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Gianoukakis, Andrew G., H. James Cao, Timothy A. Jennings, and Terry J. Smith. Prostaglandin endoperoxide H synthase expression in human thyroid epithelial cells. *Am J Physiol Cell Physiol* 280: C701–C708, 2001.—KAT-50, an established human thyrocyte cell line, expresses constitutively high levels of prostaglandin endoperoxide H synthase-2 (PGHS-2), the inflammatory cyclooxygenase. Here, we examine primary human thyrocytes. We find that they, too, express PGHS-2 mRNA and protein under control culture conditions. A substantial fraction of the basal prostaglandin E_2 (PGE_2) produced by these cells can be inhibited by SC-58125 (5 μM), a PGHS-2-selective inhibitor. Interleukin (IL)-1β (10 ng/ml) induces PGHS-2 expression and PGE_2 production in primary thyrocytes. The induction of PGHS-2 and PGE_2 synthesis by IL-1β could be blocked by glucocorticoid treatment. Unlike KAT-50, most of the culture strains also express PGHS-1 protein. Our observations suggest that both cyclooxygenase isoforms may have functional roles in primary human thyroid epithelial cells, and PGHS-2 might predominate under basal and cytokine-activated culture conditions.

prostanoid; inflammation; cyclooxygenase

THYROID TISSUE IS A FREQUENT target of neoplastic and inflammatory diseases. The high prevalence of nonendemic goiter, thyroid neoplasia, and autoimmune diseases such as Hashimoto's thyroiditis and Graves' disease has provoked substantial investigation into the pathogenesis of thyroid inflammation. However, our understanding of these diseases remains superficial and incomplete. Moreover, the inflammatory mediators expressed in thyroid tissue in physiological states and in disease have yet to be defined in detail.

The eicosanoid biosynthetic pathways are important components of the inflammatory machinery that utilize arachidonate as the principal substrate. Arachidonate is a 20-carbon polyunsaturated fatty acid liberated from plasma membrane phospholipids by the action of phospholipase A_2 (PLA_2) (28). Prostaglandins are formed by the cyclic oxygenation and subsequent peroxidation of the central 5 carbon of arachidonate (6, 27). These two rate-limiting steps are catalyzed by prostaglandin endoperoxidase H synthase (PGHS, EC 1.14.99.1), also known as cyclooxygenase (27). Two isoforms of PGHS have been identified and cloned. They are designated PGHS-1 and PGHS-2. Both isoforms are membrane bound, heavily glycosylated, and contain heme prosthetic groups. They have very similar protein X-ray crystallographic structures (19, 27). PGHS-1 is constitutively expressed in most cell types and is thought to be responsible for a substantial fraction of unprovoked prostaglandin production (26, 27). In contrast, PGHS-2, also known as the inflammatory cyclooxygenase, is not expressed ordinarily in most tissues but can be induced by growth factors, tumor promoters, and cytokines (3, 11, 13, 18, 24, 32) and, thus, is thought to be responsible for prostaglandin production in inflammation.

The pattern of expression and the functional roles of PGHS enzymes and prostaglandins in normal and diseased thyroid tissue are poorly characterized. Products of PGHS enzymes have been implicated in the mediation of certain thyrotropin actions in thyroid epithelial cells, but details concerning the expression of cyclooxygenases in these cells remain ill defined. Recent studies have suggested that the arachidonate cascade and PGHS-2 expression may have a significant involvement in the regulation of cell growth, apoptosis, and tumorgenesis in various cell types (4–6, 8, 14, 21, 23, 28, 33). Thus there is considerable reason to implicate PGHS as participating in several normal and pathological facets of thyrocyte biology.

Constitutive PGHS-2 expression has been found in few tissues and cell types. These include certain regions of the brain (35), the macula densa of the kidney (12), bronchial epithelium (1), and pancreatic beta islet (20), granulosa (16), and hepatic stellate cells (9). We have reported recently that normal human thyroid epithelium in situ and the well-differentiated human thyroid epithelial cell line, KAT-50, express high levels of PGHS-2 under basal, nonpathological conditions (25). Those studies revealed that factors usually asso-

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ciated with PGHS-2 upregulation, including interleukin (IL)-1β and serum, lower PGHS-2 expression transiently in KAT-50 cells (25). The biological implications of constitutive PGHS-2 expression in human thyroid epithelium are unclear, but findings to date suggest that the enzyme may play an important role in the healthy thyroid.

We report here the results of studies examining PGHS expression and activity in primary thyroid epithelial cell cultures derived from donors with several thyroid diseases and from normal appearing tissue. Like KAT-50 cells, these thyrocytes express PGHS-2 mRNA and protein under basal culture conditions. Moreover, an appreciable fraction of unprovoked prostaglandin E_2 (PGE_2) is attributable to the activity of PGHS-2. These observations demonstrate the complexity with which PGHS genes are expressed, regulated, and utilized in human thyroid epithelial cells.

