Pediat. Res. *12*: 1039–1044 (1978)

Amino acids
α-ketoderivatives
kidney
renal gluconeogenesis

Inhibition of Gluconeogenesis in Isolated Rat Kidney Tubules by Branched Chain α-Ketoacids

BRUNO STUMPF[35] AND HANS KRAUS

WITH THE TECHNICAL ASSISTANCE OF REGINA KASTEN AND GITTA AHRENS

Department of Paediatrics, University of Göttingen, Göttingen, West Germany

Summary

Isolated rat kidney tubules served as a model to investigate the direct effects of branched chain aminoacids, their α-ketoderivatives, and of the homolog straight chain aliphatic α-ketoacids on renal gluconeogenesis. It is demonstrated that the α-ketoderivatives, rather than the branched chain aminoacids themselves, are potent inhibitors of renal gluconeogenesis from precursors, entering the glucogenic pathway on all levels below and above triose phosphate. This inhibitory action is not specific for the branched chain α-ketoacids, since it is also observed in the presence of the homolog straight chain aliphatic α-ketoacids. The suppression of renal gluconeogenesis by α-ketoacids can not be explained by a direct inhibition of gluconeogenic reactions, by inhibition of cellular respiration, or by interference with the stimulatory action of Ca^{++}, cAMP, and L-lysine on renal gluconeogenesis. Although the point of inhibitory attack of α-ketoacids in renal gluconeogenesis could not be localized, an impairment of the kidney to respond to metabolic acidosis with an increase of gluconeogenesis was observed, since the pH optimum of renal gluconeogenesis was shifted from pH 6.8 to pH 7.7 in the presence of α-ketoisovaleric acid.

Speculation

On the basis of well known pathophysiologic parameters in human maple syrup urine disease an *in vitro* model has been developed with the purpose of elucidating the pathogenesis of metabolic acidosis and hypoglycemia in this disease.

In infancy and childhood conditions are encountered in which the intake of protein or certain aminoacids provokes hypoglycemia and/or metabolic acidosis. Such a condition is the rare inborn error of metabolism, called maple syrup urine disease (1, 3, 4, 11). In this disease the oxidative decarboxylation of the branched chain α-ketoacids is impaired (3, 4). Therefore, the plasma levels of the branched chain aminoacids, L-leucine, L-isoleucine, L-valine, and their ketoderivatives, α-ketoisocaproic acid, α-keto-β-methylvaleric acid, and α-ketoisovaleric acid, are extremely elevated (3–5, 13, 31). Neither the pathogenesis of metabolic acidosis nor that of hypoglycemia in maple syrup urine disease is completely understood. Two explanations for hypoglycemia must be considered.: 1) stimulation of insulin release from the β cells of the islets of Langerhans by leucine and α-ketoisocaproic acid as in cases of leucine sensitivity (6, 9) and 2) direct interference of branched chain aminoacids and/or their ketoderivatives with the role of liver (8, 11) and kidney in glucose homeostasis.

Inasmuch as Krebs and de Gasquet (18) had reported an inhibitory action of aromatic α-ketoacids on renal gluconeogenesis, the influence of the branched chain aminoacids and their α-ketoderivatives on glucose synthesis in isolated tubules from rat kidney cortex was investigated. In this context it is of interest that renal gluconeogenesis may serve a dual function, namely the regulation of glucose homeostasis and the regulation of acid base balance, because renal gluconeogenesis is considered to be a prerequisite for tubular ammoniagenesis (24, 27). Therefore, the kidney was given preference over the liver in the study of the direct influence of branched chain aminoacids and their α-ketoderivatives on gluconeogenesis. In addition, the kidney offers a particular advantage in the study of gluconeogenesis from different gluconeogenic precursors. Although the liver cell membrane is effectively impermeable towards citric acid cycle intermediates (28), kidney cortex has a reasonable gluconeogenic capacity for these intermediates, which seem to penetrate the tubule cell membrane without difficulty (17). Furthermore, the liver cell membrane shows only limited permeability towards ketoacids (27–29). Lactate, pyruvate, glutamine, succinate, and D-fructose were used as substrates because they enter the glucogenic pathway at different levels (29).

MATERIALS AND METHODS

Chemicals, substrates, and enzymes were obtained from the following sources: $Na_2{}^{14}CO_3$ from Amersham-Buchler (Braunschweig, Germany); sodium salts of the α-ketoacids from Sigma Chemical Company (St. Louis, MO); *2,5*-diphenyloxazole (PPO) and 1,4-bis-2[4-methyl-5-phenyl-oxazolyl]-benzene (POPOP) from W. Zinsser (Frankfurt a.M., Germany); collagenase (clostridiopeptidase A, EC 3.4.24.3, Reinheitsgrad II), glucose oxidase test combinations (GOD-Perid), hexokinase test combinations and cAMP from Boehringer (Mannheim, Germany); all other chemicals were purchased from E. Merck (Darmstadt, Germany).

ANIMALS

Male rats of the Wistar strain (200–250 g) were kept on the standard laboratory chow Altromin R from Altrogge (Lage/Lippe, Germany). They were stunned with a blow on the head and killed by bleeding. Kidneys were removed within seconds and chilled in ice-cold medium of Krebs and de Gasquet (18).

