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Intact Epidermal Cell Assay for Ornithine Decarboxylase Activity

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Summary. A procedure measuring the ornithine decarboxylase (ODC) activity and polyamine formation of intact neonatal mouse epidermal cells in culture has been developed and tested. Basal cells prepared from neonatal mouse epidermis were plated on round 15-mm Lux coverslips, placed in Costar 24 well culture clusters and grown at 32° C in M-199 + 13% fetal bovine serum. Before assay the cells were rendered permeable to ornithine ^{14}C and ODC inhibitors using the buffer described by Berger et al. [3]. The slides, covered with adhering cell layers, were then placed in vials, covered with assay buffer and assayed intact for ODC activity. The ODC reaction was terminated by addition of citric acid to the buffer and the amount of $^{14}CO_2$ released was determined by scintillation counting of a center well filled with trapping agent. The baseline ODC activity of the intact cells was 500 – 1,000 pmol $^{14}CO_2$/mg protein/45 min. The validity of this ODC assay procedure using intact neonatal mouse keratinocytes was tested by use of three specific ODC inhibitors and by measuring the formation of polyamines from uniform labeled ornithine. The results indicated that authentic ODC activity was measured and preserved in this intact neonatal mouse epidermal cell assay. This technique holds promise for future studies of epidermal cell regulation of ODC and polyamine synthesis and studies of the multiple ornithine metabolites and conjugates formed, using a highly manipulable in vitro system.

Key words: Primary keratinocytes – Intact epidermal cell ODC activity – Polyamine metabolites and conjugates – Epidermal cell culture

Introduction

In the epidermal cell, as in all other tissues, ornithine decarboxylase (ODC) (L-ornithine carboxylyase; EC 4.1.1.17) is the first and, most probably, the rate-limiting enzyme in polyamine biosynthesis [17, 19]. This unique and ubiquitous enzyme may have an important function in the regulation of epidermal cell division

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and growth and in epidermal tumor promotion [9, 12, 15, 24, 25, 27]. Besides the evidence implicating ODC and polyamines in animal model epidermal hyperproliferation and hyperplasia, the intracellular levels of the polyamines and the activity of their biosynthetic enzymes [22] may have significant correlation with the accelerated epidermal proliferation of the psoriatic epidermis [5, 26]. There is at present, however, no evidence demonstrating a direct cause and effect mode of regulation between polyamine biosynthesis (ODC induction) and initiation of epidermal cell proliferation.

The enzyme ODC is unique in a number of ways: (a) it has a biologic half-life of 10–20 min [21], which can vary depending on growth conditions [8]; (b) the induction of ODC is controlled by the production of a protein(s) that is an ODC inhibitor (ODC-antizymes) [7]; (c) the synthesis of the ODC-antizyme(s) is induced by the polyamines putrescine, spermidine, or spermine [7], i.e., there is a negative-feedback regulation of ODC by its endproducts; (d) there are two or more enzymatic forms of ODC [13]; and (e) moreover, ODC is an enzyme that can exhibit changes in its biologic half-life [4].

The usual method of assay for ODC activity is the measurement of $^{14}CO_2$ release from L-(1-^{14}C) ornithine by the ODC present in the supernatant fraction of homogenized tissue or cells [17, 19]. The ODC present in the supernatant of homogenized isolated epidermal cells is difficult to assay because of the short half-life of the enzyme [21] and because the control level of ODC in epidermal cells is so low that often the activity is below assay sensitivity.

To overcome these problems, i.e., the complex control of an indicible enzyme with multiple forms, a variable, short half-life, and low control level activity, we designed and tested the first ODC assay using intact living epidermal cell cultures [11, 20]. With the whole-cell enzyme assay we can better approximate the physiologic conditions of intact epidermis, i.e., feedback regulation, synthesis of ODC enzyme(s) and ODC-antizymes, and functional changes in ODC half-life during the assay itself instead of assaying a degrading enzyme in the absence of possibly essential control mechanisms. The complete details of this whole-cell technique are described in this report.

