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LIVER AND KIDNEY CORTEX GLUCONEOGENESIS FROM L-ALANINE IN FED AND STARVED RATS

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Abstract—1. Circulating [^{14}C]glucose 2, 5 and 10 min after intravenous injection of [U-^{14}C]-L-alanine was greater in 24 hr starved than in fed rats.

2. *In vitro* uptake of [^{14}C]alanine by liver and kidney cortex slices from 24 hr starved and fed rats rose in parallel with increased medium substrate concentration.

3. Formation of [^{14}C]glucose from 1 mM [^{14}C]alanine was similar in liver and kidney cortex slices and increased in tissues from 24 hr starved compared with fed rats. With 5 mM [^{14}C]alanine more [^{14}C]glucose was produced by liver than by kidney cortex slices from 24 hr starved rats. Liver slices always produced more [^{14}C]lactate and less [^{14}C]-CO_2 from [^{14}C]alanine than kidney cortex slices.

4. It is proposed that under physiological conditions, the kidneys cortex actively participates in glucose production from alanine.

INTRODUCTION

It is well established that the availability of substrates plays an important role in the regulation of gluconeogenesis (Exton and Park, 1967; Felig *et al.*, 1969; Mallette *et al.*, 1969) and alanine is considered the main protein-derived precursor for gluconeogenesis (Felig *et al.*, 1969; Felig, 1973; MacDonald *et al.*, 1976; Snell, 1979; Christensen, 1982) both in fed and starved subjects. The contribution of alanine to gluconeogenesis seems to be less than half that of lactate (Golden *et al.*, 1981; Huang and Lardy, 1981), but conditions in which circulating levels of alanine decrease cause hypoglycemia both in man (Garber *et al.*, 1974) and in rats (Metzger *et al.*, 1971), indicating that alanine availability is also important in maintaining glucose homeostasis. Because of the absence of mitochondrial alanine aminotransferase in the rat kidney (DeRosa and Swick, 1975), it has been proposed that this organ cannot convert alanine to glucose. This conclusion was also based on reported absence of glucose from L-alanine in different kidney preparations (Krebs *et al.*, 1963; Bowman, 1970). More recently, however, it has been demonstrated that alanine contribution to *in vivo* renal gluconeogenesis is of the same or even greater magnitude than that of other well recognized renal-gluconeogenic amino acids such as glutamate or aspartate (Kida *et al.*, 1982) and that the transamination step in alanine catabolism occurs in the cytosol of the liver cell (Groen *et al.*, 1982). As cytosolic alanine aminotransferase activity is present in rat kidney (DeRosa and Swick, 1975) it should not be ignored that under certain conditions this organ could utilize a considerable proportion of alanine as a gluconeogenic substrate. To test this possibility, in the present work we determined the effect of 24 hr starvation in the rat on *in vivo* glucose production from [U-^{14}C]-L-alanine and compared liver and kidney cortex gluconeogenesis from the same substrate in *in vitro* preparations.

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MATERIALS AND METHODS

Female Sprague–Dawley rats from our own colony were fed a Purina chow diet and maintained in a temperature (23 ± 1°C) and light (12 hr on–off cycle) controlled room. When weighing 200–220 g they were studied either fed or after a 24 hr starvation. For *in vivo* experiments, animals were given through a tail vein 10 μCi of [U-^{14}C]-L-alanine (from The Radiochemical Centre, Amersham, Bucks, U.K.) containing 0.2 mmol of L-alanine per 200 g body weight. Blood samples were collected into heparinized receptables from the tip of the tail at 2 and 5 min and animals were decapitated without anaesthesia at 10 min after tracer administration. Blood was collected from the neck wound into heparinized tubes and a piece of liver was immediately placed in liquid nitrogen. Blood aliquots were deproteinized with $Ba(OH)_2$–$ZnSO_4$ (Somogy, 1945) and supernatants were used for both glucose evaluation (Huggett and Nixon, 1957) and for [^{14}C]glucose purification by ion exchange column chromatography following the method of Herrera *et al.* (1969) slightly modified. With this technique, recuperation of [U-^{14}C]glucose added to blood before protein precipitation was above 98.7% whereas less than 0.19% of [U-^{14}C]-L-alanine was recovered. Other aliquots of blood were used for separation of plasma which was deproteinized with 10% sulfosalycylic acid. These protein-free supernatants were used for alanine quantification with a Beckman 121 MB amino acid autoanalyzer (Beckman Co., U.S.A.) and for purification of [^{14}C]alanine throughout cation microcolumns (2.5 cm × 4 mm) (AG 50 W × 8, from Bio-Rad Laboratories, Richmond, U.S.A.) eluted with 3 ml of distilled water and 3 ml of 2 N ammonia. With this technique, recuperation of [U-^{14}C]-L-alanine added to the plasma was over 92.5% whereas that of [U-^{14}C]glucose was less than 0.37%. Glycogen was purified (Good *et al.*, 1933) in aliquots of the frozen liver after alkali digestion. Purified glycogen was hydrolyzed with 5 N H_2SO_4 for 2 hr in a