PREPARATION OF KIDNEY TUBULES

Kidney tubules were isolated from kidney cortex slices by collagenase treatment as described by Guder *et al.* (10), except that the medium of Krebs and Henseleit (19) was replaced by the phosphate-buffered saline medium of Krebs and de Gasquet (18), and incubation vessels were gassed during the first 10 min of collagenase treatment with oxygen.

Incubation of kidney tubules during gluconeogenesis experiments was performed in 15-ml Warburg vessels at 37° under oxygen at a shaking rate of 120 cpm with a shaking amplitude of 4 cm. Tests contained kidney tubules corresponding to 1.0–4.0 mg of protein in 2.0 ml of the saline medium of Krebs and de Gasquet (18).

The concentrations of substrates and inhibitors are indicated in connection with the appropriate tables and figures. After a pre-

gassing period of 10 min vessels were closed and incubations were started by addition of substrates and terminated 60 min later by addition of 0.4 ml 0.33 M $HClO_4$. After neutralizing the medium with $KHCO_3$ the centrifuged supernatants were analyzed for glucose. Glucose was determined by the use of glucose oxidase test combinations or hexokinase test combinations, the latter in the presence of α-ketobutyrate. No other substance than α-ketobutyrate interfered with the glucose oxidase or peroxidase reaction, respectively. $^{14}CO_2$ incorporation into glucose was determined according to the method of Stumpf *et al.* (32). Protein content of tubule suspensions was determined by the method of Lowry *et al.* (21) after three cycles of freezing and thawing.

RESULTS

TUBULAR GLUCONEOGENESIS FROM DIFFERENT SUBSTRATES IN PRESENCE OF BRANCHED CHAIN AMINOACIDS

In maple syrup urine disease serum levels of branched chain aminoacids are elevated up to 5 mM (1, 3–5, 11, 13, 31). Therefore, this concentration was used in the experiments of Tables 1–3 in order to evaluate the action of these aminoacids on renal gluconeogenesis. L-Leucine, L-isoleucine, and L-valine showed only minor inhibitory effects on glucose synthesis from succinate and fructose. L-Isoleucine also depressed gluconeogenesis from glutamine (Table 2), whereas L-valine had additional inhibitory effects

Table 1. *The influence of L-leucine and α-ketoisocaproic acid on tubular glucose synthesis from various substrates*[1]

	Glucose synthesis (nmol · mg protein^{-1} · hr^{-1})							
	Controls	L-Leucine (5 mM)	% of controls	*P*	Controls	α-Ketoisocaproic acid (5 mM)	% of controls	*P*
Lactate (5 mM)	473 ± 0.032 (4)	467 ± 0.010 (6)	99.00	n.s.	352 ± 0.016 (4)	220 ± 0.019 (4)	63.00	<0.0025
Pyruvate (5 mM)	647 ± 0.019 (4)	637 ± 0.022 (4)	98.00	n.s.	782 ± 0.030 (5)	472 ± 0.014 (5)	60.00	<0.0005
Glutamine (5 mM)	252 ± 0.013 (5)	226 ± 0.012 (5)	90.00	n.s.	464 ± 0.008 (5)	369 ± 0.014 (5)	80.00	<0.0005
Succinate (5 mM)	737 ± 0.030 (4)	629 ± 0.019 (4)	85.00	<0.01	889 ± 0.030 (4)	619 ± 0.025 (4)	70.00	<0.0005
Fructose (5 mM)	1369 ± 0.049 (4)	959 ± 0.036 (5)	70.00	<0.0005	2058 ± 0.087 (4)	1224 ± 0.088 (5)	50.00	<0.0005

[1] Concentrations of substrates, L-leucine, and α-ketoisocaproic acid as indicated. For further experimental details see *Materials and Methods.* Indicated are mean values ± SEM (n). *P* refers to controls.

Table 2. *Influence of L-isoleucine and α-keto-β-DL-methyl-valeric acid on tubular glucose synthesis from various substrates*[1]

	Glucose synthesis (nmol · mg protein^{-1} · hr^{-1})							
	Controls	L-Isoleucine (5 mM)	% of controls	*P*	Controls	α-keto-β-DL-methylvaleric acid (5 mM)	% of controls	*P*
Lactate (5 mM)	347 ± 0.045 (4)	334 ± 0.069 (5)	96.00	n.s.	347 ± 0.003 (4)	60 ± 0.003 (5)	17.00	<0.0005
Pyruvate (5 mM)	517 ± 0.015 (5)	503 ± 0.026 (4)	97.00	n.s.	279 ± 0.009 (5)	143 ± 0.006 (5)	51.00	<0.0005
Glutamine (5 mM)	370 ± 0.007 (5)	256 ± 0.008 (5)	69.00	<0.0005	412 ± 0.011 (5)	166 ± 0.010 (5)	60.00	<0.0005
Succinate (5 mM)	232 ± 0.008 (4)	187 ± 0.007 (4)	81.00	<0.005	232 ± 0.008 (4)	119 ± 0.008 (4)	51.00	<0.0005
Fructose (5 mM)	350 ± 0.018 (5)	302 ± 0.006 (4)	86.00	<0.005	400 ± 0.017 (5)	258 ± 0.001 (5)	67.00	<0.0005

[1] Concentrations of substrates, L-isoleucine and of α-keto-β-DL-methyl-valeric acid as indicated. For further experimental details see *Materials and Methods.* Indicated are mean values ± SEM (n). *P* refers to controls.

