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# New mechanism for amino acid influx into human epidermal Langerhans cells: L-dopa/proton counter-transport system

Falck B, Bendsoe N, Ronquist G. New mechanism for amino acid influx into human epidermal Langerhans cells: L-dopa/proton counter-transport system.

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**Abstract:** We have characterized a stereospecific transport mechanism for L-dopa into human epidermal Langerhans cells (LCs). It is different from any other amino acid transport system. It is highly concentrative, largely pH-independent, and independent of exogenous  $\text{Na}^+$ , glucose and oxygen, and fuelled by a renewable intracellular energy source inhibited by iodoacetate but not by arsenate. We propose that the mechanism is a unidirectional L-dopa/proton counter-transport system. We have recently demonstrated anaerobic glycolysis in human epidermis, which substantiates the need of proton pumps for resident LCs. The findings prompt a re-evaluation of the profound changes LCs undergo when exposed to oxygen in aerobic culture. L-dopa is not metabolized by LCs but can rapidly be dislocated to the intercellular space by certain extracellular amino acids, i.e. LCs can profit by L-dopa in a dualistic way, altogether a remarkable biological phenomenon.

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**Key words:** proton counter transport – L-dopa – Langerhans cells – anaerobic glycolysis – epidermis – Birbeck granules.

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## Introduction

It is well established that epidermal Langerhans cells (LCs), but no other epidermal cells, possess uptake mechanisms allowing the aromatic amino acid, L-dihydroxyphenylalanine (L-dopa), and certain catecholamines to be accumulated and retained in high concentrations (1–3). These substances can be demonstrated with high precision and sensitivity on the cellular level with the histofluorescence method of Falck and Hillarp (4,5). It has been shown that L-dopa in LCs is located not only in the cytoplasm including the dendrites but also in the nucleus, whereas the catecholamines are confined to cytoplasmic granules (2). L-dopa uptake has been extensively used for visualizing epidermal LCs in both normal and diseased states but its mechanism has so far not been elucidated.

Three main transport systems account for most of the amino acid uptake by mammalian cells (6). System ASC preferentially transports alanine, serine and cysteine but recognizes some aliphatic

amino acids as well (7). System ASC deals with amino acid exchange rather than net uptake although it is  $\text{Na}^+$ -dependent (8). System L recognizes branched chain and aromatic amino acids (9). Similar to system ASC, system L mediates exchange rather than net uptake, but, as opposed to system ASC, system L is  $\text{Na}^+$ -independent. System A involves a transport mechanism which is  $\text{Na}^+$ -dependent and results in the net uptake of many neutral and cationic amino acids. System A also has several unusual properties including the recognition of N-methylated amino acids, tolerance of  $\text{Li}^+$ -substitution for  $\text{Na}^+$ , and sensitivity to inhibition by low extracellular pH (6). System N, which in contrast to the widely distributed systems ASC, L and A, appears more tissue-specific and catalyzes the uptake specifically of glutamine, histidine, and asparagine (10). Transport by system N is also dependent on  $\text{Na}^+$  but is strikingly sensitive to inhibition by a low external pH. In line with this sensitivity, it was found that the system N-transporter mediates proton exchange as well as  $\text{Na}^+$  cotransport (11). System T was originally described for human red blood cells (12,13). It mediates the  $\text{Na}^+$ -independent transport of

*Abbreviations:* KRP, Krebs-Ringer-phosphate buffer; LCs, Langerhans cells; BG, Birbeck granules; CMS, cytomembrane-sandwiching.

aromatic amino acids (14–16). It was initially assumed that system T was a variant of system L and had a  $\text{Na}^+$ -independent transport of neutral amino acids, including aromatic amino acids. However, subsequent studies revealed that in contrast to system L (16–18), system T accepts N-methyl analogues of the aromatic amino acids. A subclass of system T is TAT 1 (T-type amino acid transporter) exhibiting  $\text{Na}^+$ -independent and low-affinity transport of tryptophan, tyrosine and phenylalanine, as does system T. In addition, TAT 1 accepts some variations of aromatic side chains because it interacts with amino acid-related compounds such as L-dopa and 3-O-methyldopa (19). TAT 1 is not coupled with  $\text{H}^+$  transport but mediates an electroneutral-facilitated diffusion (19). A slow  $\text{Na}^+$ -independent uptake of L-dopa was also observed in human erythrocytes, tending towards a distribution ratio close to unity with a half-time to equilibrium of 1 h (20). It was concluded (20) that uptake was by carrier-mediated facilitated diffusion via the L and T systems for which L-dopa had low affinity.

The present report deals with the transport mechanism of L-dopa into human epidermal LCs. We reported previously that L-dopa is readily taken up and accumulated in that part of the LC population that we have termed L-dopa(+)LCs (21), where low extracellular concentrations of L-dopa will result in high intracellular concentrations as visualized by the histofluorescence method. As L-dopa is not a substrate amino acid for ribosomal protein synthesis, and as there is no known intracellular ligand for L-dopa, it is anticipated that L-dopa occurs freely in its intracellular context. Hence the uptake is concentrative, which in turn requires a motive force. The transport mechanism of L-dopa has so far not been explained in the context of the various amino acid transport systems of other cell types. The aim of the present work was to characterize the components of the active transport of L-dopa into LCs.

