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EFFECT OF EXTRACELLULAR pH AND INHIBITORS ON THE GLUCONEOGENESIS AND AMMONIAGENESIS RELATIONSHIP IN RAT KIDNEY CORTEX SLICES*

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1. Gluconeogenesis from glutamine, fumarate, pyruvate, glutamine plus fumarate, and glutamine plus pyruvate, was generally higher at pH 7.1 than at pH 7.4 and 7.7, whereas ammoniagenesis did not depend on the pH of the medium.

2. The intermediates of the Krebs cycle decreased ammonia formation from glutamine, raising at the same time gluconeogenesis.

3. Arsenite, malonate, maleate, hydrazine and 2,4-dinitrophenol inhibited gluconeogenesis, and enhanced simultaneously ammonia formation irrespective of the pH of the medium.

It is well established that gluconeogenesis from various substrates is increased in kidney cortex slices from rats with chronic metabolic acidosis (Goodman et al., 1966; Kamm et al., 1967). Alleyne (1970) and Hems & Brosnan (1971) demonstrated that this is due to the increased activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32). It is also believed that the enhanced utilization of dicarboxylic intermediates of the Krebs cycle during gluconeogenesis results in a lower concentration of 2-oxoglutarate and glutamate in the kidney. Since glutamate is an inhibitor of glutaminase (Goldstein & Schooler, 1967), one may anticipate that a reduction of the glutamate concentration increases glutaminase activity. According to this interpretation, changes in the rate of ammonia formation are secondary to changes in the rate of gluconeogenesis.

In 1967 Kamm et al. demonstrated that glucose formation in kidney cortex slices from normal rats could be increased or lowered by lowering or raising, re-

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respectively, the pH value of the incubation medium. This means that the step limiting gluconeogenesis responds immediately, without any lag period, to changes in the pH of the extracellular fluid.

The aim of the present work was to examine whether deviations from normal pH values affect the relationship between gluconeogenesis and ammonia formation.

MATERIALS AND METHODS

The rats used for the experiments were of the Wistar strain, weighing 170 - 220 g. They were fasted overnight and killed by decapitation. The isolated kidneys were placed in cooled (4°C) 0.9% NaCl solution containing 4.5 mm-KCl. The rat kidney cortex was sliced manually with a razor, over a period of 15 - 20 min, and the slices were incubated for 90 min at 37°C in bicarbonate buffer of pH 7.1, 7.4 or 7.7 under O₂:CO₂ (95%:5%). The pH of the incubation mixture was adjusted to the required value by adding appropriate amounts of NaHCO₃. To maintain a constant concentration of Na⁺, varying amounts of NaCl were added to the incubation media. The kidney cortex slices, in an amount corresponding to 5 - 10 mg of dry tissue weight, were incubated in 25-ml Erlenmayer flasks containing 4.0 ml of the medium consisting of 96 - 120 mm-NaCl, 12 - 35 mm-NaHCO₃, 4.5 mm-KCl, 1.3 mm-CaCl₂, 0.6 mm-MgSO₄, 1.2 mm-Na₂HPO₄. Glutamine, pyruvate and fumarate were added each at a concentration of 5 mm.

The incubation was stopped by adding 30% trichloroacetic acid. Glucose was determined by the o-toluidine method (Hultman, 1959), and ammonia in the indophenol reaction according to Okuda et al. (1965).

Chemicals used in the experiments were the analytical grade products obtained from P.O.Ch. (Gliwice, Poland).

RESULTS

In rat kidney cortex slices glutamine, in contrast to pyruvate and particularly to fumarate, is a relatively poor substrate for gluconeogenesis. The data given in Table 1 illustrate the dependence of glucose and ammonia formation from these three substrates on the pH of the incubation medium. The amount of glucose synthesized from glutamine at pH 7.7 was about a half that formed at pH 7.1 or 7.4; on the other hand, ammoniagenesis from glutamine did not depend on the pH of the incubation medium. Consequently, the ratio of ammoniagenesis to gluconeogenesis was almost twice as high at pH 7.7 as at pH 7.4 or 7.1.

Gluconeogenesis from fumarate showed a distinct pH dependence: the higher the pH value, the lower the gluconeogenesis rate. At pH 7.7 the amount of glucose formed was lower by about 35% than at pH 7.1. Formation of glucose from pyruvate was virtually unaffected by changes in pH.

On addition of fumarate or pyruvate to the glutamine-containing medium, an additive increase in glucose formation was observed at all pH values tested. Glucose
Table 1

Effect of pH on glucose and ammonia formation by rat kidney cortex slices

The net increase in glucose and ammonia is expressed as μmoles/g dry weight/90 min. The results are mean values ±S.E.M. from 6–7 experiments. All substrates were added at a concentration of 5 mM.

