuttle. In the "up -hill" transport of reducing equivalents into the mitochondria there seems to be

energy dependence which cannot be explained by the mal-OAA shuttle mechanism (9). However, the effects of a transaminase inhibitor on the redox homeostasis of intact liver could elucidate the importance of transamination in the transport of reducing equivalents.

MATERIAL AND METHODS

Male albino rats (150 to 250 g) of the Wistar strain were used in all experiments. They received ordinary laboratory diet ad libitum. No fasting period preceded the experiments.

Livers were perfused with haemoglobin-free Krebs-Ringer bicarbonate solution (10) using an apparatus which has been described earlier (10-12). The lactate/pyruvate (L/P) concentration ratio in the perfusion medium was used as an index of the redox state of the NAD/NADH couple in the extramitochondrial cytosolic compartment of the liver cell (13). The intramitochondrial redox state was measured by recording the changes in the absorbance of mitochondrial flavoproteins from an area of the liver 2 mm thick with a dual wavelength spectrophotometer (14). The CS- and ethanol-induced changes were compared to those induced by anoxia which completely reduced all the flavoproteins.

Cycloserine (CS) was used as a transaminase inhibitor. It blocks the pyridoxalphosphate group in the active site of transaminases (15). CS concentration in the perfusion medium was 20 mM, this nearly totally inhibited transamination in vitro, but had no effect on the activities on lactate dehydrogenase, malate dehydrogenase or alcohol dehydrogenase.

Lactate and pyruvate concentrations in the perfusion medium were determined enzymatically (16,17).

RESULTS AND DISCUSSION

At first we studied the effect of CS on the L/P ratio in perfused livers from normal rats 2 mM lactate as a substrate which was added at the start of perfusion. These results are seen in the upper part of Fig. 1. The L/P ratio

was greatly increased by CS. The centre section of Fig. 1 shows that CS induced an increase in flavoprotein absorbance, which indicates flavoprotein oxidation. Thus, CS induced a reduction of the cytosolic redox state but an oxidation of the mitochondrial redox state. So CS seems to affect the regulation of intracellular redox homeostasis. No noticeable changes were seen in the oxygen

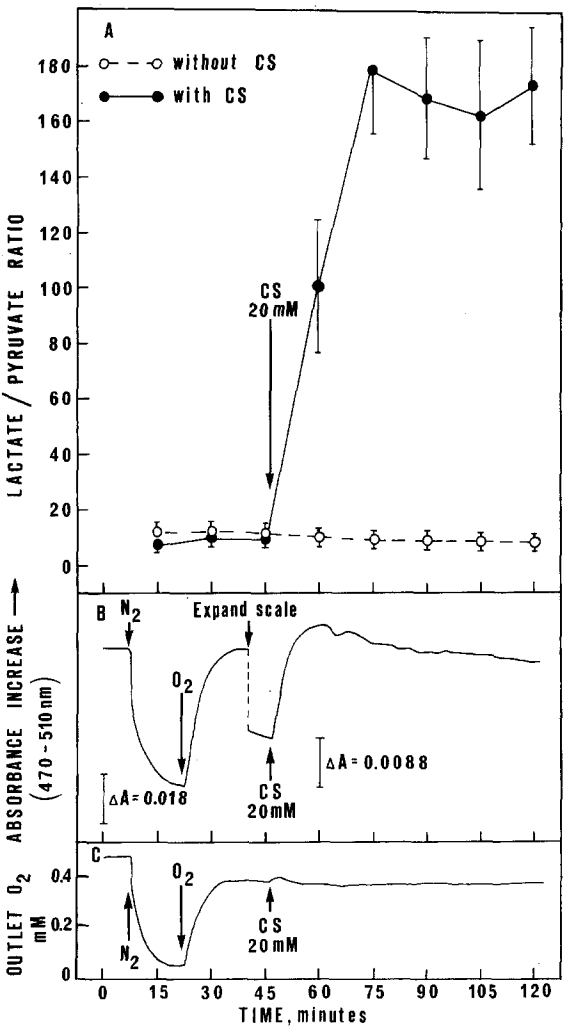

Fig. 1. Effect of cycloserine on the perfusate lactate/pyruvate ratio, flavoprotein absorbance and oxygen consumption in perfused livers from fed rats. Livers were perfused with Krebs-Ringer bicarbonate solution (pH 7.4, at 35°, flow rate 5 ml/min/g of liver wet wt). The absorbance of flavoproteins was measured with a dual wavelength spectrophotometer from a lobe of the liver. An upward deflection means oxidation of the flavoproteins. Oxygen tension in the outlet cannula was measured with an oxygen electrode. The "arterial" oxygen concentration was 1.01 mM, except in the cycle of anoxia. Lactate/pyruvate ratios were measured from 5 to 8 different perfusions.

consumption of the liver after the addition of CS (Fig. 1 the lower part). This suggests that the effects of CS on extra- and intramitochondrial redox state were not attributable to interference in the reactions of the electron tranfer chain.