Material and Method

Reagents

Neonatal mice were obtained from the Dermatology Department (University of Michigan Medical School) BALB/c colony. Medium 199 (M-199, modified with Earle's salt, with glutamine, without sodium bicarbonate) and fetal bovine serum (FBS) were purchased from Flow Laboratories, Rockville, MD, USA. Penicillin and streptomycin (P&S), phosphate buffered saline (PBS, Dulbecco's) were from Flow Laboratories, McLean, VA, USA. Dansyl hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO, USA). L-[1-^{14}C] ornithine, L-[^{14}C(U)] ornithine, and [1-^{14}C] α-ketoglutaric acid, sodium salt were obtained from New England Nuclear (Boston, MA, USA). The respective specific activities were: 49.2 mCi/mmol, 247 mCi/mmol, and 53.6 mCi/mmol. Putrescine dihydrochloride [1,4 ^{14}C](Pu) (116 mCi/mmol), [^{14}C] spermidine trihydrochloride (spd) (120 mCi/mmol), [^{14}C] spermine tetrahydrochloride (spm) (120 mCi/mmol), and the aqueous (ACS) and organic (OCS) counting scintillation fluid were purchased from Amersham, Arlington Heights, IL, USA. Dowex 50 [H^+] form was from Bio-Rad, Richmond, CA, USA. The Lux plastic coverslips were from Flow Laboratories, Newbury Park, CA, USA, and tissue culture cluster 24 from Costar, Cambridge, MA, USA. The center wells and rubber

stoppers for the ODC assay were purchased from Kontes, Vineland, NJ, USA. Thin layer chromatography (TLC) plates were purchased from Analtech, Newark, DE, USA.

Epidermal Cell Cultures

The primary neonatal mouse keratinocyte cultures were prepared as described by Marcelo et al. [11]. Cells were plated in Costar 24 well culture clusters containing 15-mm round Lux coverslips in each well and grown in 5% CO_2 in air gassing at 32°C in Medium-199, containing 50 IU/ml of penicillin and 50 µg/ml of streptomycin plus 13% FBS. Four hours after plating, the cells were washed with cold Dulbecco's PBS to remove all nonadherent keratinocytes as described by Lichti et al. [9].

Ornithine Decarboxylase Assay

The coverslips with intact adherent cell monolayers were rinsed twice with cold Dulbecco's PBS. All subsequent procedures were carried out at 4°C unless otherwise indicated. The cells were rendered permeable to L-[^{14}C] ornithine using a permeable buffer (Tris 10 mM, sucrose 250 mM, EDTA 1 mM, $MgCl_2$ 4 mM, and 2-mercapto-ethanol 30 mM) [3]. The coverslips with the cells were placed in vials and covered with 475 µl of ice-cold 50 mM sodium potassium phosphate buffer pH 7.2, dithiothreitol (1 mM), EDTA (0.1 mM), pyridoxal PO_4 (0.4 mM), and 0.9% NaCl. Cell layers on coverslips were fixed with absolute ethanol for 15 min and used as blanks for the assay. To the vials, 25 µl ornithine (1.25 µCi), 63 µl L-[^{14}C(U)] ornithine (1.25 µCi), or 1.25 µCi [1,4 ^{14}C] Pu was added. The final concentration in the reaction mixture was 56.0 µM for L-[1-^{14}C] ornithine [27], 10 µM for L-[^{14}C(U)] ornithine, or 53 µM for ^{14}C putrescine. The vials were capped with serum stoppers containing plastic center wells filled with 0.2 ml CO_2 trapping agent (1 part 2-methoxy-ethanol, 2 parts 2-aminoethanol) and immediately placed in a 37°C shaking waterbath. After 45 or 60 min, 0.5 ml 1 M citric acid was added to the vials. These were left in the shaking bath for another 30 min. The center wells containing the trapped $^{14}CO_2$ were put in 10 ml ACS and counted in a Packard Tri-Carb Scintillation counter (Packard Instrument Co. Inc., Downers Grove, IL, USA). To the coverslips (epidermal cells) and reaction mixture in the vials was added 1 ml 10% trichloroacetic acid (TCA). After scraping the cells and centrifugation, the pellet containing cellular DNA, RNA, and proteins was resuspended in 1 N NaOH and assayed for protein by the Lowry method as previously described [11] or was resuspended in 3% perchloric acid (PCA) and hydrolyzed to determine the DNA content using the Burton method [11]. The TCA supernatant was saved for polyamine qualitative analysis.

