Inhibition of diphosphatidylglycerol synthesis by u.v. A radiations in N.C.T.C. 2544 human keratinocytes

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The effects of u.v. A radiations on phospholipid synthesis were studied in the N.C.T.C. 2544 human keratinocyte cell line, by using [^{14}C]arachidonic acid, [^{14}C]oleic acid or sodium [^{32}P]-orthophosphate as precursors. Cells were irradiated in Hanks' medium with 365 nm light at doses up to 19 J/cm^2, and then phospholipid synthesis from the three precursors was studied. Under these conditions, only small alterations in the incorporation pattern of [^{14}C]arachidonic into phospholipids [phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI)] were observed, for u.v. A irradiation doses up to 19 J/cm^2. In contrast, with [^{14}C]oleic acid as precursor, two additional spots were observed, which co-migrate with pure phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG) standards. The incorporation of [^{14}C]oleic acid into PG and DPG was decreased in a dose-dependent manner after u.v. A exposure, with about 50% and 75% decreases at 9.5 J/cm^2 and 19 J/cm^2 respectively. As for arachidonic acid incorporation, no significant differences in the synthesis of the major phospholipids (PC, PE, PI) were noted upon u.v. A exposure. The dramatic and selective decrease in PG and DPG syntheses was confirmed with [^{32}P]orthophosphate as precursor. As DPG is a specific component of the mitochondrial inner membrane, it appears that one of the early kinds of damage induced by u.v. A irradiation could be the impairment of mitochondrial functions.

INTRODUCTION

U.v. radiations have been shown to be involved in degenerative processes of the skin, such as photoaging and photocarcinogenesis [1–3]. These pathological consequences of solar exposure have been mainly related to the interaction of short-wavelength radiations (u.v. B) with the skin, resulting in direct alterations of nucleic acid structure such as dimerization of pyrimidine bases [4,5] and breaking of DNA strands [6]. Thus nucleic acids, which are chromophores of short-wavelength radiations, have long been considered as the main targets involved in the harmful effects of solar exposure. However, in recent years, an increasing amount of work has dealt with the effects of long-wavelength u.v. A radiations on photoaging and photocarcinogenesis [7–10]. The u.v. A can induce damage to all kinds of cellular components (nucleic acids, proteins and lipids) via photosensitization by cellular chromophores, mainly flavins, NADH/NADPH and porphyrins [9]. As a consequence, lipid peroxidation can occur [11,12], leading to alterations in membrane structure and functions. Indeed, activation of membrane phospholipase activities [13] and modification of protein kinase C activity [14] have been reported in cells exposed to u.v. A. Secondary reactions of lipid peroxidation can also alter protein and nucleic acid structure and biological activities by formation of covalent adducts [15–17]. It can therefore be supposed that the potential alteration of a great variety of cellular targets by u.v. A radiations might play an important role in inducing or potentiating photodegenerative skin processes.

To date, there are only a few studies on the action of u.v. A radiations on cellular lipid metabolism, and especially on the dynamic aspects of phospholipid metabolism. Among recent results, it has been demonstrated that phospholipases are activated by u.v. A and u.v. B radiations in human fibroblasts and keratinocytes [13,18]. Phospholipids are well known to undergo a rapid deacylation–reacylation process involving phospholipases and acyltransferases [19]. This rapid cycle is known to play an important role in the regulation of membrane-bound enzyme activities and in signal transduction [20]. We thus investigated the effects of u.v. A radiations on phospholipid metabolism in the human keratinocyte cell line N.C.T.C. 2544, and found that u.v. A induces a marked and selective inhibition of the synthesis of phosphatidylglycerol (PG) and diphosphatidylglycerol (DPG), phospholipids which are in mammalian cells specifically located in the mitochondrial membrane. Such an alteration cannot be detected by incorporation of arachidonic acid, but is readily observed by using either oleic acid or phosphate as precursors.