boiling water bath and after neutralization, samples were used for both analysis of glucose (Huggett and Nixon, 1957) and for their radioactivity count. Values for *in vivo* production of [^{14}C]glucose were calculated by considering a glucose space of 38% body weight and all values of radioactivity were adjusted by considering 1×10^6 dpm the administered tracer per 200 g of body weight.

For *in vitro* studies, rats were decapitated and liver and kidneys were immediately placed in ice cold 0.9% NaCl. Slices of liver and kidney cortex were cut 0.5 mm wide with a tissue chopper (McIlwain, U.K.) and placed in Krebs–Ringer bicarbonate buffer pH 7.4 (Umbreit *et al.*, 1964). Then the slices were weighed and placed in counting vials (100–120 mg/vial) containing 0.5 ml of cold Krebs–Ringer bicarbonate pH 7.4 containing 1 μCi of [U-^{14}C]-L-alanine (from The Radiochemical Centre, Amersham, Bucks, U.K.) and 1 or 5 μmol of cold L-alanine. Zero time vials to which 0.1 ml of 1 N H_2SO_4 was added simultaneously with the tracer and blank vials without tissue slices were also used in each experiment. Incubations were performed as previously described (Castro *et al.*, 1972) in a O_2–CO_2 (95:5%) gas atmospliere at 37°C and with agitation (100 cycles/min). Incubations were terminated after 90 min by injecting 250 μl of hydroxyde of hyamine into plastic receptacles connected to the rubber caps of the vials and 0.1 ml of 1 N H_2SO_4 into the media for subsequent collection of [^{14}C]-CO_2 with slight agitation at room temperature for 60 min. After centrifugation of the media, aliquots of supernatants were placed on microcolumns (4 mm i.d.) filled with AG 50 W × 8 resin in its H^+ form (Bio Rad Laboratories, Richmond, U.S.A.) up to 2.5 cm level of the column and up to 5 cm with AG 1 × 8 resin in its HO^- form (Bio Rad Laboratories, Richmond, U.S.A.). Microcolumns were eluted with 3 ml of distilled water for the collection of [^{14}C]glucose, followed by introduction of 3 ml of 0.5 N formic acid for the collection of [^{14}C]lactate, and 3 ml of 2 N ammonia for the collection of [^{14}C]alanine. By this procedure, recoveries of [U-^{14}C]lactate and [U-^{14}C]-L-alanine added to non-radioactive incubation medium were always above 96.6, 88.0 and 90.1% in their respective fractions whereas contamination in the other fractions never exceeded 0.4%. Aliquots of blood collected from the neck wound of rats used in the *in vitro* experiments were processed as described above for the estimation of basal blood glucose and plasma alanine concentrations. Statistical comparisons between the groups were always done with the Student's *t*-test.

