Biosynthesis and Metabolism of 2-Iodohexadecanal in Cultured Dog Thyroid Cells*

(Received for publication, April 30, 1996, and in revised form, June 7, 1996)

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2-Iodohexadecanal (2-IHDA) is a major thyroid iodo- lipid. It mimics the main regulatory effects of iodide on thyroid metabolism: inhibition of H\textsubscript{2}O\textsubscript{2} production and of adenyl cyclase. The biosynthesis of 2-IHDA and its metabolism have been investigated in cultured dog thyroid cells maintained in a differentiated state by forskolin. Incubation of these cells with [9,10-\textsuperscript{3}H]hexadecan-1-ol or [9,10-\textsuperscript{3}H]palmitic acid labeled several phospholipids, but [9,10-\textsuperscript{3}H]hexadecan-1-ol was selectively incorporated into plasmalogenylethanolamine. In the presence of an exogenous H\textsubscript{2}O\textsubscript{2} generating system (glucose oxidase), iodide induced the production of [9,10-\textsuperscript{3}H]2-IHDA from [9,10-\textsuperscript{3}H]hexadecan-1-ol labeled cells but not from [9,10-\textsuperscript{3}H]palmitic acid labeled cells. 2-IHDA was also generated during the lactoperoxidase-catalyzed iodination of brain and heart plasmalogens, and of ethyl hexadec-1-etyl ether, a synthetic vinyl ether-containing compound. Taken together, these results show that thyroid 2-IHDA is derived from plasmalogenylethanolamine via an attack of reactive iodine on the vinyl ether group. 2-Iodohexadecan-1-ol (2-IHDO) was also detected in these studies; it was formed later than 2-IHDA, and thyroid cells converted exogenous 2-IHDA into 2-IHDO in a time-dependent way. The ratio of 2-IHDO/2-IHDA increased with H\textsubscript{2}O\textsubscript{2} production and decreased as a function of iodide concentration. An aldehyde-reducing activity was detected in subcellular fractions of the horse thyroid. No formation of 2-iodohexadecanoic acid could be detected. Reduction into the biologically inactive 2-IHDO is thus a major metabolic pathway of 2-IHDA in dog thyrocytes.

It has been known as early as 1955 that the iodinating capacity of the thyroid gland is not restricted to tyrosyl residues in thyroglobulin. Some of the unknown iodinated compounds were characterized as lipids. The major iodo-lipid formed in the horse thyroid incubated in vitro with iodide was identified as 2-iodohexadecanal (2-IHDA)\textsuperscript{1} (1). This compound was also detected in the rat, the dog, and the human thyroid (1, 2). Another a-iodoaldehyde, 2-iodoacetanal, was also detected in the rat thyroid and in the dog thyroid where it was even more abundant than 2-IHDA (1). The pleiotropic inhibitory actions of excess iodide on the thyroid are well known and constitute an homeostatic mechanism of protection against thyrotoxicosis in case of sudden exposure to an abundant supply of iodine (3). It was shown recently that 2-IHDA mimics several of the actions of iodide. In particular, it directly inhibited the H\textsubscript{2}O\textsubscript{2}-producing NADPH oxidase, which is the target of the Wolff-Chaikoff effect (4), in thyroid porcine membranes (5) and adenyl cyclase in human thyroid membranes (6). This latter effect shared several features of the inhibition of cAMP formation by iodide (7–9) in intact cells or in membranes prepared from thyroid tissue exposed to iodide. Furthermore a comparison with various 2-IHDA analogues demonstrated that the effects of 2-IHDA are highly specific and identified the critical role of two structural determinants: the aldehyde function and the iodine at C2 (6, 10). These data suggest that 2-IHDA is the mediator of the regulatory actions of iodide on the thyroid. So far little attention has been paid to the biosynthesis and metabolic fate of 2-IHDA. The attack of reactive iodine on the vinyl ether group of plasmalogens appears as a rather straightforward mechanism of 2-IHDA formation. The present study was started in order to test that hypothesis.

**EXPERIMENTAL PROCEDURES**

Materials—\textsuperscript{1}H NMR spectra were recorded in CDCl\textsubscript{3} on a Bruker WM 250 spectrometer and are reported in ppm from internal tetramethylsilane on the 8 scale. Infrared spectra were taken with a Bruker IFS 25 instrument, and the samples were examined as deposited films on NaCl discs or in chloroform solution. Electron impact mass spectra were recorded on a VG Micromass 7070 or on a FIONS VG AUTOSPEC spectrometer. In both cases, peak intensities are expressed as percentages relative to the base peak. Thin layer chromatography analyses were performed on 0.25-mm POLYGRAM silica gel SILGUV\textsubscript{p254} precoated plates (MACHEREY NAGEL). Unless otherwise stated, column chromatographies were performed over silica gel (MN Kieselgel 0.04–0.063 mm) using flash technique or over florisor 0.15–0.25 mm (Merck). Glucose oxidase from Aspergillus niger (type 5), \textalpha-amino acid oxidase from porcine kidney (type 2), bovine serum albumin (fatty acid poor), thyrotropin, standard phospholipids for TLC, and phosphatidylethanolamine (P9137) or -choline (P9813) containing 60 or 30 plasmalogens, respectively, were purchased from Sigma. Lactoperoxidase—