MATERIALS AND METHODS

Materials

The N.C.T.C. 2544 human keratinocyte cell line [21] was purchased from Flow (Paris, France). Dulbecco's modified Minimum Essential Medium (DMEM) with Earle's salts, Hanks' salts solution and fetal-calf serum were from Gibco (Grand Island, NY, U.S.A.). [^{114}C]Oleic acid (55 mCi/mmol) and [^{14}C]arachidonic acid (53 mCi/mmol) were from Amersham International (Amersham, Bucks., U.K.). Sodium[^{32}P]orthophosphate (200 mCi/mmol) was from CEA (Saclay, France). Silica-gel plates were purchased from Schleicher and Schüll (Dassel, Germany). L-[U-^{14}C]-*sn*-Glycerol phosphate (153 mCi/mmol) was obtained from NEN Products, Du Pont de Nemours (Les Ulis, France). Lipid standards for chromatography, CDP-

Abbreviations used: CDP-DG, CDP-diacylglycerol; DPG, diphosphatidylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; TBARS, thiobarbituric acid-reacting substances.

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sn-1,2-diacylglycerol (CDP-DG), L-α-glycerol phosphate and all other unlabelled chemicals were purchased from Sigma (St. Louis, MO, U.S.A.) and were of the purest available grade. Solvents for chromatography were provided by Merck (Darmstadt, Germany).

Cell culture

Cells were seeded at a density of $1.5 \times 10^4/cm^2$, and cultured in 35 mm Nunc Petri dishes in DMEM supplemented with 10% fetal-calf serum and 10 mM Hepes buffer. Cultures were maintained at 37 °C in a 5%-CO_2 humidified atmosphere. All experiments were carried out on sub-confluent cells.

Studies on lipid metabolism

(a) Experimental conditions for cell irradiation

Before u.v. A irradiation, cells were washed three times with Hanks' salt solution. Irradiation at 365 nm was performed in 1 ml of Hanks' salts devoid of any additive, by using a Vilber Lourmat (Torcy, France) table equipped with TF-20L tubes and appropriate filters. A glass window (4 mm thickness) was placed 20 mm above the lamp to absorb the remaining short-wavelength u.v. light of the commercial equipment (transmittance < 0.01% at 320 nm). Samples (plastic Petri dishes) were placed on the glass window and irradiated from the bottom. An average light intensity of 3.0 ± 0.2 mW/cm² was measured with the Vilber Lourmat UVR 365 photometer. After irradiation, cells were left in the dark for 1 h at 37 °C. Sham-irradiated cells were left under similar conditions but without u.v. A exposure.

(b) Fatty acid incorporation into lipids

After irradiation with 365 nm light, followed by a 1 h dark period at 37 °C, incorporation of [^{14}C]oleic acid or [^{14}C]arachidonic acid was measured as described by Mazière et al. [22]. Briefly, after evaporation to dryness under nitrogen, ^{14}C-labelled fatty acids were resuspended in a fatty-acid-free serum albumin solution (0.2 mg/ml), and introduced into the incubation medium at a concentration corresponding to 1 μCi/ml. After another 1 h incubation at 37 °C, cells were washed four times with a pH 7.4 phosphate-buffered solution, harvested with a rubber policeman, and centrifuged for 5 min at 400 *g*. Lipid separation and analysis were carried out by t.l.c. as described by Mazière et al. [23], with chloroform/methanol/acetic acid/water (50:30:8:4, by vol.) as solvent system [24,25]. After autoradiography, the spots corresponding to the studied lipids identified by comparison with pure lipid standards were cut out and counted for radioactivity by liquid scintillation with a Beckman 6000 SC instrument. Results, calculated as pmol of precursor incorporated per mg of cell protein, are expressed as percentages of the controls (unirradiated cells, zero time).