Table 3. *Influence of L-valine and of α-ketoisovaleric acid on tubular glucose synthesis from various substrates*[1]

	Glucose synthesis (nmol · mg protein^{-1} · hr^{-1})							
	Controls	L-Valine (5 mM)	% of controls	*P*	Controls	α-Ketoisovaleric acid (5 mM)	% of controls	*P*
Lactate (5 mM)	485 ± 0.003 (4)	475 ± 0.008 (4)	98.00	n.s.	683 ± 0.017 (5)	227 ± 0.014 (4)	33.00	<0.0005
Pyruvate (5 mM)	207 ± 0.004 (5)	176 ± 0.007 (4)	85.00	<0.0025	560 ± 0.087 (4)	259 ± 0.027 (5)	46.00	<0.0005
Glutamine (5 mM)	148 ± 0.006 (4)	101 ± 0.004 (5)	68.00	<0.0005	148 ± 0.006 (4)	67 ± 0.001 (5)	45.00	<0.0005
Succinate (5 mM)	173 ± 0.009 (4)	140 ± 0.005 (5)	81.00	<0.01	173 ± 0.009 (4)	59 ± 0.002 (4)	34.00	<0.0005
Fructose (5 mM)	1264 ± 0.039 (5)	1068 ± 0.072 (5)	85.00	<0.025	858 ± 0.017 (5)	227 ± 0.011 (4)	26.00	<0.0005

[1] Concentrations of substrates, L-valine, and α-ketoisovaleric acid as indicated. For further experimental details see *Materials and Methods.* Indicated are mean values ± SEM (n). *P* refers to controls.

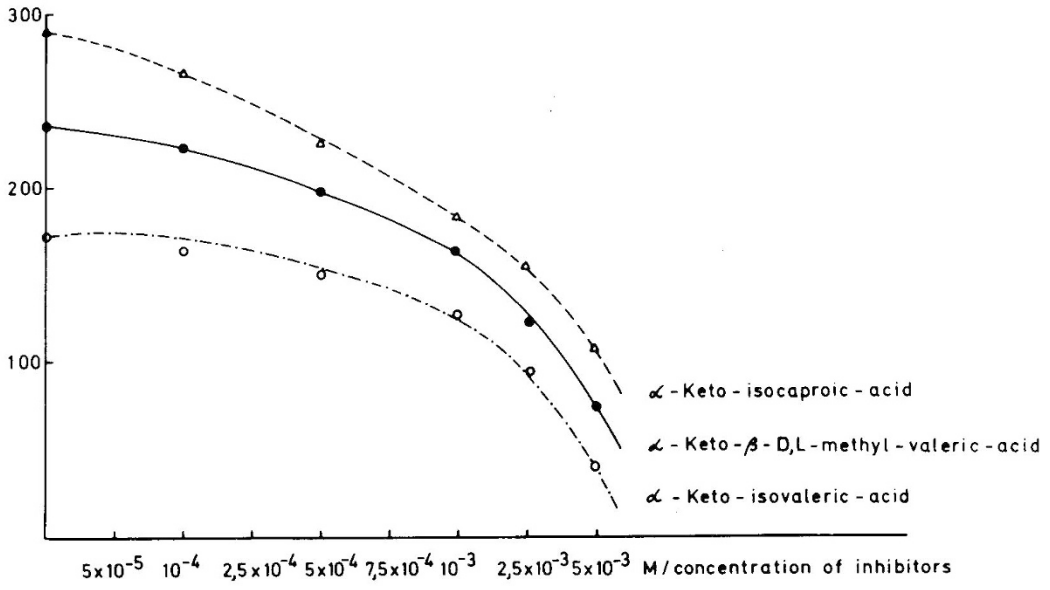

Fig. 1. Inhibition of tubular gluconeogenesis (in nanomoles per mg protein · hr) from 5 mM lactate by increasing concentrations of α-ketoisocaproic acid, α-keto-β-DL-methylvaleric acid, and α-ketoisovaleric acid. Concentrations of inhibitors are indicated on the abscissa. Indicated are the mean values of four experiments. For further experimental details see *Materials and Methods.*