\footnote{1 The abbreviations used are: 2-IHDA, 2-iodohexadecanal; BSA, bovine serum albumin; EHDE, ethyl hexadec-1-etyl ether; GC, gas chromatography; RP, reverse phase; NP, normal phase; HPLC, high performance liquid chromatography; 2-IHDO, 2-iodohexadecan-1-ol; LSIIMS, liquid secondary ion mass spectrometry; MG, monoacylglycerol; EI, electron impact; MS, mass spectrometry; PE, synthetic phosphatidylethanolamine; PLE, phosphatidylethanolamine containing 60% plasmalogenylethanolamine; HPTLC, high performance thin layer chromatography; TSH, thyrotropin.}
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Synthesis of Hexadecan-1-ol diethyl acetal (14).

CH3

OCH2

H

p-TsOH, m, solutions 4h

CH2=CH2

OCH2

H

Reaction 1. Synthesis of hexadecan-1-ol diethyl acetal.

dase was purchased from Boehringer Mannheim. [9,10-3H]Palmitic acid (50 Ci/mmole) and [1-14C]NaI were provided by Amersham Corp. Sep Pak silica gel cartridges were provided by Waters Associates. TLC aluminum sheets silica gel 60 (20 × 20 cm) for nano-TLC were provided by Merck. The various 2-iodoalkylaldehydes, 2-iodohexadecan-1-ol, and 2-iodohexadecanoic acid were synthesized according to the procedures described previously (5, 10).

Synthesis of [9,10-3H]Hexadecan-1-ol and [9,10-3H]2-Iodo-1-hexadecan-1-ol was prepared by B/H/dimethyl sulfide reduction of [9,10-3H]palmitic acid according to Yoon et al. (11). It was oxidized into [9,10-3H]hexadecan-1-ol with oxalyl chloride and dimethyl sulfoxide in anhydrous dichloromethane according to Mancuso et al. (12). [9,10-3H]2-IHDA was obtained by direct iodination of the corresponding aldehyde by a previously described procedure (5) adapted from Barluenga et al. (13). Briefly, iodine (49 mg, 0.20 mmol, 1 eq) and molecular sieves (4 Å) were added, and the mixture was refluxed until completion of Reaction 1. The reaction was monitored by TLC (hexane/dichloromethane, 1:1) or by infrared analysis (disappearance of the carbonyl band at 1732 cm⁻¹) and was completed within 24 h. The mixture was diluted with hexane (50 ml) and washed with an aqueous solution of NaOH 0.5%, and the organic layer was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (hexane/chloroform, 95:5) affording the pure acetal as an oil (294 mg, 43%).

Phospholipid Analysis—Phospholipids (PLE, Sigma P 9137) were dissolved in chloroform/methanol (2:1) and treated with 2.5 N methanolic HCl (200 ml) and then with a linear gradient to hexane/chloroform/acetic acid (20:80:0.5) over 5 min. Fractions of 0.5 or 1 ml were counted using a liquid scintillation counter or a γ-counter. The retention times of synthetic 2-IHDA, 2-IHDO, and 2-iodohexadecanoic acid were, respectively, 4.5 ± 0.8 min (mean ± S.D. of nine injections), 16.5 ± 0.9 min (mean ± S.D. of nine injections), and 6.3 ± 0.36 min (mean ± S.D. of four injections). GC-MS analyses were performed using a capillary GC (TRACOR 540) coupled to an ion trap detector mass spectrometer (FINNIGAN ITD 800) on a 1.5 m × 0.25 mm fused silica gel column (Wectos) containing chemically bonded polyethylene glycol (CP-Wax 52CB, Chrompack). The following elution program was applied: initial temperature, 100 °C (1 min); final temperature, 200 °C; rate of rise, 30 °C/min. The temperature of the transfer line was 220 °C. Helium was used as carrier gas (pressure, 15 p.s.i.). Injections were performed directly on-column. Electron impact (EI) with an electron beam energy of 70 eV or chemical ionization with ammonia were both used. 2-IHDA was analyzed either as such or after derivatization into its [(2,3,4,5,6-pentafluorobenzyl)oximyl] derivative, prepared as follows: a solution of 0.6 mg (2.4 μmol) of O-(2,3,4,5,6-pentafluorobenzyl)hydroxyamine hydrochloride in water (100 μl) was added to 2-IHDA (15 μg, 41 nmol) dissolved in ethanol (100 μl). The mixture was vortexed for 5 min and allowed to stand at room temperature for 24 min. The mixture was diluted with water (0.4 ml) and extracted with hexane (3 × 0.5 ml). The hexane phase was analyzed by GC-MS using the conditions described above.