If the solvent system described above was currently used for the measurement of fatty acid incorporation into cellular lipids, the formal identification of the labelled lipids, especially PG and DPG, has been done by comparison of their migration with that of pure standards [phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylethanolamine (PE), phosphatidic acid (PA), PG, DPG, triacylglycerol] in four different chromatographic solvent systems: a one-dimensional [chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) (see above)] and three two-dimensional solvent systems: (a) the classic solvent system described by Gray [26], using chloroform/methanol/water (65:25:4, by vol.) for the first run, and a basic solvent system (tetrahydrofuran/methylal/aq. 2 M ammonia) for the second run; (b) a basic mixture (chloroform/methanol/28% ammonia, 13:7:1, by vol.). for the first run, followed by an acidic phase (chloroform/methanol/acetone/acetic acid/water, 50:10:20:13:5, by vol.) for the second direction, as described by Nakagawa and Waku [27]; (c) a third two-dimensional solvent system, which consists of a combination of the routinely used one-dimensional solvent system chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) for the first dimension, and of the basic mixture described by Nakagawa and Waku [27] (chloroform/methanol/28% ammonia, 13:7:1, by vol.) for the second run. In all cases, the two lipid spots labelled by oleic acid but not by arachidonic acid (see below, in the Results section) co-migrated with pure PG and DPG. The use of basic solvent systems especially allows clear separation of PG and DPG from PA.

(c) Incorporation of P_i into phospholipids

The phospholipid polar head-group synthesis was studied as described by Mazière et al. [28] by using 25 μCi/ml sodium [^{32}P]-orthophosphate. The experimental conditions were the same as those described above for oleic acid incorporation, but cells were incubated with the labelled precursor for 4 h. Phospholipid separation was performed by t.l.c. as described above. Results are expressed as percentage of controls (unirradiated cells, zero time).

Assay of CDP-diacylglycerol–glycerophosphate phosphatidyltransferase and phosphatidylglycerophosphate phosphohydrolase activities

The PG synthesis from CDP-DG and glycerol 3-phosphate is achieved by the CDP-DG/glycerophosphate phosphatidyltransferase (EC 2.7.8.5), leading to phosphatidylglycerophosphate, which is subsequently cleaved into PG and P_i by a phosphatidylglycerophosphate phosphohydrolase (EC 3.1.3.27). The combination of these two enzyme activities can be measured with [U-^{14}C]-*sn*-glycerol 3-phosphate and unlabelled CDP-DG as substrates [29]. After irradiation at a u.v. A dose of 19 J/cm², followed by a 1 h incubation in the dark at 37 °C, the cells were harvested with a rubber policemen. The cell suspension was centrifuged for 5 min at 400 *g*, and then the pellet was resuspended in 0.05 M imidazole buffer (pH 7.5)/0.1 M NaF/0.001 M EDTA/0.25 M NaCl/0.005 M dithiothreitol, and sonicated for 5 s at 4 °C. The cell homogenate was incubated for 1.5 h at 30 °C in a final volume of 0.2 ml containing 0.25 M Tris/HCl, pH 7.3, 5 mM $MnCl_2$, 0.075% Triton X-100, 0.1 mM CDP-DG, and 0.2 μCi of [U-^{14}C]-*sn*-glycerophosphate diluted with unlabelled glycerol phosphate to give a final concentration of 4 mM. The reaction was stopped by addition of 4 ml of chloroform/methanol (1:1, v/v). After washing with 2×5 ml of 0.1 M KCl and then with 2.5 ml of water, the radioactivity of the chloroform fraction was measured by liquid-scintillation counting. Results are expressed as nmol/h per mg of cell protein.

Determination of TBARS (thiobarbituric acid-reacting substances)

Lipid-peroxidation products (TBARS) were measured at the end of the 1 h dark period after cell irradiation. Measurements were performed on a sample of the irradiated medium, as we previously found that most (over 80%) of the TBARS measured after u.v. A exposure are secreted by cells [11]. TBARS were determined by the fluorimetric method described by Yagi [30]. Results, calculated as malondialdehyde equivalents produced/mg of cell protein, were expressed as percentages of controls (unirradiated cells, at the beginning of the experiments).