The oxidation of ethanol in the liver is a typical metabolic situation, in which the transport of reducing equivalents from the cytosol into the mitochondria is necessary (18,19). The production of NADH in the cytosol after the

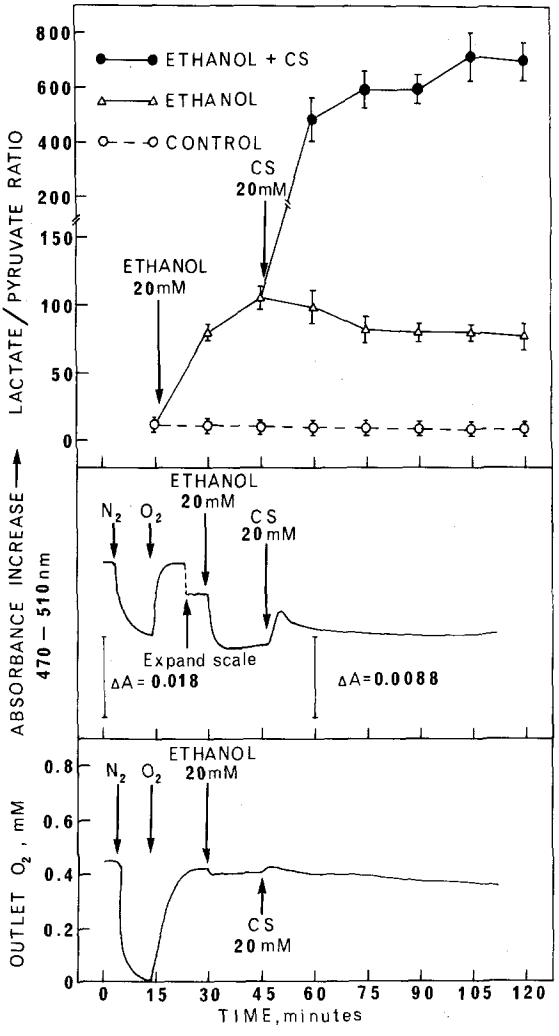

Fig. 2. Effects of ethanol and cycloserine (CS) on perfusate lactate/pyruvate ratio, flavoprotein absorbance and oxygen consumption in the livers from fed rats. Experimental conditions were as in Fig. 1. Lactate/pyruvate ratios are means ± SEM of 5 - 8 different perfusions.

addition of ethanol was reflected by an increase in the perfusate L/P ratio (Fig. 2 the upper part). Also the intramitochondrial redox state was reduced during ethanol oxidation as indicated by the decrease in flavoprotein absorbance (Fig. 2 the centre section). When CS was added after ethanol the L/P ratio was further increased but flavoproteins were oxidized (Fig. 2). These changes may indicate that the transport of reducing equivalents from the cytosol into the mitochondria is impaired by CS. Fig. 3 supports this view. In these experiments CS was added to the perfusion medium before ethanol. CS significantly decreased the ethanol-induced reduction of intramitochondrial flavoproteins. Williamson et al. have found a similar effect after addition of butylmalonate which inhibits the function of the mal-OAA shuttle by blocking malate transport (4).

The present reselts clearly show that CS potentiates the effects of ethanol on the redox state of the cytosolic NAD/NADH couple and diminishes

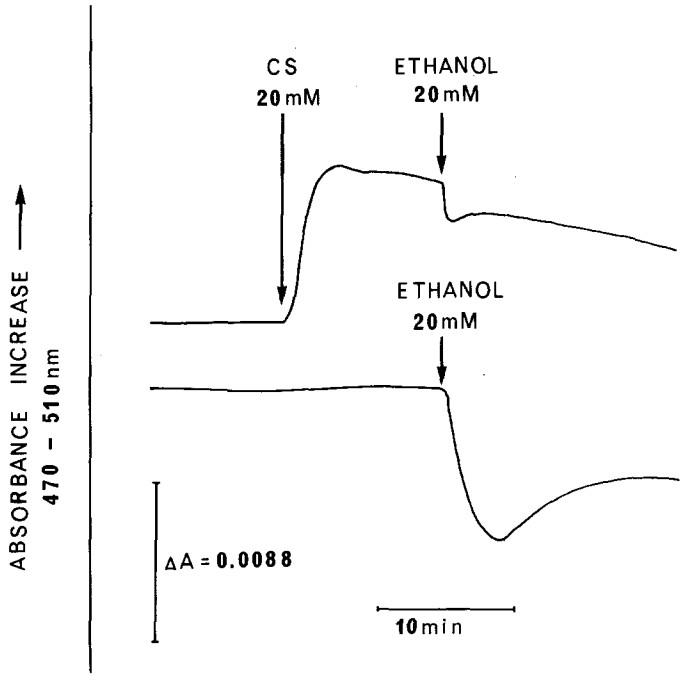

Fig. 3. Effect of cycloserine on ethanol-induced changes in flavoprotein absorbance in perfused livers from fed rats. Experimental conditions were as in Fig. 1. Results are means from 3 different perfusions.

the ethanol-induced reduction of intramitochondrial flavoproteins. The most probable explanation for these findings is that CS inhibits the transfer of reducing equivalents produced in the cytosol into the mitochondria. Because CS is a transaminase inhibitor (15), it can be assumed that the inhibition of hydrogen transfer by CS is due to the inhibition of the transamination step involved in the mal-OAA shuttle.

CS increased the L/P ratio and oxidized flavoproteins also in the livers of fed rats perfused without ethanol. This is slightly surprising, for it is usually assumed that there is normally an outward flow of reducing equivalents from the mitochondria for cytosolic reductive syntheses e.g. gluconeogenesis (4,5). If this flow were blocked, the L/P ratio ought to be decreased. However, CS is also an inhibitor of pyruvate carboxylase (15), which is one of the key enzymes of gluconeogenesis (20,21). Thus, gluconeogenesis is inhibited and the need for reducing power in the cytosol is decreased. In addition, glycolysis seems to be activated, as the sum of lactate and pyruvate was increased by 150 - 300 % after the addition of CS. Activated glycolysis produces NADH, which must be transported into the mitochondria. On this basis it is possible to explain the redox effects of CS also in the livers perfused without ethanol.

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