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α-amino-n-butyric Acid Methyl Ester Induces Concentrative Uptake of L-dopa in Human Langerhans' Cells Normally not Operative for L-dopa Transport

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We recently reported the existence of two kinds of human epidermal Langerhans' cells (LC), one which can take up and accumulate L-dopa and one which cannot. The dopa(+) LC take up L-dopa by carrier-mediated exchange diffusion, that is, the influx of L-dopa and the outflow of an intracellular substance are linked via the same carrier. The nature of the fundamental difference between L-dopa(+) and L-dopa(−) cells has not been clarified. We have now found that α-amino-n-butyric acid methyl ester (ABA-OME) penetrates into intracellular compartments, perhaps endosomes or lysosomes, of all LC, where hydrolysis results in the accumulation of the free amino acid (ABA). This accumulation causes a considerable increase in osmotic pressure of the membrane-limited organelle, leading to influx of water and swelling. Co-incubation with L-dopa revealed an influx of L-dopa into LC which normally cannot take up this amino acid. It is suggested that these LC lack the capacity to synthesize and/or store the counterpart which allows L-dopa to enter the dopa(+) LC, but that ABA in the L-dopa(−) LC can function as an equivalent counterpart.
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While human epidermal Langerhans' cells (LC) can take up and store considerable amounts of L-dopa, as demonstrated by the Falck-Hillarp fluorescence method (1), they do not seem capable of metabolizing this amino acid (2). Nevertheless, renewed interest in the L-dopa uptake mechanism arose when it was shown that there are human LC which lack the capacity to take up L-dopa (3). So far, this is the first observation that justifies the division of human LC into two functionally different populations: that which takes up L-dopa (dopa(+) LC) and that with cells lacking this capacity (dopa(−) LC).

Continued studies of prospective substances with effects on L-dopa uptake, which may further elucidate the transport mechanism, have disclosed an amino acid derivative, α-amino-n-butyric acid methyl ester (ABA-OME), with a most remarkable effect on the L-dopa uptake of the LC. It causes some of the dopa(−) LC to take up L-dopa, and, moreover, it causes dopa fluorescence to take on a ring-shaped appearance, instead of being uniformly distributed throughout the cells. The elucidation of these phenomena is described here.

MATERIAL AND METHODS

Punch biopsies (2 mm) from normal human forearm skin were obtained from 7 adults.

The biopsies were incubated in a Krebs-Ringer phosphate buffer (KRP) (4 ml per biopsy, 37°C, pH 7.4), containing either L-dopa (10 mM) or L-dopa (10 mM) and DL-ABA-OME (20 mM) according to the following experimental design: (I) pre-incubation with ABA-OME for 30 min, followed by incubation for 60 min with ABA-OME + L-dopa and a second incubation for 60 min with ABA-OME; (II) pre-incubation with ABA-OME for 30 min followed first by incubation in ABA-OME and L-dopa for 60 min and then by washing in KRP for 60 min; (III) pre-incubation for 30 min with ABA-OME, two washings in KRP for 15 min followed by incubation with L-dopa for 60 min and washing in KRP for 60 min. Controls were obtained by excluding ABA-OME. In some experiments with ABA-OME, the L-dopa was replaced by D-dopa.

The specimens were divided into halves and processed for fluorescence microscopy (1) (at least 20 serial paraffin sections were prepared) and for electron microscopy (series consisting of 30 or sometimes 200 consecutive sections were prepared).

RESULTS

Following incubation with L-dopa, epidermal LC appeared which displayed a moderate to strong fluorescence diffusely distributed all over the cells (Fig. 1). The general background fluorescence was very low. The number of fluorescent LC varied greatly between individuals, as could have been expected; it has been demonstrated that the number of dopa(+) LC shows very marked inter-individual variations, whereas the number of $T6^+$ LC is approximately the same within the forearm skin region (3).

Pre-incubation with ABA-OME (incubation procedure III) drastically changed this picture: the LC fluorescence now appeared ring-shaped (Fig. 2) and the number of fluorescent cells was increased in 5 out of 7 specimens. Remarkably, the fluorescence of the dendrites was evenly distributed, just as after exposure to L-dopa alone.

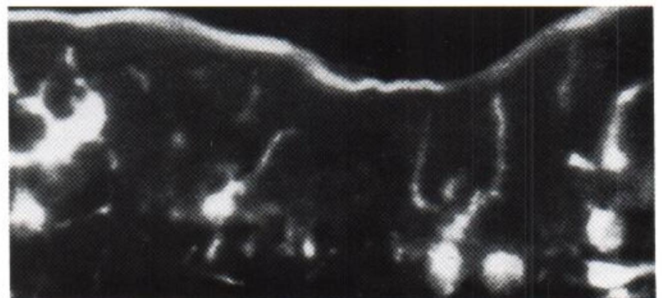

Fig. 1. Incubation with L-dopa renders dopa(+) LC strongly fluorescent.

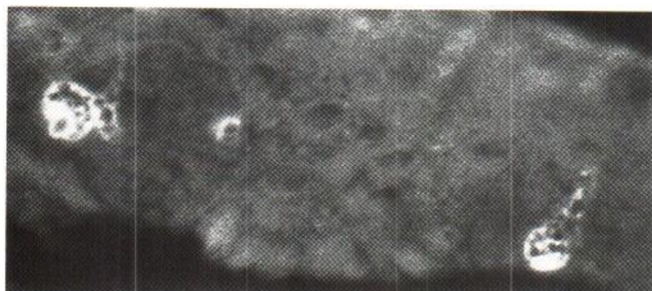

Fig. 2. Incubation with L-dopa and ABA-OME according to procedure III in Materials and Methods. Ring-shaped fluorescence in LC caused by the L-dopa which is retained in the split-up cytoplasm.