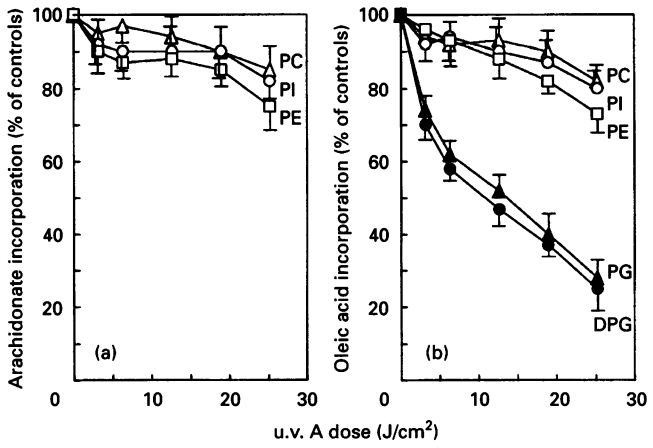

Figure 1 Effect of u.v. A exposure on [^{14}C]arachidonic acid (a) and [^{14}C]oleic acid (b) incorporation into phospholipids as a function of the irradiation dose

Cells were irradiated at 365 nm with up to 25 J/cm^2 in Hanks' salts, then left for 1 h in the dark at 37 °C. Incorporation of labelled fatty acids (1 μCi/ml) was then carried out during 4 h at 37 °C. Cells were washed three times, harvested with a rubber policeman, and lipid analysis was performed by t.l.c. as described in the Materials and methods section. Results are expressed as percentage of controls (sham-irradiated cells), as means ± S.D. of six experimental values. For arachidonic acid incorporation (pmol/mg of cell protein), 100% = 680 ± 84 (PC), 1120 ± 152 (PE), 1453 ± 126 (PI). (**b**) For oleic acid incorporation (pmol/mg of cell protein), 100% = 3370 ± 455 (PC), 1982 ± 254 (PE), 3208 ± 374 (PI), 571 ± 75 (PG), 778 ± 96 (DPG). Symbols: △, PC; ○, PI; □, PE; ▲, PG; ●, DPG.

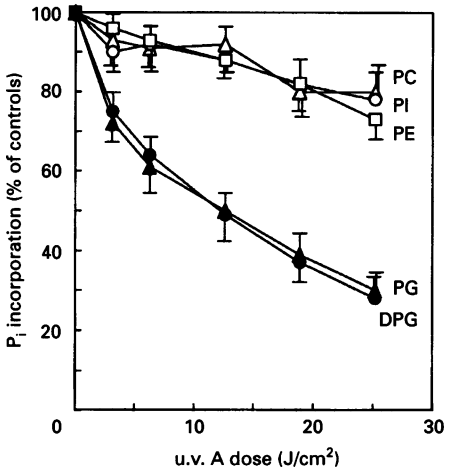

Figure 2 Effect of u.v. A exposure on [^{32}P]P$_i$ incorporation into phospholipids as a function of the irradiation dose

Cells were irradiated at 365 nm with up to 25.2 J/cm^2 in Hanks' salts, then left for 1 h in the dark at 37 °C. Incorporation of the precursor (25 μCi/ml) was carried out for 4 h at 37 °C. Cells were washed three times, harvested with a rubber policeman, and lipid analysis was performed by t.l.c. as described in the Materials and methods section. Results are expressed as percentage of controls (sham-irradiated cells), as means ± S.D. of six experimental values. The 100% values (d.p.m./mg of cell protein) were 11158 ± 1652 (PC), 4590 ± 528 (PE), 3244 ± 595 (PI), 1182 ± 168 (PG) and 742 ± 125 (DPG). Symbols: △, PC; ○, PI; □, PE; ▲, PG; ●, DPG.

Protein was determined by the Lowry method, modified as described by Peterson [31].

All experiments were performed at least in quadruplicate. Statistical analysis was performed by Student's *t* test.

