Metabolic Changes after Prior Treatment with Ethanol

EVIDENCE AGAINST AN INVOLVEMENT OF THE $\text{Na}^+ + \text{K}^+$ -ACTIVATED ATPase IN THE INCREASE IN ETHANOL METABOLISM

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In perfused rat liver, the inhibition of ethanol uptake by ouabain does not follow the rapid inhibition of the $\text{Na}^+ + \text{K}^+$ -activated ATPase as assessed by changes in perfusate $[\text{K}^+]$ (half-time, $t_{1/2} = 2\text{--}3\text{ min}$), but correlates rather with the slow inhibition of oxygen uptake (maximal inhibition = 40% in 20 min). The data indicate that ouabain exerts its effect on ethanol metabolism via the following sequence of events: inhibition of the sodium pump is followed gradually by a perturbation of the intracellular cation milieu; this leads to an inhibition of the mitochondrial respiratory chain, resulting in a diminished rate of NADH oxidation, which in turn causes an inhibition of ethanol metabolism.

When rats are given ethanol their ability to oxidize it increases markedly (Hawkins & Khanna, 1966; Videla *et al.*, 1973). This phenomenon has also been observed in some alcoholic humans (Mendelson & Mello, 1961) and has been studied extensively in liver slices (Videla *et al.*, 1973) and perfused rat livers (Thurman *et al.*, 1976; Thurman & Scholz, 1976). Bernstein *et al.* (1973) and Thurman *et al.* (1976) have demonstrated that ouabain, an inhibitor of the $\text{Na}^+ + \text{K}^+$ -activated ATPase (sodium pump), diminishes the increased ethanol utilization of liver slices caused by prior treatment with ethanol, as well as the increased hepatic oxygen uptake. They concluded that the sodium pump is involved in the mechanism of this phenomenon in the following manner: a stimulation of Na^+/K^+ transport owing to ethanol feeding in the liver would utilize ATP and subsequently increase the rate by which NADH is re-oxidized by the mitochondrial respiratory chain. This in turn would enhance the dissociation of the alcohol dehydrogenase–NADH complex, which is the rate-limiting step in the alcohol dehydrogenase reaction (Theorell & Chance, 1951). Such a hypothesis, however, rests on the fact that ouabain is a specific inhibitor of the sodium pump and on the assumption that secondary effects, e.g. cation accumulation in the cell, are not responsible for the metabolic changes after addition of ouabain. The purpose of the experiments described below was therefore to evaluate if the action of ouabain on ethanol uptake is due to the decreased energy demand for the sodium pump.

Methods and Materials

Male Wistar rats (150–180 g) receiving a chow diet *ad libitum* were treated with ethanol (20%, w/v) in a 25% (w/v) sucrose solution for 3–5 weeks (Porta *et al.*, 1968). Control animals received a 25% sucrose solution. The perfusion technique has been described elsewhere (Scholz *et al.*, 1973). The perfusion fluid was Krebs–Henseleit bicarbonate buffer, pH 7.4, saturated with O_2/CO_2 (19:1). The perfusate flowed past an oxygen electrode before it was discarded. Ethanol was infused into the perfusion fluid entering the liver, whereas ouabain was dissolved directly in the perfusion fluid. Samples of effluent perfusate were collected every 1 or 2 min and were analysed for ethanol, glucose, lactate and pyruvate enzymically (Bergmeyer, 1970). Rates of ethanol and oxygen uptake were calculated from the influent-minus-effluent concentration difference and the flow rate, and were referred to the liver wet weight. The potassium concentration in the perfusate was determined by flame photometry. Redox changes in mitochondrial flavin and mitochondrial and cytosolic nicotinamide nucleotides were monitored by surface fluorimetry (Scholz *et al.*, 1969). Enzymes, coenzymes and ouabain were purchased from Boehringer, Mannheim, Germany. All chemicals were reagent grade.

Results

The time course of a perfusion experiment with the isolated liver from a fed rat is shown in Fig. 1.

Fig. 1. *Effect of ouabain on metabolic rates and perfusate concentration of K^+ ions in perfused rat liver*

Perfusate analyses and original trace of the venous oxygen concentration are shown from a representative experiment ($n=4$) with the isolated liver from a fed rat perfused in a non-recirculating system. The infusion of ethanol (1 mM) and the addition of ouabain (1 mM) are indicated by horizontal bars. Perfusate samples were collected at 1 min intervals. Rates of glucose production (■), lactate + pyruvate production (▲), ethanol uptake (●) and oxygen uptake (trace) were calculated from the arterio-venous concentration differences and the constant flow rate. Concentrations of K^+ ions in the influent (dashed line) and effluent perfusate (○) are also shown.

After the infusion of ethanol (1 mM) a 12% increase in oxygen uptake was observed. Simultaneously, the rate of glycolysis (lactate + pyruvate production) was diminished by 60%, whereas glucose production from glycogen was increased. The adenine nucleotide-dependent interaction between glycolysis and the mitochondrial respiratory chain has been described as an explanation for the phenomenon of stimulated respiration owing to the redox-mediated inhibition of glycolysis by ethanol (Thurman & Scholz, 1977). Under these conditions, rates of ethanol uptake of about $110 \mu\text{mol} \cdot \text{h}^{-1} \cdot \text{g}^{-1}$ were observed.

