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Inhibition of γ-glutamyl transpeptidase decreases amino acid uptake in human keratinocytes in culture

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Acivicin inhibits γ-glutamyl transpeptidase activity in human keratinocytes in culture. Treatment of these cells with acivicin produces a decrease in the uptake of L-[U-^{14}C]alanine, 2-amino-[1-^{14}C]-isobutyrate, L-[U-^{14}C]leucine and 1-aminocyclopentane-1-[^{14}C]carboxylate. D-[U-^{14}C]glucose uptake is not affected by the presence of acivicin. These results support, for the first time in vitro, the hypothesis that the γ-glutamyl cycle may be involved in amino acid uptake by human cells.

Amino acid transport; γ-Glutamyl transpeptidase; Glutathione; Cell culture; Human keratinocyte

1. INTRODUCTION

The γ-glutamyl cycle, which accounts for the biosynthesis and degradation of GSH, was proposed as a mechanism for amino acid uptake by cells [1]. This is supported by the fact that tissues rich in γ-glutamyl transpeptidase (GGT) show high rates of amino acid uptake and that γ-glutamyl amino acids are formed by the interaction of GGT with intracellular GSH and extracellular amino acids [2]. γ-Glutamyl amino acids are transported by a system not shared by free amino acids and are converted, inside the cells, to free amino acids and 5-oxoproline by the action of γ-glutamyl cyclotransferase [3].

The hypothesis that the cycle is a mechanism for amino acid uptake has received serious criticisms. The bulk uptake of amino acids by yeast (*Saccharomyces cerevisiae*) is not related to GGT activity [4]. Recently, no relationship was found between GGT activity and amino acid uptake in the intestine of Atlantic salmon [5]. However, the possible role of GGT in amino acid uptake has received support in some tissues and under different experimental situations. For instance, in cerebral endothelial cells, GGT activity was related to amino acid uptake [6] and, inhibition of GGT, resulted in a decreased utilization of glutamine in kidney cells in culture [7]. We showed that amino acid uptake by the lactating mammary gland is related to GGT activity [8].

To address the possibility of a role of this cycle in human cells, we measured the uptake of L-[U-^{14}C]leucine, L-[U-^{14}C]alanine, 2-amino-[1-^{14}C]isobutyric acid and 1-aminocyclopentane-1-[^{14}C]carboxylic acid by isolated human keratinocytes when GGT activity was decreased by a specific inhibitor. We have selected this experimental model to study the γ-glutamyl cycle in human cells because (i) the epidermal keratinocytes are one of the few human diploid cell types that grow well in culture, (ii) they present high GGT activity and (iii) when they are isolated from a primary culture and suspended in adequate incubation media, they show a linear uptake of amino acids, at least during the first 20 min of incubation. In this paper, we show that inhibition of GGT leads to a decrease in the uptake of amino acids, but not of glucose, by human keratinocytes.

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2. MATERIALS AND METHODS

Human epidermal keratinocytes were cultivated according to Rheinwald and Green [9]. To obtain isolated cells, secondary cultures were trypsinized. The resulting cell suspension was centrifuged for 5 min and resuspended in Krebs-Henseleit saline to a cell density of 2 × 10^6 cells/ml.

Keratinocytes were preincubated at 37°C for 30 min in Krebs-Henseleit saline solution equilibrated with O_2/CO_2 (19:1), or with the same saline solution containing 0.35 mM acivicin (α-amino-3-chloro-4,5-dihydro-5-isoxazoleacetic acid) to inhibit GGT activity [10].

After this preincubation, they were incubated for 10 or 20 min in 2 ml of Krebs-Henseleit saline containing L-[U-^{14}C]leucine, or L-[U-^{14}C]alanine, or 2-amino-[1-^{14}C]isobutyric acid, a non-metabolizable analog of alanine, or 1-aminocyclopentane-1-[^{14}C]-carboxylic acid (cycloleucine), a leucine analog, or D-[U-^{14}C]glucose.

Incubations were terminated by addition of 5 ml of ice-cold Krebs-Henseleit saline. After washing cells 3 times, amino acid or glucose uptake was determined measuring specific activity of each metabolite.

GGT activity in the cells was estimated measuring spectrophotometrically the appearance of *p*-nitroaniline from γ-glutamyl *p*-nitroanilide in presence of glycyl-glycine [11].