Determination of Polyamine Biosynthesis

The TCA supernatants were applied to a 9 × 40 mm Dowex 50 [H^+] column. The column was washed with eight 5-ml fractions of sodium phosphate buffer (pH 8.0) and two 5-ml fractions of 1 N HCl. The polyamines were eluted from the resin with three 5-ml fractions of 6 N HCl. A neutralized 0.5 ml aliquot of each 6-N HCL fraction was counted to locate the labeled polyamines. The first 6-N HCl fraction, containing most of the ^{14}C-polyamines, was evaporated to dryness in a 60°C waterbath with a stream of air prior to dansylation. A number of samples were hydrolyzed for 16 h at 70°C to convert the polyamines to the nonderivitized forms prior to evaporation. The polyamine samples were dansylated using standard procedures [23]. To each sample was added 20 µl, containing 20 µg putrescine, spermidine, and spermine and 100 µl diluted dansly chloride (30 mg/ml acetone). The dansylated polyamines were extracted into 100 µl benzene by vortexing the samples. The polyamines were separated by thin-layer chromatography (TLC). Silica gel G plates were spotted with 40 µl of the benzene-extracted dansylated polyamines. The chromatograms were developed in two dimensions. The first solvent system containing cyclohexane – ethylacetate (1:1, v/v); the second solvent system consisted of chloroform – triethylamine (50:6, v/v). The areas containing dansylated polyamines were located by fluorescence under UV light. The polyamines were scraped from the plates and eluted from the silica gel by adding 3 ml benzene, triethylamine (95:5), and vortexing vigorously. The benzene and triethylamine were removed by evaporation under a stream of air and the residue was counted in 10 ml OCS to determine the ^{14}C content of each polyamine. One set of the evaporated 6-N HCl fraction, containing the ^{14}C-polyamines was sent to Dr. Abdel-Monem at the University of Minnesota for confirmation of our results. Standard procedure for dansylation and TLC of polyamines was done [2].

Determination of α-Ketoglutarate Decarboxylase Activity

α-Ketoglutarate decarboxylase activity in the pellet and supernatant fractions of sonicated cells was assayed using [1-^{14}C] α-ketoglutarate sodium salt as substrate; the final concentration was 0.4 mM containing 0.5 μCi.

Results

The Keratinocyte Cultures

In Fig. 1 is presented a phase-contrast light micrograph and a transmission electron micrograph of the primary keratinocyte cultures used in this study. Figure 1 A is a

