Consistent with the insulin-releasing action of orally administered glucose (Flatt & Bailey, 1981c), ob/ob mice exhibited a prompt and marked insulin response to voluntary glucose ingestion (upper section, Table 1). The mice consumed 0.34 ± 0.11 g of glucose (mean ± S.E.M.), corresponding to approx. 4 g/kg body wt. Although glucose tolerance was impaired under these conditions, it was considerably better than when half of the dose (2 g/kg body wt.) was administered by intraperitoneal injection (Flatt & Bailey, 1981a,b, 1982a). The lower section of Table 1 shows the responses to voluntary ingestion of different quantities of stock diet. Calculation of the percentage changes from basal values indicates a good correlation between the amounts of food ingested and the percentage plasma glucose responses (25%, 45% and 73% increases for 0.5 g, 1.0 g and 1.5 g of food respectively). However, there was no simple relationship between food consumption and the percentage insulin responses (33%, 80% and 259% for 0.5 g, 1.0 g and 1.5 g of food respectively). The insulin response was proportionally much greater than the increment in food consumption. Indeed, the insulin response to 1.5 g of food was more than 7 times that evoked by ingestion of 0.5 g of food.

The present study demonstrates that voluntary ingestion of glucose or stock diet evokes a marked plasma insulin response in ob/ob mice. The magnitude of the effect on insulin concentrations, combined with the inability of parenteral glucose to stimulate insulin release (Flatt & Bailey, 1981a,b, 1982a), emphasizes the importance of neuroendocrine reflexes generated by feeding in the hyperinsulinaemia of ob/ob mice (Flatt & Bailey, 1981c). The heightened sensitivity of the insulin-releasing mechanism to relatively small increments in food consumption indicates the involvement of the enteroinhibitory axis, and suggests that hyperphagia represents a key to hyperinsulinaemia in ob/ob mice.


The effects of some short-chain fatty acids on pyruvate carboxylase activity in intact isolated rat liver mitochondria

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Pyruvate carboxylase (EC 6.4.1.1) catalyses the formation of oxaloacetate from pyruvate in the mitochondrial matrix:  
\[
\text{CH}_3\text{C}O\text{CO}_2\text{H} + \text{CO}_2 + \text{ATP} \rightarrow \text{HO\textsubscript{C}-\textsubscript{C}-\textsubscript{C}-\textsubscript{C}O\textsubscript{2}\text{H} + \text{ADP} + \text{Pi}
\]

and has a major role in gluconeogenesis. Liver pyruvate carboxylase is allosterically activated by acetyl-CoA, although some mitochondrial, and propionyl-CoA also activate (Scriver & Griffiths, 1981). Some other acyl-CoA esters antagonize activation by acetyl-CoA (see Billington et al., 1978; Sherratt, 1981). Unusual acyl-CoA esters may accumulate in the mitochondrial matrix after the ingestion of certain poisons, or in some inborn errors of metabolism. Hypoglycaemia is often a feature of such disturbed metabolic states, and the present study was to determine whether inhibition of pyruvate carboxylase by some acyl-CoA esters likely to accumulate in these conditions occurs in intact liver mitochondria. Impairment of gluconeogenesis at this stage may be important in the development of hypoglycaemia.

Hypoglycaemia, isovalericacidemia and 2-methylbutyricacidemia occur in hypoglycin poisoning (Tanaka et al., 1972; Sherratt, 1981). Methylene cyclopropylacetoin-CoA, a metabolite of hypoglycin, inhibits some acyl-CoA dehydrogenases in the matrix, causing an accumulation of butyryl-CoA, 3-methylpentyl-CoA (isovaleryl-CoA) and 2-methylbutyryl-CoA, which are then deacetylated to give an organic acidemia. Isovalericacidemia is also found in an inborn error of leucine metabolism, associated with occasional hypoglycaemic crises (Kreiger & Tanaka, 1976). Pent-4-enoate and cyclopropane-carboxylate are structurally related to methylene cyclopropylacetate, but only pent-4-enoate is hypoglycaemic in the rat (Senior & Sherratt, 1969). Administration of large amounts of glycine alleviates the symptoms of hypoglycin poisoning in rats (Al-Bassam & Sherratt, 1981) and of congenital isovalericacidemia (Kreiger & Tanaka, 1976; Cohn et al., 1978). Conjugation of accumulated acyl-CoA esters with glycine is catalysed by glycine N-acetylase (EC 2.3.1.13), located in the mitochondrial matrix after the ingestion of certain poisons, or in some inborn errors of metabolism. Hypoglycaemia is often a feature of such disturbed metabolic states, and the present study was to determine whether inhibition of pyruvate carboxylase by some acyl-CoA esters likely to accumulate in these conditions occurs in intact liver mitochondria. Impairment of gluconeogenesis at this stage may be important in the development of hypoglycaemia.

