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Utilization of alanine for glucose formation in isolated rabbit kidney-cortex tubules

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In kidney cortex tubules isolated from fed rabbits L-alanine is not utilized as glucose precursor, when added as a sole substrate. However, this amino acid decreases gluconeogenesis from low (up to 1 mM) 2-oxoglutarate concentrations and stimulates this process at higher (2.5–10 mM) ketoacid contents in the suspension medium. Aminooxyacetate, an inhibitor of aminotransferases, abolishes both inhibitory and stimulatory effects of L-alanine on glucose formation. The addition of 2-oxoglutarate increases the incorporation of L-[U-^{14}C]alanine to glucose from 8- to 123-fold, depending upon the ketoacid and alanine concentrations used. In contrast, nonlabelled L-alanine decreases the incorporation of low [U-^{14}C)2-oxoglutarate concentrations into glucose, while it does not affect contribution of 5 mM ketoacid to gluconeogenesis. The data indicate that (i) in the presence of 2-oxoglutarate L-alanine is utilized as glucose precursor in rabbit renal tubules and (ii) this amino acid may decrease the contribution of low extracellular concentrations of the ketoacid to gluconeogenesis.

Alanine; Gluconeogenesis; (Rabbit renal tubule)

1. INTRODUCTION

Although alanine aminotransferase is located in the straight portion of proximal nephron [1] it has been shown that in contrast to the rat perfused liver [2] and isolated hepatocytes [3,4], L-alanine is not used as glucose precursor in rat kidney cortex slices [5] and isolated renal cortex tubules derived from rat [6] and guinea pig [7]. Alanine does, however, exert a stimulatory effect on glucose synthesis from pyruvate and lactate in rat renal cortical tubules, probably due to an inhibition of pyruvate kinase activity by this amino acid [6], a potent inhibitor of the enzyme [8].

It is established that the major role of alanine in renal metabolism reside in its production by renal tubules [9]. However, Michoudet et al. [10] have recently reported that in guinea pig renal cortex tubules alanine may be converted into glutamine with the use of pyruvate carboxylase which catalyses the fixation of CO_2 on pyruvate to give oxaloacetate for synthesis of citrate and then of aconitate and 2-oxoglutarate. Moreover, as concluded from ^{13}C-NMR studies with the use of renal epithelial cells [11], ^{13}C label from L-[^{13}C]alanine may enter tricarboxylic acid cycle through both pyruvate dehydrogenase and pyruvate carboxylase pathways, giving rise to ^{13}C-labelled glutamate. Since pyruvate carboxylase is the first enzyme of gluconeogenesis, studies were undertaken to establish conditions for glucose formation from alanine in rabbit kidney cortex tubules.

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2. MATERIALS AND METHODS

2.1. *Animals*

Termond strain, male white rabbits (2–3 kg body weight) were used throughout. Animals were maintained on standard rabbit chow.

2.2. *Preparation and incubation of kidney-cortex tubules*

Renal cortex tubules from rabbits were isolated using basically the procedures of Burg and Orloff [12] and Guder et al. [13] as described previously [14]. The dry weight of tubules was determined according to Krebs [15]. Isolated kidney tubules (10–13 mg dry weight) were incubated at 37°C in 2 ml of Krebs–Henseleit bicarbonate solution in 25 ml plastic Erlenmeyer flasks with the rubber stoppers under the atmosphere of 95% O_2 + 5% CO_2. Substrates were used at concentrations given in legends to tables and figure. Reactions were stopped by the addition of 35% $HClO_4$ (0.1 volume of suspension).

The isotopic studies used for investigation of the contribution of alanine and 2-oxoglutarate to glucose formation were performed according to Exton and Park [3] and Pilkis et al. [16] following the treatment of samples with glutaminase.

2.3. *Determination of metabolites*

Glucose, pyruvate, 2-oxoglutarate and alanine were determined in the deproteinized and neutralized samples. Glucose was measured with either glucose oxidase and peroxidase [17] or hexokinase and glucose 6-phosphate dehydrogenase [18]. 2-Oxoglutarate was estimated spectrophotometrically according to Williamson and Corkey [19]. For assay of pyruvate the standard enzymatic method was used [18] while alanine determination was performed with the T339 Mikrotechna amino acid analyzer (Czechoslovakia).

