ALANINE RELEASE BY RAT ADIPOSE TISSUE IN VITRO

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SUMMARY: Isolated rat fat pads incubated in vitro release alanine into the medium at increased rates in the presence of glucose, glutamate, leucine or valine. The increased release was inhibited by aminooxyacetate indicating that alanine release is due to intracellular formation by pyruvate transamination. Starvation and diabetes are associated with increased alanine release and evidence is given for the first time that amino acid carbon and nitrogen may be used for alanine formation in adipose tissue, so that this tissue is capable of making a net contribution to body glucogenic substrate provision.

INTRODUCTION

The release of alanine by muscle tissues has attracted much attention, particularly in view of the role of this amino acid as a substrate for hepatic gluconeogenesis (1-3, for reviews). The alanine released is formed de novo by transamination of pyruvate, rather than as the result of proteolysis and release of alanine residues from muscle proteins (4-7). A large part of the pyruvate for alanine formation is derived from glycolysis (5,8) and in these circumstances the alanine released into the blood stream is serving a nitrogen transport function to transfer amino groups, derived from muscle amino acid oxidation, to the liver for conversion to urea. In certain circumstances, such as starvation, pyruvate may be derived from the carbon of amino acids (including valine and isoleucine) (7,9,10) and in this case alanine release serves as a means of transferring nitrogen and glucogenic carbon, for net body glucose synthesis, from muscle to the liver. Branched-chain amino acids have been considered especially in this respect because of their high rate of oxidation in muscle (5,11).

The regulation of protein and amino acid metabolism in adipose tissue has received little attention even though it is capable of rapid rates of protein turnover which show hormone sensitivity (12). It has been shown that

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amino acids, in particular the branched-chain amino acids, are rapidly oxidised by adipose tissue and can also serve as substrates for lipogenesis (13,14). Utilisation of amino acid carbon for either of these purposes requires disposal of the amino nitrogen generated. We give evidence here that this is achieved, as in muscle tissues,by the formation of alanine by pyruvate transamination in adipose tissue. We also show that starvation or diabetes is associated with increased alanine release and, in these situations associated with enhanced gluconeogenesis, amino acid carbon may be utilised for alanine formation so that adipose tissue could make a net contribution to the replenishment of body carbohydrate.

METHODS

Male rats of an albino Wistar strain were fed ad libitum, or starved for the periods of time indicated, and were used at a body wt. of 70-100 g. Fed rats were made diabetic by an intraperitoneal injection of alloxan monohydrate (100 mg/kg) and were used 48 h later when plasma glucose concentrations exceeded 20 mM. Rats were killed by cervical disolocation and the epididymal fat pads were rapidly dissected out, weighed and immediately transferred to 4 ml of Krebs-Henseleit bicarbonate saline (15) for preincubation at 37°C for 30 min under O_2 + CO_2 (95:5%). Individual fat pads were then transferred into flasks containing fresh saline, with additions as indicated, at a concentration of 50 mg tissue/ml of medium and incubated for a further 2 h as above. At the end of this period fat pads were removed and the medium, after deproteinisation with 20% $HClO_4$ and neutralisation, was assayed for alanine (16) and lactate (17) by enzymatic methods.

RESULTS AND DISCUSSION

Alanine release by epididymal fat pads from fed or starved rats was stimulated by glucose and by glutamate, leucine or valine (Table 1). The stimulation of alanine release by valine in the presence of glucose (3.6-fold, Table 2) is very similar to the increase in intracellular alanine concentration (3.7-fold) found by Taylor and Halperin (18) under very similar conditions, suggesting that increased release is the result of intracellular alanine formation. Alanine formation is due to pyruvate transamination, rather than protein breakdown, because valine-stimulated release is inhibited by 82% in the presence of 1 mM aminooxyacetate (not shown), a known inhibitor of alanine aminotransferase (19). Thus adipose tissue is capable of alanine formation from pyruvate and glutamate (derived by transamination of amino