Synthesis of Ethyl Hexadec-1-enyl Ether (EHDE)—The following two-step sequence was used to prepare the mixture of E/Z isomers of ethyl hexadec-1-enyl ether starting from hexadecan-1-ol. 517 mg (2.15 mmol) of hexadecanal diethyl acetal was prepared using the conditions described below.

Reaction 2. Synthesis of ethyl hexadec-1-enyl ether (14).
FIG. 1.  a, lactoperoxidase-catalyzed iodination of vinyl ether-containing compounds. Synthetic EHDE, bovine brain PLE, synthetic PE, or bovine heart phosphatidylcholine containing 30% plasmenvylcholine (PLC) (100 μg/ml) were incubated at room temperature for 30 min, with lactoperoxidase (7 μg/ml), [125I]NaI (30 μCi/ml, 16 μCi), and H2O2 (0.26 mM). Neutral lipids were extracted, prepurified on silica gel cartridges, and analyzed on HPTLC silica gel plates by development in n-hexane/diethyl ether/acetic acid (80:20:0.1). The results represent the autoradiography of the TLC plate. The nonradioactive 2-IHDA standard was visualized by phosphomolybdic acid coloration of the plate. b, RP-HPLC analysis of phospholipids.
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Production of 2-IHDA by cultured dog thyroid cells in the presence or the absence of an H2O2 generating system. Dog thyrocytes, cultured as described under “Experimental Procedures,” were incubated for 3 h at 37 °C in 3 ml of H2O2-buffered medium containing BSA (0.5 mg/ml), TSH (1 milliunit/ml), [125I]NaI (15 μCi/ml) with or without glucose oxidase (2.4 units/ml). The reaction was stopped by scraping the cells in 2 ml of glacial phosphate-buffered saline, centrifugation of the cells, and homogenization of the resulting pellet in methanol/chloroform (2:1) as described under “Experimental Procedures.” After extraction of the lipids following Bligh and Dyer (20) and addition of synthetic 2-IHDA (7 μg), the neutral lipids were partitioned with chloroform from a silica gel cartridge and submitted to RP-HPLC as described under “Experimental Procedures.” Fractions of 0.5 ml were collected, and radioactivity was counted in a solid scintillation counter. The arrows indicate the retention time of synthetic 2-IHDA. Because the runs were stopped after 15 min, the 2-iodoctadecanal peak (retention time, 17 min) is not visible on these particular chromatograms.

FIG. 2. Production of 2-IHDA by cultured dog thyroid cells in the presence or the absence of an H2O2 generating system. Dog thyrocytes were pre-equilibrated at 37 °C in a HEPES-buffered medium: 20 mM HEPES (pH 7.4), 3 mM NaCl, 5 mM KCl, 1 mM MgCl2, 1 mM CaCl2, 10 mM glucose. After 30 min, the medium was replaced by 3 ml of fresh buffer containing BSA (0.5 mg/ml) with or without TSH (1 milliunit/ml). Two types of experiments were performed. In some cases, the incubation medium contained [3H]hexadecan-1-ol as described above, incubated with or without KI (100 μM), glucose oxidase (2.4 units/ml), and methimazole (500 μM). The incubation was stopped after 3 h; cells were rinsed and scraped in 2 ml of glacial phosphate-buffered saline. Lipid extraction and isopodilid separation and detection were performed as described above.

Measurement of Aldehyde Reductase and Alcohol Dehydrogenase Activity

Aldehyde reductase and alcohol dehydrogenase activities were determined from horse thyroid subcellular fractions by monitoring the decrease in the absorbance at 343 nm due to the oxidation of NADP+ in the presence of 2-IHDA. Horse thyroid membranes (6) (30,000 × g for 10 min) or the supernatant of this preparation solubilized by Triton X-100 (1%) inactivated or not by incubation 3 min at 100 °C were incubated with 0.5 mg protein/ml in 500 μl of a medium containing bovine serum albumin (0.5 mg/ml), NADP (1 mM, pH 7.4) containing 1 mM EDTA and 0.1 mM NADH (for the assay of alcohol dehydrogenase) or 0.1 mM NADPH (for the assay of aldehyde reductase) in the presence of 20 μM 2-IHDA, 2-IIIOD, 2-iododecanal, 2-iodocanonal, or octanal. The kinetics of NADH or NADPH disappearance were performed over 30 min at 37 °C on a Uvikon 930 (Kontron Instruments) spectrophotometer. The molar extinction coefficient of NADPH at 343 nm is 6200 M−1 cm−1.

