STIMULATION OF RAT KIDNEY GLUCONEOGENIC ABILITY BY INHIBITION OF LIVER GLUCONEOGENESIS

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1. Introduction

It is generally recognized that the liver is the major gluconeogenic tissue concerned with the regulation of glycemia because of the relative organ size as compared to that of the kidney cortex. In fact, glucose formed by the kidneys should provide only a small fraction of the blood glucose under normal physiological conditions, considering the renal removal of lactate, the main gluconeogenic precursor [1]. However, Owen et al. have reported that renal glucose production after prolonged fasting in obese patients approached that of the liver, which is decreased in these conditions [2]. On the other hand, Niederland et al. showed that kidneys of partially hepatectomized rats synthetized more glucose both in vitro and in vivo than the sham-operated animals [3] and Jones et al. reported a significant increase in net renal glucose production in fasted anesthetized dogs in which liver gluconeogenesis was inhibited by ethanol administration [4].

In previous work from this laboratory it has been reported that a stimulation of rat kidney phosphoenolpyruvate carboxykinase activity in experimental liver disease induced by galactosamine when hepatic gluconeogenesis was severely impaired. In these conditions, the rise in renal phosphoenolpyruvate carboxykinase activity was accompanied by a slight but significant increase in renal gluconeogenic ability [5].

Taking these findings altogether it could be assumed that kidneys play an important role in glucose homeostasis when liver functional capacity diminishes. In this context we have undertaken the study of the renal gluconeogenic response to the selective inhibition of liver gluconeogenesis in order to avoid the generalized cellular damage which occurs during galactosamine treatment.

2. Experimental

Female rats of the Wistar strain weighing 150–200 g were used. 5-methoxy indole-2-carboxylic acid (50 and 200 mg/kg body weight) was administered as a neutral solution 3 h before killing. Triamcinolone acetonide (2.5 mg per rat) was injected 3 h before killing. Actinomycin D (125 μg/kg body weight) was administered 3.5 h before killing and cycloheximide (1 mg per rat) was injected 5 h and 2.5 h before killing. All the agents were intraperitoneally injected and were substituted by saline solution in the controls. The rats were killed by cervical dislocation.

Liver gluconeogenic capacity was measured by perfusion with 10 mM L-lactate. The perfusion method has been described by Hems et al. [6]. The perfusate consisted of Krebs–Henseleit physiological saline [7], bovine serum albumin powder fraction V and washed human red cells stored 30 days at 4°C in citrate dextrose anticoagulant solution. Glucose production by renal cortical slices was estimated according to Krebs et al. [8] by incubating the washed cells in saline medium to which the substrate has been added in a 10 mM concentration, at 40°C for 1 h, with O_2 + CO_2 (95:5) as the gas phase. Glucose was determined by the glucose oxidase method [8,9].

Phosphoenolpyruvate carboxykinase activity was

measured spectrophotometrically in the direction of oxaloacetate synthesis in the presence of an excess of malate dehydrogenase at 30°C, as described previously [10]. Protein was determined by the method of Lowry et al. [11], with bovine serum albumin as standard.

Lactate dehydrogenase was measured by the method of Bergmeyer et al. [12]. Glucose 6-phosphatase was determined as described by Harper [13] and fructose diphosphatase as described by Pontremoli et al. [14], by using 0.04 M glycine buffer, pH 9.4.

3. Results and discussion

Among the several inhibitors of liver gluconeogenesis which have been extensively studied [15] we have tested the effect of 5-methoxy indole-2-carboxylic acid (MICA) on rat kidney cortex gluconeogenesis in vitro, since it is known that this compound, which inhibits gluconeogenesis mainly by blocking mitochondrial utilization of pyruvate, has little or no effect on kidney mitochondria [16]. As expected, the addition of MICA to the incubation medium produced only a slight inhibition of cortical gluconeogenesis both in well fed and starved rats whereas the same concentrations of the inhibitor strongly decreased liver gluconeogenesis when added to the perfusion medium in starved rats (table 1).