Table 1 Effect of glucose and amino acids on alanine release by fat pads

Additions to incubation medium	Alanine release (μmol/2h per g of tissue)	
	fed	starved
none	0.10 ± 0.02 (14)	0.46 ± 0.09 (12)
10 mM glucose	0.31 ± 0.04 (3)*	0.68 ± 0.10 (3)*
3 mM glutamate	1.16 ± 0.17 (3)***	1.35 ± 0.27 (3)***
3 mM leucine	0.71 ± 0.09 (3)***	0.91 ± 0.13 (3)**
3 mM valine	0.58 ± 0.12 (5)**	0.72 ± 0.24 (3)**

Epididymal fat pads from fed or 42-h starved rats were preincubated in saline for 30min before incubation in the absence or presence of substrate (as indicated) for a further 2 h. After removal of the fat pad, medium was analysed for alanine as described in Methods. Values represent the means of measurements on different animals and are given together with S.E.M. and the number of observations in parentheses. Statistical analysis (t test) is on paired observations (incubations with and without substrate).
* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Table 2 Effect of alloxan diabetes on alanine and lactate release by fat pads

Additions to incubation medium	Metabolite release(μmol/2h per g of tissue)	
	Alanine	Lactate
Normal, fed		
10 mM glucose	0.21 ± 0.07 (6)	1.32 ± 0.27 (6)
10 mM glucose + 3 mM valine	0.76 ± 0.09 (6)***	1.02 ± 0.22 (6)
Diabetic, fed		
10 mM glucose	1.54 ± 0.36 (5)	9.63 ± 1.75 (5)
10 mM glucose + 3 mM valine	2.78 ± 0.75 (5)*	11.54 ± 3.26 (5)

Details as in Table 1. Statistical analysis (t test) is on paired observations (incubations containing glucose with and without valine).
* $p < 0.05$; *** $p < 0.001$

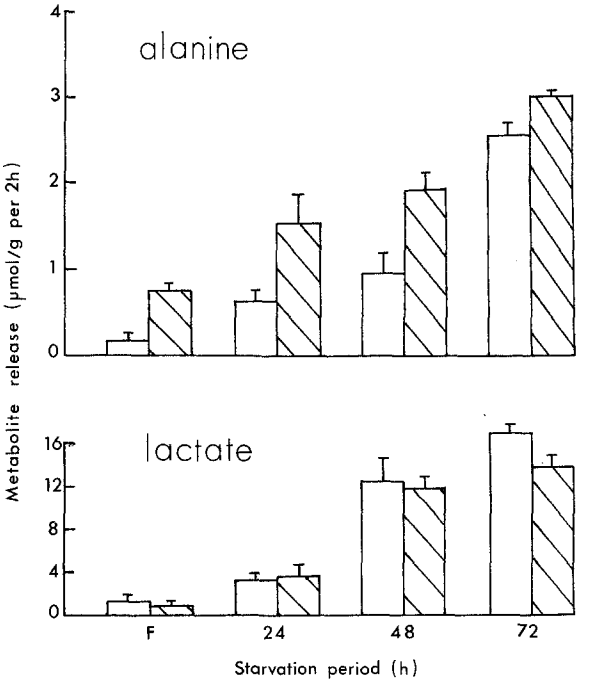

Fig. 1. Effect of starvation on alanine and lactate release by fat pads

Epididymal fat pads were preincubated in saline for 30 min before incubation in medium containing 10 mM glucose alone (open columns) or 10 mM glucose + 3 mM valine (hatched columns). After removal of the fat pad, medium was analysed for alanine and lactate. Columns represent the means of 3 - 6 measurements on different animals and vertical bars represent S.E.M.

acids with 2-oxoglutarate). In this way the utilisation of the carbon skeleton of amino acids, either as an oxidative fuel or as a substrate for fatty acid synthesis, can occur with alanine formation serving as a means of removing the liberated amino groups from the tissue to the liver for disposal via urea formation.