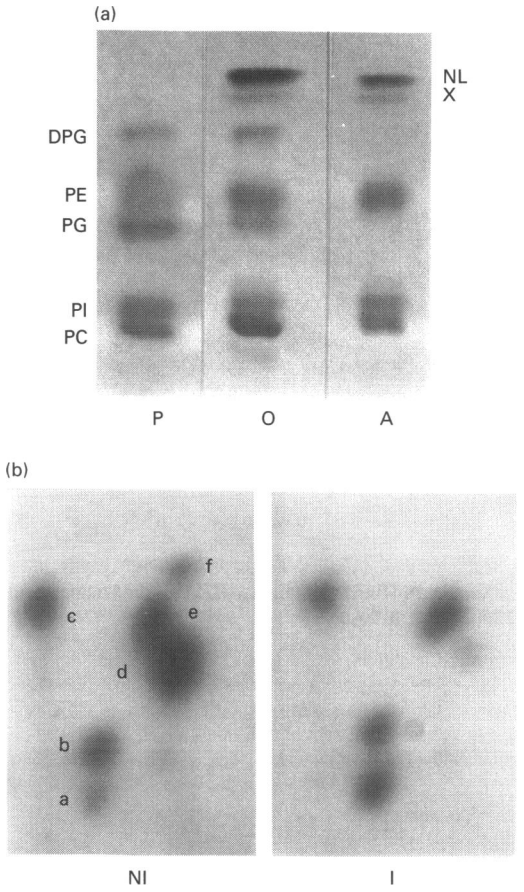

Figure 3 Autoradiograms after t.l.c. displaying typical patterns of the incorporation of labelled precursors into phospholipids in N.C.T.C. 2544 human keratinocytes

Experimental conditions for precursor incorporation were the same as described in the legends to Figures 1 and 2, except for (**b**), which displays results obtained with short-time (30 min) incorporation of [^{32}P]P$_i$ into cellular lipids. (**a**) One-dimensional t.l.c. in chloroform/methanol/acetic acid/water (25:15:4:2, by vol.) as solvent system: lane P, [^{32}P]P$_i$; lane O, [^{14}C]oleic acid; lane A, [^{14}C]arachidonic acid as precursors respectively. Abbreviations: X, unidentified; NL, neutral lipids. (**b**) Two-dimensional separation of phospholipids after incorporation of [^{32}P]P$_i$ by sham-irradiated cells (NI) or by cells irradiated with a u.v. A dose of 13 J/cm^2 (I). The solvent mixtures used were those described in the Materials and methods section (two-dimensional solvent system c). Spots: a, PC; b, PI; c, PA; d, PG; e, PE; f, DPG.

RESULTS

Results in Figure 1(a) show that no significant decrease in [^{14}C]arachidonic acid incorporation into phospholipids was observed upon u.v. A irradiation, up to doses of 19 J/cm^2. At 25 J/cm^2, a slight decrease (20–30%) in the precursor incorporation was noted, mainly into PE. In these experiments, the main phospholipids identified in our solvent system were PC, PI and PE. By contrast, with [^{14}C]oleic acid as precursor, two additional spots co-migrating in various solvent systems (see the Materials and methods section) with pure standards of PG and DPG appeared. Figure 1(b) shows that the oleic acid incorporation into these two lipids was strongly decreased by u.v. A, in a dose-dependent manner: decreases of about 50 and 75% were observed after irradiation with doses of 12.5 and 25 J/cm^2 respectively, whereas the precursor incorporation into PC, PE and PI was only slightly decreased.