RESULTS

In vivo *formation of glucose from alanine*

As shown in Table 1, plasma basal (time 0) concentration of alanine appeared significantly lower in 24 hr starved rats than in fed animals. Values of plasma alanine concentrations were greatly augmented at 5 min after intravenous administration of 0.2 mmol of [U-^{14}C]-L-alanine in both fed and starved rats and differences between the two groups disappeared (Table 1). Plasma levels of alanine decreased similarly from 5 to 10 min after the injection in both fed and starved animals. Similar results were also found in plasma [^{14}C]alanine and [^{14}C]alanine specific acitivity, demonstrating the rapid disappearance of the administered substrate from circulation and the lack of difference in this parameter in fed and starved rats. Blood glucose concentrations were always lower in starved than in fed rats (Table 1) whereas the appearance of [^{14}C]glucose in circulation was greater in the 24 hr starved rats than in the fed ones from the 2nd to the 10th min after tracer administration (Table 1). Blood [^{14}C]glucose specific activity progressively increased after delivery of the tracer and was always greater in starved than in fed animals (Table 1). There were no significant differences between the two groups in liver glycogen radioactivity levels 10 min after the tracer (88 ± 3 dpm/g in fed animals and 81 ± 3 in 24 hr starved) and, as liver glycogen concentration was much lower in starved rats (0.004 ± 0.001 mg/100 mg) than in fed ones (3.22 ± 0.15), [^{14}C]liver glycogen specific activity appeared significantly ($P < 0.05$) greater in the former (2237 ± 620 dpm/mg) than in the latter group (3 ± 0).

In vitro *experiments*

To obtain an index of the comparative contributions of liver and kidney cortex gluconeogenesis to the overall *in vivo* glucose production, slices of both organs from other fed and 24 hr starved rats were incubated *in vitro* for 90 min in the presence of either 1 or 5 mM [U-^{14}C]alanine. As shown in Table 2, the uptake of [^{14}C]alanine, quantified as the difference between labelled alanine levels in the medium at the onset and end of incubation, increased 3.81–5.48 times when the tracer concentration was increased from 1 to 5 mM. Values per gramme of fresh tissue weight were always similar in liver and kidney cortex slices and in both organs alanine uptake was always slightly lower in slices from starved than from fed rats, with a statistically significant difference only for liver slices incubated in the presence of 1 mM alanine. The *in vitro* production of [^{14}C]glucose was also increased by raising the substrate concentration in the medium and values were always significantly greater in tissues from

Table 1. Effect of 24 hr starvation in the rat on *in vivo* utilization of [U-^{14}C]-L-alanine for the synthesis of glucose

	Time after tracer (min)	Alanine (μM)	[^{14}C]alanine (dpm/ml)	[^{14}C]alanine specific activity (dpm/μmol)	Glucose (mg/dl)	[^{14}C]glucose (dpm-10^{-2}/200 g body wt)	[^{14}C] glucose specific activity (dpm/mg)
Fed rats	0	579 ± 52	—	—	98 ± 5	—	—
	2	—	—	—	97 ± 11	179 ± 8	289 ± 7
	5	5908 ± 1867	$20{,}699 \pm 6561$	3376 ± 332	83 ± 6	193 ± 13	316 ± 4
	10	1000 ± 48	1215 ± 57	1225 ± 65	108 ± 2	362 ± 38	427 ± 51
24 hr Starved rat	0	427 ± 32*	—	—	76 ± 5*	—	—
	2	—	—	—	63 ± 7**	274 ± 18**	605 ± 53**
	5	5668 ± 1889	$21{,}339 \pm 8750$	3628 ± 217	66 ± 5*	414 ± 67*	952 ± 129**
	10	1150 ± 51	1471 ± 97	1283 ± 77	86 ± 4**	1036 ± 171**	1613 ± 244**

Rats were intravenously injected with 10 μCi/0.2 mmol of [U-^{14}C]-L-alanine/200 g body weight and blood was collected at different times thereafter. Means ± SEM of 5–10 rats/group. Statistical comparisons between values from starved and fed rats are indicated by asterisks: * = $P < 0.05$; ** = $P < 0.01$.

Table 2. *In vitro* utilization of [U-^{14}C]-L-alanine by liver and kidney cortex slices from fed and 24 hr starved rats