RESULTS

The incubation of a crude preparation of bovine brain PLE or of bovine heart phosphatidylcholine containing plasmalogen with lactoperoxidase, radioiodide, and H2O2 generated an iodinated material co-migrating with synthetic 2-IHDA in HPTLC (Fig. 1a) and in RP-HPLC (not shown). This material

before (A) and after (B) lactoperoxidase-catalyzed iodination. PLE was incubated at room temperature for 30 min with lactoperoxidase (7 μg/ml), NaI (16 μM), and H2O2 (0.26 mM). Phospholipids were extracted with ethyl acetate and purified on silica gel cartridges by elution with 10 ml of chloroform/methanol (1:1). RP-HPLC analysis were performed using the conditions described under “Experimental Procedures.” A, RP-HPLC analysis of the PLE mixture. The two major peaks (1 and 2) were identified by LSIMS analysis (data not shown). The LSIMS analysis of peak 1 (tR = 46.4 min, 18% of total phospholipids) revealed that this peak was constituted of diacyl derivatives. The mass spectrum in the positive mode presented an ion at 792 Da corresponding to the quasi-molecular ion of a diacyl 40:6 derivative and an ion at 744 Da corresponding to the quasi-molecular ion of a diacyl 36:2 derivative. Other characteristic fragments were present at m/z 651 (loss of phosphorylethanolamine from 792), 603 (loss of phosphorylcholine from 744), 385 (MG: 22:6), 370, 341, 339 (MG: 18:0) and 339 (MG: 18:1). The mass spectrum in the negative mode presented ions at m/z 790 (M−H) diacyl 40:6, 742 (M−H) diacyl 36:2, 478, 283 (stearate anion), 281 (oleate anion), and 273. The LSIMS analysis of the peak 2 (tR = 55.3 min, 10% of total phospholipids) revealed that this peak was constituted of alkylacyl derivatives. The mass spectrum in the positive mode presented an ion at 728 Da corresponding to the quasi-molecular ion of an alkylacyl 36:2 derivative and an ion at 702 Da corresponding to the quasi-molecular ion of an alkylacyl 34:1 derivative. Other characteristic fragments appeared at m/z 587 (loss of phosphorylcholine from 728), 561 (loss of phosphorylethanolamine from 702), 404, 359, and 339 (MG: 18:1). The mass spectrum in the negative mode presented ions at m/z 726 (M−H) alkylacyl 36:2, 700 (M−H) alkylacyl 34:1, 462, 281 (oleate anion), and 273. B, RP-HPLC analysis of PLE after lactoperoxidase-catalyzed iodination. Peak 1 (tR = 46.0 min) was composed of two components. Lysophosphatidylcholine resulting from the cleavage of the alkyl chain had a retention time less than 10 min and therefore is not apparent on the chromatogram. The LSIMS analysis of phospholipids remaining after iodination showed that the major peak (tR = 46.0 min) was constituted of diacyl derivatives. C, RP-HPLC analysis of PLE after acid methanolysis. C, GC-MS characterization of the 2-IHDA generated by lactoperoxidase iodination of EHDE. 2-IHDA, purified by NP-HPLC, was analyzed by gas chromatography coupled to a Finnigan ion trap detector mass spectrometer, either as such (A) or after derivatization into its O-pentafluorobenzoyloxy derivative (B and C). A capillary column (CP-Wax 52CB, 1.5 μm × 0.25 mm) was used with a temperature gradient from 100 to 200 °C at a rate of 30 °C/min, as described under “Experimental Procedures.” A, the mass spectrum of 2-IHDA in the EI mode presented an ion at m/z 434 corresponding to the loss of iodine from the molecular ion and an ion at m/z 170 resulting from a MacLafferty rearrangement (40). B, the mass spectrum of the O-pentafluorobenzoyloxy derivative of 2-IHDA in the EI mode presented an ion at m/z 434 corresponding to the loss of molecular ion and an ion at m/z 181 corresponding to the pentfluorotramipol cation. C, the spectrum in the chemical ionization mode (NH3 as collision gas) of this derivative presented a quasi-molecular ion at 562 Da and a strong fragment peak at m/z 434.
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Dog thyrocytes, cultured as described under "Experimental Procedures," were labeled with [9,10-3H]hexadecan-1-ol (100 μCi/dish) or [9,10-3H]palmitic acid (100 μCi/dish) for the last 24 h of the culture. The cells were scraped in 2 ml of glacial phosphate-buffered saline, centrifuged, and homogenized in methanol/chloroform (2:1) as described under "Experimental Procedures." After extraction of the lipids following Bligh and Dyer (20), the total extract was analyzed on HPTLC plates by the two-step elution described under "Experimental Procedures." Before the second elution, some plates were dried and submitted to a 15-min HCl vapor treatment. After the second elution, the standards, co-eluting with the sample, were revealed by iodine vapor, cut, eluted by methanol/water (2:1), and counted in a liquid scintillation counter. Results are expressed as percentages of the total radioactivity incorporated in the cell extract, before and after HCl treatment. LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; PE, phosphatidylethanolamine; PA, phosphatidic acid; DAG, diacyl glycerol; TG, triglycerides; NL, neutral lipids.

