The uptake of basic amino acids into fibroblasts was enhanced by PCA.

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SYNOPSIS

Previously, we reported that L-PCA enhanced blood circulation by modulating constitutive NO production. It was that L-PCA increased L-Arg uptake into endothelial cell, followed by the enhancement of NO production. Then we recommended the use of L-PCA for cosmetics, not only as humectants but also as enhancer of blood circulation.

Since L-Arg is transported into endothelial cells by CAT (cationic amino acid transporter), it is expected that L-PCA also increase the uptake of basic amino acid, L-Lys. In this study, the uptakes of some amino acids into cells were evaluated by using ^{3}H-labelled amino acid. Then we found the tendency that the uptake of L-Lys into endothelial cells was also enhanced by L-PCA. And the evident effect was observed in the epidermal fibroblasts, which had also CAT. Furthermore, it was found that the transportation of the other type of amino acids were not enhanced by L-PCA.

That is to say, a famous moisturizer, L-PCA, has some effects on basic amino acid transport into cells.

KEYWORDS

L-2-pyrrolidone-5-carboxylic acid (L-PCA), cationic amino acid transporter (CAT), L-arginine (L-Arg), amino acid transport

INTRODUCTION

PCA is one of the key component of natural moisturizing factor (NMF) to regulate hydration of the stratum corneum. PCA and sodium PCA are used as skin and hair conditioning agents for their moisturizing effect [1].

Previously, we reported that L-PCA enhanced blood circulation by modulating constitutive NO production [2]. It was that L-PCA (5 to 10 mM) increased L-Arg uptake into endothelial cells, followed by the enhancement of NO production. It is well known that amino acids enter mammalian cells via several transport systems, each being distinguished by its substrate specificity and requirement for a sodium gradient to drive uptake. Since L-Arg is transported to endothelial cells by CAT (cationic amino acid transporter), it is expected that L-PCA also increases the uptake of

basic amino acid, L-Lys. And the same effect is also expected in the other cells, which has also CAT. Here we have investigated the effect of L-PCA on the uptake of amino acid into cells.

MATERIAL AND METHODS

Cell Culture

Human aortic endothelial cells (HAEC) and normal human dermal fibroblasts (NHDF) were purchased from Kurabo co. Ltd. HAEC were routinely cultured in HuMedia-EG2, which was also from Kurabo co. Ltd., and used in passages 1-5. NHDF were routinely cultured in DMEM, which included 10% FBS, 50 U/ml penicillin and 50 μg/ml streptomycin. Subcultured cells were used in passages 5-15. The conditions of culture were: pH 7.4, atmosphere 5% CO_2 in air, temperature 37 ^{o}C.

Determination of transportation of L-Lys into HAEC

The method to measure the transportation of L-Lys into HAEC is shown here, but it is the same in the case of the other amino acids and the other kind of cells.

L-Lys uptake was determined by measuring the influx of radio-labeled L-Lys into HAEC. The experiments were made on HAEC subcultures resulting from 1 X 10^5 cells seeded onto 2-cm^2 wells of disposable 24-well trays (Falcon). After cells were rinsed with HEPES buffer (140 mM choline chloride, 5.0 mM KCl, 1 mM $MgCl_2$, 0.9 mM $CaCl_2$, 5.6 mM D-glucose, 25 mM HEPES; pH 7.4), another HEPES buffer including [^{3}H]L-Lys (50 μM, 1 μCi) and L-PCA (0-10 mM) was added, and transport was measured over the next 10 min at 37 ^{o}C. Transport activity was terminated by aspirating the medium and rapidly washing HAEC with ice-cold HEPES buffer. The cells were allowed to dry and were solubilized by the addition of 0.1 M NaOH (500 μl). Extracts (350 μl) were collected, and radioactivity was monitored using liquid scintillation spectrometry. The remaining extract was used for the determination of protein content using Lowry method [3] with bovine serum albumin as the standard.

Statistical Analysis

Student's t-test was used for statistical analysis.

RESULTS AND DISCUSSION

Effect of L-PCA on basic amino acid transportation

The transportation of L-Arg and L-Lys into HAEC are shown in **Fig. 1**. Treatment of HAEC with L-PCA (0-10 mM) under the existence of L-Arg (50 μM) resulted in an increase in L-Arg transport. This increase was significant at the $P<0.01$ level. It is already confirmed that this increase was due to the L-Arg uptake via CAT pathway [2] . Treatment of HAEC with the same concentration of L-PCA also showed an incremental tendency in L-Lys transport.