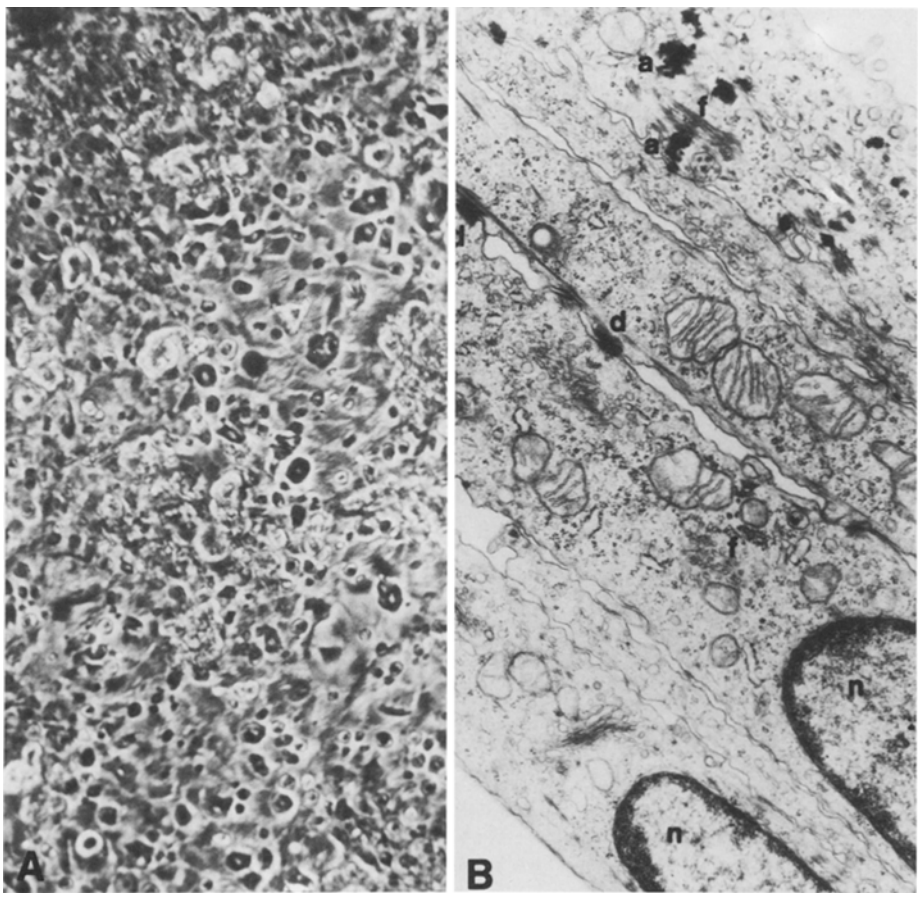

Fig. 1 A, B. Phase-contrast light micrograph and transmission electron micrograph. **A** Phase-contrast light micrograph of day-4 primary neonatal epidermal cell culture in M-199 with 13% FBS and penicillin and streptomycin. The multilayered nature of the cultures give the phase-contrast micrographs an unusual appearance. × 200. **B** Transmission electron micrograph of day-6 keratinocyte culture showing cell layers. The bottom and third cell layers have a nucleus (*n*). Desmosomal complexes (*d*) can be seen between the third and fourth cell layers and fibrils (*f*) and dense amorphous aggregates (*a*) are present in the upper cell layers. × 5,018

phase-contrast light micrograph of a culture 4 days after plating and shows the multilayered and specialized state of the cultures. The cultures, originating as a basal cell in monolayer, undergo a growth pattern in which proliferation, stratification, and differentiation occur concomitantly. The proliferation of these primary keratinocyte cultures demonstrated an oscillating semisynchronous pattern over a 3–4-week period and have neither a plateau nor a logarithmic growth-phase [11]. In an autoradiographic study, only 10%–30% of the cells proliferated over any 20-h period within the first 3 weeks of culture [11]. As the cultures proliferate and stratify, differentiated cells are shed into the medium [11]. The electron micrograph in Fig. 1B better depicts the extent of stratification and differentiation: the desmosomes, fibers, and electron-dense amorphous aggregates are characteristic of the epidermal keratinocytes of the skin.

ODC Activity of Primary Keratinocyte Cultures

Comparison of Various Preparations. One-day-old primary epidermal cell cultures were assayed for baseline ODC activity using different procedures (Table 1). In one procedure, the cells were scraped from six coverslips, homogenized in the ODC assay buffer, and centrifuged at $500 \times g$. The pellet and supernatant were then each assayed for ODC or α-ketoglutarate decarboxylase activity. In the second procedure, the cultures remained intact on the coverslips and were used in the ODC assay with, and without, exposure to the permeable buffer. The cultures were exposed to this permeable buffer to facilitate the entry of ODC inhibitors and other drugs into the cells. Our studies (data not presented) showed that there was no difference in ODC activity between the permeable-buffer-treated and nontreated cells, although the values for ODC activity in the cells exposed to the permeable buffer showed less variance.

