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# INHIBITION OF GLUCONEOGENESIS AND OF CELL RESPIRATION BY 1-(2-PYRIMIDINYL)-4-IMINO-1,4-DIHYDROPYRIDINIUM CHLORIDE IN PERFUSED GUINEA PIG LIVER*

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**Abstract**—1-(2-Pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride, a hypoglycemic agent, inhibits gluconeogenesis from lactate in the perfused guinea pig liver. The alterations in the concentrations of hepatic metabolites resemble those observed in the presence of phenylethylbiguanide and 1-methyl-4(3-methyl-5-isoxazolyl)-pyridinium chloride; reducing equivalents are increased while the ATP/ADP ratio and the hepatic cell respiration are decreased.

1-(2-Pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (PIDH) is a new hypoglycemic compound which has been found to lower the serum glucose concentration in the fasting state and to reduce hyperglycemia in man [1]. The mode of action of this compound has not been clarified so far. Similar effects of phenylethylbiguanide (DBI) and of PIDH on hyperglycemia and insulin response in human subjects after an oral glucose load as well as on glucose absorption from the intestinal tract of rats have been observed [1]. Since the hypoglycemia caused by DBI is at least partly due to an inhibition of gluconeogenesis [2], we investigated the influence of PIDH on the glucose formation of perfused guinea pig livers.

## EXPERIMENTAL

*Materials.* All enzymes and coenzymes were purchased from Boehringer Mannheim (D-68 Mannheim), L-lactate from Serva (D-69 Heidelberg), bovine serum albumin from Behring-Werke AG (D-3550 Marburg), antifoam from Beckman Instruments (D-8 Munic), all materials for gas chromatography from Hewlett-Packard (D-703 Böblingen) and all other chemicals from Merck AG (D-61 Darmstadt). 1-(2-Pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride was a generous gift from Merck, Sharp and Dohme Research Laboratories (Westpoint, Pennsylvania, U.S.A.). Male, albino guinea pigs (White Pirpright strain, Versuchstierzucht K.M. Peters, D-3255 Lauenau), weighing 300–350 g were fed for 8 days on a standard diet (ssniff, Intermast GmbH, D-463 Bochum-Hövel) and then fasted 48 hr prior to the perfusion experiments.

*Perfusion procedure.* The perfusion was carried out according to Miller *et al.* [3] and Schimassek [4] as described in detail previously [5, 6]. The perfusate (100 ml) contained 30 g/l bovine serum albumin, 13 mg/l sodium ampicillin and bovine erythrocytes washed three times and taken up in Krebs–Ringer bicarbonate solution (hemoglobin concentration: 50 g/l). The perfusion lasted 90 min. 1-(2-Pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride was added to the medium after 45 min and lactate after 60 min.

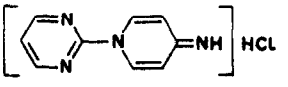


Fig. 1. Structural formula for 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride.

The liver metabolites were measured enzymatically in a perchloric acid extract as described previously [7]. The concentration of glycogen was determined by the method of Keppler and Decker [8] and that of 3-hydroxybutyrate and acetoacetate as reported by Bergmeyer and Bernt [9]. The $CO_2$ and $O_2$ concentrations were determined in perfusate samples taken from the hepatic in- and outflow [6] by gas chromatography as reported recently [10].

## RESULTS

Glucose formation from lactate was reduced by 80 per cent by 0·5 m-mole/l 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride in perfused guinea pig livers (Fig. 2).

The concentrations of hepatic metabolites (Table 1) are similar to those observed with phenylethylbiguanide and 1-methyl-4(3-methyl-5-isoxazolyl)-pyridinium chloride under similar experimental conditions [6, 10, 11]:

(i) A decrease of the hepatic glycogen concentration observed in the presence of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride can be related to the inhibition of gluconeogenesis.

(ii) The concentration of intrahepatic lactate plus pyruvate was increased due to the elevated lactate concentration. The citrate and 2-oxoglutarate concentrations, however, were reduced. The intrahepatic concentrations of phosphoenolpyruvate, 2-phosphoglycerate and 3-phosphoglycerate were elevated while

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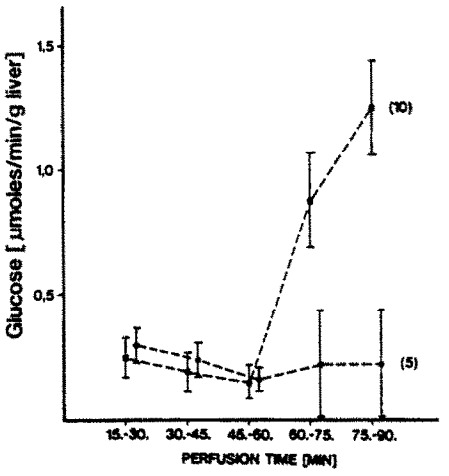


Fig. 2. The influence of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride on glucose formation from lactate in perfused guinea pig livers. ●----● 0·5 m-mole/l 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (n = 5), ■----■ controls (n = 10). Vertical bars represent standard deviations.

those of the triose phosphates and hexosemonophosphates were decreased in the presence of the compound studied (Table 1).