## Materials and methods

### Chemicals

Amino acids, amino acylesters, porcine trypsin, iodoacetate and sodium arsenate were obtained from Sigma Chemical Company (St. Louis, MO, USA). All chemicals were of the purest grade commercially available.

### Biopsies

Thin-skin biopsies containing the epidermis and upper dermis were obtained by punching (2 mm) without anaesthesia from the volar forearm skin of healthy adults.

### Incubation procedures

Unless otherwise stated the *in vitro* procedure was as follows. Incubations were carried out in the presence of L-dopa at 37°C, pH 7.4, usually for 60 min (in studies of sodium independence, 30 min). The basic incubation medium was 4 ml of plain Krebs-Ringer-phosphate buffer (KRP) with or without glucose (10 mmol/l). In some experiments potential competitive amino acids and potential other effectors were included, and, in those cases, preceded by a preincubation step in their presence. Post-incubation was generally performed in isotonic sodium chloride solution for 30 min to remove the high L-dopa concentration in the intercellular fluid that would give rise to a high background fluorescence which could mask the fluorescence of the L-dopa taken up and retained by the LC. To exclude any appreciable degradation of L-dopa in the LC, some biopsies were incubated as detailed earlier and then kept in Krebs-Ringer-bicarbonate buffer (KRB) for 2 and 3 h, respectively, before processing for fluorescence histochemistry.

### Fluorescence histochemistry

After incubation the biopsies were placed into 0.05% porcine trypsin in KRP at 4°C. The length of time for trypsin exposure, before separation of the epidermis was possible, was 25–50 min. The epidermis was gently teased off the dermis and processed according to the Falck-Hillarp formaldehyde method (22). Briefly, the epidermal sheets were placed on a slide, corneal surface downwards, dried overnight in a vacuum chamber, then exposed to formaldehyde gas for 1 h at 80°C and mounted in liquid paraffin. This method selectively visualizes a population of epidermal LCs capable of taking up and accumulating L-dopa.

### Uptake assay

Biopsies were coded and viewed in the fluorescence microscope on at least two different occasions by two independent observers unaware of the type of experiment concerned. Intracellular L-dopa uptake was evaluated from the fluorescence intensity expressed on an arbitrary stepwise scale from 0 (no visible uptake) to 3 (maximum uptake) including ½ steps (Fig. 1 a–c).

## Results

### Characteristics of the uptake process

**Concentration and time dependence.** Figure 2 illustrates the concentration dependence of the L-dopa uptake in the LCs. A Michaelis-Menten-like kinetics of the transport process was observed, with an apparent  $K_m$  of approximately 2 mmol/l.

As seen from Fig. 3 L-dopa uptake was a function of time. Among factors affecting the time to reach saturation was the concentration of L-dopa. The method we now used is based on microscopic estimation of fluorescence intensity. This may result in the maximum level being a somewhat broader zone rather than a sharp point.

**Dependence of temperature, pH and sodium ions (not shown in Figures or Tables).** Uptake of L-dopa (10 mmol/l, 60 min) was tested at 4°, 15–16° and 23°C ( $n = 6$  per temperature point). There was no uptake at the two lower temperatures, and only a relatively low uptake was seen at 23°C (Fig. 1a).