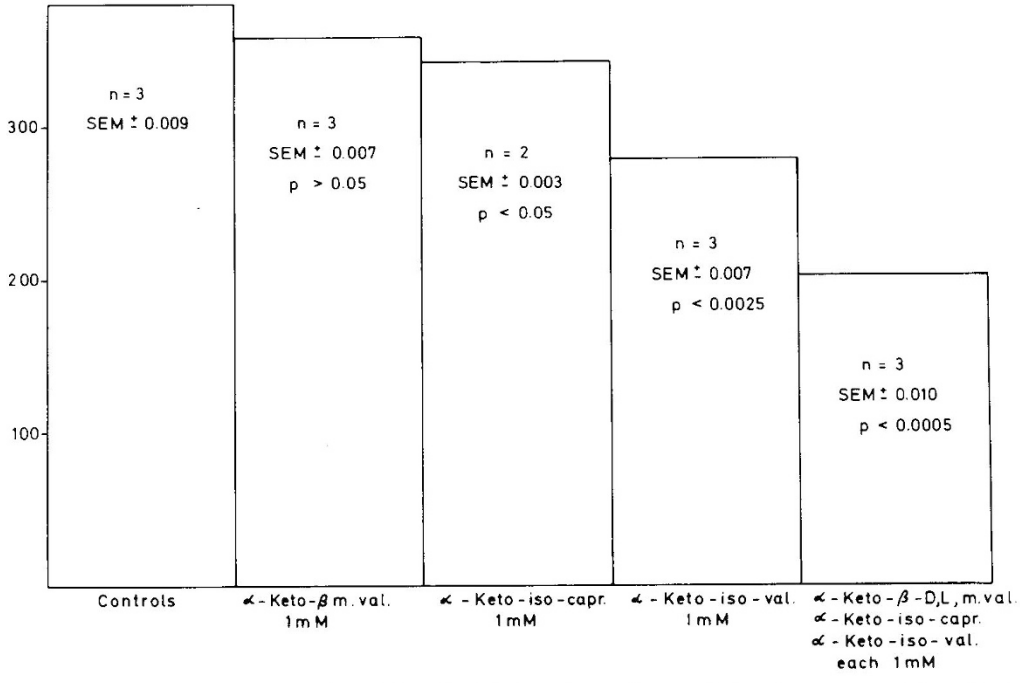

Fig. 2. The additive behavior of the inhibitory action of branched chain α-ketoacids on tubular gluconeogenesis from 5 mM lactate. α-Keto-β-m.val.: α-keto-β-DL-methylvaleric acid; α-Keto-iso-capr.: α-ketoisocaproic acid; α-Keto-iso-val.: α-ketoisovaleric acid. Inhibitor concentrations were 1 mM. Indicated is the glucogenic rate in nanomoles · mg protein^{-1} · h^{-1}. *P* refers to controls. For further experimental details see *Materials and Methods.*

on glucose synthesis in the presence of glutamine and pyruvate (Table 3). The inhibitory effects in these experiments ranged between 14% and 32% of the control values. Gluconeogenesis from lactate was not impaired by any of the aminoacids investigated.

TUBULAR GLUCONEOGENESIS FROM DIFFERENT SUBSTRATES IN PRESENCE OF BRANCHED CHAIN α-KETOACIDS

In contrast to the branched chain aminoacids the corresponding α-ketoacids proved to be potent inhibitors from all substrates. The glucogenic rates were suppressed to values ranging between 17% and 80% of controls. The inhibitors showed no preference for a definite substrate (Tables 1–3). The inhibitory action of α-ketoisocaproic acid, α-keto-β-DL-methylvaleric acid, and α-ketoisovaleric acid was visible in the range of $5 \cdot 10^{-4}$ M (Fig. 1).

The suppressive effects of the branched chain α-ketoacids on tubular gluconeogenesis from 5 mM lactate were additive. When 5 mM concentrations were employed the combined actions of the three α-ketoacids resulted in almost complete suppression of glucose synthesis (not shown). α-Ketoacid concentrations in the range of those encountered in maple syrup urine disease, namely 1 mM (4, 5, 13), still inhibited the synthetic rate by 50% (Fig. 2).

TUBULAR GLUCONEOGENESIS IN PRESENCE OF NONBRANCHED ALIPHATIC α-KETOACIDS

The inhibitory effect on renal gluconeogenesis was not exclusively observed in the presence of branched chain α-ketoacids,

since the straight chain homologs, α-ketobutyric acid, α-ketovaleric acid, and α-ketocaproic acid, also potently suppressed the conversion of lactate to glucose (Table 4). When α-ketobutyric acid was studied in the presence of pyruvate, succinate, and fructose, it depressed the glucogenic rates by 63, 61, and 68%, respectively (not shown).

$^{14}CO_2$ INCORPORATION DURING TUBULAR GLUCONEOGENESIS FROM LACTATE IN PRESENCE OF α-KETOACIDS

$^{14}CO_2$ incorporation may be used as an indicator of the activity of pyruvate carboxylase, the first key enzyme in gluconeogenesis from C_3 precursors, like lactate and pyruvate (32). Both α-keto-β-DL methylvaleric acid and the unbranched α-ketovaleric acid inhibited glucose synthesis and $^{14}CO_2$ incorporation into glucose during renal gluconeogenesis from lactate (Table 5).

ACTION OF EFFECTORS OF RENAL GLUCONEOGENESIS IN PRESENCE OF α-KETOISOVALERIC ACID

Ca^{++}, H^+, cAMP, and L-lysine are positive effectors of renal gluconeogenesis (10, 20, 22, 23, 32). Neither the stimulatory action of Ca^{++} nor the accelerating effects of cAMP and L-lysine on renal gluconeogenesis from lactate were abolished by the presence of 5 mM α-ketoisovaleric acid (not shown).