Prolonged exposure of the LC to ABA-OME (incubation procedures I and II) did not lead to increased L-dopa uptake. On the contrary, it produced marked differences in fluorescence intensity between the LC, varying from weak to strong, and a disturbing background fluorescence which almost disguised the weakly fluorescent cells. Moreover, the LC appeared enlarged and the number of fluorescent dendrites was greatly reduced; commonly no or only 1–2 short dendrites were seen. Ring-shaped fluorescence was sometimes seen in coarse proximal dendritic parts.

Co-incubation with D-dopa and ABA-OME did not render the dopa(−) LC fluorescent, demonstrating that the uptake mechanism for dopa is stereospecific as in dopa(+) cells under normal conditions (2). Surprisingly, more D-dopa than L-dopa was retained in the epidermis during washing, as evidenced by a final higher general background fluorescence.

The findings on electron microscopy were striking. All LC were filled with huge vacuoles, many of which were surrounded by a trilaminar membrane, structurally similar to the plasma membrane (Fig. 3). The LC cytoplasm was split into a continuous system of strands and tiny islets containing all the cell components found in a normal LC. These components were structurally intact, even after the longest incubation times.

In some LC discrete plasma membrane parts showed ruffling, which was sometimes intense. Studies of serial sections from ruffling membrane areas and adjacent cytoplasm did not disclose any incorporation of membrane-limited vesicles.

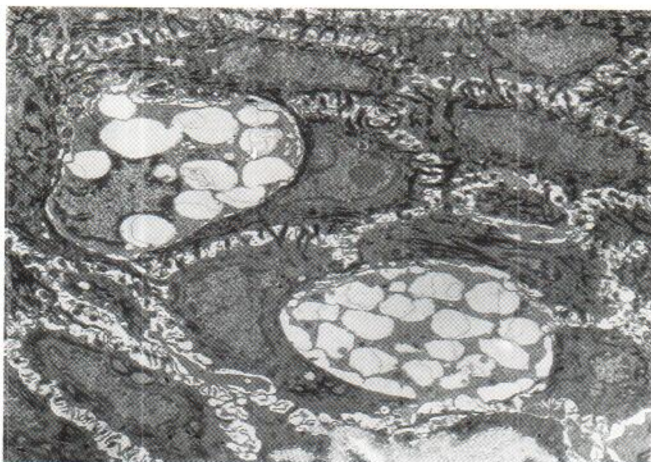

Fig. 3. Part of the same specimen as shown in Fig. 2. The cytoplasm is split into strands and tiny islets. This structural disconfiguration explains the ring-shaped fluorescence seen in Fig. 2.

The ABA-OME effect was highly selective within the epidermis, in that only rarely was a vacuole seen in occasional keratinocytes. The massive vacuolization made it very easy to observe the cells under low-power magnification.

DISCUSSION

The mechanism behind the vacuolization of the LC caused by ABA-OME is currently under study in this laboratory. ABA-OME probably penetrates into intracellular compartments, perhaps endosomes or lysosomes, where the free amino acid, which cannot diffuse freely out of the membrane-limited organelle, is formed by hydrolysis. The accumulation of ABA causes a considerable increase in the osmotic pressure of these compartments, leading to influx of water and swelling (cf. 4). The great reduction in the dendritic tree of the LC following prolonged exposure to ABA-OME may be explained by an increase in the volume of the perikaryon that takes place at the expense of the plasma membrane of dendrites. Consumption of plasma membrane protrusions is known to occur during the cell swelling induced by exposing cells to a hypo-osmotic environment (5, 6).

This mode of action, leading to vacuolization, however, does not explain the fact that LC that are originally dopa(−) display fluorescence after exposure in vitro to L-dopa and ABA-OME. ABA-OME also induced plasma membrane ruffling which did not appear to result in concomitant formation of pinocytotic vesicles. Thus, it seems unlikely that L-dopa is transported into L-dopa(−) LC by fluid-phase endocytosis. This possibility is also excluded by the result obtained with the second experimental set-up (see Materials and methods), which demonstrates that dopa(−) LC also take up L-dopa after ABA-OME has been removed and membrane ruffling has ceased.

There are several components operating in the uptake and storage of L-dopa in dopa(+) LC: a plasma membrane-bound carrier, counter-transport of an intracellular substance and a binding mechanism (2). It can be speculated that when dopa(−) LC are seemingly transformed into dopa(+) LC, ABA-OME initiates the formation of the carrier protein or the binding mechanism. It is more reasonable to assume that ABA, formed by demethylation within the LC, can act as a counterpart which allows L-dopa to enter the LC by exchange diffusion, provided that no new and hitherto unknown uptake mechanism of L-dopa exists in LC. The rapid vacuolization produced by ABA-OME is followed by a relatively slow reflux, and the normal morphology of the LC is restored (unpublished observations). This process reflects a slow escape of ABA from the membrane-limited organelles, thus making ABA accessible for translocation to the exterior of the cells. This would mean that under normal conditions dopa(−) LC possess the stereospecific carrier but cannot take up L-dopa because they lack the capacity to synthesize and/or store an appropriate counterpart amino acid. Whatever the explanation, the fact that LC which are originally dopa(−) display

fluorescence after exposure in vitro to L-dopa and ABA-OME is of great importance to the understanding of both the uptake process and the possibly different functions of the two LC populations, the dopa(−) and dopa(+).

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