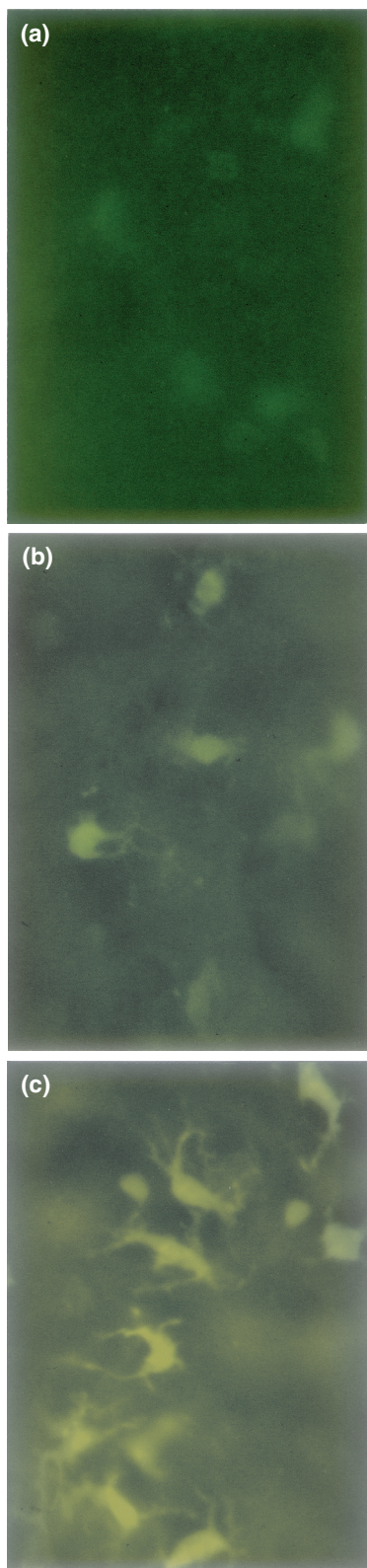


Figure 1. (a–c) L-dopa uptake in Langerhans cells estimated by fluorescence intensity using an arbitrary stepwise scale from 0 to 3. (a) low, (b) intermediate, and (c) maximum fluorescence intensity with L-dopa after incubation in Krebs-Ringer-phosphate buffer at 37°C, pH 7.4, with (from a to c) 1, 4, 10 mmol/l of L-dopa for 30, 40 and 60 min, respectively.

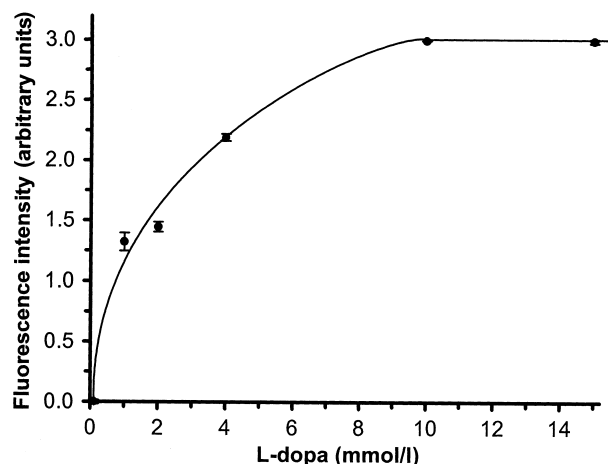


Figure 2. Concentration dependence of L-dopa uptake in LCs as measured by fluorescence intensity at 60 min of incubation, 37°C. Each point represents the mean value of at least 6 observations (the lowest concentration used was 0.1 mmol/l).

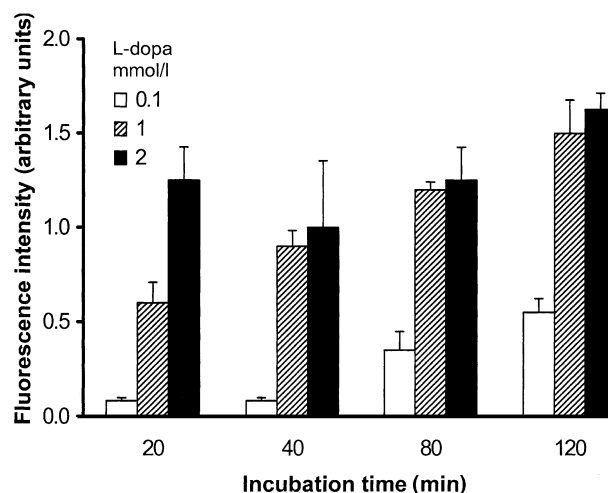


Figure 3. Uptake of L-dopa in Langerhans cells as measured by fluorescence intensity at three different concentrations as a function of time. Each column represents the mean value of at least five observations.

L-dopa transport (10 mmol/l, 60 min, 37°C) was independent of pH within the 4.8–8.5 range ( $n = 12$  at each of seven evenly spaced pH levels).

$\text{Na}^+$  was not obligatory for operation of the L-dopa transport system. Thus, a substantial uptake was noted when the  $\text{Na}^+$  was replaced by  $\text{K}^+$  in the KRP ( $n = 9$ ). Also, a clear-cut uptake was seen in isotonic KCl and in the absence of monovalent cations as well, i.e. in isotonic sucrose as the incubation medium ( $n = 6$ ). In contrast to the incubations in  $\text{K}^+$ -substituted or in plain KRP, some of the LCs appeared more or less poor in dendrites after 60 min of exposure to only isotonic KCl and sucrose, respectively.



**Stereospecificity.** D-dopa was ineffective as a substrate and no uptake was observed under experimental conditions similar to those used with L-dopa ( $n=9$ ).

Prolonged postincubation for up to 3 h after the L-dopa incubation resulted in no, or a very slight, decrease in fluorescence intensity and no change in fluorescence pattern, showing that L-dopa is not metabolized in LCs nor is it released under these conditions.

#### Competition with other amino acids

Physiological amino acids in excess did not interfere with the L-dopa uptake into the LCs when coincubated with L-dopa for 30–60 min. Further, to rule out the involvement of system A, N-methyl-aminoisobutyric acid (MeAIB) was tested. When concentrations exceeded that of L-dopa, no inhibition was observed (Table 1).