| Hexadecan-1-ol labeling | Palmitic acid labeling |
|-------------------------|------------------------|
| HCl treatment           |                        |
| -                       | +                      | +                     |
| LPC                     | 2.8 ± 0.2              | 4.1 ± 3.4             | 5.0 ± 0.0              | 4.1 ± 1.0              |
| PC                      | 46.1 ± 0.1             | 44.1 ± 0.7            | 56.5 ± 7.8            | 50.8 ± 13             |
| PS + PI                 | 6.5 ± 1.4              | 5.0 ± 0.4             | 9.0 ± 0.5             | 6.2 ± 0.8             |
| PE                      | 27.5 ± 1.3             | 10.0 ± 1.2            | 14.5 ± 0.2            | 13.0 ± 0.1            |
| PA                      | 1.0 ± 0.1              | 1.1 ± 0.1             | 1.0 ± 0.0             | 1.4 ± 0.0             |
| DAG, TG, NL             | 16.2 ± 3.3             | 35.7 ± 4.7            | 13.8 ± 7.0            | 24.4 ± 14             |

Fig. 3. Iodination of [9,10-3H]hexadecan-1-ol-labeled material in dog thyroid cells. Dog thyrocytes, cultured as described under "Experimental Procedures," were labeled with [9,10-3H]hexadecan-1-ol (100 μCi/dish) for the last 24 h of the culture in the presence or the absence of methimazole (500 μM). The cells were incubated for 3 h at 37°C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), TSH (1 mIU/ml), glucose oxidase (2.4 units/ml) and methimazole (500 μM). The radiolabeled lipids produced by the thyrocytes were extracted, prepurified as described in the legend to Fig. 2, and submitted to RP-HPLC. Fractions of 0.5 ml were collected, and radioactivity was counted in a liquid scintillation counter. The arrows indicate the retention time of synthetic 2-IHDA. The results of this experiment are representative of four.

was not produced when synthetic PE was incubated under the same experimental conditions (Fig. 1a). The iodination of the PLE preparation by lactoperoxidase was associated with the consumption of plasmenylethanolamine and not phosphatidylethanolamine (Fig. 1b, B). To establish the specific role of the vinyl ether bond of plasmalogens in the generation of 2-IHDA, EHDE (23) was synthesized. Incubation of EHDE with lactoperoxidase and radiiodide generated an iodinated material co-eluting with synthetic 2-IHDA in HPTLC (Fig. 1a) and in RP-HPLC (not shown). The identity of this compound as 2-IHDA was confirmed by GC-MS (Fig. 1c).

When dog thyrocytes in culture were incubated with radiiodide, they produced two iodolipids co-eluting in RP-HPLC with synthetic 2-IHDA (Fig. 2) and 2-iodoctadecanal (data not shown). This production was amplified in the presence of the H2O2 generating system glucose-glucose oxidase (Fig. 2): 2.4 units/ml glucose oxidase induced a 11.7 ± 5.0-fold increase (mean ± S.D. of five experiments) of the radioactivity incorporated into the neutral lipids fraction containing 2-IHDA. As control, no radioactivity was found in the lipid extract when nonthyroid cells (COS-7 cells) were incubated with radiiodide and glucose oxidase (data not shown). Using [9,10-3H]hexadecan-1-ol to label plasmalogens in the sn1 position (19), we investigated if they were the source of 2-IHDA in dog thyrocytes. When the cells were incubated with [9,10-3H]hexadecan-1-ol or [9,10-3H]palmitic acid, several classes of lipids were labeled (Table I). The labeling patterns were similar, except for a larger incorporation of [9,10-3H]hexadecan-1-ol in the fraction co-migrating with the PE standard. This difference was abolished following exposure of the plates to HCl vapor, which is known to cleave the vinyl ether bond of plasmalogens (22). The RP-HPLC chromatogram of phospholipids remaining after acid methanolysis of the PLE mixture was similar to the chromatogram obtained after lactoperoxidase-catalyzed iodination of the PLE mixture with disappearance of the same plasmalogen peaks (Fig. 1b, C). The results are thus consistent with a selective incorporation of [9,10-3H]hexadecan-1-ol at the sn1 position of plasmenylethanolamine. On the basis of the differ-
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Fig. 4. Comparison of the iodination of [9,10-3H]hexadecan-1-ol and [9,10-3H]palmitic acid-labeled material in dog thyroid cells exposed to iodide. Dog thyrocytes, cultured as described under “Experimental Procedures,” were labeled with [9,10-3H]hexadecan-1-ol (100 μCi/dish) or [9,10-3H]palmitic acid (100 μCi/dish) for the last 24 h of the culture. The cells were then incubated for 3 h at 37 °C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), TSH (1 milliunit/ml), KI (100 μM), and glucose oxidase (0.8 unit/ml). The radiolabeled lipids produced by the thyrocytes were extracted, prepurified as described in the legend to Fig. 2, and submitted to RP-HPLC. Fractions of 0.5 ml were collected, and radioactivity was counted in a liquid scintillation counter. The arrows indicate the retention time of synthetic 2-IHDA. The results of this experiment are representative of two.