It is well known that acidification of the incubation medium enhances renal gluconeogenesis. Therefore, under normal conditions the pH optimum of gluconeogenesis of kidney cortex is in the acidotic range (15, 22, 27). However, in the presence of 5 mM α-ketoisovaleric acid the pH optimum of the suppressed glucose synthesis from lactate was shifted from pH 6.8 to pH 7.7. Thus a situation appeared in which isolated kidney tubules were no longer able to respond with increased glucogenic rates in metabolic acidosis (Fig. 3).

Table 4. *Inhibition of tubular glucose synthesis from lactate by various straight chain α-ketoacids*[1]

Glucose synthesis from 5 mM lactate (nmol · mg protein^{-1} · hr^{-1})			
Controls	α-Ketobutyric acid (5 mM)	% of controls	*P*
285 ± 0.006 (3)	76 ± 0.009 (4)	27.00	0.0005
	α-Ketovaleric acid (5 mM)		
369 ± 0.031 (4)	129 ± 0.004 (5)	35.00	0.0005
	α-Ketocaproic acid (5 mM)		
369 ± 0.031 (4)	144 ± 0.003 (5)	39.00	0.0005

[1] Concentrations of lactate, α-ketobutyric acid, α-ketovaleric acid and of α-ketocaproic acid as indicated. For further experimental see *Materials and Methods.* Indicated are mean values ± SEM (n). *P* refers to controls.

DISCUSSION

In maple syrup urine disease neither the pathogenesis of metabolic acidosis nor that of hypoglycemia is completely understood. Hypoglycemia could be secondary to a stimulation of insulin release, as seen in patients with leucine-sensitive hypoglycemia (5, 9). This assumption, however, seems unlikely, since Haymond *et al.* (11) reported a case of the classic type of maple syrup urine disease, in whom hyperinsulinism was not a causative factor. In 1966 Greenberg and Reaven (8) were able to demonstrate an

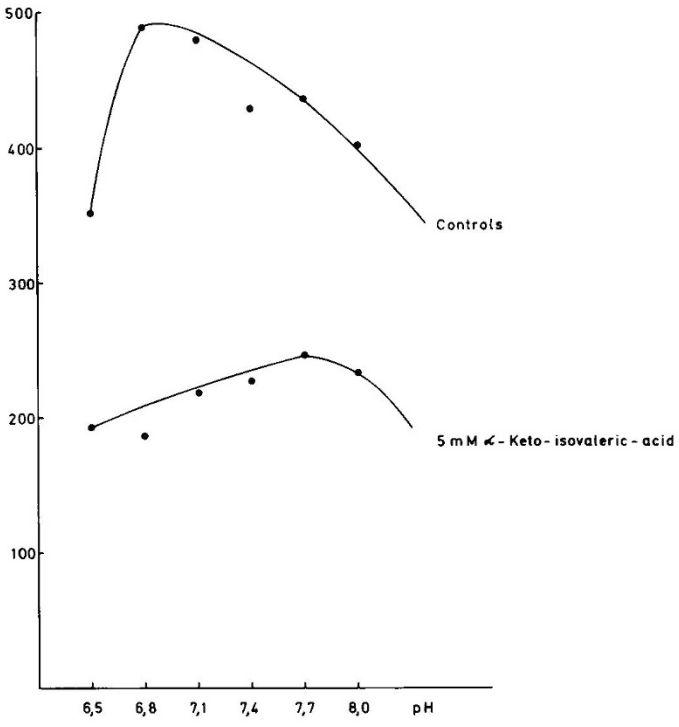

Fig. 3. pH dependence of tubular glucose synthesis (in nanomoles per mg protein · hr) from 5 mM lactate in the presence of 0 and 5 mM α-ketoisovaleric acid. The pH values are indicated on the abscissa. For further experimental details see Materials and Methods.

Table 5. *Inhibition of glucose synthesis and $^{14}CO_2$ incorporation during tubular gluconeogenesis from lactate by α-keto-β-DL-methylvaleric acid and α-ketovaleric acid*[1]

	Glucose synthesis (nmol · mg protein^{-1} · hr^{-1}) and $^{14}CO_2$ incorporation (cpm · mg protein^{-1} · hr^{-1})			
	Controls	α-Keto-β-methylvaleric acid (5 mM)	% of controls	*P*
Glucose synthesis	447 ± 0.01 (4)	198 ± 0.007 (4)	44.00	0.0005
$^{14}CO_2$ incorporation	50,770 ± 1,391 (4)	36,460 ± 1,672 (4)	72.00	0.0005
		α-Ketovaleric acid 5 (mM)		
Glucose synthesis	295 ± 0.010 (4)	151 ± 0.008 (4)	51.00	0.0005
$^{14}CO_2$ incorporation	32,480 ± 1,543 (4)	14,470 ± 465 (4)	46.00	0.0005

[1] Concentrations of α-keto-β-DL-methyl-valeric acid and of α-ketovaleric acid as indicated. Concentration of lactate (5 mM) and of [^{14}C]bicarbonate (10 mM). Specific radioactivity of [^{14}C]bicarbonate: 0.1 μCi/μmol. For further experimental details see *Materials and Methods.* Indicated are mean values ± SEM (n). *P* refers to controls.

inhibitory action of high concentrations (75 mM) of L-leucine on the incorporation of C_3 precursors into glucose and glycogen by mouse liver slices. Therefore, these authors postulated a direct inhibitory effect of this aminoacid on hepatic gluconeogenesis.