#### Energetics

The L-dopa transport was independent, not only of the  $\text{Na}^+$  gradient (see earlier), but also of glucose in the medium. We recently demonstrated that D-methionine efficiently depletes intracellular L-dopa, possibly via a trans-stimulatory action (unpublished data). Therefore uptake-depletion cycles of L-dopa could be carried out. Repeated incubations of 30 min each with L-dopa in the absence of glucose, followed by washings in the presence of D-methionine for up to five cycles

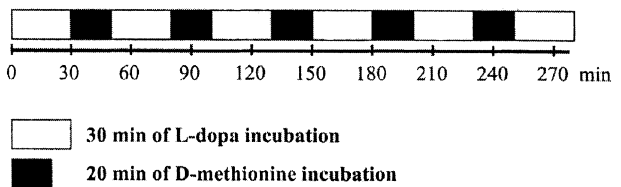


Figure 4. Protocol of chase experiments.

did not result in any fading of L-dopa influx. The protocol of these chase experiments is given in Fig. 4.

Preincubation in KRP without glucose for 7 h followed by incubation with L-dopa (10 mmol/l, 60 min) resulted in an unchanged L-dopa uptake. Such long preincubations in the total absence of oxygen (argon environment) also did not impair the uptake of L-dopa ( $n=6$ ).

A series of experiments was carried out involving iodoacetate, a well-known inhibitor of glycolysis. Based upon detailed preliminary experiments we chose an experimental design consisting of preincubation (30–90 min) and incubation (30 or 60 min) with 1 mmol/l of the inhibitor. The concentration of L-dopa in the incubation medium was 4 mmol/l, i.e. rather close to the apparent  $K_m$  value for L-dopa transport (see Fig. 2). Under these conditions a pronounced to almost total inhibition of the L-dopa uptake into the LCs was achieved both in the presence ( $n=6$ ) and absence ( $n=15$ ) of glucose. Fluorescence intensity in these cases was at most not more than that shown in Fig. 1(a) and there was often a somewhat less pronounced decrease in the number of LCs.

When iodoacetate was exchanged for sodium arsenate (5 mmol/l;  $n=17$ ) in otherwise similar experiments, no influence of arsenate on L-dopa uptake was observed regardless of phosphate being present.

#### Induction of L-dopa uptake into L-dopa(–) LCs

There are two types of human LCs, one that readily takes up and accumulates L-dopa (L-dopa(+) LCs) and the other that does not take up demonstrable amounts of L-dopa (L-dopa(–) LCs) (21,23). Exposure to  $\alpha$ -methyl-n-aminobutyryl methyl ester (AnBMe) most probably leads to ester hydrolysis in the LC and the release of free amino n-butyric acid (AnB) and protons. Simultaneously, L-dopa(–) LCs are transformed to L-dopa(+) LCs but their final fluorescence is considerably lower than that exhibited by L-dopa(+) LCs (23). Thus, after incubation with AnBMe and L-dopa all or almost all LCs take up L-dopa although the formerly L-dopa(–) LCs do so to a

Table 1. Lack of inhibitory effect on L-dopa transport into Langerhans cells by other amino acids. The first 12 amino acids are transported mainly by system A and the next four mainly by system L, as defined by Christensen (6). N-methyl- $\alpha$ -aminoisobutyric acid is a model amino acid for system A

Amino acid added	Amino acid: L-dopa ratio (mmol/l)	Inhibitory effect	<i>n</i>
L-alanine	40:4	0	10
$\alpha$ -aminoisobutyric acid	10:10	0	5
$\alpha$ -aminobutyric acid	10:2 & 40:4	0	7
glycine	40:4	0	3
L-histidine	40:4	0	3
L-homocysteine	15:4	0	3
L-methionine	10:10	0	3
D-methionine	10:10 & 40:4	0	7
L-norleucine	40:4	0	7
Sarcosine	40:4	0	8
L-serine	40:4	0	3
L-threonine	40:4	0	8
L-leucine	40:4	0	7
D-leucine	40:4	0	7
L-phenylalanine	40:4	0	10
L-valine	40:10 & 40:4	0	6
MeAIB	20:2	0	3

*n* = number of experiments carried out on different occasions and individuals.  
MeAIB, N-methyl- $\alpha$ -aminoisobutyric acid.

Table 2. Ability of different amino acid esters to transform L-dopa(–) Langerhans cells to L-dopa(+) Langerhans cells

Amino acylester (10 mmol/l)	Amino acid formed by hydrolysis	Inductive activity/n
AnBMe	AnB	+/9
L-aspartic acyldimethylester	Aspartic acid	+/3
Glutamic acyldiethylester	Glutamic acid	+/3
D-methionine acylmethylester	D-methionine	+/3*
L-proline acylmethylester	L-proline	+/3*
L-alanine methylester	L-alanine	+/3*

\*One of three cases.

AnBMe,  $\alpha$ -methyl-n-aminobutyl methyl ester.

lesser extent than do L-dopa(+) LCs. We have now extended these studies to five other methyl esters whose amino acid moieties are improbable candidates for exchange with L-dopa. As seen from Table 2, all these esters could induce L-dopa uptake into L-dopa(–) LCs.