It seemed then that MICA was, at least to a certain extent, a selective inhibitor of liver gluconeogenesis unlike quinolinic acid or pent-4-enoic acid, which were known to be powerful agents in also preventing kidney gluconeogenesis as shown by Kalhr and Schoolwerth [17] and by Senior and Sherratt [18] respectively. Consequently we have studied the effect of MICA administration on liver and kidney gluconeogenesis.

The livers from 48 h starved rats treated with MICA produced about half the amount of glucose than controls when perfused with 10 mM lactate, the differences regarding the doses of the inhibitor being small (table 2). On the contrary, the production of glucose by kidney cortical slices from treated animals was significantly higher than controls both in well fed and in starved rats when the gluconeogenic substrates were lactate, pyruvate or glutamine (table 3). On the other hand, there were no significant differences when fructose was used as carbon precursor.

The stimulation of kidney production of glucose as a consequence of MICA administration sharply contrasts with the results obtained by Senior and Sherrat using pent-4-enoic acid as gluconeogenic inhibitor. In this case the renal gluconeogenic ability was also reduced after the administration of this compound [18]. It provides further support to the hypothesis that MICA acts in vivo as a rather selective inhibitor of liver gluconeogenesis.

It is interesting to point out the higher stimulation of kidney gluconeogenic ability which takes place in well fed animals. This finding seems to suggest that a similar mechanism would account for the stimulation of gluconeogenesis in starvation and in the conditions created by the treatment with MICA. On the other hand, the lack of effect of MICA administration on kidney gluconeogenesis when

Table 1
Effect of MICA on rat liver and kidney cortex gluconeogenic ability in vitro

	Well fed rats	48 h starved rats	
	Kidney	Kidney	Liver
Control	113.2 ± 2.1 (17)	235.4 ± 4.1 (5)	294.4 ± 25.2 (6)
0.2 mM MICA	93.6 ± 4.4 (7)	208.7 ± 4.2 (5)	129.1 ± 6.2 (7)
0.8 mM MICA	90.1 ± 4.1 (7)	177.9 ± 3.1 (5)	−4.0 ± 6.2 (3)

Gluconeogenesis ability is expressed in μmoles of glucose produced per h per g tissue dry wt. from 10 mM lactate. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

Table 2
Effect of MICA administration on gluconeogenic ability in perfused liver from 48 h starved rats

Control	396.0 ± 26.0 (4)
MICA (50 mg/Kg)	221.6 ± 8.5 (8)
MICA (200 mg/Kg)	198.7 ± 6.0 (7)

Gluconeogenic ability is expressed in μmoles of glucose produced per h per g liver dry wt. from 10 mM lactate. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

fructose was the carbon precursor suggested to us that the primary factor responsible for the stimulation of glucose production was the activation of phosphoenolpyruvate carboxykinase, in a similar way than in rats treated with galactosamine [5]. Actually, as shown in table 4, among the enzyme activities tested in kidney cortex after MICA administration only phosphoenolpyruvate carboxykinase exhibited a significant increase. This effect was higher in fed animals than in starved ones in good agreement with the results regarding gluconeogenic ability (table 3).

The rise in the activity of renal phosphoenolpyruvate carboxykinase promoted by MICA seems to be due to de novo synthesis of the enzyme since this effect was fully counteracted by treatment with actinomycin D or cycloheximide (table 5), antibiotics which are known to inhibit protein synthesis [19,20]. The doses used were able to overcome the well known induction of phosphoenolpyruvate carboxykinase by glucocorticoids [21–23].

The results described in this paper are closely related to those obtained in rats treated with galactosamine [5] although the inhibition of liver gluconeogenesis is quite different in both situations. Thus, in rats with liver damage induced by galactosamine all the key gluconeogenic enzymes are decreased in their activities, specially phosphoenolpyruvate carboxykinase [24,25]. However, none of the enzymes tested in the livers of the rats treated with MICA (glucose 6-phosphatase, fructose diphosphatase, phosphoenolpyruvate carboxykinase and lactate dehydrogenase) showed significant differences to the controls both in fed and starved animals (results not detailed in the text). Hence, if one assumes that a similar mechanism would account for the induction of renal phosphoenolpyruvate carboxykinase activity and gluconeogenic ability in both situations, the impairment of liver gluconeogenesis should be the common starting factor involved. The nature of this mechanism and the in vivo significance of the adaptative metabolic response of the kidney to the experimental impairment of liver gluconeogenesis are at present under investigation.