Fig. 2 shows the transportation of L-Arg and L-Lys into NHDF. The transportation of those amino acids was also enhanced by the addition of L-PCA. It is reasonable because NHDF also has CAT, which can be affected by L-PCA.

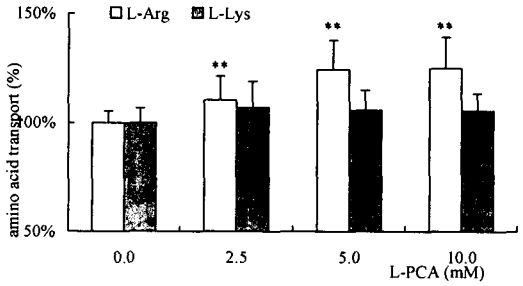

Fig.1 The effect of L-PCA on the transportation of L-Arg and L-Lys into HAEC (**; p<0.01).

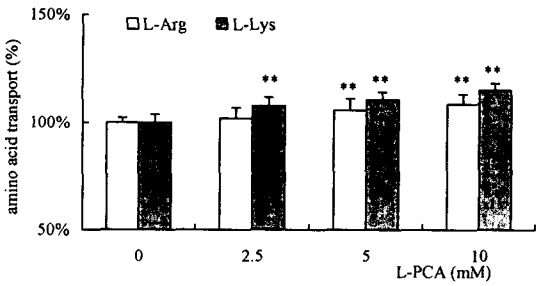

Fig.2 The effect of L-PCA on the transportation of L-Arg and L-Lys into NHDF (**; p<0.01).

Effect of L-PCA on other amino acid transportation

Then we examined the effect of L-PCA on the transportation of neutral (L-Gln, L-Pro, L-Gly and L-Leu) and acidic amino acids (L-Glu). As shown in **Fig. 3**, the transportation of L-Gln, L- Pro and L-Leu into NHDF under the existence of individual amino acid (50 μM) were not affected by the addition of L-PCA . On the other hand, treatment of NHDF with L-PCA resulted in a slight suppression in the transportation of L-Gly and L-Glu (**Fig. 4**).

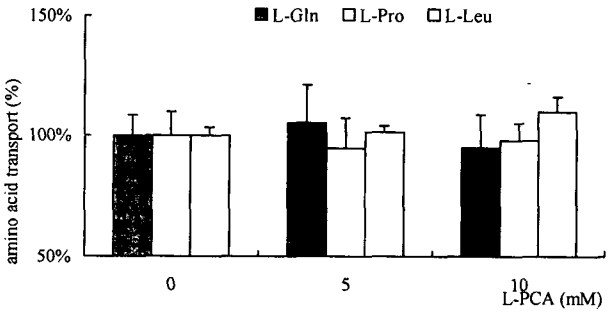

Fig.3 The effect of L-PCA on the transportation of neutral amino acid into NHDF.

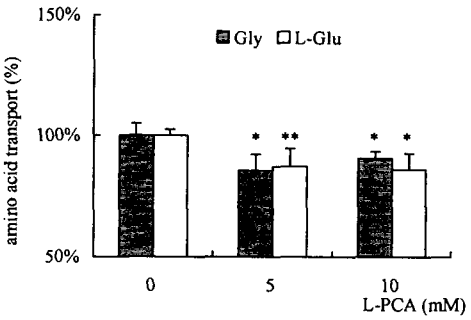

Fig.4 The effect of L-PCA on the transportation of Gly and L-Glu into NHDF (*; p<0.05, **; p<0.01).

CONCLUSION

In this study, we recognized the tendency that the uptake of L-Lys into endothelial cells was also enhanced by L-PCA. And the same effect was observed in the epidermal fibroblasts, which had also CAT. The uptake of neutral amino acids such as L-Gln, L-Pro, and L-Leu were not enhanced by L-PCA, while L-PCA slightly suppressed the uptake of Gly and L-Glu,

L-Arg increased hydroxyproline and collagen levels of skin in case of wound healing [4]. One of the characteristic features of collagen is the presence of relatively large amounts of hydroxyproline. In addition to that, hydroxylysine, which is enzymatically synthesized from L-Lys, is another amino acid characteristic of collagen. These hydroxylized amino acid play a critical role

in the formation of cross-links that stabilize the extracellular collagen matrix [5]. In view of this aspect, L-Arg and L-Lys are essential to dermis fibroblasts. In addition to moisturizing property in stratum corneum, L-PCA may participate in homeostasis of skin dermis.

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