## Discussion

The present work was carried out with the explicit aim to avoid perturbation of the biological system under study. Therefore, L-dopa transport was studied with the LCs still resident in their epidermal microenvironment. Cultured LCs undergo certain changes (24), which may be the result, at least in part, of the fact that they are exposed to an oxygen-containing atmosphere. The LCs are a small subpopulation constituting only 3% or less of all cells in the living epidermis, and the total amount of L-dopa taken up by the cells present in a 2-mm epidermal biopsy is exceedingly small. This, and the fact that L-dopa analysis of the epidermis is so far impossible (see later), using conventional biochemical methods makes the histofluorescence method (4,5) appropriate to examine L-dopa transport. The various aspects of the transport system were easily observable because L-dopa yields intense fluorescence and the LCs are the only epidermal tissue components that can take up and retain L-dopa. Moreover, conversion of L-dopa to catecholamines does not occur in LCs, which is easily established because this would have given rise to a different fluorescence pattern (2).

The Michaelis-Menten-like kinetics of the uptake process indicated a carrier-mediated transport. There were no grounds for the assumption that the L-dopa carrier was identical with the known carriers of other physiological amino acids. This proposal is based on the following findings. The transport was operative over an unusually broad pH range and was independent of sodium ions and any other monovalent cation in the incubation

medium. Nor could any competitive inhibition be revealed by any of a broad variety of physiological and non-physiological amino acids, present in excess relative to L-dopa, having affinity for not only the A and L systems, but also for other systems transporting neutral amino acids, the gly<sup>+</sup> and ASC systems. Of special interest was the inability of MeAIB to compete for uptake. N-methylaminoisobutyric acid is considered to be a specific substrate for system A (6). Although system T was initially considered as a variant of system L, it became apparent that it was a distinct system (16). System T was ruled out because phenylalanine was inefficient as a competitive inhibitor (L-dopa : phenylalanine, 4 : 40 mmol/l). A subclass of system T is TAT 1 exhibiting Na<sup>+</sup>-independent and low-affinity transport of tryptophan, tyrosine and phenylalanine, conforming with system T. TAT 1 is strongly expressed in intestine, placenta and liver with a concentration in the basolateral membrane of the epithelial cells of the rat small intestine (19). It suggests a role of TAT 1 in the transepithelial transport of aromatic amino acids (19). There are no reports of the existence of TAT 1 in the epidermis or in epidermal LCs. Thus, there was no apparent candidate functioning in a symport or antiport context with L-dopa for its concentrative uptake.

Viruses exploit cell-surface molecules as receptors for bonding to and entry into host cells. Two studies of the *ecoR* gene, which codes for the receptor of the ecotropic murine leukemia virus, have discovered that the receptor molecule is most likely the classic Ly<sup>+</sup> amino acid transporter. This gene encodes a protein of 622 amino acid residues with 14 domains with potentially membrane-spanning characteristics. This transmembrane topology was similar to several membrane transporter proteins, especially ones for arginine and histidine in yeast (25,26). These authors further showed that *Xenopus* oocytes injected with *ecoR* messenger RNA display increased amino acid uptake corresponding closely to System Ly<sup>+</sup>. These findings are a landmark because they represent the first mammalian amino acid transporter to be cloned and the first instance of a virus subverting a transmembrane transport protein as a receptor (27). So far, no amino acid transport systems have been the subject of study in LCs besides that described by us, and accordingly, this new transport system has not been characterized in molecular terms.

The chase experiments (Fig. 4), originally expected to result in an exhaustive uptake pattern as a result of energy substrate depletion, revealed an undisturbed transport capacity for at least 4 h (five cycles), pointing to a renewable source acting as a motive force. Also, the uptake of

L-dopa was not compromised under strictly anaerobic conditions for an extended period of time. Other experiments in our laboratory have demonstrated that the human epidermis has the capacity to produce lactic acid continuously, regardless of the presence of oxygen (28). Accordingly, the unknown counterpart of a proposed antiport system could well be the continuously produced protons of glycolysis, as long as glucose equivalents are supplied either from the external compartment or by glycogenolysis. A sustaining effect of the glycogen store in the epidermis was consistent with our observation that after incubation in buffer for 7 h without glucose, the transport system was still fully functional. Moreover, introducing iodoacetate (a well-known inhibitor of glycolysis) into the experimental system caused a diminished uptake of L-dopa into the LCs, strongly supporting the idea of metabolic coupling in LCs between glycolytic flux and L-dopa influx. It is of importance in this context that arsenate in the incubation medium did not inhibit L-dopa uptake in the LCs. The working principle of this metallomolecule is enzymatic oxidative arsenolysis of D-glyceraldehyde 3-phosphate. Hence, arsenate may substitute for phosphate in the oxidative phosphorylation of glyceraldehyde 3-phosphate, giving rise to 1-arseno, 3-phosphoglyceric acid. This intermediate is a highly unstable compound that immediately and spontaneously decomposes into 3-phosphoglycerate (an ordinary metabolite of anaerobic glucose oxidation) and arsenate in aqueous systems. Note that in the presence of arsenate no high-energy phosphate compound is generated by the dehydrogenase although overall oxidoreduction takes place. Arsenate can thus uncouple oxidation from phosphorylation. Hence, the glycolytic flux ending up in lactate formation is not retarded, although energy metabolism is compromised. Thus, L-dopa uptake in LCs is dependent on lactate formation rather than on ATP formation.