The ODC activity of homogenized cells is much lower than the activity of the intact cells (Table 1). The posibility that ^{14}C-ornithine was being shunted into the Krebs cycle [14], thus releasing $^{14}CO_2$ from reactions other than ODC activity, was tested. We assayed the pellet from the homogenized epidermal cells to determine the degree of ^{14}C-ornithine decarboxylation activity of the membrane fractions of the cells; ODC is a cytoplasmic enzyme. The results, as presented in Table 1, show

Table 1. Comparison of ODC activity in intact and fractionated epidermal cells, and the integrity of the Krebs cycle in the pellet of fractionated cells

Substrate	Homogenates		Whole cells	
	Pellet	Supernatant	(+)Buffer	(–) Buffer
L-(1-^{14}C) ornithine	3.30	15.75	564 ± 6.9	730 ± 267.7
α-(1-^{14}C) ketoglutarate	6,643.61	225.19	$N=4$	$N=2$

Day-1 cultures on coverslips were assayed for ODC activity with (+) and without (–) exposing the intact cells to permeable buffer
ODC activity = mean ± SEM in pmol $^{14}CO_2$/mg protein/45 min
α-Ketoglutarate decarboxylase activity is in pmol $^{14}CO_2$/mg protein/45 min

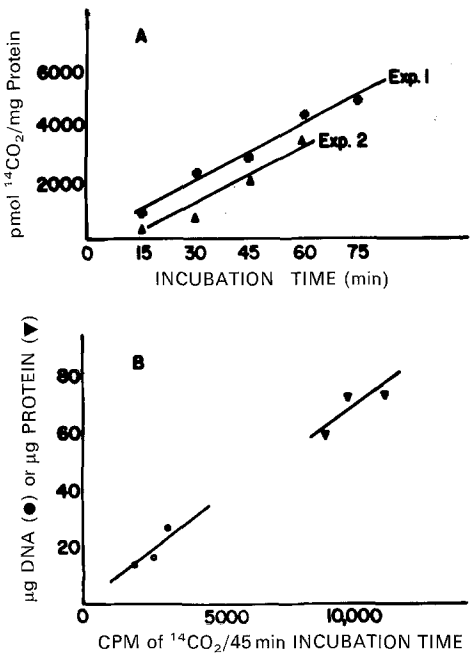

Fig. 2A, B. Linearity of ODC activity. **A** With time, each assay was done in duplicate. **B** With the amount of protein ▼, with the amount of DNA ●. 1-^{14}C-ornithine or ^{14}C(U) ornithine was used as substrate

negligable decarboxylation of ^{14}C-ornithine although, significantly, decarboxylation of the Krebs cycle substrate α-[1-^{14}C] glutarate occurred, indicating a viable cell pellet.

In the intact cell assay, if 1,4 ^{14}C putrescine was used as substrate instead of labeled ornithine no release of $^{14}CO_2$ occurred. This indicated that diamine oxidase activity and subsequent decarboxylation reactions exhibit minimal activity in these assay conditions.

Linearity of the ODC Activity. The ODC activity of 1-day-old cultures was determined. The assay was stopped 15, 30, 45, 60, and 75 min after initiation. As seen in Fig. 2, the ODC activity of intact living cells is linear with time for at least 60 min and is linear with respect to protein or DNA concentrations.

Inhibition of ODC Activity of Whole Epidermal Cells

The specificity of the ODC assay of living whole cells was tested by addition of three specific inhibitors of ODC activity. α-Methyl ornithine (αMe-O) and α-hydrazino ornithine (αHO) are known potent competitive inhibitors of ODC activity [1, 6], whereas LD-α-difluoromethyl ornithine (αDFMO) is an irreversible inhibitor of ODC [10]. The data presented in Table 2 indicate that all three inhibitors at 0.5 – 5 mM concentrations decreased the ODC activity from 40% to 80%. At the higher concentrations, these three substances may also inhibit a variety of other decarboxylases [6].