<table>
<thead>
<tr>
<th>Addition</th>
<th>pH 7.1</th>
<th>pH 7.4</th>
<th>pH 7.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ glucose</td>
<td>ΔNH₄⁺</td>
<td>Δ glucose</td>
</tr>
<tr>
<td>None</td>
<td>19±3</td>
<td>111±14</td>
<td>17±5</td>
</tr>
<tr>
<td>Glutamine</td>
<td>79±4</td>
<td>1045±45</td>
<td>72±9</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>180±14</td>
<td>50±8</td>
<td>180±21</td>
</tr>
<tr>
<td>Fumarate</td>
<td>267±28</td>
<td>94±15</td>
<td>235±19</td>
</tr>
<tr>
<td>t-Glutamin + pyruvate</td>
<td>226±11</td>
<td>500±62</td>
<td>238±17</td>
</tr>
<tr>
<td>t-Glutamine + fumarate</td>
<td>377±19</td>
<td>778±42</td>
<td>319±20**</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.02, as compared with the value obtained at pH 7.1.

Formation from glutamine and fumarate was distinctly pH-dependent (at pH 7.1 it was nearly twice as high as at pH 7.7). The increase in glucose formation was accompanied by a decrease in ammonia formation. The addition of fumarate or pyruvate reduced ammoniagenesis by 20 and 50%, respectively. This effect was not related to the pH of the incubation medium. Moreover, pyruvate by itself lowered by about 70% endogenous formation of ammonia, and this effect increased with the pH value of the medium. The analogous effect of fumarate was much less pronounced and was distinctly notable only at pH 7.7.

Table 2

Effect of inhibitors on gluconeogenesis and ammoniagenesis in rat kidney cortex slices in the presence of glutamine and pyruvate

Kidney cortex slices were incubated at pH 7.1 or 7.4 in the presence of 5 mM-glutamine and 5 mM-pyruvate, and the inhibitor indicated. The net increase in glucose and ammonia formation is expressed as μmoles/g dry weight/90 min. The results are mean values ±S.E.M. from 6–7 experiments.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>pH 7.1</th>
<th>pH 7.7</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ glucose</td>
<td>ΔNH₄⁺</td>
</tr>
<tr>
<td>None, without substrates</td>
<td>25±4</td>
<td>87±6</td>
</tr>
<tr>
<td>None, with substrates</td>
<td>254±15</td>
<td>516±57</td>
</tr>
<tr>
<td>Arsenite, 0.05 mM</td>
<td>100±4**</td>
<td>630±54</td>
</tr>
<tr>
<td>2,4-DNP, 0.05 mM</td>
<td>93±6**</td>
<td>607±74</td>
</tr>
<tr>
<td>Hydrazine, 3 mM</td>
<td>22±2**</td>
<td>649±63</td>
</tr>
<tr>
<td>Malonate, 2 mM</td>
<td>193±13**</td>
<td>554±29</td>
</tr>
<tr>
<td>Maleate, 2 mM</td>
<td>40±4**</td>
<td>797±60**</td>
</tr>
</tbody>
</table>

** P < 0.02 as compared with the control value (with substrates, without inhibitor added).

Application of inhibitors enabled to define more precisely the relation of the two processes at different pH values. As shown in Table 2, arsenite, which inhibits oxidative metabolism in the Krebs cycle, and dinitrophenol, which stimulates this
Table 3

Effect of inhibitors on gluconeogenesis and ammoniagenesis in rat kidney cortex slices in the presence of glutamine and fumarate

Kidney cortex slices were incubated at pH 7.1 or 7.4 in the presence of 5mm-glutamine and 5mm-fumarate, and the inhibitor indicated. The net increase in glucose and ammonia formation is expressed as μmoles/g dry weight/90 min. The results are mean values ±S.E.M. from 6-7 experiments.