With these background findings, it seemed obvious to further study the effects of AnBMe (23). Exposure of epidermal tissue to this lipophilic ester leads to rapid penetration across the plasma membrane, most probably followed by intracellular hydrolysis of the ester linkage, giving rise to two possible intracellular counterparts in the antiport, viz. protons and the free amino acid. Concomitantly, formerly L-dopa(−)LCs develop the capacity to take up and accumulate L-dopa (23). At the time when these results were published, the liberated amino acid was considered a possible counterpart. However, in the present experiments we tested some other esters that, besides the obligatory release of protons, produce

various amino acids which can be ruled out as counterparts. Thus, glutamic acid and aspartic acid are negatively charged because of the extra carboxylic group and therefore are not counterparts of the neutral L-dopa. The present L-dopa transport system is strictly stereospecific (D-dopa is invalid as a substrate amino acid) and hence L-dopa should not interfere with D-methionine. Proline and alanine are the preferred substrates for Na<sup>+</sup>-dependent systems and are therefore less likely exchange partners. Nevertheless, these esters transformed the L-dopa(−)LCs to L-dopa(+)LCs. The intracellular protons generated by the hydrolysis, analogous to protons generated by glycolysis, are suggested as the motive force of the L-dopa uptake by migration downhill. At first glance, it may appear somewhat puzzling that we did not observe any influence of a lowered extracellular pH upon the L-dopa uptake into the LCs. However, it should be kept in mind that the epidermal cell microenvironment was not representative of that in most other parenchymal cells because of the high anaerobicity and compromised perfusion possibilities that create the prerequisites for an acidic environment.

The human epidermis displays an anaerobic production of lactate (28), and, obviously, LCs residing in the epidermis must have effective mechanisms for extruding protons. The L-dopa/proton counter-transport system we now describe seems to be both energy-saving and potent. It is an antiport system, which implies that it can be regarded as an integral part of glycolysis in epidermal LCs. Hence, we have now provided evidence, based on several different approaches, that intracellular proton production in LCs is the motive force for the concentrative uptake of L-dopa in these cells.

A conceptual consequence of the proposed transport mechanism is that L-dopa(+)LCs and L-dopa(−)LCs should not be considered as functionally different subpopulations. Instead, L-dopa(−)LCs are temporarily inactive cells with a low metabolic rate. Clearly L-dopa(−)LCs also must be able to eliminate intracellularly formed protons. Whether this is performed via the L-dopa pump but at a rate too slow to be visualized with the method used or via some other mechanism is not yet known.

Langerhans cells are major histocompatibility complex (MHC) class II-positive dendritic cells that act as antigen-presenting cells for T-cell-dependent immune responses. The question might therefore be well founded whether a functional coupling exists between L-dopa(+)LCs cells and the antigen-presenting ability. We believe they represent different properties, as the conversion



from L-dopa(-) LCs to L-dopa(+) LCs is momentaneous and dependent on the release of protons while the capturing and processing of antigens in the skin and subsequent activation of naive T cells is a complicated process. e.g. in the case of protozoan parasites of the genus *Leishmania* it was shown that *i.a.* newly synthesized MHC class II molecules were required for *Leishmania* major antigen presentation by LCs (29), which means that this process is not at all momentaneous.

L-dopa is not metabolized by the LCs but can, after dislocation, again engage the carrier. We have indeed shown (unpublished observations) that a number of physiological amino acids that can be expected to appear in the epidermal intercellular fluid, rapidly induce an exodus of intracellular L-dopa in LCs. Thus, it is possible that LCs profit from L-dopa in a dualistic way, an altogether remarkable biological mechanism.

The transport mechanism probably accepts no closely related molecules other than L-dopa, and, to a certain extent, metatyrosine (unpublished observations). Of the two, only L-dopa can be produced in the epidermis, namely by the melanocytes. Extensive pigment research has shown so far that insurmountable difficulties prevent the demonstration of the presence of L-dopa in human epidermis, possibly because of the existence of components that cause unspecific oxidation of epidermal tyrosine to dopa in the homogenization step (Rosengren and Rorsman, personal communication). It has, however, been shown that epidermal melanocytes in pigmented animals produce considerable amounts of L-dopa (30–32).