MATERIALS AND METHODS

Materials. Dispase I was purchased from Boehringer Mannheim (Mannheim, Germany), and collagenase was from Worthington Biochemical (Lakewood, NJ). The basic DAB detection kit, mouse anti-human pan-keratin antibody (clones AE1/AE3/PCK26), anti-human actin antibody (clone HUC 1–1), and mouse Ig-negative reagent (clone MOPC 21) were purchased from Ventanna Medical Systems (Tucson, Arizona). The mouse anti-human CD31 antibody (clones SC/70A) was purchased from Dako (Carpiteria, CA). Arachidonate and dexamethasone (1,4 pregnadien-9-fluoro-16α-methyl-11β, 17α,21-triol-3,20-dione) were from Sigma (St. Louis, MO). Anti-PGHS-1 and anti-PGHS-2 monoclonal antibodies were obtained from Cayman (Ann Arbor, MI) and IL-1β from Biosource (Camarillo, CA). SC-58125 was a generous gift of Searle/Monsanto, and PGHS-1 and -2 cDNAs were generously provided by Dr. D. A. Young (Univ. of Rochester, Rochester, NY).

Primary thyrocyte isolation and culture. These activities were approved by the Institutional Review Board of the Albany Medical Center. Thyroid tissue was obtained as surgical waste from patients undergoing thyroidectomies for the treatment of a variety of thyroid conditions. These included Graves' disease, Hashimoto's thyroiditis, multinodular goiter, and thyroid adenomas and carcinomas. Tissue from glands harboring distant neoplasms was normal appearing on gross examination. Thyroid specimens were incubated in Hanks' balanced salt solution (HBSS) on ice and processed within 1 h of harvest. Tissues were trimmed of connective tissue, finely minced, and washed four times with HBSS. Minced tissue was incubated at 37°C in HBSS that contained collagenase type A (130 U/ml) and Dispase grade I (0.5 U/ml) for 1 h. The tissue remaining intact was reincubated in fresh enzyme mixture for 30-min intervals until all fragments were digested. The cellular mixture that contained liberated follicles was centrifuged at 200 *g* for 2 min, and the resulting pellet was resuspended in RPMI medium and allowed to sediment with gravity for 1 h. The supernatant (containing single cells) was discarded, and the sedimented follicles were collected, pooled, resuspended in RPMI, and filtered through a 200-μm nylon mesh. The twice-washed follicles were resuspended in 4 ml medium and incubated overnight in plastic culture plates coated with agar (2%; Sigma). Intact follicles were then pipetted into 25-mm plastic flasks. Follicles attached to the flask surface, and monolayer cultures developed. Cells were maintained in a humidified 5% CO_2 incubator at 37°C covered with RPMI supplemented with 10% fetal bovine serum (FBS), penicillin (50 U/ml), and streptomycin (50 μg/ml). Medium was changed every 3–4 days, and cells were passaged with gentle trypsin treatment and utilized between the second and sixth passages. Experiments were performed on confluent monolayers, in most cases, within 1 wk of passage and seeding. KAT-50 cells were a generous gift from Dr. K. Ain (Univ. of Kentucky, Lexington, KY). These were provided to us at *passage 8* in January 1997 and were covered with MEM and utilized between *passages 10* and *35*.

Immunohistochemistry. A sample of the cultured primary thyrocytes was trypsinized and washed in PBS. Cells were then fixed in 95% ethyl alcohol for 30 min and embedded in paraffin. Four micrometers of cell block sections were cut and placed in the reaction chamber of a Ventanna ES automated immunohistochemistry system (Ventanna Medical Systems) that uses an indirect biotin-avidin detection method. Endogenous peroxidase activity was blocked with 1% H_2O_2. The slides were incubated with a primary antibody for 32 min at 37°C followed by a biotinylated secondary antibody for 8 min at 37°C. Avidin-horseradish peroxidase and diaminobenzidine were followed by copper sulfate enhancement and counterstaining with hematoxylin. To confirm the specificity of the primary antibody, negative control slides were run using a negative control reagent for the same incubation time as the primary antibody. Slides were dehydrated, coverslipped, and evaluated by light microscopy.

Western blot analysis. Standard immunoblot techniques were utilized to determine cyclooxygenase protein levels. Confluent monolayers in 60-mm-diameter plates were shifted to medium that contained 1% FBS for 16 h before initiation of experimental treatments. After incubation with test compounds, plates were rinsed with PBS and harvested in a buffer that contained 15 mM 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate, 1 mM EDTA, 20 mM Tris, 10 μg/ml soybean trypsin inhibitor, 5 μl/ml Nonidet P-40, and 10 μM phenylmethylsulfonyl fluoride. Lysates were taken up in Laemmli buffer and subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and the separated proteins were transferred to a polyviniledene difluoride membrane (Millipore, Bedford, MA). The membrane was incubated with monoclonal anti-human PGHS-1 or -2 antibodies at RT for 2 h, washed, reincubated with peroxidase-labeled secondary antibodies for 2 h at RT, and rewashed. The ECL-Plus system (Amersham Pharmacia Biotech, Piscataway, NJ) was then used to generate the specific signals, which were captured on X-OMAT film (Kodak, Rochester, NY) and quantitated densitometrically with a BioImage (Milligen, Ann Arbor, MI) scanner.