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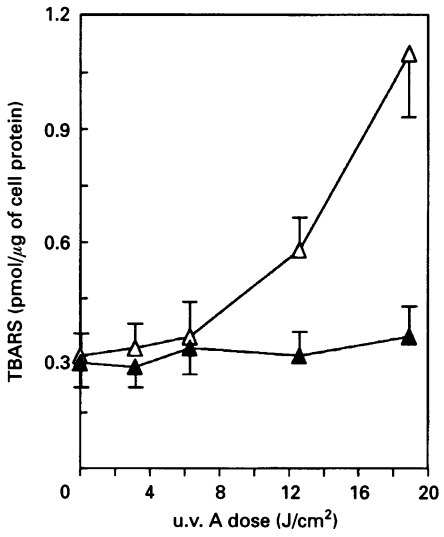

Figure 4 Kinetics of TBARS formation in N.C.T.C. 2544 human keratinocytes as a function of u.v. A dose

Cells were irradiated at 365 nm, left for 1 h at 37 °C in the dark, and then the TBARS level was measured in the incubation medium by the fluorimetric method described by Yagi [30] (see the Materials and methods section). Key: △, irradiated cells; ▲, sham-irradiated controls. Results are means ± S.D. of four experimental values.

Table 1 Influence of the antioxidants vitamin E and vitamin C on the effects of u.v. A radiations on TBARS formation and oleic acid incorporation into DPG in N.C.T.C. 2544 human keratinocytes

Cells were pre-cultured for 24 h in the absence or in the presence of vitamin E (50 μM) and vitamin C (1 mM), then washed three times and irradiated with u.v. A at a dose of 19 J/cm². The TBARS generation was measured as specified above. The oleic acid incorporation into DPG was assessed as described in the Materials and methods section after incubation of cells for 4 h with the ^{14}C-labelled fatty acid (1 μCi/ml). Results are means ± S.D. of four experimental values.

	TBARS formation (nmol/mg of cell protein)	[^{14}C]Oleic acid incorporated into DPG (pmol/mg of cell protein)
Without antioxidants		
Sham-irradiated	0.28 ± 0.05	745 ± 82
Irradiated	0.93 ± 0.17	179 ± 33
With antioxidants		
Sham-irradiated	0.22 ± 0.05	780 ± 98
Irradiated	0.34 ± 0.10	218 ± 36

N.C.T.C. 2544 cells has also been studied by incorporation of [^{32}P]P_i. Figure 2 shows that u.v. A exposure resulted in a marked and dose-dependent decrease in the precursor incorporation into PG and DPG, with about 50 and 70 % decreases by doses of 12.5 and 25 J/cm² respectively. These values are very similar to those observed with oleic acid as precursor. PC, PI or PE syntheses were only slightly affected.

A typical pattern of the incorporation of the three precursors into N.C.T.C. 2544 cell phospholipids is displayed in Figure 3(a), which clearly illustrates the absence of PG and DPG labelling when [^{14}C]arachidonic acid was used as a precursor (lane A),

Table 2 Effect of u.v. A radiation (19 J/cm²) on PG synthesis, measured on cell homogenates

After irradiation at a u.v. A dose of 19 J/cm², followed by a 1 h incubation in the dark at 37 °C, the cells were harvested with a rubber policeman. The cell suspension was centrifuged for 5 min at 400 *g*, then the pellet was resuspended in 0.05 M imidazole buffer, pH 7.5, containing 0.1 M NaF, 0.001 M EDTA, 0.25 M NaCl and 0.005 M dithiothreitol, and sonicated for 5 s at 4 °C. The PG synthesis was measured in the cell homogenate with 0.1 mM CDP-DG and 0.2 μCi of [U-^{14}C]-*sn*-glycerophosphate as substrates, as described in the Materials and methods section. Results are expressed as nmol/h per mg of cell protein, and are means ± S.D. of six experimental values.

Cells	PG synthesis *in vitro* (nmol/h per mg of cell protein)
Sham-irradiated	88.9 ± 10.9
u.v. A-treated	74.7 ± 12.7

whereas synthesis of these two phospholipids was observed when cells were incubated with [^{14}C]oleic acid (lane O) or [^{32}P]P_i (lane P). Figures 3(a) (one-dimensional t.l.c.) and 3(b) (two-dimensional t.l.c.) show the marked decrease in [^{32}P]P_i incorporation into PG and DPG after a u.v. A dose of 13 J/cm² as compared with sham-irradiated controls. It must be noted that in Figure 3(b) the precursor incorporation had been performed for a short time (30 min); as shown there, under these conditions PG was labelled much more than DPG. It may also be observed in Figure 3(b) that u.v. A radiation did not significantly affect [^{32}P]P_i incorporation into the other phospholipid classes.