Table 2. Inhibition of ODC activity

Control	α-Methyl-ornithine ($1-5\times10^{-3}$ M)	α-Hydrazino ornithine ($0.5-5\times10^{-3}$ M)	α-Difluoro methyl ornithine ($1-5\times10^{-3}$ M)
100%	55.45% ± 8.4% $N=7$	16.70% ± 5.69% $N=6$	59% ± 17.55% $N=2$

Values are in percent control
Four hours after plating the cultures were rinsed with cold Dulbecco's PBS
The inhibitors were added to the medium 14 h prior to ODC assay; the same concentrations were added to the permeable buffer and the ODC assay buffer
ODC activity was measured on day 1 of culture

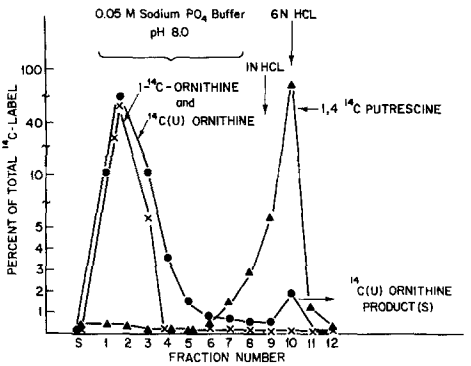

Fig. 3. Elution of Dowex 50 (H^+) column of the reaction substrate and products from cells assayed with 1-^{14}C-ornithine, ^{14}C(U) ornithine, and 1,4 ^{14}C putrescine. Labeled ornithine is eluted in the sodium phosphate buffer, while labeled putrescine, polyamines, and polyamine-like compounds are eluted in the 6-N HCl fraction

Evaluation of Products of ODC Activity in Whole-Cell Cultures

Column Chromatography. When 1-^{14}C-ornithine is used as a substrate for ODC, the products are $^{14}CO_2$ and unlabeled putrescine and other polyamines. Use of ^{14}C(U) ornithine as a substrate yields $^{14}CO_2$- and ^{14}C(U)-labeled putrescine and other labeled polyamines. The ^{14}C content of the fractions obtained by Dowex 50 (H^+) column chromatography of the incubation mixture [1-^{14}C-ornithine, ^{14}C-(U)-ornithine, or 1,4 ^{14}C putrescine as substrate] following termination of the whole-cell assay with citric acid is presented in Fig. 3. Hydrolysis of the samples prior to chromatography did not change the results.

With 1-^{14}C-ornithine as substrate, 0.18% of the total counts were released as $^{14}CO_2$. Chromatography of the incubation mixture showed that 99% of the added counts were eluted in the sodium phosphate buffer fractions which contain the unreacted 1-^{14}C-ornithine. The polyamines formed with 1-^{14}C-ornithine as the substrate were unlabeled, and, therefore, there were few or no counts present in the fractions eluted with 6 N HCl.

When ^{14}C(U) ornithine was used as a substrate, ^{14}C labeled products were found in the 6 N HCl and 88% of the total counts were eluted by the phosphate buffer (unreacted ^{14}C-ornithine). When 1,4 ^{14}C putrescine was used as substrate, most of the radioactivity was eluted with 6 N HCl. These results indicate that intact

