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Factors Influencing Rates of Ethanol Oxidation in Isolated Rat Hepatocytes

KATHRYN E. CROW, KATHRYN M. NEWLAND AND RICHARD D. BATT

Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand

CROW, K. E., K. M. NEWLAND AND R. D. BATT. *Factors influencing rates of ethanol oxidation in isolated rat hepatocytes.* PHARMACOL BIOCHEM BEHAV **18:** Suppl. 1, 237–240, 1983.—The stimulation of ethanol oxidation by fructose which has frequently been observed in isolated hepatocytes was found to occur only in unsupplemented cells. In the presence of other substrates (lactate, pyruvate) which accelerate ethanol oxidation, fructose had no additional effect. Acceleration of ethanol oxidation by fructose was not directly related to the ATP demand created by fructose. The effects of fructose on ethanol oxidation rates were not due to changes in acetaldehyde concentration. In cells from fed animals, acetaldehyde concentrations rose as high as 200 μM in some incubations, and therefore became a significant factor limiting ethanol oxidation rates. In hepatocytes isolated from starved rats incubated with pyruvate, where acetaldehyde concentrations were very low, (1–2 μM) it was possible to assess the effect of changes in [lactate]/[pyruvate] (and hence free cytosolic NADH) on rates of ethanol oxidation. The results showed that the increase in free cytosolic [NADH] usually found during ethanol oxidation *in vivo* would inhibit rates of ethanol clearance by a maximum of 20%.

Isolated rat hepatocytes Rates of ethanol oxidation Fructose effect Acetaldehyde Lactate/pyruvate ratio

THE effects of fructose, concentrations of acetaldehyde, and changes in cytosolic redox state have been measured in isolated rat hepatocytes metabolising ethanol.

METHOD

Hepatocytes were isolated from the livers of male Sprague-Dawley rats using the method of Berry and Friend [2] as modified by Cornell *et al.* [8]. Incubations were carried out as described previously [8, 9, 14]. Ethanol was assayed by a modification (N. W. Cornell, personal communication) of the method of Dickinson and Dalziel [11]. Other metabolites were assayed by the following procedures: acetaldehyde [26]; pyruvate [20]; lactate [20]; glucose [1]; and ATP [16, 21].

RESULTS AND DISCUSSION

1. The Effect of Fructose on Ethanol Oxidation Rates

It has often been reported that fructose causes marked acceleration of ethanol oxidation in perfused liver and isolated hepatocytes [3, 10, 13, 15, 24]. However, administration of fructose *in vivo* gives little, if any, acceleration of ethanol metabolism [6, 12, 17, 19, 22, 25, 27]. The results in Table 1 show that ethanol oxidation rates in isolated hepatocytes are: (a) much lower in unsupplemented cells than *in vivo* (4.0 μmoles per minute per g, [4]; (b) accelerated by fructose in cells from fed or starved rats; (c) also accelerated by lactate or pyruvate and (d) not further increased by fructose in the presence of lactate or pyruvate. These results explain the differing effectiveness of fructose *in vivo* and *in vitro*. Low ethanol oxidation rates in unsupplemented cells are due to metabolite depletion during cell preparation [7]. When rates are restored to the levels found *in vivo* by addition of other substrates, fructose becomes ineffective.

It has been suggested previously [23,24] that fructose accelerates ethanol oxidation by increasing the rate of intramitochondrial reoxidation of NADH due to an increased ATP demand for glucose synthesis. Table 2 shows that the ATP concentration is decreased and glucose synthesis increased in all incubations containing fructose. This indicates that the rate of intramitochondrial reoxidation of NADH is increased in the presence of fructose. However, as shown in Table 1, the presence of fructose did not result in increased rates of ethanol oxidation in all incubations. These results indicate that the rate of intramitochondrial reoxidation of NADH is not a limiting factor for ethanol oxidation.

2. Acetaldehyde Concentrations During Ethanol Oxidation

Acetaldehyde concentrations were measured at 20, 40 and 60 minute intervals during incubation of hepatocytes with various substrate combinations (Table 3). The results show that acetaldehyde concentrations were consistently higher in cells from fed rats than in those from starved rats. The maximum concentrations observed (~200 μM) were much lower than those previously reported by Lindros [18] for cells isolated from fed rats. The high concentrations observed by Lindros were probably due to an inhibitor of aldehyde dehydrogenase in the rats diet [18]. The acetaldehyde concentrations observed in several of the present experiments would be sufficiently high to cause inhibition of rat liver alcohol dehydrogenase [7]. However, differences found in acetaldehyde concentrations could not provide an explanation of the fructose effect, since incubations containing fructose had either the same or higher acetaldehyde concentrations than those without. Acetaldehyde concentrations decreased with increasing incubation time, and varied widely between individual cell preparations. In these re-

TABLE 1

EFFECT OF FRUCTOSE, LACTATE AND PYRUVATE ON ETHANOL OXIDATION RATES IN HEPATOCYTES ISOLATED FROM FED AND STARVED RATS

	Ethanol oxidation rates‡ (μmol per min per g wet wt liver)			
	Fed rats		Starved rats	
Substrates*	−Fructose	+ Fructose	− Fructose	+ Fructose
Ethanol only	1.87 ± 0.13	2.54 ± 0.18	1.35 ± 0.15	3.5 ± 0.16
Ethanol + lactate	2.59 ± 0.15	2.61 ± 0.14	3.01 ± 0.15	3.6 ± 0.07
Ethanol + pyruvate	2.98 ± 0.17	2.83 ± 0.14	4.07 ± 0.08	4.01 ± 0.13

*Concentrations of substrates: ethanol, 10 mM; lactate, 10 mM; pyruvate, 5 mM; fructose, 10 mM.
‡Results are means±SEM for 6 (starved) or 9 (fed) experiments.