During increasing periods of starvation, there is a progressive increase in the release of both alanine and lactate by fat pads incubated with glucose alone as substrate or glucose + valine (Fig. 1). This progressive increase is still apparent when calculations are made on a unit body weight basis. Alanine release(but not lactate)is greater with glucose + valine compared to

glucose alone in the medium for all starvation periods. The increasing release of alanine and lactate with progressive starvation is consistent with the conclusion drawn by Blackshear et al. (20), on the basis of plasma substrate accumulations in hepatectomized rats, that during starvation there is an approximate doubling in the flux of these glucogenic substrates from peripheral tissues to the liver. Probably the quantitative contribution by adipose tissue to the total substrate supply will be quite small since this tissue occupies only about 7% of the total body weight. In order to make a net contribution to body glucose formation the pyruvate required for alanine (and lactate) formation by adipose tissue must be derived from non-carbohydrate sources, such as protein-derived amino acids. Evidence for such a pathway in diaphragm muscle in which branched-chain amino acid carbon is converted to oxaloacetate and then to pyruvate via the enzymes phosphoenolpyruvate carboxykinase and pyruvate kinase has been given (7) and was based on the use of 3-mercaptopicolinate (a known inhibitor of phosphoenolpyruvate carboxykinase). In the present study 1 mM 3-mercaptopicolinate diminished valine-stimulated alanine release by 32% with fat pads from fed rats and by 89% with fat pads from 48 h-starved rats (not shown). In contrast, leucine-stimulated alanine release was not affected by the presence of 1 mM 3-mercaptopicolinate with fat pads from either fed or 48h-starved rats. The inhibitory effect of this agent on alanine release is therefore specific to valine stimulation and an indirect effect of 3-mercaptopicolinate on alanine production via diminished protein breakdown is unlikely. A pathway may therefore operate in adipose tissue for the conversion of valine carbon to phosphoenolpyruvate and pyruvate for alanine formation. Phosphoenolpyruvate carboxykinase activity is increased in adipose tissue during starvation (21), however conversion of phosphoenolpyruvate so-formed to pyruvate will be limited by the enhanced pathway for conversion to glycerol for the increased rates of fatty acid re-esterification observed in starved rats (22, 23). Nevertheless, the activation state of pyruvate dehydrogenase in this situation (24) will

preferentially divert any pyruvate formed towards alanine and lactate formation rather than oxidative metabolism. Valine itself may contribute to this redirection of pyruvate metabolism since the product of valine transamination, 3-methyl-2-oxobutyrate, is a competitive inhibitor of adipose tissue pyruvate dehydrogenase (18).

Fat pads from alloxan diabetic rats showed an increased release of alanine and lactate in incubations with glucose alone or glucose + valine (Table 2). The increases were also significant when calculated on a unit body weight basis. The hyperglycaemia of alloxan diabetes is the result of overproduction of glucose as well as diminished utilisation and the present results suggest that, as in starvation, the increase in body glucose formation is the result not only of enhanced hepatic gluconeogenesis but also of increased supply of glucogenic substrates from peripheral tissues. Valine-stimulated, but not leucine-stimulated, alanine release by fat pads from diabetic rats was diminished by 85% by 1 mM 3-mercaptopicolinate (not shown), so that a pathway for the conversion of valine carbon to pyruvate via phosphoenolpyruvate carboxykinase may operate in diabetes. Again phosphoenolpyruvate carboxykinase activity is elevated in diabetic rats (22), but competition by the glyceroneogenic pathway may limit the formation of pyruvate by this pathway. However, the decrease in the proportion of active pyruvate dehydrogenase found in adipose tissue from diabetic rats (24) will tend to divert any pyruvate formed towards alanine and lactate formation.

We have shown that adipose tissue, like muscle, is among the peripheral tissues which contribute to the production of alanine in the body. Situations such as starvation or diabetes, which are associated with enhanced gluconeogenesis *in vivo*, lead to an increased release of alanine by adipose tissue. The carbon for alanine may be provided from valine (and presumably other amino acids) by a pathway involving phosphoenolpyruvate carboxykinase, however valine carbon metabolised in this way may alternatively be used for glycerol

synthesis for fatty acid re-esterification and in this latter case the release of alanine serves as a means of transporting amino groups from adipose tissue to the liver rather than serving any net glucogenic role.

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