(iii) Reducing equivalents were accumulated as shown by the elevated lactate/pyruvate and 3-hydroxybutyrate/acetoacetate ratios. In relation to the increase of reducing equivalents a slight rise of the intrahepatic malate concentration was observed (Table 1).

(iv) The intrahepatic ATP/ADP ratio was decreased by 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (Table 1).

(v) The hepatic oxygen uptake and $CO_2$ formation as well as the utilization of lactate were also significantly reduced (Table 2).

(vi) The uptake of potassium ions was significantly inhibited by 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (Fig. 3). The relation of this effect to the respiratory state and the intracellular ATP potential has been discussed recently [5]. The uptake of phosphate ions was not significantly reduced by this compound.

Table 1. The influence of 1-(2-pyrimidinyl)-4-imino-1,4 dihydropyridinium chloride (0·5 m-mole/l.) on hepatic metabolite concentrations (µmoles/g liver wet wt) in the presence of lactate (15 m-moles/l.)

| | Controls $\bar{x} \pm$ S.D. (n = 7) | 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride $\bar{x} \pm$ S.D. (n = 4) |
|---|---|---|
| ATP | 2·00 ± 0·18 | 1·64 ± 0·24 |
| ADP | 0·66 ± 0·13 | 1·18 ± 0·06 |
| ATP/ADP | 3·14 ± 0·65 | 1·40 ± 0·20 |
| 3-OH-Butyrate | 0·56 ± 0·13 | 0·63 ± 0·11 |
| Acetoacetate | 1·32 ± 0·22 | 0·82 ± 0·28 |
| 3-OH-Butyrate/acetoacetate | 0·43 ± 0·08 | 0·78 ± 0·35 |
| Lactate* | 1·09 ± 0·39 | 2·49 ± 0·75 |
| Pyruvate | 0·16 ± 0·03 | 0·16 ± 0·05 |
| Citrate | 0·60 ± 0·20 | 0·33 ± 0·11 |
| 2-Oxoglutarate | 0·74 ± 0·14 | 0·06 ± 0·03 |
| Malate | 0·51 ± 0·09 | 0·65 ± 0·41 |
| Phosphoenolpyruvate | 0·26 ± 0·06 | 0·39 ± 0·08 |
| Glycerate-2-phosphate | 0·06 ± 0·02 | 0·22 ± 0·16 |
| Glycerate-3-phosphate | 0·49 ± 0·08 | 0·79 ± 0·08 |
| Glyceraldehyde-3-phosphate | 0·02 (n = 2) | 0·01 ± 0·005 |
| Dihydroxyacetone-3-phosphate | 0·04 ± 0·01 | 0·03 ± 0·01 |
| Fructose-1,6-diphosphate | 0·01 ± 0·005 | 0·01 ± 0·005 |
| Fructose-6-phosphate | 0·02 ± 0·005 | < 0·01 |
| Glucose-6-phosphate | 0·10 ± 0·01 | 0·01 ± 0·01 |
| Glycogen | 3·22 ± 2·94 | 1·67 ± 1·64 |

* Intracellular concentration, corrected for extracellular lactate as described elsewhere [10].
All liver samples were taken at the 90th minute of the perfusion experiment.

Table 2. The influence of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (0·5 mmole/l.) on hepatic lactate consumption, glucose formation, $O_2$ uptake and $CO_2$ release in the presence of lactate (15 m-moles/l.)

| | Controls $\bar{x} \pm$ S.D. (n)* | 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride $\bar{x} \pm$ S.D. (n) |
|---|---|---|
| Lactate (used) | 2·61 ± 0·72 (10)‡ | 0·39 ± 0·41† (4) |
| Glucose (formed) | 1·25 ± 0·19 (10)‡ | 0·22 ± 0·26† (5) |
| Lactate (used)/glucose (formed) | 2·1 | 1·8 |
| $O_2$ uptake | 4·56 ± 1·13 (6)‡ | 2·61 ± 0·86† (5) |
| $CO_2$ release | 4·28 ± 1·22 (6)‡ | 2·92 ± 0·64† (5) |

* (n) refers to the number of contributing values.
† Differs significantly from its corresponding control value ($P < 0·05$; $t$-test).
‡ µmoles/min per g liver wet wt.