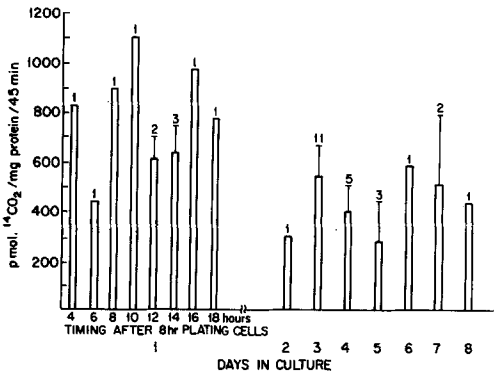

Fig. 4. Baseline ODC activity of primary neonatal mouse epidermal cells in culture from day 1 to day 8 of culture is from 500 to 1,000 pmol/mg protein/45 min. Differences in baseline ODC activity between day-1 and day-2–8 cells in culture probably reflected slight variations in dissociation procedures (i.e., slightly older mice, batch differences in trypsin activity or in fetal bovine serum, etc.). In one experiment, there was no more than 10% variation between triplicate or quadruplicate control and treated cells

living cells convert ornithine to polyamines or polyamine-like compounds as ascertained by fractionation of the incubated materials by Dowex 50 column chromatography.

Thin Layer Chromatography. Doctor Abdel-Monem analyzed the contents of the 6-N HCl fractions of the chromatographed samples for the presence of polyamines after dansylation of the fraction. It was found that 60% of the radioactivity in this fraction was extractable into toluene as dansylated polyamines. The other 40% of the counts remained in the aqueous fraction and the identity of these polyamine-like compounds is uncertain. The extractable radioactive compounds, when separated on silica gel G or alumina TLC plates, had the same mobility as dansyl putrescine. No radioactivity was detected in the areas corresponding to spermidine, spermine, N^1-acetyl spermidine, or N^8-acetyl spermidine.

Thus, the whole epidermal cells synthesize polyamines and polyamine-like compounds that are eluted from Dowex 50 columns with 6 N HCl. One such polyamine is probably putrescine, although other as yet unidentified compounds with properties similar to polyamines are also synthesized. The ratio of $^{14}CO_2$ to ^{14}C in the polyamine fraction was equal to or less than 1.0 (data not presented).

Constancy of the ODC Activity in Whole Keratinocyte Cultures

The ODC activity of primary epidermal cell cultures was determined over a daytime period after plating in 35 separate experiments. On the first day after plating, the activity was slightly higher (about a 50% increase) than on subsequent days. The data given in Fig. 4 show that the baseline activity of ODC ranged from 500 to 1,000 pmol/mg protein/45 min over the first 8 days after plating.

Discussion

Several years ago we began a study of the interaction of ornithine decarboxylase activity and polyamines with epidermal cell growth [20]. As our work progressed, we found that to obtain enough homogenate supernatant with which to assay ODC

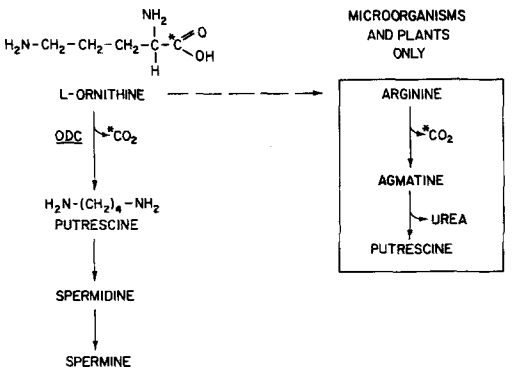

Fig. 5. Metabolic pathways of ornithine. The conversion of arginine into agmatine and CO_2 occurs only in microorganisms and plants

activity, we required $15-25 \times 10^6$ cells per time point, i.e., three to five 60-mm petri dishes.

Even then, the values that we obtained for ODC activity were very low, often times giving raw cpm numbers equivalent to the blank values (100 – 150 cpm). We overcame the time, cell number, and low-assay-sensitivity restraints and circumvented other problems due to the short half-life of this enzyme [8, 21] – the potentially variable and complex feedback control of ODC [7] and the existence of ODC in various growth-related states – by developing the whole living cell assay for ODC activity [20], which we are now completely describing in this report. Recently, similar results using the whole-cell assay of cell lines in culture have been reported [16].