After the addition of ouabain (1 mM), a rapid but transient (half-time, $t_{1/2} = 2-3$ min) increase in perfusate K^+ concentration of about 0.3 mequiv./litre was observed (Fig. 1), owing to the efflux of K^+ from the hepatocyte. After 10 min, K^+ concentrations tended to return to the concentration in the influent perfusate. The K^+ efflux during a 36 min infusion period with ouabain was about $20 \mu\text{equiv./g}$ of liver. At 8–10 min after the changes in K^+ concentration became detectable, the rate of oxygen uptake began to decline. Maximal inhibition of more than 40% was reached after a further 20 min. With a similar time course, rates of ethanol uptake were

decreased by 60%, whereas rates of glycolysis and glycogenolysis were increased. When the infusion of ouabain was terminated, a rapid but transient decrease in the K^+ concentration was observed. The influx of K^+ calculated over a 30 min period was less than $10\mu\text{equiv./g}$. After a time lag, the rates of ethanol and oxygen uptake slowly returned to the original values.

After the infusion of ethanol, a reduction of flavin

and nicotinamide nucleotides was detected by surface fluorimetry as reported previously (Williamson *et al.*, 1969). After new steady-state conditions were reached, ouabain was added (Fig. 2). Initially, ouabain had no effect on these parameters. However, 5–8 min later, when the rate of oxygen uptake began to decline, a further reduction of both flavin and nicotinamide nucleotides was observed which had a time course similar to the changes in respiration. These phenomena were observed in livers from both ethanol-treated and control rats, although rates of oxygen and ethanol uptake were over 50% greater as a result of treatment with ethanol (results not shown).

When the experimental protocol described in Fig. 1 was performed with 0.1 mM-ouabain, no effects on uptake of oxygen and ethanol and on glycolysis and glycogenolysis were observed (results not shown). On the other hand, the K^+ concentration changed with a time course similar to that observed with 1 mM-ouabain; the potassium efflux over 36 min of ouabain perfusion, however, was less than $7\mu\text{equiv./g}$ (i.e. about 30% of the efflux observed with 1 mM-ouabain).

Discussion

In the last decade, evidence has accumulated to support the hypothesis that ethanol oxidation is regulated indirectly by the rate of various ATP-requiring processes in the liver (Scholz & Nohl, 1976). The coupling between ATP utilization and generation controls the respiratory rate, and consequently the rate of re-oxidation of NADH. Changes in the demand for ATP could therefore be followed by changes in the cytosolic NADH concentration, which would lead to changes in the rate of ethanol oxidation. Furthermore, chronic treatment with ethanol results in an enhancement of hepatic uptake of both oxygen and ethanol (Videla *et al.*, 1973; Thurman *et al.*, 1976), suggesting that an increase in some ATP-utilizing system(s) is the primary event. One of the first adaptations thought to be responsible for this enhanced ethanol metabolism is an increase in the activity of the sodium pump, from the observation that rubidium transport is greater and utilization of ethanol and oxygen is more sensitive to ouabain in liver slices and perfused liver from ethanol-treated rats than in controls (Bernstein *et al.*, 1973; Thurman *et al.*, 1976).

The experiments with perfused rat livers described in the present paper, however, indicate that the action of ouabain (1 mM) is not restricted to an inhibition of ATP-dependent Na^+ and K^+ transport. The kinetics of inhibition of uptake of oxygen and ethanol, as well as the kinetics of the redox changes and the stimulation of glycolysis, were clearly slower than the effects of ouabain on the

Fig. 2. Effect of ouabain on surface fluorescence of flavin and nicotinamide nucleotides and on oxygen uptake. Nicotinamide nucleotide fluorescence (excitation 366 nm, emission 450 nm) and flavin fluorescence (excitation 436 nm, emission 570 nm) were determined simultaneously from the surface of the perfused liver. The effluent oxygen concentration was determined polarographically and was converted into rates of oxygen uptake (ordinate, lower panel). The onset of ouabain infusion (1 mM) is indicated by the arrow. Original traces are shown from a representative experiment, which has been repeated six times.

sodium pump. Most likely, an inhibition of the sodium pump causes a change in the internal cation milieu of the liver cell. This in turn, after a time lag, when a critical alteration of the ionic composition has been achieved, affects metabolic flux rates. The slight inhibition of the sodium pump by 0.1 mM-ouabain, however, was apparently not sufficient to change the internal ionic milieu considerably, since the metabolic parameters were not affected.

The changes in the mitochondrial redox state as reflected by flavin fluorescence also indicate that the respiratory chain was inhibited gradually in the presence of ouabain. It is therefore unlikely that a diminished ATP utilization after addition of ouabain (e.g. owing to a diminished demand of ATP for the sodium pump) is the primary event responsible for the decreased rate of oxygen uptake. This inhibition of the respiratory chain, however, has to be indirect, since respiration of isolated mitochondria was unaffected by ouabain (results not shown).

In summary, our data suggest that, as a result of the rapid inhibition of the sodium pump by ouabain, metabolic processes are affected progressively by changes in the internal cation milieu. The time course of the inhibition of ethanol utilization was similar to the decline in respiration, changes in the redox state and stimulation of glycolytic flux. We conclude, therefore, that it is the non-specific inhibition of the respiratory chain that is responsible for the ouabain-sensitivity of ethanol oxidation. This casts doubts on the hypothesis that the primary event in the liver responsible for the increase in ethanol metabolism after ethanol treatment is an increased activity of the sodium pump.

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