2.4. *Enzymes and chemicals*

Collagenase (EC 3.4.24.3), type IV, was obtained from Sigma Chemical Co. (St. Louis, MO, USA). L-Alanine was from Reanal

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Factory of Laboratory Chemicals (Budapest, Hungary). L-[U-^{14}C]-alanine was from Amersham Earle Corporation (England) whereas [U-^{14}C]2-oxoglutarate was produced from L-[U-^{14}C]glutamate (Institute for Research Production and Uses of Radioisotopes, Prague, Czechoslovakia) with the use of aspartate aminotransferase (EC 2.6.1.1, Boehringer, FRG) and limited amounts of oxaloacetate. The reaction mixture consisted of 50 mM triethanolamine-HCl buffer (pH 7.4), 1 mM L-[U-^{14}C]glutamate (50 mCi/mmol), 0.5 mM oxaloacetate and 50 units of aspartate aminotransferase/ml. On 2 h incubation at room temperature the reaction was terminated by the addition of 1 M HCl. The protein was removed by centrifugation and supernatant was subjected to cation-exchange chromatography on Dowex 50W-X8, H^+, as described by Elsas et al. [20] for radiolabelled branched-chain 2-ketoacids. Column effluent fractions were collected and the ^{14}C fractions were pooled. The [^{14}C]2-oxoglutarate contained in the pooled fraction was then converted to its sodium salt, lyophilized and stored desiccated at -20°C. A single vial was reconstituted for each experiment and discarded after use.

Other chemicals were of analytical grade.

3. RESULTS AND DISCUSSION

3.1. *Glucose synthesis and substrate utilization*

In agreement with data for guinea pig renal cortex tubules [7,10] and perfused rat kidney [5] in isolated rabbit renal cortex tubules there is no glucose production from alanine added as a sole substrate (cf. table 1). However, in contrast Friedrichs and Schoner's [6] findings in rat renal tubules, isolated rabbit renal tubules, just like dog [9] and guinea pig kidney cortex tubules [7,10], utilize alanine from the incubation medium, while aminooxyacetate, a potent inhibitor of aminotransferases [21], abolishes this process. Since alanine is considered to be an important substrate for glutamine formation [7,10], it seems likely that no glucose accumulation occurring during the metabolism of alanine in rabbit renal tubules might be due to utilization of pyruvate arising from the transamination of alanine as the source of 2-oxoglutarate required for the transamination of alanine. This implies that, under these conditions, pyruvate carboxylase which catalyses the fixation of CO_2 on pyruvate to give oxaloacetate needed for the synthesis of citrate and then of aconitate and 2-oxoglutarate, is involved in the conversion of alanine into glutamine rather than in the conversion of alanine into glucose [10]. Accumulation of both glutamate and glutamine in the rabbit renal tubule suspension incubated with alanine, stimulated 2-3-fold on the addition of 5 mM 2-oxoglutarate (results not presented), is in agreement with this proposal.

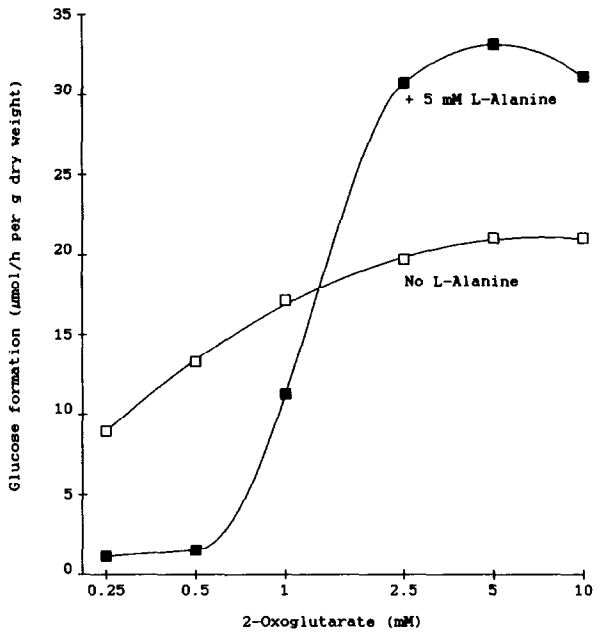

Fig.1. Effect of 5 mM alanine on glucose formation in rabbit renal tubules incubated in the presence of various concentrations of 2-oxoglutarate. Data represent values of a typical experiment.