The ODC activity of the intact cell cultures, as measured by the amount of $^{14}CO_2$ from L-[^{14}C] ornithine, ranged between 3,500 to 6,000 raw cpm, the exact count depending on the number of cells on the coverslip and the age of the culture (Figs. 2, 4). These baseline values were obtained using $0.5-2.0 \times 10^6$ epidermal cells per coverslip. Correction for background, i.e., approximately 350 cpm, did not significantly effect the final value for ODC activity since assay background was only 5 % – 10 % of the control raw cpm. This is not the case when the homogenate is used as the source of ODC. Often the background was equal to, or only slightly less than the control cpm, i.e., the blank value was 60 % – 100 % of the control value; when the ODC activity of the cultures was stimulated [20] the background blank (100 cpm) was often 20 % – 40 % of the stimulated ODC activity number (250 – 500 cpm). It was very difficult for us to interpret this data since we often had no, or a very low, control ODC value with which to evaluate our stimulated ODC samples. Using the intact-cell assay, stimulated values were often 10,000 – 15,000 cpm [20]; comparing control and experimental values with this ODC assay technique gave us more reliable information.

Since in the intact-cell assay the baseline (unstimulated) ODC activity was a constant and easily assayed value, it was possible to determine the effect of known inhibitors of ODC activity using the intact-cell assay technique. Three inhibitors of ODC activity in other cells were used. These were α-methyl-ornithine, α-hydrazino

ornithine, and α-difluoromethyl ornithine [1, 6, 10]. All three inhibitors decreased the ODC activity of intact, living epidermal cells between 40%–85% (Table 2). Actual assay of the polyamine levels of cultures treated with these metabolites for 6–7 days indicated that the synthesis of putrescine was decreased 40%–50% in these cells by these inhibitors (Roseeuw, unpublished data).

The intact-cell assay gave greater values for ODC activity than those seen with the usual supernatant ODC assay (Table 1). This is not surprising when the unique labile nature of the ODC enzyme is considered. However, we used a number of criteria besides the decreased $^{14}CO_2$ release by classic ODC inhibitors (Table 2) to verify that we were indeed measuring only authentic ODC activity. We found that ODC activity was located primarily in the cell cytoplasm. When both the pellet and supernatant from cell homogenates were assayed for ODC activity, the decarboxylase activity was located primarily in the supernatant fraction (Table 1), although the pellet membranes were viable as shown by the presence of extensive Krebs cycle activity (Table 1). Thus, using whole cells containing intact cytoplasmic membrane structures does not contribute nonspecific decarboxylase activity to the assay reading. We used Dowex columns to identify the ornithine and ornithine metabolites in the assay mixture. The results showed that polyamines were being synthesized by the cells using the labeled ornithine as substrate (Fig. 3). Equally important is that there was an approximate 1:1 ratio between the $^{14}CO_2$ released and the ^{14}C polyamines present in the assay buffer, indicating that no decarboxylation of uniformally labeled ^{14}C ornithine was occurring through alternate pathways, i.e. the Krebs cycle. One other possible alternate pathway of ornithine decarboxylation was through the formation of agmatine (Fig. 5). In the epidermal cell, this would not occur since this pathway is present only in plants and microorganisms [18].

The results of our tests of this procedure, the decreased amount of $^{14}CO_2$ released from labeled ornithine caused by three ODC inhibitors and decreased putrescine synthesis in cells treated with these three inhibitors for 5–7 days (Roseeuw, manuscript submitted for publication), are strong evidence that we were measuring authentic ODC activity. This conclusion is the same as that reported for a whole-cell assay of several cell lines in which the assay under anaerobic conditions was also used to verify this type of assay system.

The intact-cell ODC assay holds promise for future polyamine studies since this technique preserved and measured ODC activity in whole cells, thereby allowing all the fine tuning of the polyamine metabolite pathway to be expressed as the assay occurred. This data showed that the effect of changes in epidermal cell growth, and its relation to ODC activity and polyamine synthesis, can be better studied using this technique. In addition, the whole-cell assay technique will allow future study of the multiple ornithine metabolites and conjugates formed as a result of ODC activity in a totally manipulable in vitro system.

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