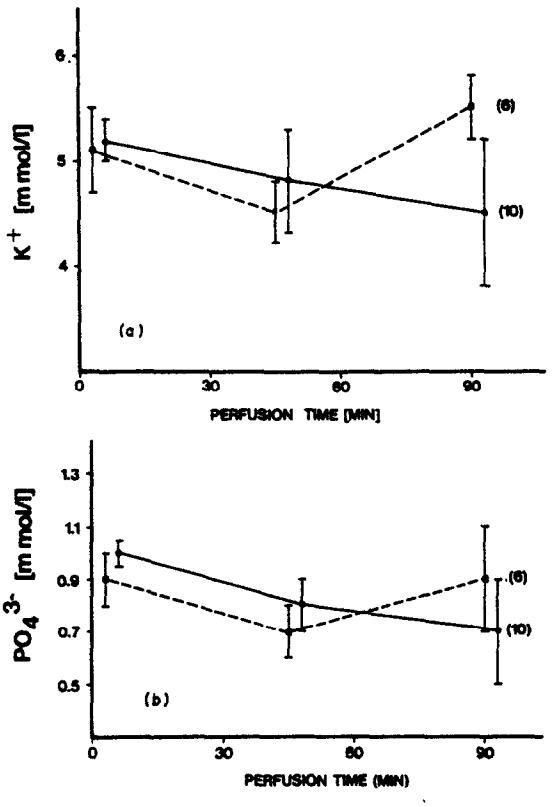


Fig. 3. The influence of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride on the perfusate concentration of potassium (a) and phosphate ions (b). Substrate, lactate. ■----■ 0·5 m-mole/l 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (n = 6), ●——● controls (n = 10). Vertical bars represent standard deviations.

## DISCUSSION

The results indicate a similar mode of action for biguanides, 1-methyl-4(3-methyl-5-isoxazolyl-)pyridinium chloride and 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride in the perfused guinea pig liver.

The inhibition of cell respiration by phenylethylbiguanide is well known [6, 12–14]. In the perfused guinea pig liver oxygen uptake is reduced also by 1-methyl-4(3-methyl-5-isoxazolyl-)pyridinium chloride [10] and 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride (Table 2). The decreased cell respiration explains the decline of the ATP/ADP ratio and the accumulation of reducing equivalents.

The results indicate that pyruvate oxidation was reduced by 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride. The hepatic lactate concentration was significantly increased. A rise of pyruvate, however, may have been prevented by the concomitant accumulation of reducing equivalents. The decrease of the citrate and 2-oxoglutarate concentrations and of the hepatic $CO_2$ production (Tables 1 and 2) points to an inhibition of the citric acid cycle.

In analogy to the effects of phenylethylbiguanide which were discussed in detail elsewhere [6], we assume that cross-over phenomena at steps coupled to the ATP/ADP ratio, such as the glycerate kinase or the fructose-1,6-diphosphatase reactions do not reflect a regulation of the flow rate at these points. The exact step at which the flow rate is affected by these compounds cannot be detected from the concentrations of gluconeogenic metabolites. It cannot be excluded that the inhibitory effect of these agents on hepatic oxygen uptake causes the reduction of gluconeogenesis. The ATP/ADP ratio could drop as a consequence of the reduced cell respiration. However, several authors have questioned whether this ratio can be an effective regulator of the gluconeogenic flow rate [6, 15, 16].

It has been discussed elsewhere [2] that inhibition of the Cori cycle in diabetes treated with biguanides could not be established conclusively. However, it can be assumed that in those patients with lactate-acidosis [2], phenylethylbiguanide inhibits the conversion of lactate into glucose. Under these conditions renal insufficiency has probably led to accumulation of the drug and to concentrations which may be comparable with those used in liver perfusion experiments [6].

This argument can also be applied to the action of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride on the Cori cycle. We have shown that in the perfused guinea pig liver, both drugs have the same effects on cell respiration, gluconeogenesis from lactate and on various metabolite concentrations (especially on lactate accumulation). 1-(2-Pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride should therefore only be administered to diabetic patients with renal insufficiency under careful observation for lactacidosis.

The dose of 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride used to lower hyperglycemia (400 mg per os) was double that of phenylethylbiguanide needed to produce comparable effects in diabetic patients [1]. In the perfused guinea pig liver the same concentration ratio (2:1) had to be used to lower the hepatic glucose output to 20 per cent of the control value.

The effects observed with 1-(2-pyrimidinyl)-4-imino-1,4-dihydropyridinium chloride are not specific as identical alterations of hepatic metabolite concentrations were found with phenylethylbiguanide and 1-methyl-4(3-methyl-5-isoxazolyl-)pyridinium chloride in the perfused guinea pig liver [6, 10, 11].

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