	Alanine in medium at onset of incubation (mM)	[^{14}C]alanine uptake (μmol/hr per g)	[^{14}C]glucose production (μmol/hr per g)	[^{14}C]lactate production (μmol/hr per g)	[^{14}C]-CO_2 production (μmol/hr per g)
Liver slices					
Fed rats	1	4.17 ± 0.35	0.12 ± 0.03	1.04 ± 0.12	0.51 ± 0.02
24 hr Starved rat	1	2.55 ± 36	0.31 ± 0.07	0.27 ± 0.02	0.47 ± 0.08
P		< 0.01	< 0.05	< 0.001	NS
Fed rats	5	16.25 ± 0.75†††	0.40 ± 0.05†††	2.12 ± 0.19†††	1.25 ± 0.12†††
24 hr Starved rats	5	14.40 ± 1.06†††	2.05 ± 0.27†††	0.86 ± 0.07†††	1.93 ± 0.23†††
P		NS	< 0.001	< 0.001	< 0.05
Kidney cortex slices					
Fed rats	1	3.80 ± 0.12	0.14 ± 0.01	0.15 ± 0.01***	1.42 ± 0.10***
24 hr Starved rats	1	3.46 ± 0.25	0.29 ± 0.02	0.16 ± 0.02**	1.23 ± 0.11***
P		NS	< 0.001	NS	NS
Fed rats	5	16.38 ± 1.98†††	0.32 ± 0.02†††	0.53 ± 0.02***†††	2.74 ± 0.13***†††
24 hr Starved rats	5	13.87 ± 1.98†††	0.49 ± 0.02***†††	0.51 ± 0.05***†††	2.69 ± 0.23*†††
P		NS	< 0.001	NS	NS

Incubations were performed for 90 min at 37°C. Results are expressed per g of fresh tissue weight as means ± SEM of 5–9 rats/group. *P* values correspond to the comparison of fed and 24 hr starved rats while comparisons of values from kidney cortex and liver slices are denoted by asterisks and those of incubations with 5 mM alanine and with 1 mM by daggers: * or † = $P < 0.05$, ** or †† = $P < 0.01$ and *** or ††† = $P < 0.001$.

starved than from fed rats (Table 2) although these two concomitant effects were much more pronounced in liver slices than in kidney cortex due to their different [^{14}C]glucose production which was statistically significant for tissues from 24 hr starved rats incubated with 5 mM alanine. [^{14}C]Lactate formation from labelled aianine also changed in the same direction as substrate concentration in the medium and it was always significantly higher in liver than in kidney cortex slices (Table 2). This parameter decreased in liver slices from starved versus fed rats whereas it did not change in kidney cortex slices (Table 2). Contrary to [^{14}C]lactate, the production of [^{14}C]-CO_2 from labelled alanine was significantly lower in liver than in kidney cortex slices. [^{14}C]-CO_2 increased in both liver and kidney cortex slices when alanine concentration in the medium rose from 1 to 5 mM and no differences between tissues from fed and starved animals were found except for a slight but significant increase in [^{14}C]-CO_2 production by liver slices from starved versus fed rats when incubated with 5 mM alanine (Table 2).

The lack of parallel changes in alanine uptake and the resulting metabolic products made it necessary to calculate the actual conversion of alanine to the products as a percentage of uptake for individual values. This data is presented in Table 3 where it is seen that in tissues from fed animals the percentual conversion of the alanine being taken up and converted into [^{14}C]glucose was similar in incubations performed with 1 and 5 mM of substrate and did not differ between liver and kidney cortex slices. This parameter rose significantly in tissues from starved versus fed rats but the effect was much greater in liver than in kidney preparations, making absolute values statistically lower in kidney cortex than in liver slices from starved animals. The percentual formation of [^{14}C]lactate from captured [U-^{14}C]-L-alanine was much greater in liver than in kidney cortex slices and starvation reduced this parameter only in liver slices but did not affect the kidney cortex. Contrary to lactate, the percentual formation of [^{14}C]-CO_2 from recovered [U-^{14}C]-L-alanine was lower in liver than in kidney cortex slices and starvation augmented this

Table 3. Percentual *in vitro* formation of [^{14}C]glucose, [^{14}C]lactate and [^{14}C]-CO_2 from recovered [U-^{14}C]-L-alanine by liver and kidney cortex slices from fed and 24 hr starved rats

	Alanine in medium at onset of incubation (mM)	[^{14}C]glucose (% of [U-^{14}C]alanine uptake	[^{14}C]lactate (% of [U-^{14}C]alanine uptake)	[^{14}C]-CO_2 (% of [U-^{14}C]alanine uptake)
Liver slices				
Fed rats	1	2.92 ± 0.20	24.82 ± 1.74	12.20 ± 1.09
24 hr Starved rat	1	11.74 ± 0.82	10.10 ± 0.71	17.67 ± 1.24
P		< 0.001	< 0.001	< 0.01
Fed rats	5	2.46 ± 0.17	13.04 ± 0.91††	7.69 ± 0.69††
24 hr Starved rats	5	14.22 ± 1.00	6.00 ± 0.42†††	13.43 ± 0.94†
P		< 0.001	< 0.001	< 0.001
Kidney cortex slices				
Fed rats	1	3.60 ± 0.25	3.91 ± 0.27***	37.28 ± 2.61***
24 hr Starved rats	1	7.85 ± 0.55**	4.39 ± 0.31***	33.77 ± 2.36***
P		< 0.001	NS	NS
Fed rats	5	1.97 ± 0.27†††	3.26 ± 0.23***	16.75 ± 1.17***†††
24 hr Starved rats	5	2.98 ± 0.23***†††	3.70 ± 0.26***	19.39 ± 1.36***†††
P		< 0.001	NS	NS