Since u.v. A exposure can result in cellular lipid peroxidation [11,12], we also studied the kinetics of TBARS formation in irradiated N.C.T.C. 2544 cells. Figure 4 shows that no significant appearance of TBARS was noted with irradiation doses up to 6 J/cm², as compared with controls (sham-irradiated cells). With increasing u.v. A doses, dose-dependent TBARS formation was observed. Moreover, whereas a 24 h preculture with 50 μM vitamin E + 1 mM vitamin C decreased by about 70 % the amount of TBARS formed in cells exposed to 19 J/cm² u.v. A, no significant protective effect against the u.v. A-induced decrease in oleic acid incorporation into PG or DPG was observed (Table 1).

In order to specify the mechanism(s) by which PG (and DPG) synthesis might be affected by u.v. A radiations, we also investigated the activities of CDP-DG/glycerophosphate phosphatidyltransferase and phosphatidylglycerophosphate phosphohydrolase on cell homogenates. Results in Table 2 show that a u.v. A dose of 19 J/cm² did not significantly affect PG synthesis *in vitro*.

DISCUSSION

Among the few studies on the effects of long-wavelength radiations on lipid metabolism in cultured cells, most of them have been concerned with the activation of membrane phospholipases. Hanson and DeLeo [13] described the activation of phospholipases A_2 and C by u.v. A in human keratinocytes. Punnonen et al. [12] also reported arachidonic acid release in N.C.T.C. 2544 cells exposed to u.v. A radiations, and suggested that the fatty acid originated mainly from PE cleavage. They also showed that u.v. A radiations induce a decrease in the percentage of labelled arachidonic acid found in PE and a significant increase in the precursor incorporation into neutral lipids [12]. However, these experiments have been carried out on cells prelabelled with arachidonic acid for a long period (24 h) and then submitted to

u.v. A irradiation [12]. These results were thus mainly representative of the effects of light on the 'static' distribution of the precursor between cell lipids at a given time, and did not provide information about the biosynthetic pathways. In the present work, the consequences of the u.v. A irradiation on phospholipid syntheses have been studied by short-time incubation of cells with the precursors after exposure to u.v. A radiations. Under these experimental conditions, we show that, although the syntheses of the main phospholipids (PC, PI and PE) are only slightly affected, dramatic and dose-dependent decreases in PG and DPG synthesis from oleic acid or P_i are observed. This effect of u.v. A cannot be detected with arachidonic acid as precursor, probably because PG and DPG only contain a small percentage of this fatty acid, oleic and linoleic acids being the main abundant fatty acids in these phospholipids [32].

DPG is an acidic phospholipid which, in mammalian cells, is mainly localized in the mitochondrial inner membranes [33]. Its involvement in the respiratory-chain function has been suggested by numerous works. For example, a de-differentiation of mitochondria occurs in hepatoma cells, in which the content of DPG is decreased about 2-fold as compared with normal liver cells [34]. A decrease in the DPG content accompanies the repression of the yeast respiratory chain under anaerobiosis [35]. It has also been demonstrated that DPG is the most effective phospholipid in restoring the electron-transport activity in lipid-depleted mitochondria [36]. Since DPG binds firmly to cytochrome *c*, it has been suggested that this phospholipid may be involved in the anchorage of cytochrome *c* to the mitochondrial inner membrane [37]. A role of DPG in maintaining mitochondrial NADH oxidase and ATPase activities has also been evoked [38,39].