As shown in fig.1, alanine alters the rate of gluconeogenesis in the presence of 2-oxoglutarate depending upon the concentration of this ketoacid in the reaction mixture. At low concentrations of 2-oxoglutarate (between 0.25 and 1.0 mM) 5 mM L-alanine decreases the rate of glucose formation, whereas at concentrations of 2.5–10 mM this amino acid significantly stimulates this process. Moreover, aminooxyacetate abolishes both the inhibitory and stimulatory effects of alanine on glucose

Table 1

Glucose formation and substrate utilization in rabbit renal tubules incubated with L-alanine and/or 2-oxoglutarate (μmol/h per g dry weight)

L-Alanine (5 mM)	2-Oxoglutarate (mM)	AOA (0.2 mM)	Glucose formation	L-Alanine utilization	2-Oxoglutarate uptake
+	–	–	0.9 ± 0.2 (7)	180.0 ± 17.0 (3)	–
–	0.5	–	16.8 ± 2.8 (5)	–	83.4 ± 11.4 (4)
–	5.0	–	22.6 ± 4.4 (7)	–	146.7 ± 9.4 (4)
+	–	+	0.4 ± 0.1 (3)	13.0 ± 2.1 (3)[a]	–
+	0.5	–	4.1 ± 1.1 (5)[b,c]	182.6 ± 23.2 (3)	91.2 ± 12.3 (4)
+	0.5	+	18.4 ± 2.7 (3)[a]	21.0 ± 2.7 (3)[a]	–
+	5.0	–	32.5 ± 4.9 (7)[b,c]	381.8 ± 13.2 (3)	479.4 ± 18.6 (4)[c]
+	5.0	+	25.2 ± 3.0 (3)[a]	23.0 ± 4.9 (3)[a]	–

[a] $P<0.01$ versus corresponding control without AOA
[b] $P<0.01$ versus corresponding control with alanine alone
[b] $P<0.01$ versus corresponding control with 2-oxoglutarate alone

Table 2

The effect of 2-oxoglutarate on the incorporation of L-[U-^{14}C]alanine into glucose (A) and the influence of unlabelled L-alanine on the incorporation of [U-^{14}C]2-oxoglutarate into glucose (B)

Expt	^{14}C-labelled substrate	Unlabelled substrate	AOA (0.2 mM)	[^{14}C]Glucose (% of control)
A	5.0 mM [U-^{14}C]Alanine	0.5 mM 2-Oxoglutarate	–	812 ± 77 (3)[a]
	5.0 mM [U-^{14}C]Alanine	0.5 mM 2-Oxoglutarate	+	111 ± 9 (2)
	5.0 mM [U-^{14}C]Alanine	5.0 mM 2-Oxoglutarate	–	2655 ± 389 (3)[a]
	5.0 mM [U-^{14}C]Alanine	5.0 mM 2-Oxoglutarate	+	84 ± 11 (2)
B	0.5 mM [U-^{14}C]2-Oxoglutarate	5.0 mM Alanine	–	35 ± 2 (3)[a]
	0.5 mM [U-^{14}C]2-Oxoglutarate	5.0 mM Alanine	+	105 ± 12 (2)
	5.0 mM [U-^{14}C]2-Oxoglutarate	5.0 mM Alanine	–	110 ± 8 (3)
	5.0 mM [U-^{14}C]2-Oxoglutarate	5.0 mM Alanine	+	99 ± 6 (2)