<table>
<thead>
<tr>
<th>Inhibitor (mM)</th>
<th>pH 7.1</th>
<th></th>
<th>pH 7.7</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Δ glucose</td>
<td>ΔNH₄⁺</td>
<td>Δ glucose</td>
<td>ΔNH₄⁺</td>
</tr>
<tr>
<td>None, without substrates</td>
<td>27±4</td>
<td>100±4</td>
<td>20±2</td>
<td>118±5</td>
</tr>
<tr>
<td>None, with substrates</td>
<td>374±25</td>
<td>757±36</td>
<td>230±39</td>
<td>797±68</td>
</tr>
<tr>
<td>Arsenite, 0.05 mM</td>
<td>265±23**</td>
<td>985±93**</td>
<td>168±9</td>
<td>1140±121**</td>
</tr>
<tr>
<td>2,4-DNP, 0.05 mM</td>
<td>140±28</td>
<td>1200±118**</td>
<td>76±11**</td>
<td>1375±114**</td>
</tr>
<tr>
<td>Hydrazine, 3 mM</td>
<td>129±13**</td>
<td>1110±161**</td>
<td>79±6**</td>
<td>1060±96*</td>
</tr>
<tr>
<td>Malonate, 2 mM</td>
<td>279±34*</td>
<td>834±51</td>
<td>187±18</td>
<td>765±87</td>
</tr>
<tr>
<td>Maleate, 2 mM</td>
<td>196±20**</td>
<td>945±70**</td>
<td>223±29</td>
<td>930±94</td>
</tr>
</tbody>
</table>

* P < 0.05; ** P < 0.02 as compared with the control value (with substrates, without inhibitor added).

metabolism, inhibited glucose formation at pH 7.1 and 7.7 by about 60%. Malonate and maleate, the two other known inhibitors of the Krebs cycle, inhibited glucose formation to a somewhat greater extent at pH 7.1 than at pH 7.7. Hydrazine, a non-specific inhibitor of phosphoenolpyruvate carboxykinase (Ray et al., 1970), completely inhibited gluconeogenesis from glutamine and pyruvate. Each of the

Fig. 1. Effect of inhibitors on the interrelation between gluconeogenesis and ammoniagenesis in rat kidney cortex slices. The slices were incubated in the presence of glutamine and pyruvate (A) or glutamine and fumarate (B), at pH 7.4. Other additions: ○, none; ○, 0.05 mM-arsenite; ▲, 0.05 mM-2,4-dinitrophenol; ▲, 3 mM-hydrazine; □, 2 mM-malonate; ■, 2 mM-maleate. The results are mean values from 7-9 experiments.
inhibitors tested, irrespective of the site of its action or the extent of inhibition of gluconeogenesis, slightly stimulated ammonia formation at both pH values tested. When glutamine and fumarate served as substrates for gluconeogenesis and ammoniagenesis in kidney cortex slices (Table 3), arsenite inhibited gluconeogenesis only by 25 - 30%, malonate showed a still lower inhibitory effect, and maleate showed about 50% inhibition only at pH 7.1. In the glutamine-fumarate system, inhibition of gluconeogenesis was also accompanied by stimulation of ammonia formation. Arsenite, dinitrophenol and hydrazine increased ammoniagenesis by 25 - 50% irrespective of the pH of the medium. The addition of malonate was ineffective, and that of maleate gave a negligible effect.

The relationship between glucose and ammonia formation at pH 7.4 in the presence of various inhibitors is illustrated in Fig. 1. The negative correlation observed was more pronounced in the glutamine-fumarate system than in the system containing glutamine and pyruvate.

![Graph](image)

**Fig. 2.** Effect of inhibitors on the ratio of ammoniagenesis to gluconeogenesis in rat kidney cortex slices. The slices were incubated in the presence of glutamine and pyruvate (A) or glutamine and fumarate (B) at the indicated pH values. Other additions as in Fig. 1.

The ratio of ammoniagenesis and gluconeogenesis in these two systems ranged from 2.0 to 2.5 both at pH 7.4 and 7.1 (Fig. 2). At pH 7.7, due to the decreased glucose formation, this ratio was raised to 3.5 - 4.0. The addition of each of the inhibitors tested resulted in a severalfold increase in the ammoniagenesis-to-gluconeogenesis ratio. The highest ratio was found at pH 7.7, except with maleate, where this ratio was distinctly higher at pH 7.1 than at pH 7.7.
DISCUSSION

The results presented indicate that gluconeogenesis in rat kidney cortex slices responds rapidly to changes in the pH of the extracellular fluid. On the other hand, ammoniaogenesis is not affected even by wide-range fluctuations in the pH of the environment. Variations in the rate of gluconeogenesis by 30-50% are not accompanied by any changes in ammonia formation. The earlier studies which testified to the close interdependence of gluconeogenesis and ammonia formation were performed exclusively with glutamine or glutamate as substrates (Alleyne, 1970; Goodman et al., 1966). An increase in utilization of the carbon skeleton of these compounds under conditions of metabolic acidosis must be obviously associated with "overproduction" of ammonia.