Table 3
Effect of MICA administration on rat kidney cortex gluconeogenic ability

Experimental conditions	Control	MICA (50 mg/Kg)	MICA (200 mg/Kg)
Well fed rats			
Pyruvate	219.4 ± 6.6 (9)	305.8 ± 6.5 (9)	320.6 ± 11.3 (4)
L-lactate	110.5 ± 1.9 (17)	156.4 ± 5.9 (8)	189.9 ± 5.1 (7)
Glutamine	115.4 ± 2.5 (7)	160.6 ± 1.7 (3)	175.5 ± 4.3 (8)
Fructose	456.5 ± 31.8 (4)	447.5 ± 9.8 (4)	460.3 ± 16.1 (9)
48 h starved rats			
Pyruvate	261.1 ± 8.9 (7)	300.4 ± 12.9 (9)	321.5 ± 1.9 (4)
L-lactate	218.8 ± 3.6 (10)	252.2 ± 2.8 (8)	261.2 ± 3.5 (4)
Glutamine	155.5 ± 2.1 (8)	184.3 ± 6.3 (3)	195.6 ± 6.8 (3)
Fructose	506.3 ± 2.7 (3)	511.5 ± 4.7 (6)	508.7 ± 2.1 (4)

Gluconeogenic ability is expressed in μmoles of glucose produced per h per g kidney cortex dry wt. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

Table 4
Effect of MICA administration on lactate dehydrogenase, phosphoenolpyruvate carboxykinase, fructose diphosphatase and glucose 6-phosphatase activities in rat kidney cortex

Experimental conditions	Control	MICA 50 mg/Kg	MICA 200 mg/Kg
Well fed rats			
Lactate dehydrogenase	3.04 ± 0.332 (7)	2.75 ± 0.28 (10)	3.64 ± 0.30 (6)
Phosphoenolpyruvate carboxykinase	34.08 ± 1.99 (9)	62.24 ± 3.28 (6)	66.34 ± 2.27 (5)
Fructose diphosphatase	156.90 ± 7.02 (10)	150.36 ± 10.00 (11)	161.66 ± 7.17 (6)
Glucose 6-phosphatase	16.92 ± 1.99 (7)	18.22 ± 1.01 (7)	18.84 ± 2.39 (6)
48 h starved rats			
Lactate dehydrogenase	4.63 ± 1.08 (4)	4.87 ± 0.48 (3)	4.30 ± 0.63 (8)
Phosphoenolpyruvate carboxykinase	79.28 ± 3.97 (7)	94.89 ± 3.22 (5)	95.91 ± 3.33 (6)
Fructose diphosphatase	186.00 ± 19.28 (3)	193.66 ± 6.35 (3)	162.80 ± 13.92 (5)
Glucose 6-phosphatase	19.65 ± 1.69 (7)	20.26 ± 2.07 (5)	18.33 ± 0.96 (5)

Lactate dehydrogenase activity is expressed in μmoles of pyruvate transformed/min per mg of protein. Phosphoenolpyruvate carboxykinase and fructose diphosphatase activities are expressed in nmoles of substrate transformed/min per mg of protein. Glucose 6-phosphatase activity is expressed in μmoles of substrate transformed/min per g tissue wet wt. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

Table 5
Effect of actinomycin D acid cycloheximide on the stimulation of renal phosphoenolpyruvate carboxykinase activity in rats treated with MICA