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Pyruvate carboxylase activity (McClure & Lardy, 1971) was measured in the mitochondrial fractions from rat livers (Holland & Sherratt, 1973). Mitochondria (4–6 mg of protein) were incubated in 2.0 ml of medium containing 120 mm-KCl, 10 mm-Hepes (4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid), 2.5 mm-phosphate and 20 mm-KHCO3 (specific radioactivity 0.5 µCi/mmol), pH 7.6, at 30°C. After 5 min preincubation with the carboxylic acids, the reaction was started with 5 mm-pyruvate, and stopped at 2 min intervals up to 10 min with 0.5 ml of 1.0 M-HClO4; unfixed CO2 was removed by the addition of a small piece of solid CO3. The remaining radioactive pyruvate was determined after acetylation and extraction with hexane (Flatt & Bailey, 1981a,b, 1982a). The rate of CO2 fixation was used to estimate the rate of pyruvate carboxylation. All rates were linear with time and mitochondrial protein concentration, and these are expressed as percentages of the control rates, which were in the range 130–200 mmol/min per mg of protein. When 3-methylcrotonate was added, the results were corrected for CO2 fixation catalysed by 3-methylcrotonyl-CoA carboxylase by using an appropriate blank incubation. 

Table 1. Effect of short-chain fatty acids on the rate of pyruvate carboxylase activity in intact mitochondria

<table>
<thead>
<tr>
<th>Carboxylic acid added (0.2 mM)</th>
<th>Basal medium</th>
<th>5 mm-Glycine</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Methylene cyclopropylacetate</td>
<td>48</td>
<td>77</td>
</tr>
<tr>
<td>Pent-4-enoate</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Cyclopropane-carboxylate</td>
<td>13</td>
<td>51</td>
</tr>
<tr>
<td>3-Methylbutyrate</td>
<td>33</td>
<td>59</td>
</tr>
<tr>
<td>3-Methylcrotonate</td>
<td>61</td>
<td>100</td>
</tr>
<tr>
<td>2-Methylbutyrate</td>
<td>68</td>
<td>100</td>
</tr>
</tbody>
</table>

1983
mitochondrial matrix (Gateley & Sherratt, 1979), and has a low affinity for glycine (apparent \( K_m \) 3.3 mm) (Bartlett & Gompertz, 1974).

Methylene-cyclopropylacetate, pent-4-enoate, cyclopropane-carboxylate, 3-methylbutyrate, 3-methylcrotonate and 2-methylbutyrate inhibited pyruvate carboxylation (Table 1). These inhibitions could be due to antagonism of the activation of pyruvate carboxylase by acetyl-CoA by other acyl-CoA esters, or by depletion of acetyl-CoA concentrations, or by a combination of both effects. Indeed activation of purified pig liver pyruvate carboxylase by acetyl-CoA is competitively inhibited by pent-4-enoyl-CoA (apparent \( K_i \) 20 \( \mu \)m) (Billington et al., 1978). Acetyl-CoA could be formed from pyruvate by the action of pyruvate dehydrogenase or by the \( \beta \)-oxidation of endogenous fatty acids. The addition of 0.5 mm-arsenite, which inhibits pyruvate dehydrogenase, suppressed pyruvate carboxylation. This effect was reversed by the addition of 1 mm-L-acetylcarnitine, which restores the matrix concentration of acetyl-CoA. Evidence for a direct inhibitory effect of some acyl-CoA esters was provided by the partial relief of inhibition in the presence of 5 mm-glycine, except for that caused by pent-4-enoate (Table 1). This correlates with the specificity of glycine N-acylase for acyl-CoA esters (Bartlett & Gompertz, 1974; Sherratt, 1981), and is presumably due to lowering of the steady-state concentration of acetyl-CoA esters of the added fatty acids.