This assumption found additional support by the recent clinical investigations of Haymond *et al.* (11). By the use of fructose and alanine tolerance tests these authors demonstrated inhibited hepatic gluconeogenesis from substrates entering the glucogenic pathway below the levels of triose phosphate. Inasmuch as blood glutamine showed a sustained rise after intravenous alanine loading, Haymond *et al.* (11) concluded that there existed a preferential shunting of C_3 precursors via α-ketoglutarate and the glutamate dehydrogenase reaction into glutamate and glutamine, thus resulting in a drop of the gluconeogenic intermediate oxaloacetate. This proposal was supported by *in vitro* experiments with purified bovine liver glutamate dehydrogenase, which was activated by exogenous leucine and the patient's plasma (11). The work presented in this paper extends the investigations on the metabolic effects of branched chain aminoacids and their α-ketoderivatives on renal gluconeogenesis.

Under most physiolôgic states the kidney plays only a limited role in the regulation of glucose homeostasis, inasmuch as renal gluconeogenic flux is rarely more than 10% of the body's total glucose synthesis (24, 27, 29). Under certain conditions, however, renal gluconeogenesis becomes increasingly important, namely during metabolic and respiratory acidosis, when it is supposed to be a prerequisite for tubular ammoniagenesis (15, 27), and after prolonged starvation, when it covers about 50% of the body's total glucose synthesis (24).

In the experiments of Tables 1–3 L-leucine, L-isoleucine, and L-valine showed only minor inhibitory effects on glucose synthesis from pyruvate, glutamine, succinate, and fructose. All branched chain α-ketoacids, on the contrary, proved to be potent inhibitors of gluconeogenesis from all substrates, including lactate. Although in these experiments rather high inhibitor concentrations, namely 5 mM, were employed, the inhibitory action of the three branched chain α-ketoacids was already visible in the range of 5×10^{-4} M (Fig. 1). This concentration is about 50 times higher than normal serum levels of the branched chain α-ketoacids (14), but still below those of children with maple syrup urine disease (4, 5, 13).

Of special importance is the observation that these inhibitory effects were additive. Thus, α-ketoacids in the range of those reported for maple syrup urine disease (4, 5, 13), namely 1 mM, were able to suppress the synthetic rate by 50% (Fig. 2). Therefore, it may be assumed that branched chain α-ketoacids effectively suppress renal gluconeogenesis in untreated cases of maple urine disease.

The data presented in this paper demonstrate that the α-ketoderivatives rather than the branched chain aminoacids themselves are potent inhibitors of renal gluconeogenesis. This effect is not specific for branched chain α-ketoacids. It is a common feature of a larger group of α-ketoacids, such as phenylpyruvic acid, *p*-hydroxyphenylpyruvic acid (17), and branched and straight chain aliphatic α-ketoacids (Tables 1–4).

Neither by the use of various precursors entering the glucogenic pathway at different levels (Tables 1–3) nor by measuring the rate of $^{14}CO_2$ incorporation into glucose (Table 5) were we able to localize the site of the inhibitory action of α-ketoacids in renal gluconeogenesis. Although a suppression of $^{14}CO_2$ incorporation into glucose during glucose synthesis from lactate seems to be in accord with an inhibition of pyruvate carboxylase by α-ketoacids such as phenylpyruvic acid, as previously reported by Seubert and Huth (30), this does not explain inhibition of gluconeogenesis from substrates like glutamine, succinate, and fructose, which all enter the glucogenic pathway above the pyruvate carboxylase reaction (29). Inasmuch as none of the glucogenic reactions leading from triosephosphate to glucose was impaired in the presence of α-ketoacids (19, 33), some other process fundamental to gluconeogenesis from all glucogenic substrates, entering the pathway below and above triose phosphate, must have been affected.

Although various investigators were able to demonstrate inhibitory effects of maple syrup urine disease metabolites on the oxidation of glucose, pyruvate, and α-ketoglutarate by rat brain preparations (7, 25, 26), as well as on pyruvate kinase (7), mitochondrial pyruvate transport (2), pyruvate dehydrogenase (4, 7, 13, 16, 25), and α-ketoglutarate dehydrogenase (7, 13, 16, 26) in different mammalian tissues, oxygen consumption of isolated kidney tubules was not inhibited by α-ketoisovaleric acid during gluconeogenesis from lactate (33).

Neither the stimulatory action of Ca^{++} nor the accelerating effects of cAMP and L-lysine were abolished by α-ketoisovaleric acid. Therefore, an interference of α-ketoacids with the action of these positive effectors seems not to be the reason of inhibition of renal gluconeogenesis.