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Table I

Effect of acivicin on GSH levels, γ-glutamyl transpeptidase (GGT) activity and glucose uptake in isolated human keratinocytes in culture

	GSH (nmol × 10^{-6} cells)	GGT activity (nmol PNA × 10^{-6} cells × min^{-1})	Glucose uptake (nmol × 10^{-6} cells)	
			10 min	20 min
Control	20.8 ± 9.2 (3)	7.1 ± 2.8 (3)	1.0 ± 0.12 (3)	2.3 ± 0.35 (3)
Acivicin	21.4 ± 10.4 (3)	3.0 ± 1.0 (3)*	1.0 ± 0.06 (3)	2.2 ± 0.23 (3)

PNA = *p*-nitroaniline. Glucose concentration in the suspension medium was 5 mM. Results are means ± SE, with the numbers of observations in parentheses. Values that are significantly different from controls are shown: * $P<0.05$.

Table II

Effect of inhibition of γ-glutamyl transpeptidase by acivicin on amino acid uptake in isolated human keratinocytes from secondary culture

		Leucine (nmol × 10^{-6} cells)	Cycloleucine	Alanine	AIB
Control	10 min	3.7 ± 1.3 (3)	25.6 ± 9.1 (3)	69.1 ± 12.2 (3)	-
	20 min	7.2 ± 2.2 (3)	35.7 ± 14.4 (3)	138.1 ± 33.5 (3)	7.6 ± 1.4 (6)
Acivicin	10 min	2.6 ± 1.0 (3)*	15.5 ± 5.4 (3)	42.8 ± 7.6 (3)	-
	20 min	5.0 ± 1.6 (3)*	21.2 ± 10.2 (3)*	81.4 ± 10.6 (3)*	5.3 ± 0.8 (6)*

AIB = 2-amino isobutyric acid. Cycloleucine = 1-aminocyclopentane-1-carboxylic acid. The concentration of amino acids or their analogs was 1 mM. Results are means ± SE, with the numbers of observations in parentheses. Values that are significantly different from controls are shown: *$P<0.05$.

3. RESULTS AND DISCUSSION

Table I shows that addition of 0.35 mM acivicin to isolated human keratinocytes resulted in a 53% decrease in γ-glutamyl transpeptidase activity. This is in agreement with previous findings in other organs which showed that acivicin is a good inhibitor of GGT [12]. Inhibition of GGT has no effect on D-[U-^{14}C]glucose uptake by isolated human keratinocytes after 10 or 20 min of incubation (Table I).

We have studied the uptake of physiological amino acids and of their analogs by isolated human keratinocytes. Table II shows that the uptake of L-[U-^{14}C]-leucine or of 1-aminocyclopentane-1-[^{14}C]carboxylic acid (cycloleucine), which are transported inside the cells mainly by the L system, were significantly lower in keratinocytes incubated with acivicin than in controls. Table II also shows that the uptake of L-[U-^{14}C]alanine or of 2-amino-[1-^{14}C]isobutyric acid, which can be carried by two systems (A and ASC), was also significantly lower in keratinocytes incubated with acivicin than in controls.

All our previous experiments on the involvement of the γ-glutamyl cycle on amino acid uptake had been carried out in vivo. In lactating mammary gland, we have shown that this cycle is involved in amino acid translocation by measuring arterio-venous differences of amino acids across the gland under different experimental conditions [8,13]. In pregnant rats, inhibition (79%) of placental GGT activity by acivicin results in a 50% decrease in placental transfer of L-[U-^{14}C]-alanine and a 70–80% decrease in its incorporation into the placental and fetal proteins [14]. Recently, we have proposed that the γ-glutamyl cycle may not be an amino acid transport system, but rather a mechanism to generate a signal that activates either amino acid uptake by the conventional systems for amino acid transport [15,16] or amino acid metabolism. Furthermore, we have provided some evidence showing that γ-glutamyl amino acids and/or oxoproline may be such signals [16].

Results reported in this paper support the hypothesis that the γ-glutamyl cycle is involved in amino acid transport in human cells. These results are compatible with the idea [16] that the cycle is not a system for amino acid transport, but rather a mechanism for the control of amino acid uptake by systems like the L, the A or the ASC that we have tested here.

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