Addition of 3 mm-ATP caused a 20-30% increase in the control rate of pyruvate carboxylation, and the carboxylic acids were also inhibitory under these conditions (results not shown). Liver mitochondria had an impaired respiratory control rate (stimulation of the basal rate by about 1.3) in the incubation medium used (Table 1) when compared with that in HCO\(_3\)-free medium usually used for mitochondrial incubations (Holland & Sherratt, 1973), and suggests a decreased efficiency of ATP synthesis.

Measurements of the activity of pyruvate carboxylase in intact mitochondria are difficult to interpret, since it is not possible to control the concentrations of all the substrates, effectors and inhibitors in the mitochondrial matrix. Nevertheless, these results provide evidence for a significant inhibition by some acyl-CoA esters that can be formed in vivo. They also have implications for understanding the mechanisms of metabolic disorders caused by some hypoglycaemic poisons, and of those associated with abnormal accumulations of acyl-CoA esters found in some inborn errors of metabolism.

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**The effects of valproate (2-n-propylpentanoate) on intermediate metabolism in isolated rat hepatocytes and intact rats**

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Valproate, administered as either sodium valproate or free valproic acid, is a widely used anticonvulsant that has been associated with hepatic failure (Editorial, 1980). The mechanism of valproate-associated hepatic failure is unknown. Valproate is a simple branched-chain fatty acid, and a superficial analogy has been drawn with the effects of the toxic hypoglycaemic compounds hypoglycin and pent-4-enoate. It was suggested that valproate, or its metabolites, may cause a similar disturbance of intermediary metabolism. We therefore investigated the effects of valproate, in concentrations similar to those found in man during valproate therapy, on intermediary metabolism in isolated rat hepatocytes. The metabolic response of rats to a single dose of valproate was also studied.

Hepatocytes were prepared from the livers of fed male Wistar rats (Seglen, 1972). Pyruvate oxidation was determined by the production of \( ^{14} \)CO\(_2\) from 10 mm-\( [1-^{14} \)C]pyruvate (specific radioactivity 0.05 \( \mu \)Ci/mm mol). Palmitate oxidation was measured by using 1.0 mm-\( [1-^{14} \)C]palmitate (specific radioactivity 1 \( \mu \)Ci/mm mol) and the acid-soluble radioactivity formed was determined. Urea synthesis was determined by using 4 mm-L-ornithine and 10 mm-L-glutamine as substrates in the presence of 5 mm-glucose. Gluconeogenesis was measured by determining the amount of \( ^{14} \)C-glucose formed from 2 mm-L-\( [1-^{14} \)C]alanine after a 20 min incubation (Felius et al., 1976).

Male Wistar rats (200 g), fed or starved for 18 h, were given an intraperitoneal injection of sodium valproate (1000 mg/kg body wt. as a 0.14 m solution). Blood was taken from the tail vein for the determination of glucose, 3-hydroxybutyrate, pyruvate, lactate, alanine and glycerol by automated enzymic analysis (Lloyd et al., 1978), and of acetocetate by manual enzymic assay (Williamson et al., 1962). Plasma valproate concentration was determined by the method of Chard (1976).

In isolated hepatocytes the oxidation of \( [1-^{14} \)C]pyruvate and of \( [1-^{14} \)C]palmitate and urea synthesis were inhibited by concentrations of valproate in the therapeutic range (Figs. 1 and 2). Gluconon increased the incorporation of \( [1-^{14} \)C]alanine into glucose (Fig. 2). Both basal and glucagon-stimulated gluconeogenesis were inhibited by valproate in a concentration-dependent manner (Fig. 2).

Valproate (100 mg/kg body wt.) decreased total ketone-body concentrations, both in fed and starved rats, with decreases in the 13-hydroxybutyrate/\([\text{acetocetate}]/\text{valproate}\) ratios. Valproate also caused decreases in blood glucose concentrations in starved rats. There were no significant changes in the concentrations of pyruvate, lactate, glyceral and alanine. The maximum decreases in blood glucose and ketone-body concentrations coincided with, or occurred slightly after, the maximum blood concentration of valproate.

Pyruvate and palmitate oxidation, urea synthesis and gluconeogenesis were inhibited by 30-80% by valproate at