The pH optimum of renal glucose synthesis from lactate, however, was shifted from pH 6.8 to pH 7.7 in the presence of 5 mM α-ketoisovaleric acid. Thus a situation appeared in which isolated kidney tubules were no longer able to respond with increased glucogenic rates to metabolic acidosis. Since Kamm *et al.* (15) and Kurokawa and Rasmussen (20) were able to demonstrate stimulation of renal gluconeogenesis from pyruvate, oxaloacetate, glutamine, glutamate, α-ketoglutarate, succinate, citrate, and glycerol by acidification of the incubation medium this alteration of the pH optimum to more alkalotic values might in part explain the impairment of tubular gluconeogenesis in the presence of α-ketoacids. The mechanism of this phenomenon, however, remains unexplained.

On the basis of the present results it is not clear whether the proposal of Haymond *et al.* (11) is also applicable to the kidney. This proposal is as follows: in maple syrup urine disease L-leucine induces a shift of C_3 precursors via glutamate into glutamine, thus resulting in a lowering of intramitochondrial oxaloacetate levels and, in consequence of this, in an inhibition of gluconeogenesis. Inhibition of renal gluconeogenesis and hence of tubular ammoniagenesis, however, might explain the sustained increase of blood glutamine levels after iv alanine loading observed by these authors (11).

Inasmuch as tubular ammoniagenesis depends on intact renal gluconeogenesis (24, 27), inhibition of the latter process by branched chain ketoacids might well explain the appearance of metabolic acidosis in maple syrup urine disease.

In contrast to its important function in the control of acid base balance, however, the kidney plays only a limited role in the regulation of the body's glucose homeostasis under most physiological conditions (24, 27). Therefore, the present results do not necessarily explain the pathogenesis of hypoglycemia in maple syrup urine disease.

The question arises as to the relative contributions of the liver and the kidney to glucose homeostasis by gluconeogenesis in this disease. The liver contains only little transaminases of the branched chain aminoacids in contrast to kidney and muscle (12). Hepatic gluconeogenesis may therefore be rather unaffected, the more so since the liver cell membrane shows a limited permeability towards the ketoacids (27, 28).

Although earlier experiments by Greenberg and Reaven (8) and the clinical investigations of Haymond *et al.* (11) are consistent with an inhibition of hepatic gluconeogenesis in branched chain ketoaciduria the mechanisms operating in liver and kidney may be quite different. This will be the subject of further investigations.

CONCLUSION

The authors wish to emphasize that their results are derived from an artificial *in vitro* system employing material from animals without a metabolic disturbance. Whether these results are also applicable to human pathophysiologic conditions characterized by metabolic acidosis and hypoglycemia has to be proven by further clinical investigations.

REFERENCES AND NOTES

1. Auerbach, V. H., and Di George, A. M.: Maple syrup disease. In: F. A. Hommes and C. J. van den Berg: Inborn Errors of Metabolism, pp. 337–354 (Academic Press, New York, 1973).