Northern blot analysis. To determine the steady-state levels of PGHS mRNAs, confluent thyrocyte monolayers in 100-mm-diameter plates were shifted to medium supplemented with 1% FBS for 16 h before experimental manipulation. Plates were then rinsed, and cellular RNA was extracted, precipitated, and solubilized in diethyl pyrocarbonate-treated water using the RNA isolation system (ULTRASPEC; Biotecx, Houston, TX) as we have described previously (25). Equal amounts of RNA (usually 30 μg) were electrophoresed in 1% agarose (GIBCO BRL)-formaldehyde gels and transferred to Zetaprobe membrane (Bio-Rad). Before transfer, ethidium bromide staining was performed to verify the integrity of the electrophoresed RNA. ^{32}P-labeled PGHS-1 and PGHS-2 cDNA probes were generated using a random primer labeling kit (Bio-Rad). The membrane was incubated with the probe in a hybridization solution [20× SSC (standard sodium citrate), 5× Denhardt's solution, 50% formamide, 1 M phosphate buffer, 10% SDS, and 0.25 mg/ml

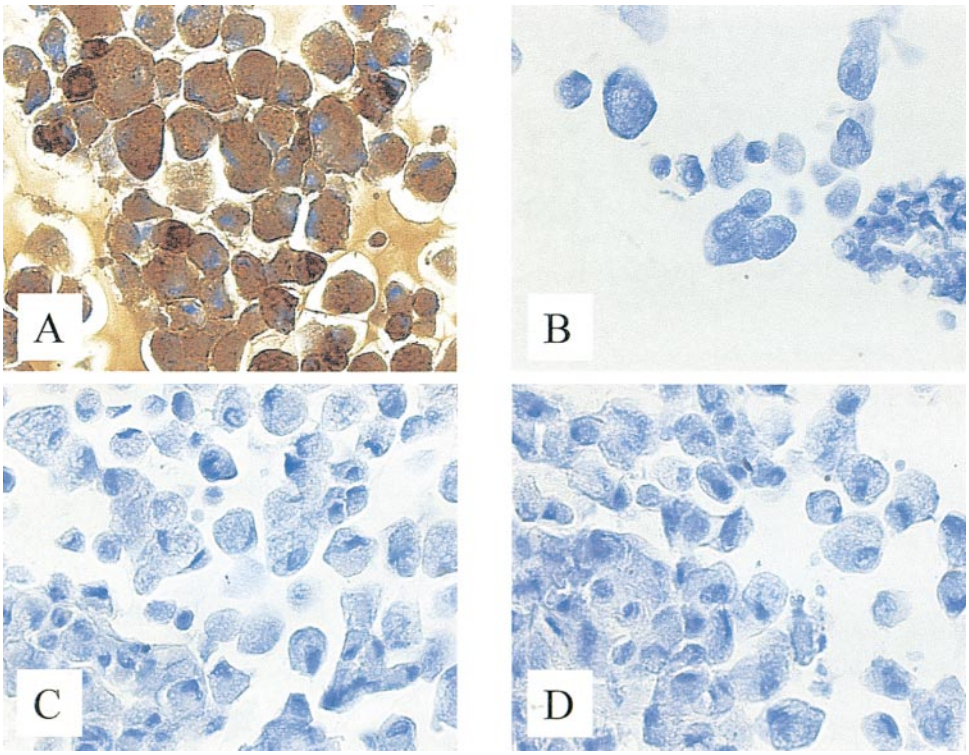

Fig. 1. Thyrocyte immunohistochemical staining. Thyrocytes were cultured as described in MATERIALS AND METHODS. They were then trypsinized, collected, and stained with a primary antibody followed by a biotinylated secondary antibody. The cells, in this case from a patient with Hashimoto's thyroiditis (*A*), demonstrate strong, diffuse cytoplasmic immunoreactivity for cytokeratin. *B*: negative control. *C*: cells do not stain for CD31. *D*: fewer than 5% of cells stain for actin.

sheared, denatured salmon sperm DNA (Amresco, Solon, OH)] at 42°C overnight. After high-stringency washing, membranes were exposed to X-OMAT AR film at −20°C. To normalize the amount of RNA transferred, the membranes were stripped according to the manufacturer's guidelines and rehybridized with a radiolabled human glyceraldehyde-3-phosphate dehydrogenase probe.

PGE_2 assay. PGE_2 levels were determined using a radioimmunoassay (Amersham Pharmacia Biotech). Confluent monolayers in 24-well plates were shifted to 1% FBS for 16 h before experimental manipulations. All treatments were performed in triplicate culture wells. Half an hour before culture harvest, medium was removed and replaced with PBS supplemented with the respective test compounds. PBS was removed and subjected to PGE_2 assay following the manufacturer's instructions. Data are expressed as means ± SE of PGE_2 levels from triplicate cultures.

RESULTS

Primary thyrocyte immunohistochemical analysis. The strategy employed to isolate primary thyrocytes yielded cells of a consistent morphology that were capable of replication in culture. A typical preparation is shown in Fig. 1. We next determined whether the cultures of primary thyrocytes contained other cell types. Cells were stained as described in MATERIALS AND METHODS. As is evident in the photomicrograph contained in Fig. 1*A*, the primary thyrocytes, in this case from a patient with Hashimoto's thyroiditis, stained strongly with antibodies against the epithelial cell marker, cytokeratin. Conversely, the cells failed to exhibit immunoreactivity for the endothelial cell marker, CD31 (Fig. 1*C*). Less than 5% of cells stained for muscle-specific actin (Fig. 1*D*), likely signifying the rare presence of myofibroblasts. These cells could be passaged several times and retained the phenotype that we observed immediately following their isolation from the tissue. Moreover, the growth patterns were invariant with regard to whether they derived from normal or diseased thyroid tissue.

