STIMULATION BY ACETATE OF GLUCONEOGENESIS IN HEPATOCYTE SUSPENSIONS

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1. Introduction

The effects of acetate on tissue metabolism are of interest for a variety of reasons:

(i) Acetate is present in blood [1,2];
(ii) Regulatory effects of long-chain free fatty acids are well-established in the liver, e.g., stimulation of gluconeogenesis [3–6];
(iii) Short-chain fatty acids stimulate gluconeogenesis in the kidney cortex [7];
(iv) Acetate is a key intermediate in the assimilation of ethanol [8–10], which has complex effects on metabolism.

The existence of significant quantities of acetate in blood is of particular interest, its concentration being of the same general order as that of long-chain free fatty acids. Thus blood levels in ruminants and non-ruminants are in the μM to mM range [1,2]. There is about 200 μM acetate in arterial blood of the fed rat and this concentration rises in diabetes and falls on starvation [1,2]. The acetate concentration in the hepatic portal vein of both the fed and starved rat is considerably higher than that in either arterial or hepatic vein blood, whereas in diabetes the concentration of acetate in the hepatic portal vein is similar to that in the hepatic vein but double that of arterial blood [1].

In the light of the above facts, the effect of acetate on hepatic gluconeogenesis has been studied in isolated hepatocytes. A significant and potent stimulation of gluconeogenesis was observed.

2. Materials and methods

Male Porton-Wistar rats (200 g) were starved for 48 h from 10.00 h.

Isolated hepatocytes were prepared by perfusion of the liver with collagenase, essentially as in [11] with minor modifications [12]. No glucose was added to the perfusion fluid. Viability of cells was assessed in several ways [12].

Cells were incubated in duplicate for 40 min at 37°C as in [12]. In one group of experiments phosphorylase *a* activity was measured in the cells after 30 min preincubation with 20 mM glucose and then 2 min with acetate. Further incubation and assay details are described in [12,13] except that in these experiments hepatocytes were homogenised after addition of 1 vol. ice-cold medium containing 100 mM glycylglycine and 200 mM NaF (pH 7.4) and 100 μl sample assayed for phosphorylase *a* activity.

Methods for measuring glucose in the incubation medium [14] and glycogen in hepatocytes [12,14] have been described. Glycogen was determined in hepatocytes before incubation and a value of 4.5 ± 1.3 [10] μmol glycogen-glucose/g dry cells (mean ± SEM) was obtained.

Chemicals were of the highest grade available. Collagenase (Grade II) was from P-L Biochemicals, (Milwaukee, WI 53205).

3. Results

Hepatic gluconeogenesis from lactate was stimulated by acetate over range 100 μM to 5 mM (fig.1). Acetate did not stimulate gluconeogenesis from any other substrate tested, except serine (table 1), where the rate increased by about 1/3 rd and the absolute increment in glucose produced was much less than when lactate was the substrate.

In general, compounds or hormones which stim-

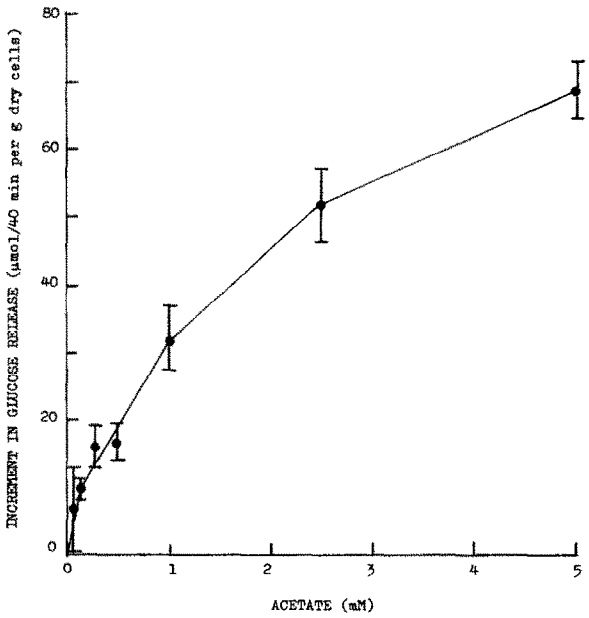

Fig.1. Concentration dependence of the effect of acetate on gluconeogenesis from lactate. Hepatocytes were prepared in the standard way. Lactate (10 mM) and various concentrations of acetate were added at zero time and incubation was for 40 min. Results are expressed as the increase in glucose release, in response to acetate; the rate from lactate alone was 73.6 ± 2.8 μmol/40 min/g dry cells. Results are means ± SEM from 4 cell preparations. Values, where acetate was 0.1 mM or greater, are significantly different from the control value ($P < 0.01$) as assessed by the paired two-tailed 't' test.

ulate hepatic gluconeogenesis exert related catabolic effects; thus they promote glucose release from both glycogen and gluconeogenic sources. Hence it was relevant to test the effect of acetate on glycogen phosphorylase activity. No significant activation of phosphorylase was observed in the presence of 5 mM acetate: control phosphorylase *a* activity was 7.4 ± 0.3 and that in the presence of acetate was 8.8 ± 1.3 μmol/min g dry wt cells (means ± SEM of 4 cell preparations).