Incubations were carried out for 60 min. The rates of incorporation of the ^{14}C-labelled substrates into glucose are expressed as means of percentage of control without 2-oxoglutarate (A) and without L-alanine (B) of number experiments shown in parentheses. The values for the basal rates of [^{14}C]glucose production from [U-^{14}C]alanine and from 0.5 and 5 mM [U-^{14}C]2-oxoglutarate added as the sole substrates were 0.4 ± 0.1, 15.5 ± 2.1 and 21.2 ± 2.2 μmol/h per g dry weight, respectively

[a] $P<0.01$ versus corresponding control with no unlabelled substrate

production in the presence of 2-oxoglutarate as well as alanine utilization from the reaction medium (table 1). An inhibition by alanine of gluconeogenesis from 0.5 mM 2-oxoglutarate results probably from a competition for 2-oxoglutarate between alanine aminotransferase and 2-oxoglutarate dehydrogenase and/or utilization of oxaloacetate generated via pyruvate carboxylase predominantly for citrate and then 2-oxoglutarate synthesis and not for phospoenolpyruvate production. In contrast, at 5 mM 2-oxoglutarate concentration glucose may be produced from both exogenous 2-oxoglutarate as well as pyruvate generated by alanine aminotransferase. An increase of pyruvate content in the reaction medium from 1.1. ± 0.9 in the presence of alanine alone up to 4.0 ± 0.3 and 200.3 ± 20.6 μmol/g dry weight upon 60 min of incubation of tubule suspension with 0.5 and 5 mM 2-oxoglutarate, respectively, is in agreement with this suggestion. Moreover, 2-oxoglutarate added at 0.5 mM concentration does not affect alanine utilization from the reaction medium, whereas in the presence of 5 mM 2-oxoglutarate the uptake of both ketoacid and alanine is markedly enhanced.

3.2. *The contribution of alanine and 2-oxoglutarate to gluconeogenesis*

In order to prove the suggestion that alanine may be converted to glucose in the presence of 2-oxoglutarate we have checked incorporation of both L-[U-^{14}C]alanine and [U-^{14}C]2-oxoglutarate into glucose under conditions studied. As shown in table 2, 2-oxoglutarate at both 0.5 and 5 mM concentrations stimulates the incorporation of 5 mM [U-^{14}]alanine to glucose 8- and 26-fold, respectively, whereas aminooxyacetate abolished this process. On the other hand, nonlabelled alanine decreases an incorporation of 0.5 mM [U-^{14}C]2-oxoglutarate into glucose by about 65%, probably due to utilization of 2-oxoglutarate predominantly for both glutamate and glutamine synthesis. At 5 mM concentration of [U-^{14}C]2-oxoglutarate alanine does not affect the contribution of ketoacid to gluconeogenesis in both the absence and presence of aminooxyacetate.

In view of these results it is possible to suggest that (i) in isolated rabbit renal tubules incubated with alanine as the sole substrate this amino acid is not used for

Table 3

Glucose formation and substrate utilization in rabbit renal tubules incubated with plasma alanine concentrations and/or various low 2-oxoglutarate concentrations[a]

2-Oxoglutarate (mM)	Alanine (0.4 mM)	Glucose formation	Alanine uptake	2-Oxoglutarate uptake
0.05	–	0.2 ± 0.1	–	5.4 ± 1.0
0.05	+	0.4 ± 0.1[b]	19.7 ± 1.7	9.2 ± 0.3[b]
0.1	–	0.5 ± 0.1	–	7.8 ± 0.8
0.1	+	1.1 ± 0.1[b]	28.7 ± 2.9	14.6 ± 1.4[b]
0.2	–	1.0 ± 0.1	–	17.3 ± 1.5
0.2	+	2.4 ± 0.2[b]	35.0 ± 2.7	29.8 ± 2.4[b]

[a] Data are expressed as means ± SE of 3 separate experiments; μmol/g dry weight per 10 min

[b] $P<0.01$ versus corresponding control with no alanine

Table 4

The effect of low 2-oxoglutarate concentrations on the incorporation of L-[U-^{14}C]alanine used at physiological concentration (A) and the influence of unlabelled L-alanine on the incorporation of [U-^{14}C]2-oxoglutarate used at the same conditions (B)