PGHS-1 and PGHS-2 mRNA expression in cultured primary thyroid follicular cells and KAT-50 cells. Near-confluent monolayers of primary thyrocytes were examined by Northern blot analysis for the expression of PGHS-1 and PGHS-2 mRNAs. Both transcripts could be detected under basal culture conditions in these cells (Fig. 2). This constitutive expression of mRNAs for both isoforms is consistent with our findings in KAT-50 cells (25). PGHS-1 mRNA levels were unaffected by the addition of IL-1β (10 ng/ml) or dexamethasone (10 μM) to the medium for 6 h. In contrast, the levels of PGHS-2 mRNA were upregulated dramatically by IL-1β. Cytokine treatment for 6 h resulted in a 24-fold increase in the levels of this transcript, which migrated as a 5.0-kb band, in primary thyrocytes. The induction of PGHS-2 mRNA contrasts with our findings in KAT-50 cells, where IL-1β downregulates the high constitutive levels of expression (25). Dexamethasone failed to influence the basal PGHS-2 mRNA levels when added alone. When the steroid was added in combination with IL-1β, the cytokine's induction of PGHS-2 mRNA was attenuated by >50% (Fig. 2). In KAT-50 cells, dexamethasone diminished significantly the basal PGHS-2 mRNA levels (25).

PGHS-1 and PGHS-2 protein expression in cultured primary thyroid follicular cells and KAT-50 cells. We next examined whether the pattern of PGHS mRNA expression observed in thyrocytes corresponded with

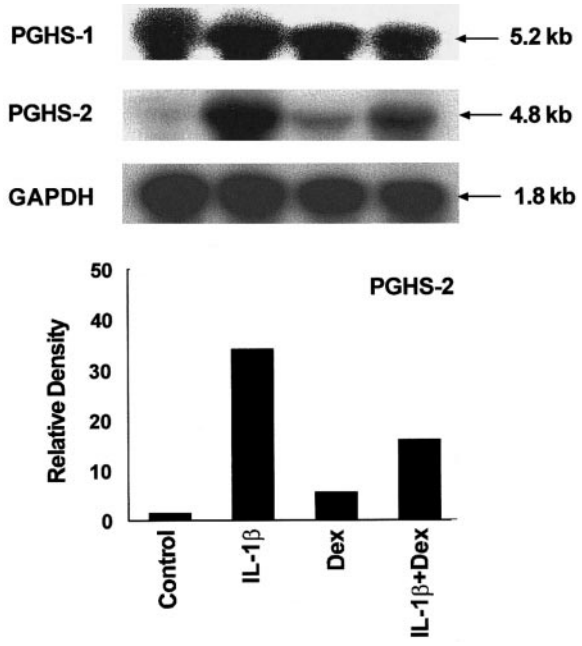

Fig. 2. Effects of interleukin (IL)-1β and dexamethasone (Dex) on the expression of prostaglandin endoperoxide H synthase (PGHS)-1 and PGHS-2 mRNA levels in primary thyrocytes. Thyrocytes, in this case from a patient with multinodular goiter, were allowed to proliferate to near confluence in RPMI medium containing 10% fetal bovine serum (FBS). They were then shifted to medium with 1% serum for 16 h and then nothing (control), IL-1β (10 ng/ml), or dexamethasone (10 μM) alone or in combination with IL-1β for 6 h. Monolayers were rinsed and cellular RNA was extracted as described in MATERIALS AND METHODS and subjected to Northern hybridization with cDNA probes for PGHS-1 and PGHS-2. RNA/DNA hybrids were analyzed with autoradiography, and the membranes were stripped and rehybridized with a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe. *Bottom*: the PGHS-2 signals normalized to the respective GAPDH densities.

that of the encoded cyclooxygenase proteins. Near-confluent monolayers were shifted to medium supplemented with 1% FBS without or with the test compounds. Cell proteins were subjected to SDS-PAGE and examined by Western blot analysis for expression of PGHS-1 and PGHS-2. We found that KAT-50 cells fail to express PGHS-1 protein under any culture conditions, despite high levels of PGHS-1 mRNA (25). In contrast, some of the primary thyrocytes express abundant PGHS-1 protein under these same basal culture conditions (Fig. 3*A*). Moreover, these levels are unaltered by IL-1β (10 ng/ml) or dexamethasone (10 μM).