A similar experiment between the radioactivity present before and after HCl treatment, we computed that 12.3 ± 3.8% of the cell-associated hexadecan-1-ol label was incorporated into plasmenylethanolamine (mean ± S.D. of seven different experiments). That difference was only 4.2 ± 2.6% (p < 0.0001, paired Student’s t test on 19 samples, seven experiments) in the case of plasmenylecholine.

Incubation of [9,10-3H]hexadecan-1-ol-labeled dog thyrocytes with both iodide (100 μM) and glucose oxidase (2.4 units/ml) led to the generation of a tritium-labeled material co-eluting with synthetic 2-IHDA in RP-HPLC and representing 0.11–0.56% of the cell-associated [9,10-3H]hexadecan-1-ol labeling (range of five experiments) (Fig. 3). Production of this material was inhibited by methimazole at a concentration (500 μM) that is known to inhibit iodolipid formation in horse thyroid slices (1). That compound was not detectable when [9,10-3H]palmitic acid-labeled dog thyrocytes were incubated with iodide and glucose oxidase (Fig. 4). In these experiments, the radioactivity recovered in the total lipid extract was 22 ± 106 and 41 ± 7 106 cpm, respectively, with hexadecan-1-ol and palmitic acid labeling, indicating that the negative result with [9,10-3H]palmitic acid was not due to a lower incorporation into the thyrocytes.

To confirm its identity to 2-IHDA, the tritium-containing peak co-eluting with synthetic 2-IHDA in RP-HPLC was rechromatographed in NP-HPLC (Fig. 5a) and submitted to HPTLC on silica gel plates (Fig. 5c). NP-HPLC resolved two radioactive peaks, one of which co-eluted with a 2-IHDA standard (Fig. 5a). In the same way, two spots were obtained in TLC, one of which had the same Rf as 2-IHDA (Fig. 5c). A similar result was obtained when analyzing directly in NP-HPLC (Fig. 5b) or TLC (Fig. 5c); the radiodiodinated product formed in dog thyrocytes incubated with [125I]NaI. The same amount of 2-IHDA was produced when TSH was omitted from the incubation medium (data not shown). The second peak (or spot) had a behavior identical to that of synthetic standard of 2-IHDO in three different chromatographic systems: RP-HPLC, NP-HPLC, and TLC. The 2-IHDO/2-IHDA ratio was variable from one experiment to the other (10.2 ± 5 with 2.4 units/ml glucose oxidase, mean ± S.D. of seven experiments) and was critically dependent on the experimental conditions (see below). In none of the experiments was it possible to detect a radioactive peak at the retention time (6.7 min) of 2-iodohexadecanoic acid (Fig. 5b); this compound was also undetectable in RP-HPLC where its retention time was 8.1 ± 0.7 min (Fig. 2).

Two types of experiments were performed to determine if 2-IHDO is formed by reduction of 2-IHDA; the kinetics of appearance of the two lipids were compared, and the conversion of exogenous [9,10-3H]2-IHDA into [9,10-3H]2-IHDO by thyrocytes was monitored. The production of [125I]2-IHDA by dog thyrocytes incubated with radioiodide in the presence of glucose oxidase was maximal at the earliest time studied (10 min) and decreased over time. In parallel to this decrease, there was a progressive increase in 2-IHDO (Fig. 6a). When...
[9,10-3H]2-IHDA was added to the thyrocytes at a final concentration of 0.1 μM, it was slowly converted into [9,10-3H]2-IHDO, resulting in a progressive increase of the 2-IHDO/2-IHDA ratio (Fig. 6b). No conversion into [9,10-3H]2-IHDA was obtained when synthetic [9,10-3H]2-IHDA was incubated in the same conditions but in the absence of cells (data not shown). The 2-IHDO/2-IHDA ratio decreased with iodide concentration (Fig. 6c) and increased with glucose oxidase activity (Fig. 6d).