Results correspond to those shown in Table 2 but are expressed as percentage of the [^{14}C]alanine uptake in each sample. Statistical comparisons are as indicated for Table 2.

parameter in liver but not in kidney cortex slices (Table 3). Increase of the alanine concentration in the medium from 1 to 5 mM caused notable reduction in the percentual formation of [^{14}C]-CO_2 which was greater in kidney cortex than in liver slices.

DISCUSSION

Present results showed that 24 hr starvation in the rat increase *in vivo* production of glucose from L-alanine. An enhancement in the *in vitro* production of glucose from L-alanine was also found in liver and kidney cortex slices from starved rats, suggesting that both organs participate in the *in vivo* change. The ^{14}C-labelled L-alanine administered to the rats was diluted with enough cold alanine to eliminate basal differences in circulating alanine concentration between fed and starved animals. In this way, results obtained in starved animals correspond to an enhancement in overall gluconeogenetic capacity produced by alanine when compared to fed rats in agreement with the proposed role of alanine as the main muscle-derived amino acid utilized as a gluconeogenetic substrate in the fasted animals (Felig, 1973; MacDonald *et al.*, 1976; Snell, 1980). Previous studies have emphasized the role of liver as the unique receptor organ of alanine derived from muscle in the glucose–alanine cycle (Felig and Warhen, 1971). Our finding that with nearly physiological concentrations of alanine in the incubation medium (1 mM), kidney cortex slices converted as much substrate to glucose per unit of fresh weight as did liver slices and that they reacted in a similar way to starvation, indicate that kidneys also participate in the endogenous production of glucose from alanine. This conclusion contrasts with the difficulties reported by some investigators in recovering glucose formed from L-alanine in *in vitro* rat kidney preparations (Krebs *et al.*, 1963; Bowman, 1970) but coincides with others studies (Kida *et al.*, 1982) describing an alanine contribution to *in vivo* renal gluconeogenesis even greater than that observed with L-aspartate or L-glutamate which have traditionally been considered major kidney gluconeogenetic amino acids (Krebs *et al.*, 1963; Bowman, 1970). In contrast with our experiments much higher concentrations of L-alanine in the medium were utilized in studies in which no glucose formation from alanine was detected in kidney preparations and this difference could partially explain the contradictory results. We observed that unlike results in liver slices, glucose production from alanine in kidney cortex slices from starved rats did not increase in proportion to the rise in substrate concentration in the medium, and when values were calculated as a percentage of the alanine uptake by the tissues, glucose production was much lower from 5 mM than from 1 mM alanine (Table 3). The limiting factor for glucose production at high concentrations of alanine by kidney cortex seem to be not its uptake, which was similar to that of liver, but rather the intracellular alanine catabolism. Independent of the experimental condition studied we have always found a lower [^{14}C]lactate and a higher [^{14}C]-CO_2 production from [U-^{14}C]-L-alanine in kidney cortex than in liver slices and this difference could be a consequence of the different intracellular distribution of alanine aminotransferase in these two organs in the rat (DeRosa and Swick, 1975). The role of extracellular alanine concentration in alanine utilization has been proposed by McGivan *et al.* (1981) for hepatocytes from fed rats. Thus apparently the concentration effect of driving alanine catabolism is more sensitive in kidney cortex than in liver and this factor has prevented other investigators from detecting the limited production of glucose from alanine in *in vitro* preparations of kidney in the presence of over-physiological concentrations of the substrate in medium. Present results show that gluconeogenesis from alanine occurs in the kidney cortex at physiological concentrations of circulating alanine and its contribution to the overall glucose production *in vivo* may be higher than previously estimated.

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