2. Clark, J. B., and Land, J. M.: Differential effects of 2-oxo-acids on pyruvate utilization and fatty acid synthesis. Biochem. J., *140:* 25 (1974).
3. Dancis, J., and Levitz, M.: Abnormalities of branched chain amino acid metabolism. In: J. B. Stanbury, J. S. Wyngaarden, and D. S. Frederickson: The Metabolic Basis of Inherited Disease, pp. 426–439 (McGraw-Hill, New York, 1972).
4. Dent, C. E., and Westall, R. G.: Studies in maple syrup urine disease. Arch. Dis. Childhood, *36:* 259 (1961).
5. Dreyfus, P. M., and Prensky, A. L.: Further observations on the biochemical leason in maple syrup urine disease. Nature, *214:* 276 (1967).
6. Frerichs, H., and Creutzfeld, W.: Die Leucin-Hypoglykämie. Deut. Med. Wochenschr., *90:* 960 (1965).
7. Gibson, G. E., and Blass, J. P.: Inhibition of acetylcholine synthesis and of carbohydrate utilization by maple syrup urine disease metabolites. J. Neurochem., *26:* 1073 (1976).
8. Greenberg, R., and Reaven, G.: The effect of L-leucine on hepatic glucose formation. Pediatrics, *37:* 934 (1966).
9. Grumbach, M. M., and Kaplan, S. L.: Aminoacid and ketoacid-induced hyperinsulinism in the leucine-sensitive type of infantile and childhood hypoglycemia. J. Pediat., *57:* 346 (1960).
10. Guder, W., Wiesner, W., Stukowski, B., and Wieland, O: Metabolism of isolated kidney tubules: Oxygen consumption, gluconeogenesis and the effect of cyclic nucleotides in tubules from starved rats. Hoppe-Seyler's Z. Physiol. Chem., *352:* 1319 (1971).
11. Haymond, M. W., Karl, J. E., Feigin, R. D., de Vivo, D., and Pagliara, A. S.: Hypoglycemia and maple syrup urine disease: Defective gluconeogenesis. Pediat. Res., *7:* 500 (1973).
12. Ichihara, A., and Koyama, E.: Transaminase of branched chain amino acids. I. Branched chain amino acids-α-ketoglutarate transaminase. J. Biochem., *59:* 160 (1966).
13. Johnson, W. A., and Connelly, J. L.: Studies on the mutual influences of substrates on bovine α-ketoacid metabolism. Biochemistry, *11:* 2416 (1972).
14. Kaeser H., Kaeser, R., and Lestradet, H.: Separation and quantitative estimation of new alpha ketoacids in human blood by paper chromatography. Metabolism, *9:* 926 (1960).
15. Kamm, D. E., Fuisz, R. E., Goodman, A. D., and Cahill, G. F., Jr.: Acid base alterations and renal gluconeogenesis: Effect of pH, bicarbonate concentration, and pCO_2. J. Clin. Invest., *46:* 1172 (1967).
16. Kanzaki, T., Hayakawa, T., Hamada, M., Fukuyoshi, Y., and Koike, M.: Mammalian α-ketoacid dehydrogenase complexes. IV. Substrate specificities and kinetic properties of the pig heart pyruvate and 2-oxoglutarate dehydrogenase complexes. J. Biol. Chem., *244:* 1183 (1969).
17. Krebs, H. A., Bennett, D. A. H., de Gasquet, P., Gascoyne, T., and Yoshida, T.: Renal gluconeogenesis: The effect of diet on the gluconeogenic capacity of rat kidney-cortex slices. Biochem. J., *86:* 22 (1963).
18. Krebs, H. A., and de Gasquet, P.: Inhibition of gluconeogenesis by α-oxo-acids. Biochem. J., *90:* 149 (1964).
19. Krebs, H. A., and Henseleit, K.: Untersuchungen über die Harnstoffbildung im Tierkörper. Hoppe-Seyler's Z. Physiol. Chem., *210:* 33 (1932).
20. Kurokawa, K., and Rasmussen, H.: Ionic control of renal gluconeogenesis. I. The interrelated effect of calcium and hydrogen ions. Biochim. Biophys. Acta, *313:* 17 (1973).
21. Lowry, O. H., Rosebrough, H. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
22. Nagata, N., and Rasmussen, H.: Renal gluconeogenesis: Effects of Ca^{2+} and H^+. Biochim. Biophys. Acta, *215:* 1 (1970).
23. Nagata, N., and Rasmussen, H.: Parathyroid hormone, 3'5'-AMP, Ca^{2+} and renal gluconeogenesis. Proc. Natl. Acad. Sci. U. S. A., *65:* 368 (1970).
24. Owen, O. E., Felig, P., Morgan, A. F., Wahren, J., and Cahill, G. F., Jr.: Liver and kidney metabolism during prolonged starvation. J. Clin. Invest., *48:* 574 (1969).
25. Patel, M. S., Auerbach, V. H., Grover, W. D., and Wilbur, D. O.: Effect of the branched-chain α-ketoacids on pyruvate metabolism by homogenates of human brain. J. Neurochem., *20:* 1793 (1973).
26. Patel, M. S.: Inhibition by the branched-chain 2-oxo acids of the 2-oxoglutarate dehydrogenase complex in developing rat and human brain. Biochem. J., *144:* 91 (1974).
27. Pogson, C. J., Longshaw, J. D., Roobol, A., Smith, S. A., and Alleyne, C. A. O.: Phosphoenolepyruvate carboxykinase and renal gluconeogenesis. In: R. W. Hanson and M. A. Mehlmann: Gluconeogenesis, pp. 335–368 (John Wiley & Sons, New York, 1976).
28. Ross, B. D., Hems, R., and Krebs, H. A.: The rate of gluconeogenesis from various precursors in the perfused rat liver. Biochem. J., *102:* 942 (1967).
29. Scrutton, M. C., and Utter, M. F.: The regulation of glycolysis and gluconeogenesis in animal tissues. Ann. Rev. Biochem., *37:* 249 (1968).
30. Seubert, W., and Huth, W.: On the mechanism of gluconeogenesis and its regulation. II. The mechanism of gluconeogenesis from pyruvate and fumarate. Biochem. Z., *343:* 176 (1965).
31. Snyderman, S. E., Norton, P. M., Roitman, E., and Holt, E., Jr.: Maple syrup urine disease, with particular reference to dietotherapy. Pediatrics, *34:* 454 (1964).
32. Stumpf, B., Boie, A., Leimcke, H., and Seubert, W.: On the mechanism of gluconeogenesis and its regulation. VIII. Differentiation of regulatory attacks of glucocorticoids, L-lysine and cyclic AMP on renal gluconeogenesis. Hoppe-Seyler's Z. Physiol. Chem., *355:* 205 (1974).
33. Stumpf, B., and Kraus, H., Unpublished results.
34. This research was supported by grants of the Maizena Gesellschaft, Hamburg.
35. Requests for reprints should be addressed to: Dr. B. Stumpf, Universitäts-Kinderklinik, Humboldtallee 38, D-3400 Göttingen (West Germany).
36. Received for publication October 10, 1977.
37. Accepted for publication February 8, 1978.

0031-3998/78/1211-1039$02.00/0