Primary thyrocytes express PGHS-2 protein under basal culture conditions (Figs. 3 and 4). The levels of expression are considerably lower than those observed in KAT-50 cells (Figs. 3*C* and 4). When thyrocytes are treated with IL-1β (10 ng/ml), the levels of PGHS-2 are induced dramatically. Adding the cytokine in combination with dexamethasone (10 μM) resulted in a markedly attenuated IL-1β induction of PGHS-2. The steroid had little effect on constitutive PGHS-2 protein expression in these thyrocytes (Fig. 3*A*). The induction of PGHS-2 protein by IL-1β was time dependent. As can be seen in Fig. 3*B*, by 6 h of cytokine addition to the medium, there is a 100-fold increase in PGHS-2 expression that is maintained for at least 48 h, the duration of the study. In contrast, IL-1β transiently attenuated constitutive PGHS-2 expression in KAT-50 cells (Fig. 3*C*). The levels were decreased by 70% after

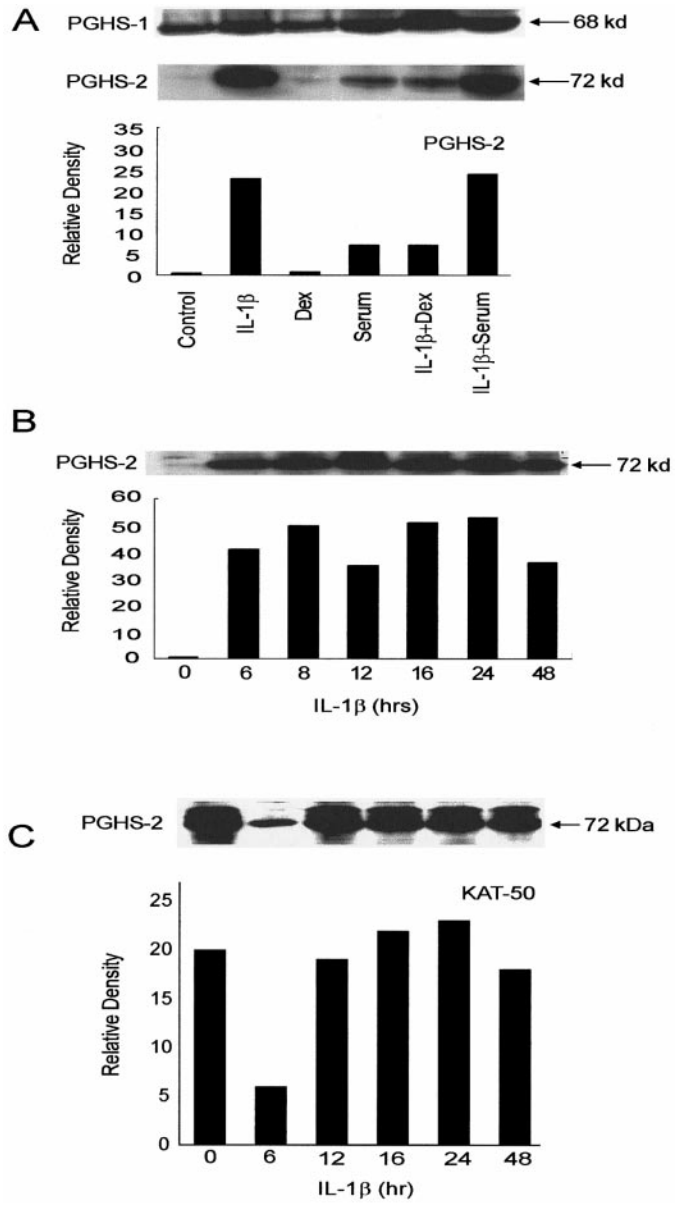

Fig. 3. Effects of IL-1β, dexamethasone, and 10% FBS on PGHS-1 and PGHS-2 protein expression in primary thyrocytes. Near-confluent monolayers were shifted to RPMI medium containing 1% FBS for 16 h. After incubation with the test compounds indicated, they were washed, and cell proteins were solubilized, subjected to SDS-PAGE, and examined by Western blot analysis for expression of PGHS-1 and -2 protein. Signals were generated using the enhanced chemiluminescence (ECL) system and captured on film, and the resulting band intensities were determined by scanning densitometry. *A*: thyrocytes were derived from a patient with Graves' disease and treated for 12 h with IL-1β (10 ng/ml), dexamethasone (10 μM), or 10% FBS alone or in combination. *B*: primary thyrocytes derived from a patient with Graves' disease were treated with IL-1β (10 ng/ml) for specified periods up to 48 h. *C*: KAT-50 cells treated with IL-1β (10 ng/ml) results in transient downregulation of constitutive PGHS-2 expression.

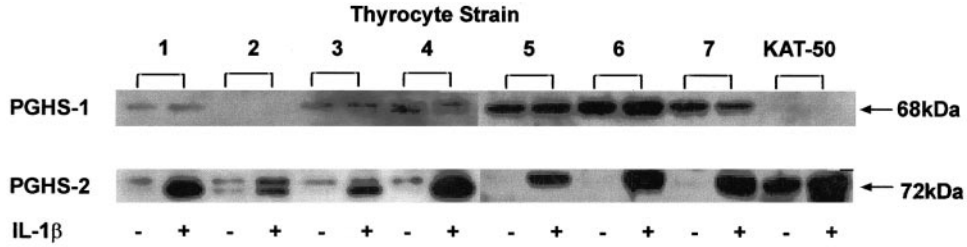

Fig. 4. Survey of basal and IL-1β-stimulated PGHS-1 and PGHS-2 expression in primary thyrocytes and KAT-50. Near-confluent monolayers were shifted to RPMI medium that contained 1% FBS for 16 h. After treatment with IL-1β (10 ng/ml) for 12 h, monolayers were washed, and cell proteins were solubilized, subjected to SDS-PAGE, and examined by Western blot analysis for expression of PGHS-1 and PGHS-2 protein. Signals were generated using the ECL system and captured on X-ray film. *Strains 1*, *2*, and *7* are thyrocytes derived from 3 patients with multinodular goiter. *Strain 3* was from normal appearing tissue in a patient with papillary cancer. *Strain 4* was from a patient with Hashimoto's thyroiditis. *Strains 5* and *6* were derived from 2 patients with Graves' disease.