Expt	^{14}C-labelled substrate	Unlabelled substrate	[^{14}C]Glucose (% of control)
A	0.4 mM [U-^{14}C]L-Alanine	0.05 mM 2-Oxoglutarate	2239 ± 270 (3)[a]
	0.4 mM [U-^{14}C]L-Alanine	0.1 mM 2-Oxoglutarate	5240 ± 662 (3)[a]
	0.4 mM [U-^{14}C]L-Alanine	0.2 mM 2-Oxoglutarate	12331 (1)
B	0.05 mM [U-^{14}C]2-Oxoglutarate	0.4 mM L-Alanine	36 ± 8 (3)[a]
	0.1 mM [U-^{14}C]2-Oxoglutarate	0.4 mM L-Alanine	50 ± 10 (3)[a]
	0.2 mM [U-^{14}C]2-Oxoglutarate	0.4 mM L-Alanine	78 (1)

Incubations were carried out for 10 min. The rates of incorporation of ^{14}C-labelled substrates into ^{14}C-labelled glucose are expressed as means ± SE of percentage of corresponding control with either no unlabelled 2-oxoglutarate (Expt A) or without unlabelled L-alanine (Expt B). The values for the basal rates of [^{14}C]glucose production from [U-^{14}C]alanine and from 0.05, 0.1 and 0.2 mM [U-^{14}C]2-oxoglutarate added as the sole substrates were 0.02 ± 0.01, 0.16 ± 0.01, 0.50 ± 0.04 and 0.97 μmol/10 min per g dry weight, respectively

[a] $P<0.001$ vs corresponding control with either no unlabelled 2-oxoglutarate (Expt A) or no unlabelled L-Alanine (Expt B)

glucose synthesis since oxaloacetate provided via pyruvate carboxylase is predominantly used for citrate and 2-oxoglutarate synthesis via the tricarboxylic acid cycle [10] and (ii) in the presence of exogenous 2-oxoglutarate alanine is utilized as a glucose precursor.

According to Bauer et al. [22] alanine concentration in rabbit serum ranges from 152.6 to 793.4 μmol/l and is generally higher in starved animals [23]. In order to check whether alanine may contribute to renal gluconeogenesis at low physiological concentrations of this amino acid, we have studied the effect of 0.4 mM alanine on glucose formation from low 2-oxoglutarate concentrations. Since to our knowledge there are no data available for 2-oxoglutarate concentrations in rabbit serum, we have applied 0.05, 0.1 and 0.2 mM 2-oxoglutarate, i.e., concentrations similar to those of pyruvate, estimated by Goldstein [24] for the whole-blood in the abdominal aorta of rat. It appeared that although alanine was not used for gluconeogenesis when added as a sole substrate, on 10 min incubation of rabbit renal tubules this amino acid increases both gluconeogenesis and 2-oxoglutarate uptake about 2-fold (table 3). Moreover, the incorporation of [^{14}C]alanine to glucose is about 22-, 52- and 123-fold higher in the presence of 0.05, 0.1 and 0.2 mM 2-oxoglutarate, respectively, while [^{14}C]ketoacid incorporation into glucose is significantly decreased by alanine under conditions studied (table 4). Thus, the contribution of alanine to renal glucose synthesis in the presence of low extracellular 2-oxoglutarate, considered to be one of the major substrates for gluconeogenesis in kidney [25] may be of physiological importance, especially in liver insufficiency or intoxication, since renal glucose formation was found to be increased under conditions of hepatic failure [26].

Similar to alanine, glycerol at concentrations up to 10 mM is not utilized for gluconeogenesis in rabbit renal tubules, when applied as the sole substrate [27,28]. However, in the presence of other glucose precursors it is incorporated into glucose and is able to alter the rate of renal glucose production [28]. In view of these observations it seems possible that the contribution of various substrates to glucose formation in kidneys established with the use of glucose precursors applied as sole substrates might be different from that estimated at physiological substrate concentrations found in the blood in the abdominal aorta.

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