DISCUSSION

We have previously proposed the hypothesis that 2-IHDA is formed as the result of the attack of a reactive iodine species (I or I°) on the vinyl ether group of plasmalogens (1). This hypothesis was supported by preliminary data showing the formation of 2-IHDA from synthetic [9,10-3H]2-IHDA in tissue culture, and the results of the present study confirm this hypothesis. The 2-IHDO/2-IHDA ratio was found to be proportional to the activity of the H2O2-generating system, indicating that the reaction is catalyzed by a peroxidase enzyme. The consumption of NAD(P)H by subcellular fractions of horse thyroid tissue was measured spectrophotometrically in the presence or the absence of 2-IHDA. Table II shows that 2-IHDA induced a consumption of NADPH by the supernatant (30000 x g for 10 min) of an homogenate of horse thyroid but not by the membranes of the same preparation, whereas with NADH an activity was found in both fractions. The consumption of NAD(P)H was strongly reduced after boiling and was not observed in the presence of 2-IHDA, indicating the role of the aldehyde function (Table II). The activity was not specific for 2-IHDA; at equimolar concentrations, 2-IHDA, 2-iodododecanal, and 2-iodooctanal induced a very similar consumption of NAD(P)H by the thyroid membranes and supernatant, whereas that consumption was about 50% lower with octanal and dodecanal (data not shown).

Fig. 6. a, kinetics of [125I]2-IHDA and [125I]2-IHDO production by cultured dog thyroid cells incubated with radioiodide. Dog thyrocytes, cultured as described under “Experimental Procedures,” were incubated for various lengths of time (10, 20, 30, or 60 min or 3 h) at 37°C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), TSH (1 milliunit/ml), [125I]NaI (23 μCi/ml) with glucose oxidase (2.4 units/ml). The radiolabeled lipids produced by the thyrocytes were extracted, purified as described in the legend to Fig. 2, and submitted to NP-HPLC. Fractions of 0.5 ml were collected, and radioactivity was counted in a solid scintillation counter. The figure represents the radioactivity eluted at the retention time of 2-IHDA (●) or 2-IHDO (○) standard as a function of the incubation time. The results of this experiment are representative of two. b, kinetics of transformation of synthetic [9,10-3H]2-IHDA in [9,10-3H]2-IHDO by dog thyroid cells in culture. Dog thyrocytes, cultured as described under “Experimental Procedures,” were incubated for various lengths of time (5 or 60 min or 3 h) at 37°C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), TSH (1 milliunit/ml), and synthetic [9,10-3H]2-IHDA (3 μCi/dish, 0.1 μM). The radiolabeled lipids of the thyrocytes were extracted, purified as described in the legend to Fig. 2, and submitted to NP-HPLC. Fractions of 0.5 ml were collected, and radioactivity was counted in a liquid scintillation counter. The figure represents the radioactivity eluted at the retention time of the 2-IHDO standard divided by the radioactivity eluted at the retention time of the 2-IHDA standard, as a function of the incubation time. The results of this experiment are representative of three. c, dog thyrocytes, cultured as described under “Experimental Procedures,” were incubated for 3 h at 37°C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), TSH (1 milliunit/ml), [125I]NaI (33 μCi/ml), glucose oxidase (2.4 units/ml), and various KI concentrations (0.01, 0.1, 1, and 10 μM (final)). d, dog thyrocytes, cultured as described under “Experimental Procedures,” were incubated for 2 h at 37°C in 3 ml of HEPES-buffered medium containing BSA (0.5 mg/ml), [125I]NaI (33 μCi/ml), and various glucose oxidase concentrations (0, 0.0024, 0.024, and 2.4 units/ml). The radiolabeled lipids produced by the thyrocytes were extracted, purified as described in the legend to Fig. 2, and submitted to NP-HPLC. Fractions of 0.5 ml were collected, and radioactivity was counted in a solid scintillation counter. The figures represent the radioactivity eluted at the retention time of the 2-IHDO standard divided by the radioactivity eluted at the retention time of the 2-IHDA standard. The results of this experiment are representative of two.
Biosynthesis and Metabolism of 2-IHDA in Dog Thyroid Cells

TABLE II

Aldehyde reductase activity in the horse thyroid

Horse thyroid membranes or the supernatant of the membrane preparation (0.5 mg protein/ml) were incubated at 37°C for 30 min in the presence of NADH or NADPH (0.1 mM) with or without 2-IHDA or 2-IHDO (20 µM). The disappearance of NAD(P)H was followed at 343 nm. The activity was calculated from the negative slope of NAD(P)H disappearance, which was linear and expressed as nmol NAD(P)H consumed/min · mg protein. As control, 2-IHDA did not interfere with the measure. ND, not determined.