6 h of cytokine treatment but returned to basal values by 12 h.

We next examined multiple thyrocyte strains derived from individuals with a variety of thyroid diseases. As the Western analysis in Fig. 4 indicates, PGHS-2 protein is expressed constitutively in all strains examined. The levels of expression varied widely but were uniformly lower than those found in untreated KAT-50. When treated with IL-1β (10 ng/ml) for 12 h, PGHS-2 levels were induced in all strains examined, but the magnitude of increase varied widely. When the cell lysates from the same strains were examined for PGHS-1 protein levels, most exhibited easily detectable signals that were invariant with cytokine treatment. Of considerable interest was *strain 2*, in which there was no detectable PGHS-1 protein. *Strain 1* expressed very low levels of PGHS-1. Interestingly, both cultures were from individuals with multinodular goiter. Thus the expression of PGHS-1 protein appears highly variable in different strains of primary thyrocytes.

PGE_2 synthesis in cultured primary thyroid follicular cells and KAT-50 cells. The distinct profiles of PGHS expression observed in primary thyrocytes and KAT-50 cells suggest that the synthesis of prostanoids in these two cell types might differ. Specifically, the PGHS isoform responsible for most of the PGE_2 production under basal and cytokine-treated conditions might differ in primary thyrocytes and KAT-50 cells. We evaluated the production of PGE_2 by quantitating its release from these cells into the culture medium under basal and cytokine-treated conditions. Primary thyrocytes produce modest levels of PGE_2 under unprovoked culture conditions, similar to those found in KAT-50 cells. Addition of exogenous arachidonate resulted in a concentration-dependent increase in PGE_2 release from the thyrocytes (Fig. 5). At a concentration of 0.625 μM, PGE_2 levels were increased fourfold over controls, while at 2.5 μM, prostanoid levels increased 14-fold above controls. Maximal PGE_2 production occurred with a concentration of 5 μM where levels were 50-fold above basal. Indomethacin (5 μM), a nonselective PGHS inhibitor, inhibited PGE_2 production nearly completely in the thyrocytes (Fig. 6). IL-1β (10 ng/ml) exerted a substantial upregulatory effect on PGE_2 production, both in the absence and presence of exogenous arachidonate (Fig. 6). SC-58125 (5 μM), a PGHS-2-selective inhibitor, reduced basal and IL-1B-induced PGE_2 synthesis by ~50% (Fig. 6). These results with SC-58125 suggest that PGHS-2 contributes substantially to both basal and cytokine-provoked PGE_2 production in thyrocytes. Dexamethasone did not appear to affect PGE_2 synthesis, consistent with our findings concerning PGHS-2 levels. Both PGHS inhibitors exerted equivalent blockade of PGE_2 production in primary thyrocytes and KAT-50 cells (Fig. 6*C*).

We next compared the PGE_2 synthesis profiles in two strains of thyrocytes, one with undetectable PGHS-1 expression and the other with high basal levels of the cyclooxygenase. As Fig. 7 suggests, *strain 2*, the thyrocytes with undetectable PGHS-1, also exhibited a considerably lower basal PGE_2 production than *strain 5*, which expresses PGHS-1. PGE_2 production increased eightfold in *strain 2* with IL-1β treatment for 16 h. This was a considerably smaller fractional increase than that in *strain 5*. Of particular interest, SC-58125 inhibited basal PGE_2 to below the levels of detection in

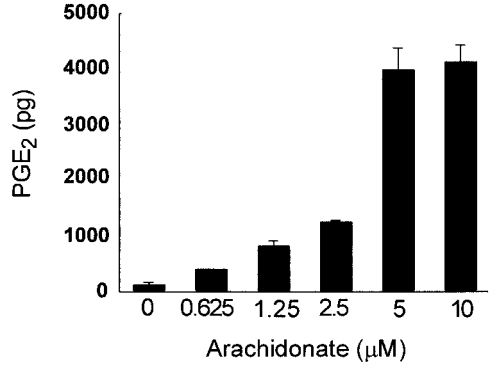

Fig. 5. Exogenous arachidonate increases prostaglandin E_2 (PGE_2) production in primary thyrocytes. Thyrocytes, in this case from a patient with multinodular goiter, were seeded in 24-well plates and allowed to proliferate to near confluence. They were shifted to medium that contained 1% FBS for 16 h and treated with arachidonate at the concentrations indicated for 16 h. For the final 30 min, medium was removed, and PBS containing the respective concentrations of arachidonate was added. PBS was collected and subjected to radioimmunoassay for PGE_2 as described in MATERIALS AND METHODS. Data are expressed as means ± SE from triplicate wells.