Research on LCs has been extensive throughout many decades but so far no one has contemplated the possibility that they are confined to a preferentially anaerobic energy metabolism when resident in the epidermis. This and the fact that human LCs possess at least one plasma membrane-bound proton extruding system may throw a different light on various biological phenomena concerning LCs. It is of interest in this context that LCs, when transferred to an aerobic culture medium, undergo profound changes (24,33,34). For example, Birbeck granules (BGs), a unique and functionally enigmatic organelle in LCs, disappear more or less completely within a few days, especially from freshly isolated LCs kept under aerobic conditions. Birbeck granules are formed by plasma membrane parts folding upon themselves with the cytosol side facing outwards (a process that has been named cytomembrane-sandwiching or CMS), the interlinking force between the zippered membranes being  $\text{Ca}^{2+}$ -dependent (35,36). Most importantly, Valladeau et al. (37) recently identified a

$\text{Ca}^{2+}$ -dependent lectin, Langerin, which induces BGs formation because of its capacity to produce membrane superimposition and zippering. The functions of BGs are not yet fully clarified, but it is likely that they are capable of endocytosis (38) involved in antigen processing (24,38), and represent a link between the exterior of the cell and the endosomal compartment, perhaps even sometimes transforming into endosomes (38). The CMS process implies internalization of the proton pumps, which could carry protons into the interior of the BG for further transport to the cell surface (or for establishment of endosomal activity). The hypothetical possibility that BGs act as proton reservoirs supporting elimination of protons derived from the anaerobic glycolysis is consistent with the finding that suprabasal LCs contain significantly more BGs than do basal LCs (39), which, being the closest to the capillary network, probably respire under higher oxygen tension. This could explain the phenomenon that BGs disappear when the facultatively anaerobic LCs are exposed to oxygen in culture. Also, BGs could represent the acid compartment, explaining why catecholamines taken up by human LCs are visualized as granular fluorescence.

## References

1. Falck B, Agrup G, Jacobsson S, et al. Uptake of l-dopa and functionally related aromatic amino acids into the Langerhans cells. *J Invest Dermatol* 1976; 66: 265.
2. Axelsson S, Elofsson R, Falck B, Sjöborg S. In vitro uptake of l-dopa and catecholamines into the epidermal Langerhans cell. *Acta Derm Venereol* 1978; 79 (Suppl 58): 31–35.
3. Sjöborg S, Axelsson S, Falck B, Jacobsson S, Ringberg A. A new method for the visualization of the epidermal Langerhans cell and its application on normal and allergic skin. *Acta Derm Venereol* 1978; 79 (Suppl 58): 23–29.
4. Falck B. Observations on the possibilities of the cellular localization of monoamines by fluorescence method. *Acta Physiol Scand* 1962; 197 (Suppl 56): 2–25.
5. Falck B, Hillarp N-Å, Thieme G, Torp A. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J Histochem Cytochem* 1962; 10: 348–354.
6. Christensen H N. Kinetics in transport and effect of solute structure on transport. In: Christensen HN, ed. *Biological Transport*, edn 2. Reading, Massachusetts: W. A. Benjamin Inc., 1975: 166–231.
7. Christensen H N, Liang M, Archer E G. A distinct  $\text{Na}^+$ -requiring transport system for alanine, serine, cysteine, and similar amino acids. *J Biol Chem* 1967; 242: 5237–5246.
8. Christensen H N. Interorgan amino acid nutrition. *Physiol Rev* 1982; 62: 1193–1228.
9. Thomas E L, Christensen H N. Nature of the cosubstrate action of  $\text{Na}^+$  and neutral amino acids in a transport system. *J Biol Chem* 1971; 246: 1682–1688.