TABLE 2

GLUCOSE SYNTHESIS RATES AND ATP CONCENTRATIONS

	Fed		Starved	
Substrates*	− Fructose	+ Fructose	− Fructose	+ Fructose
	ATP† (μmol per g wet weight liver)			
Ethanol only	1.72 ± 0.07	0.54 ± 0.08	1.95 ± 0.07	0.64 ± 0.03
Ethanol + lactate	2.02 ± 0.05	0.60 ± 0.06	2.10 ± 0.09	0.55 ± 0.05
Ethanol + pyruvate	2.00 ± 0.05	0.70 ± 0.08	2.01 ± 0.12	0.66 ± 0.08
	Glucose Synthesis‡ (μmol per min per g wet weight liver)			
Ethanol only	1.13 ± 0.15	2.57 ± 0.30	0.04 ± 0.02	2.69 ± 0.17
Ethanol + lactate	1.43 ± 0.17	3.02 ± 0.19	0.33 ± 0.03	2.75 ± 0.16
Ethanol + pyruvate	1.59 ± 0.19	2.65 ± 0.29	0.94 ± 0.02	3.00 ± 0.24

*Concentrations of substrates: ethanol, 10 mM; lactate, 10 mM; pyruvate, 5 mM; fructose, 10 mM.
†Results are means±SEM for 6 (starved) or 9 (fed) experiments.
‡Results are means±SEM for 3 (starved) or 4 (fed) experiments.

TABLE 3

ACETALDEHYDE CONCENTRATIONS*

		Acetaldehyde concentration (μM)†		
Time (min)	Ethanol	Ethanol + fructose	Ethanol + pyruvate	Ethanol + fructose + pyruvate
Fed rats				
20	0	100 ± 15	194 ± 27	219 ± 26
40	0	45 ± 13	179 ± 22	194 ± 28
60	0	26 ± 12	111 ± 15	98 ± 20
Starved Rats				
20	<1	14 ± 6	2.3 ± 0.2	85 ± 29
40	<1	7 ± 2	1.1 ± 0.4	70 ± 26
60	<1	3 ± 0.6	1.3 ± 0.1	17 ± 8

*Substrate concentrations as for Tables 1 and 2.
†Results are means±SEM for 5 (starved) or 6 (fed) experiments.

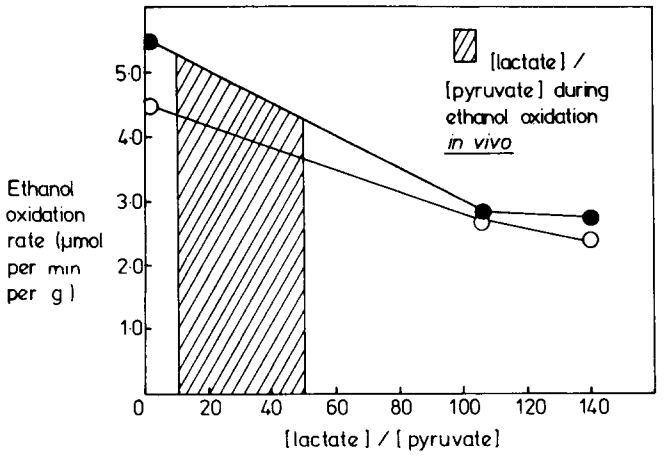

FIG. 1. Rates of ethanol oxidation and [lactate]/[pyruvate]. Experimental rates of ethanol oxidation (●) were obtained from 20, 40, and 60 minute incubations of hepatocytes from starved rats in the presence of 5 mM pyruvate. Theoretical values (○) were calculated using published kinetic parameters for rat liver alcohol dehydrogenase [7], [NADH] calculated from experimental [lactate]/[pyruvate] assuming free [NAD^+] to be 0.5 mM, and experimental [ethanol] and [acetaldehyde].

spects, the results agree with those reported previously for perfused liver [5] or for rats *in vivo* [4]. These results show that it is essential to measure acetaldehyde in experiments where isolated hepatocytes are used to study factors affecting rates of ethanol oxidation.

3. Changes in Cytosolic Redox State

In hepatocytes isolated from starved rats and incubated with pyruvate, acetaldehyde concentrations were consistently very low (Table 3), so that it was possible to directly assess the interaction between cytosolic redox state and rates of ethanol oxidation without the complicating factor of acetaldehyde inhibition. Figure 1 shows that as the [lactate]/[pyruvate] ratio increases, reflecting an increase in free cytosolic [NADH], the rate of ethanol oxidation decreases. The experimentally determined ethanol oxidation rates agree reasonably well with rates calculated using kinetic parameters for rat liver alcohol dehydrogenase [7]. The shaded area on the figure indicates the change in [lactate]/[pyruvate] that occurs during ethanol oxidation *in vivo* [4]. With the maximum change *in vivo* (from about 10 in the absence of ethanol to 50 in the presence of ethanol) the rate of ethanol oxidation is inhibited by only 20%. From this, it can be concluded that the maximum increase in the rate of ethanol oxidation that could be obtained by increasing rates of NADH reoxidation *in vivo* should be about 20%. In other words, as is also suggested by the results of the fructose experiments in part 1, the rate of reoxidation of NADH is not a major factor limiting the rate of ethanol oxidation in rats *in vivo*. In a recent paper in which rates of ethanol clearance were carefully measured in human subjects in the presence and absence of fructose [19], an increase in rate of 14% was found in the presence of fructose. This is within the range indicated by the above results, and suggests that findings of the present study using isolated rat hepatocytes may also be applicable to human subjects. If this is the case, there is little point in searching for drugs that will increase rates of ethanol clearance by increasing rates of NADH reoxidation *in vivo*.

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