Indeed, the important role of DPG in the integrity of the mitochondrial respiratory chain is well recognized. As a consequence, the marked decrease in DPG synthesis induced by u.v. A in human keratinocytes could result in alterations of the energy metabolism, leading, for example, to a decrease in the intracellular pool of ATP. This hypothesis is now under investigation. It can also be conceived that u.v. A radiations induce modifications of the mitochondrial membrane structure, as evidenced by preliminary microspectrofluorometric studies using Rhodamine 123 as a mitochondrial marker (results not shown). It is of note that the u.v. A doses producing about 50% decrease in oleic acid or P_i incorporation into DPG are rather low (about 10 J/cm^2). Such doses do not significantly alter cell viability, as assessed by the Neutral Red assay or by the Trypan Blue exclusion test. However, preliminary results from our laboratory indicate that the respiratory-chain activity was inhibited by about 30% after a 10 J/cm^2 u.v. A exposure, as assessed by O_2 consumption of the N.C.T.C. 2544 keratinocytes (results not shown).

The decrease in oleic acid and P_i incorporation into PG is consistent with that observed into DPG. Indeed, PG is a direct precursor of DPG, and is converted into the latter via an enzymic pathway utilizing CDP-diglyceride [40]. PG is present in much smaller amount in the mitochondrial membranes, and seems not to play a specific role in the mitochondrial machinery, except for being the direct precursor of DPG.

The mechanisms by which PG and DPG biosyntheses can be inhibited by u.v. A exposure remain unclear. The activities of the acyltransferases involved in the incorporation of the fatty acid into phospholipids were not significantly affected when measured *in vitro*, by using the pre-activated substrate oleoyl-CoA (results not shown). It is thus likely that acyltransferases are not direct targets of u.v. A radiations under our experimental conditions (low irradiation doses and absence of any exogenous photosensitizer). This suggests that u.v. A irradiation more probably results in functional alterations of some enzymic pathways controlling PG and DPG synthesis. It has been shown that the activity of key enzymes of phospholipid metabolism such as cytidylyltransferase, phosphatidate phosphohydrolase or diacylglycerol kinase might be regulated by translocation from cytosol to membranes, the latter process being influenced by protein kinase C effectors [41,42]. As it has previously been reported that u.v. A induces protein kinase C activation [14], it may be suggested that some regulatory steps of DPG metabolism could be affected by cell irradiation. In all the cases, we were unable to demonstrate *in vitro* a significant impairment of PG synthesis from [U-^{14}C]-*sn*-glycerol 3-phosphate and unlabelled CDP-DG as substrates in keratinocyte homogenates obtained by mild sonication (see Table 2). Such a discrepancy between results *in vivo* and *in vitro* supports the hypothesis of an impairment of the regulatory processes of PG (and DPG) synthesis, which could not be evidenced in cell homogenates.

It is of note that most of the enzymes involved in the synthesis of phospholipids are bound to cellular membranes. Their activities may be influenced by variations in the membrane physicochemical characteristics [43]. Thus it is conceivable that the previously reported oxidation of cellular lipids in u.v.-irradiated cells could potentially affect the activity of membrane-bound enzymes. However, from a kinetic point of view, the formation of TBARS under u.v. A exposure did not appear to be correlated with the observed decrease in PG or DPG synthesis, since a marked decrease in oleic acid or [^{32}P]P_i incorporation into these lipids was already observed at u.v. A doses which did not lead to significant TBARS formation. Moreover, the lack of protective effect of vitamins E and C against the decrease in DPG formation strongly suggests that, within the time scale of the experiment, there is no direct relationship between lipid peroxidation and the observed alterations in phospholipid metabolism.

In conclusion, exposure of N.C.T.C. 2544 human keratinocytes to low doses of u.v. A leads to a marked and selective decrease in DPG biosynthesis as assessed with either oleic acid or [^{32}P]P_i as precursors. This may potentially result in alterations of mitochondrial membrane structure and function. As mitochondria are a key metabolic compartment of the mammalian cells, such a phenomenon could be of definite importance in the u.v. A-induced degenerative processes of the skin.

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