|          | Membranes       | Membranes   | Inactivated membranes | Inactivated membranes | Supernatant | Supernatant | Inactivated supernatant | Inactivated supernatant |
|----------|-----------------|-------------|-----------------------|-----------------------|-------------|-------------|------------------------|------------------------|
|          | (n = 7)         | (n = 2)     | (n = 2)               | (n = 2)               | (n = 2)     | (n = 2)     | (n = 2)                | (n = 2)                |
| Membranes| 0.35 ± 0.03     | 0.33 ± 0.03 | ND                    | ND                    | 0.32 ± 0.1  | 0.39 ± 0.07 | 0.23 ± 0.1             |
| Inactivated membranes| ND | ND | ND | ND | ND | ND | ND | ND |
| Supernatant| 0.45 ± 0.07     | 0.32 ± 0.1  | 0.09 ± 0.04           | 0.47 ± 0.20           | 0.12 ± 0.03 |
| Inactivated supernatant| ND | ND | 0.18 ± 0.05           | ND | 0.18 ± 0.05 |

\[ [9,10-^3H] \text{hexadecan-1-ol} \]

\[ \text{plasmenylethanolamine} \]

\[ \text{lactoperoxidase} \]

\[ \text{iodination} \]

\[ \text{lysophosphatidylethanolamine} \]

\[ \text{2-iodohexadecanal} \]

\[ \text{2-iodohexadecan-1-ol} \]

\[ \text{aldehyde reductase} \]

\[ \text{H}_2\text{O}_2 \]

\[ \text{HO}-\text{CH}_2-\text{CH}-(\text{CH}_2)_{13}\text{CH}_3 \]

\[ \text{HO}-\text{CH}_2-\text{CH}-(\text{CH}_2)_{13}\text{CH}_3 \]

\[ \text{HO}-\text{CH}_2-\text{CH}-(\text{CH}_2)_{13}\text{CH}_3 \]

\[ \text{HO}-\text{CH}_2-\text{CH}-(\text{CH}_2)_{13}\text{CH}_3 \]

FIG. 7. Biosynthesis and catabolism of 2-iodohexadecanal. Hexadecan-1-ol is selectively incorporated at the sn1 position of plasmenylethanolamine during its de novo biosynthesis. Iodination of the vinyl ether group of plasmenylethanolamine generates an unstable iodinated derivative, which breaks into lysophosphatidylethanolamine and 2-iodohexadecanal. 2-Iodohexadecanal is reduced into 2-iodohexadecan-1-ol by a mechanism that is down-regulated by iodide and enhanced by H2O2.
There are several pathways of aldehyde metabolism in mammalian cells. They can be oxidized into the corresponding carboxylic acids by various aldehyde dehydrogenases and aldehyde oxidases. Multiple forms of these enzymes have been identified that differ inter alio by their subcellular localization, cytosol versus mitochondrion (32, 33). They can also be conjugated to glutathione by cytosolic glutathione transferases (34, 35). Reduction into alcohol by enzymes such as aldo-keto reductases or alcohol dehydrogenase represents a third possibility. For instance, the lens aldose reductase has a lower $K_m$ for 4-hydroxynonenal, a product formed during lipid peroxidation, than for glucose (36). The relative importance of these pathways is variable from one cell type to the other. For instance, whereas glutathione transferase represents the major pathway in normal hepatocytes, aldehyde dehydrogenase plays a major role in some hepatoma cell lines; other lines have prominent NADPH-dependent aldehyde reductase activity and/or NADH-dependent alcohol dehydrogenase activity (37). Reduction into the corresponding alcohol is clearly the major metabolic transformation of 2-IHDA in the thyroid, and there is no evidence of a significant oxidation into 2-iodohexadecanoic acid. This reduction seems to involve both a soluble NADPH-dependent aldehyde reductase and a NADH-dependent alcohol dehydrogenase present in membranes as well as in supernatant. These activities are not specific for $\alpha$-idoaldehydes; indeed octanal and dodecanal were also reduced, although at a lower rate than 2-iodoocanal, 2-iodododecanal, or 2-IHDA. Because unlike 2-IHDA, 2-IHDO is biologically inactive both on $\mathrm{H}_2\mathrm{O}_2$ production (5, 6) and on adenyl cyclase (10), reduction clearly represents an inactivating pathway. One intriguing finding was the increased conversion of 2-IHDA into 2-IHDO as a function of the rate of $\mathrm{H}_2\mathrm{O}_2$ generation. This is reminiscent of the observation that the lens aldose reductase is activated by reactive oxygen species (38, 39). The physiological significance of this effect in the thyroid remains unclear.

Acknowledgments—We thank J. E. Dumont and C. Lejeune for helpful discussions and L. Collyn for precious technical advice during TLC analysis of phospholipids. We thank José Carlos García Borrón and A. Pereira for helpful discussions. We thank Dr M. Kaisin and C. Moulard for the LSIMS measurements.

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J. Biol. Chem. 1996, 271:23006-23014.
doi: 10.1074/jbc.271.38.23006

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