However, the results reported in the present paper show that the increase in gluconeogenesis on addition of a glucogenic substrate to the kidney cortex slices incubated with glutamine is not paralleled by the increase in ammoniaogenesis, irrespective of the pH of the extracellular fluid (Table 1). Similar results were obtained with other tissues by Preuss (1969), Pilkington & O'Donovan (1971) and Irias & Greenberg (1972). Moreover, Preuss (1971) demonstrated that in isolated renal tubules the Krebs cycle intermediates, especially pyruvate, lowered ammonia formation from glutamate. He interpreted this observation in terms of substrate competition in oxidative metabolism and impaired accessibility of nicotinamide adenine nucleotides to glutamate dehydrogenase. This is consistent with the fact that inhibition of oxidation prevents lowering of ammoniaogenesis by the Krebs cycle intermediates. However, the simple explanation forwarded by Preuss (1971) is not supported by our experimental data.

Lowering of glutamine metabolism by the competitive mechanism at the glutamate step is hardly possible in view of the additive increase in gluconeogenesis, i.e. unchanged utilization of glutamine in the presence of the Krebs cycle intermediates. It should rather be expected that these intermediates affect glutamine metabolism not directly related to gluconeogenesis, e.g. formation of 2-oxoglutarate in the transamination reaction (Duffy et al., 1974).

Arsenite and malonate inhibit glucose formation from glutamine + fumarate to the level obtained with fumarate alone. Assuming that both these compounds inhibit oxidation of glutamine at the 2-oxoglutarate or succinate step, and that fumarate utilization for gluconeogenesis remained unchanged, a decrease in ammonia formation should be expected. However, an opposite effect was observed: under these conditions ammonia formation was increased. Similar results were reported by Preuss (1969) who studied the effect of malonate and arsenite on glucose and ammonia formation from glutamate.

Both arsenite and maleate inhibited to a much larger extent gluconeogenesis from glutamine + pyruvate than from glutamine + fumarate, due probably to the inhibition of pyruvate and oxoglutarate dehydrogenases. Lower utilization of the glutamine carbon chain was associated with the decreased carboxylation of pyruvate, presumably because of a limiting concentration of acetyl-CoA (Schärer et al., 1972).
The inhibition by maleate was distinctly lower at pH 7.7 than at 7.1, which could be related to the alkalizing effect of maleate in kidney cells, postulated by Hoppe et al. (1976). Hydrazine, a non-specific inhibitor of phosphoenolpyruvate carboxykinase (Ray et al., 1970) inhibited gluconeogenesis with simultaneous stimulation of ammonia formation, independently of the pH of the extracellular fluid. This indicates that inhibition of gluconeogenic pathway at the step outside the tricarboxylic acid cycle has a stimulatory rather than an inhibitory effect on ammonia formation. A similar interpretation may apply to the data of Preuss (1971) on the effect of iodoacetate in isolated renal tubules, and those of Ross (1976) obtained with perfused rat kidney. Ross found that 3-mercaptoethylamine, a specific inhibitor of phosphoenolpyruvate carboxykinase, distinctly inhibits glucose formation and stimulates ammoniagenesis by 26%.

In kidney slices incubated with glutamine and Krebs cycle substrates, inhibition of gluconeogenesis by dinitrophenol is obviously due to uncoupling of oxidative phosphorylation. At the same time, however, ammoniagenesis is markedly elevated although oxidation of the Krebs cycle intermediates is stimulated, and not inhibited, by dinitrophenol.

The results presented in this work indicate that over a wide range of pH values of the extracellular fluid there is no direct relationship between gluconeogenesis and ammonia formation in the rat kidney cortex slices. It appears that ammoniagenesis is dependent mainly on the kind and concentration of the substrates taken up by the kidney from the general circulation system.

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REFERENCES

Wpływ zewnętrzkomórkowego pH oraz inhibitorów metabolicznych na związek między glukoneogenezą i amoniogenezą w skrawkach kory nerek szczurów

Streszczenie

1. Glukoneogeneza z glutaminy, fumaratu, pirogronianu oraz glutaminy i fumaratu lub glutaminy i pirogronianu była z reguły wyższa w środowisku o pH 7,1 niż w pH 7,4 lub 7,7, podczas gdy amoniogeneza nie ulegała zmianie.

2. Substraty cyklu Krebsa zmniejszały produkcję amoniaku z glutaminy, powodując jednocześnie addyzyjny wzrost produkcji glukozy.

3. Arsenin, malonian, maleianian, 2,4-dwunitrofenol i hydrazy na hamowały glukoneogenezę w skrawkach kory nerki, zwiększając jednocześnie produkcję amoniaku niezależnie od pH środowiska inkubacyjnego.

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