Experimental conditions	Actinomycin D	Cycloheximide
Control	33.44 ± 2.39 (6)	32.16 ± 1.67 (6)
Antibiotic	32.51 ± 2.32 (6)	32.89 ± 1.99 (6)
Triamcinolone	66.59 ± 2.77 (7)	64.4 ± 2.47 (6)
MICA (50 mg/Kg)	65.58 ± 2.24 (7)	64.92 ± 2.18 (7)
MICA + Antibiotic	31.30 ± 2.75 (6)	33.42 ± 2.41 (7)
Triamc. + Antibiotic	35.26 ± 2.02 (7)	31.08 ± 1.22 (7)

Phosphoenolpyruvate carboxykinase activity is expressed in nmoles of oxalacetate formed at 30°C per min per mg of protein. The results are given as means ± S.E.M., with the number of observations in parentheses. Experimental details are given in the text.

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References

[1] Judkin, J. and Cohen, R. D. (1975) Clin. Sci. Mol. Med. 48, 121–131.
[2] Owen, O. E., Felig, P., Morgan, A. P., Wahren, J. and Cahill, G. F. Jr. (1969) J. Clin. Invest. 48, 574–583.
[3] Niederland, T. R., Dzúric, R. and Gregorová, B. (1971) Physiol. Bohemoslov. 20, 589–593.
[4] Jones, V. D., Spalding, Ch. T. and Jenkins, M. L. (1971) Res. Commun. Chem. Pathol. Pharm. 2, 67–77.
[5] García-Ruiz, J. P., Moreno, F., Sánchez-Medina, F. and Mayor, F. (1973) FEBS Lett. 34, 113–116.
[6] Hems, R., Ross, B. D., Berry, M. N. and Krebs, H. A. (1966) Biochem. J. 101, 284–292.
[7] Krebs, H. A. and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33–66.
[8] Krebs, H. A., Bennet, D. A. H., de Gasquet, P., Gascoyne, T. and Yoshida, T. (1963) Biochem. J. 86, 22–27.
[9] Krebs, H. A., Dierks, C. and Gascoyne, T. (1964) Biochem. J. 93, 112–121.
[10] Sánchez-Medina, F., Sánchez-Urrutia, L., Medina, J. M. and Mayor, F. (1971) FEBS Lett. 19, 128–130.
[11] Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) J. Biol. Chem. 193, 265–275.
[12] Bergmeyer, H. U., Bernt, E. and Hess, B. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U. ed.), pp. 736–743, Academic Press, New York and London.
[13] Harper, A. E. (1965) in: Methods in Enzymatic Analysis (Bergmeyer, H. U., ed.), p. 788–792, Academic Press, New York and London.
[14] Pontremoli, S. Traniello, S. Luppis, B. and Wood, W. A. (1965) J. Biol. Chem. 240, 3459–3463.
[15] Lardy, H. A. (1969) in: Inhibitors Tools in Cell Research (Bucher, Th. and Sies, H., eds.) p. 374–391, Springer Verlag, Berlin.
[16] Reed, J. and Lardy, H. A. (1970) J. Biol. Chem. 245, 5297–5303.
[17] Kalhr, S. and Schoolwerth, A. C. (1972) Biochem. Biophys. Acta. 279, 157–162.
[18] Senior, A. E. and Sherratt, H. S. A. (1968) Biochem. J. 110, 521–527.
[19] Goldberg, I. H., Rabinowitz, M. and Reich, E. (1962) Proc. Natl. Acad. Sci. USA 48, 2094–2101.
[20] Ennis, H. L. and Lubin, M. (1964) Science, 146, 1474–1476.
[21] Henning, H. V., Stumpf, B., Ohly, B. and Seubert, W. (1966) Biochem. Z. 344, 274–288.
[22] Flores, H. and Alleyne, G. A. O. (1971) Biochem. J. 123, 35–39.
[23] Iynedjian, P. B., Ballard, F. J. and Hanson, R. W. (1975) J. Biol. Chem. 250, 5596–5603.
[24] Monier, D. and Wagle, S. R. (1971) Proc. Soc. Exptl. Biol. Med. 136, 377–380.
[25] Record, C. O., Alberti, K. G. M. M. and Williamson, D. H. (1972) Biochem. J. 130, 37–44.