4. Discussion

These data show that acetate at low concentrations, such as are present in blood, stimulates hepatic gluconeogenesis specifically from lactate. This effect is as big as that observed with acetate in kidney cortex [7] and is larger than that observed in liver with long-chain fatty acids [5].

Table 1
Stimulation by acetate of hepatic gluconeogenesis from various substrates

Substrate	Glucose release (μmol/40 min/g dry cells)	
	Release due to substrate	Increment in the presence of acetate
Lactate	75.0 ± 4.1	68.3 ± 4.1[a]
Fructose	333.9 ± 23.3	4.9 ± 9.5
Glycerol	112.8 ± 11.7	−3.1 ± 2.9
Serine	39.6 ± 2.7	13.7 ± 3.1[b]
Pyruvate	91.2 ± 5.5	−5.7 ± 3.2

[a] $P < 0.01$ or [b] $P < 0.05$ as assessed by the paired two-tailed 't' test, (significance of the increment due to acetate)

Hepatocytes were prepared in the standard manner. Substrates (10 mM) and acetate (5 mM) were added at zero time and incubation was for 40 min. Results are means ± SEM of 4 cell preparations and are expressed as glucose release in 40 min/g dry wt of cells; the basal glucose release was 15.4 ± 3.4 for the 4 preparations

The concentration of acetate in the hepatic portal vein blood of fed animals is 800 μM which is halved on starvation (probably due to decreased output by the gut flora) but doubled in diabetes [1]. Since the minimum effective concentration of acetate shown to stimulate hepatic gluconeogenesis was only 100 μM it is likely that this stimulation has a role in all the above-mentioned nutritional and pathological states. Depending on circumstances, hepatic gluconeogenesis can contribute to glucose or glycogen production in the 'fed' animal and it is feasible that stimulation by acetate is relevant in this state, particularly in the maintenance of the Cori cycle.

The question arises of the mechanism underlying this stimulation of lactate-dependent gluconeogenesis by acetate. The fact that the stimulation was only obtained with lactate as precursor, and not with pyruvate, strongly suggests that stimulation of the reaction catalyzed by lactate dehydrogenase is implicated. One possibility is that acetate may convert to acetaldehyde through the action of aldehyde dehydrogenase (reverse of the pathway of ethanol metabolism). This would directly oxidise the ratio of (free NAD^+) / (free NADH) in the cytoplasm, according to conventional equilibrium theory applied to

cytoplasmic dehydrogenase reactions; the increase in available NAD^+, a substrate for another cytoplasmic dehydrogenase, i.e., lactate dehydrogenase, would lead to an increase in pyruvate availability for conversion to oxaloacetate. Such a mechanism would be the direct converse of the mechanism whereby ethanol inhibits lactate-dependent gluconeogenesis [15].

Another related possibility is that acetyl CoA production would lead to hepatic fatty acid synthesis (e.g., [16]), thereby lowering NADPH levels in the cytoplasm [17] and indirectly oxidising the NAD^+ couple.

Stimulation by acetate of hepatic gluconeogenesis from serine (present work) or alanine [18], albeit less marked than that of lactate-dependent gluconeogenesis, could imply that the effect of acetate may not solely involve alterations in the cytoplasmic NAD^+ couple. For example, it is possible that oxidation of acetate can redirect lactate from the oxidation pathway, for conversion to glucose, in the manner observed in kidney cortex slices [7]. It was proposed however, that the stimulation of gluconeogenesis in the kidney cortex by short-chain fatty acids was mainly due to activation of pyruvate carboxylase by acetyl CoA [7]. Inhibition of pyruvate oxidation at the dehydrogenase step by acetyl-CoA would also be likely to be involved in such a regulatory response to exogenous short-chain fatty acids.

The stimulation of hepatic gluconeogenesis by long-chain fatty acids has been similarly explained, i.e., activation of pyruvate carboxylase and inhibition of pyruvate dehydrogenase by acetyl CoA [5,6]. Although this mechanism may also partly explain the effect of acetate on hepatic gluconeogenesis it does not confer sufficient specificity, i.e., stimulation from lactate but not from pyruvate. In addition acetyl CoA synthase is considered to be mainly a cytoplasmic enzyme in the rat [2] and so the major site of synthesis of acetyl CoA from acetate would not be mitochondrial.

The effects of acetate may also be compared with those of ethanol. Ethanol in general inhibits hepatic gluconeogenesis [15,19,20] but in some circumstances stimulation has been observed [10,15,19]. It would seem possible that in these instances, ethanol stimulates gluconeogenesis at least partly via acetate, a main intermediate in its assimilation.

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