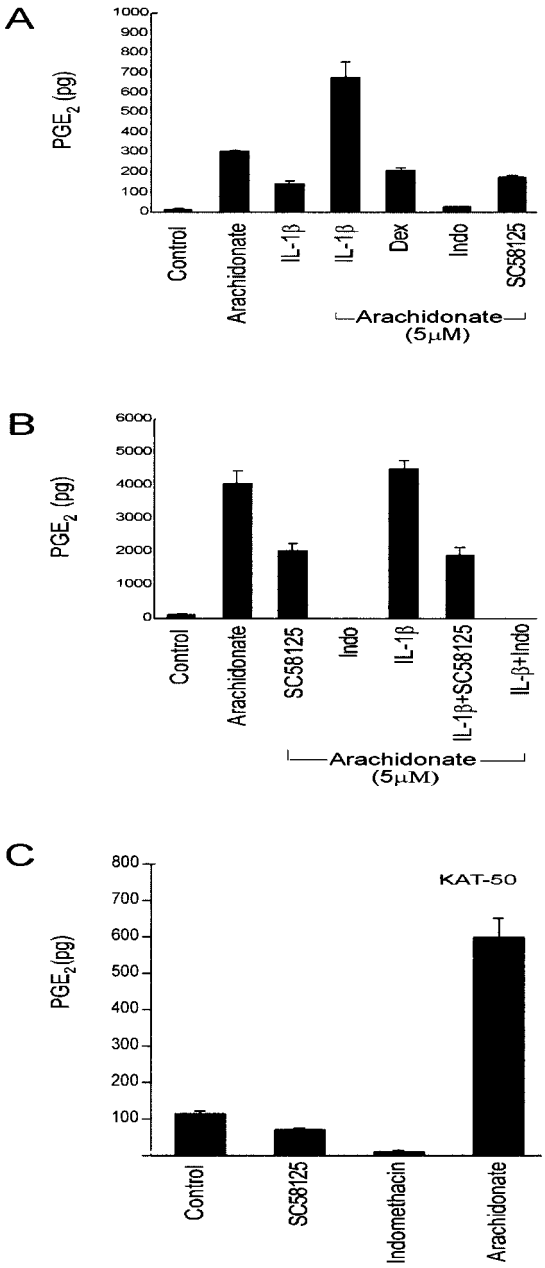

Fig. 6. Thyrocytes treated with IL-1β produce increased amounts of PGE_2. This synthesis can be inhibited by SC-58125. Thyrocytes were seeded in 24-well plates and allowed to proliferate to near confluence. They were shifted to medium with 1% FBS for 16 h and treated with the test compounds indicated for 16 h. PGE_2 levels were determined as described in the legend to Fig. 5. Thyrocytes obtained from a patient with Graves' disease (*A*) and thyrocytes from a patient with multinodular goiter (*B*) were treated with arachidonate (5 μM) or IL-1β (10 ng/ml) alone or in combination with dexamethasone (10 μM), indomethacin (5 μM), and SC-58125 (5 μM). *C*: KAT-50 cells were treated with arachidonate (5 μM), indomethacin (5 μM), or SC-58125 (5 μM) for 16 h.

strain 2, whereas production in *strain 5* was only partially inhibited. The IL-1β-provoked increase in synthesis exhibited by *strain 2* could be inhibited ~50% by SC-58125, leaving a substantial level of residual prostanoid generation. This result suggests some contribution to PGE_2 synthesis from PGHS-1 following cytokine treatment despite the failure to detect the isoform.

DISCUSSION

Cyclooxygenases and their products play essential roles in normal cellular physiology as well as in the mediation of inflammatory states. PGHS-2 was originally characterized as an oncogene-responsive enzyme (11), and significant PGHS-2 overexpression has been observed in colorectal cancers, squamous carcinomas, and other solid tumors (7, 14, 15). In cultured colonic epithelial cells, overexpression of PGHS-2 has been linked to reduced apoptosis and increased cell life span, theoretically leading to enhanced tissue growth potential (28, 33, 36). Nonsteroidal anti-inflammatory agents such as indomethacin and SC-58125 reversed these effects (8, 31). In animal studies, mice with a genetic susceptibility to colonic neoplasia exhibited a decreased propensity for polyp development after treatment with cyclooxygenase inhibitors (17, 22). In addition, epidemiological studies have shown a diminished incidence of colonic neoplasia associated with chronic ingestion of aspirin and related compounds (10, 30). A question emerging from the current studies is the potential for involvement of PGHS-2 in the development of thyroid neoplasia. This has not been assessed and remains an intriguing question.