10. Kilberg M S, Handlogten M E, Christensen H N. Characteristics of an amino acid transport system in rat liver for glutamine, asparagine, histidine, and closely related analogs. *J Biol Chem* 1980; 255: 4011–4019.
11. Kilberg M S, Handlogten M E, Christensen H N. Characteristics of system asc for transport of neutral amino acids in the isolated rat hepatocyte. *J Biol Chem* 1981; 256: 3304–3312.
12. Rosenberg R, Young J D, Ellory J C. L-Tryptophan transport in human red blood cells. *Biochim Biophys Acta* 1980; 598: 375–384.
13. Vadgama J V, Christensen H N. Discrimination of  $\text{Na}^+$ -independent transport systems L, T, and asc in erythrocytes.  $\text{Na}^+$  independence of the latter a consequence of cell maturation? *J Biol Chem* 1985; 260: 2912–2921.
14. Vadgama J V, Christensen H N. A new  $\text{Na}^+$ -independent transport system for dipolar amino acids apparently corresponding to systems persisting after erythrocyte maturation in some mammalian genotypes. *Ann N Y Acad Sci* 1985; 456: 454–456.
15. Vadgama J V, Castro M, Christensen H N. Characterization of amino acid transport during erythroid cell differentiation. *J Biol Chem* 1987; 262: 13273–13284.
16. Van Winkle L J, Campione A L, Gorman J M.  $\text{Na}^+$ -independent transport of basic and zwitterionic amino acids in mouse blastocysts by a shared system and by processes which distinguish between these substrates. *J Biol Chem* 1988; 263: 3150–3163.
17. Van Winkle L J, Mann D F, Campione A L, Farrington B. Transport of benzenoid amino acids by system T and four broad scope systems in preimplantation mouse conceptuses. *Biochim Biophys Acta* 1990; 1027: 268–277.
18. Lopez-Burillo S, Garcia-Sancho J, Herreros B. Tryptophan transport through transport system T in the human erythrocyte, the Ehrlich cell and rat intestine. *Biochim Biophys Acta* 1985; 820: 85–94.
19. Kim D K, Kanai Y, Chairoungdua A, Matsuo H, Cha Sh, Endou H. Expression cloning of a  $\text{Na}^+$ -independent aromatic amino acid transporter with structural similarity to  $\text{H}^+$ /monocarboxylate transporters. *J Biol Chem* 2001; 276: 17221–17228.
20. Floud A, Fann S. L-DOPA (L-3,4-dihydroxyphenylalanine) uptake by human red blood cells. *Biochim Biophys Acta* 1981; 645: 165–169.
21. Warfvinge K, Mikulowska A, Falck B. Functionally different Langerhans cells in human epidermis. *Acta Derm Venereol (Stockh)* 1991; 71: 429–430.
22. Falck B, Björklund A, Lindvall O. Recent progress in aldehyde fluorescence histochemistry. *Brain Res Bull* 1982; 9: 3–10.
23. Falck B, Andersson A, Mikulowska A, Ronquist G.  $\alpha$ -Amino-n-butyric acid methyl ester induces concentrative uptake of l-dopa in human Langerhans cells normally not operative for l-dopa transport. *Acta Derm Venereol (Stockh)* 1993; 73: 197–199.
24. Schuler G, Romani N, Stössel H, Wolff K. Structural organization and biological properties of Langerhans cells. In: Schuler G, ed. *Epidermal Langerhans Cells*. Boca Raton: CRC Press, 1991: 87–137.
25. Kim J W, Closs E I, Albritton L M, Cunningham J M. Transport of cationic amino acids by the mouse ecotropic retrovirus receptor. *Nature* 1991; 352: 725–728.
26. Wang H, Kavanaugh M P, North R A, Kabat D. Cell-surface receptor for ecotropic murine retroviruses is a basic amino-acid transporter. *Nature* 1991; 352: 729–731.
27. Vile R G, Weiss R A. Virus receptors as permeases. *Nature* 1991; 352: 666–667.
28. Ronquist G, Andersson A, Bendsoe N, Falck B. Human epidermal energy metabolism is functionally anaerobic. *Exp Dermatol* 2003; 12: 572–579.
29. Flohe S, Lang T, Moll H. Synthesis, stability, and subcellular distribution of major histocompatibility complex class II molecules in Langerhans cells infected with *Leishmania major*. *Infect Immun* 1997; 65: 3444–3450.
30. Cegrell L, Falck B, Rosengren A-M. Extraction of dopa from the integument of pigmented animals. *Acta Physiol Scand* 1970; 78: 65–69.
31. Rorsman H, Rosengren A-M, Rosengren E. Determination of 5-S-cysteinyl-dopa in melanomas. *Yale J Biol Med* 1973; 46: 516–522.
32. Hansson C, Agrup G, Rorsman H, Rosengren E. Dopa and 5-S-cysteinyl-dopa in the serum of albino, black, and red guinea-pigs. *Acta Derm Venereol (Stockh)* 1980; 60: 155–156.
33. Teunissen M B M, Wormmeester J, Krieg S R, et al. Human epidermal Langerhans cells undergo profound morphologic and phenotypic changes during in vitro culture. *J Invest Dermatol*, 1990; 94: 166–173.
34. Rambukkana A, Bos J D, Irik D, et al. *In situ* behavior of human Langerhans cells in skin organ culture. *Lab Invest* 1995; 73: 521–531.
35. Bartosik J, Andersson A, Axelsson S, Falck B, Ringberg A. Direct evidence for the cytomembrane derivation of Birbeck granules: The cyto-membrane-sandwich effect. *Acta Derm Venereol (Stockh)* 1985; 65: 157–160.
36. Andersson L, Bartosik J, Bendsoe N, et al. Formation and unzipping of Birbeck granules in vitro. In: Thivolet J, Schmitt D, eds. *The Langerhans Cell*. London, Paris: Colloque INSERM/John Libbey Eurotext Ltd., 1988: 172: 185–191.
37. Valladeau J, Ravel O, Dezutter-Dambuyant C, et al. Langerin, a novel C-type lectin specific to Langerhans cells, is an endocytic receptor that induces the formation of Birbeck granules. *Immunity* 2000; 12: 71–81.
38. Bartosik J. Cytomembrane-derived Birbeck granules transport horseradish peroxidase to the endosomal compartment in the human Langerhans cells. *J Invest Dermatol* 1992; 99: 53–58.
39. Bartosik J, Lamm C-J, Falck B. Quantification of Birbeck's granules in human Langerhans cells. *Acta Derm Venereol (Stockh)* 1991; 71: 209–213.