A role for PGHS-2 in normal thyroid function has been suggested by earlier studies performed in the rat thyroid cell line FRTL-5 (29). These demonstrated that thyrotropin may upregulate arachidonate release and

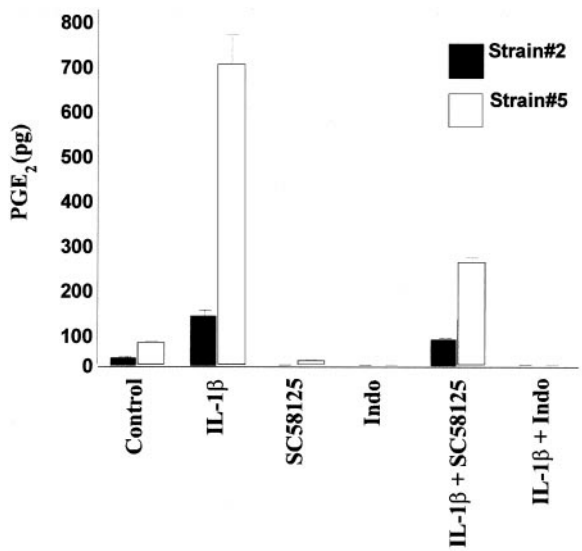

Fig. 7. Levels of basal and cytokine-stimulated PGE_2 synthesis differ among thyrocyte strains. *Strain 2* is derived from a patient with multinodular goiter and not expressing detectable PGHS-1 protein. *Strain 5*, derived from a patient with Graves' disease, is characterized by high basal PGHS-1 protein expression. Thyrocytes were seeded in 24-well plates and allowed to proliferate to near confluence in medium with 10% FBS. They were shifted to medium with 1% FBS for 16 h and treated with IL-1β (10 ng/ml), SC-58125 (5 μM), or indomethacin (10 μM) as indicated for 16 h. All wells were treated with arachidonate (1 μM). PGE_2 levels were determined as described in the legend to Fig. 5.

cyclooxygenase activity. In addition, thyroid-stimulating immunoglobulins have been shown to enhance FRTL-5 cell proliferation in vitro through increases in PLA_2 activity and arachidonate release (4). These effects on cell division proved susceptible to inhibition by indomethacin (4). Similarly, IgGs from individuals with Graves' disease have been shown to stimulate PLA_2 activity in cultured primary human thyrocytes (2). These observations imply that the prostanoid biosynthetic pathways might be involved in normal and disease-related thyroid epithelial cell growth and function.

The constitutive expression of PGHS-2 mRNA and protein exhibited by untreated primary thyrocytes is consistent with our earlier observations in KAT-50 cells (25). Moreover, they are entirely congruent with the PGHS-2 protein detected in situ in normal thyroid epithelium (25). Both primary thyroid and KAT-50 cells express PGHS-2 protein and mRNA constitutively, but the levels of this isoform appear to be considerably higher in KAT-50. This difference may reflect a compensatory mechanism by which cells can adapt to an apparent absence of PGHS-1 protein (25), where PGHS-2 may represent the only functional cyclooxygenase. Furthermore, regulation of PGHS-2 appears to differ substantially in the two types of cells. In KAT-50, PGHS-2 mRNA (25) and protein expression are downregulated by IL-1β, while the cytokine upregulates the cyclooxygenase in primary thyroid cells. Although the molecular basis for the differential impact of IL-1β in these two cell types remains uncertain, these findings underscore the cell type-specific nature of PGHS-2 gene regulation. It is unlikely that the constitutive expression of PGHS-2 is a consequence of the low serum concentration (1%) present in the culture medium, although some small impact on cyclooxygenase levels cannot be excluded. This level of serum failed to induce PGHS-2 in fibroblasts (32) and actually lowered its expression in KAT-50 cells (25).

PGHS-1 is expressed by most cell types and is generally unaffected by regulatory factors that influence PGHS-2 (27). PGHS-1 can be regulated modestly in certain situations, such as in cultured endothelial cells, where phorbol esters enhance PGHS-1 gene transcription (34). Most primary thyrocytes, unlike KAT-50, also express PGHS-1 protein. However, one strain of primary thyrocytes lacked constitutive PGHS-1 expression and a second exhibited a basal PGHS-1 level considerably lower than that found in the other strains. These two strains were derived from patients with multinodular goiter. A third strain, also derived from a multinodular gland, expressed high basal levels of PGHS-1 as did all other strains examined. Furthermore, the strain that lacked constitutive PGHS-1 expression appeared to express high basal levels of PGHS-2. The variability in PGHS-1 and -2 expression in these thyrocyte strains may signify a complementary role for the two isoforms. This is exemplified in KAT-50 where the absence of PGHS-1 protein is accompanied by high levels of PGHS-2 expression. These differences in PGHS profile expression could result from adaptive changes from cultivation in culture. What emerges from the current studies is the uncertain relationship that the two PGHS isoforms share in thyrocytes as well as in the other cell types expressing both cyclooxygenases constitutively.

Primary thyrocytes exhibit substantial basal prostaglandin production when provided exogenous arachidonate. In the absence of exogenous arachidonate, PGE_2 production is modest. This suggests that arachidonate availability may be rate limiting, at least in certain situations. Clearly, details concerning the levels of PLA_2 expression and activity in these cells might provide insight into the mechanisms regulating eicosanoid production. Both basal and IL-1β-dependent PGE_2 synthesis in primary thyrocytes can be blocked in part by SC-58125. This finding implies an important contribution of PGHS-2 activity to prostanoid generation in resting and cytokine-activated thyrocytes.

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