Prostaglandin-endoperoxide H Synthase-2 Expression in Human Thyroid Epithelium

EVIDENCE FOR CONSTITUTIVE EXPRESSION IN VIVO AND IN CULTURED KAT-50 CELLS

Terry J. Smith‡‡, Timothy A. Jennings‡, Daniela Sciaky‡, and H. James Cao‡

From the ‡Division of Molecular and Cellular Medicine, Department of Medicine, the §Department of Biochemistry and Molecular Biology, and the ¶Department of Pathology, Albany Medical College and the Samuel S. Stratton Veterans Affairs Medical Center, Albany, New York 12208

Prostaglandin-endoperoxide H synthase (PGHS) (EC 1.14.99.1) expression was examined in human thyroid tissue and in KAT-50, a well differentiated human thyroid epithelial cell line. PGHS-1 is found constitutively expressed in most healthy tissues, whereas PGHS-2 is highly inducible and currently thought to be expressed, with few exceptions, only in diseased tissues. Surprisingly, PGHS-2 mRNA and protein were easily detected in normal thyroid tissue. KAT-50 cells express high levels of constitutive PGHS-2 mRNA and protein under basal culture conditions. Compounds usually associated with PGHS-2 induction, including interleukin-1β (IL-1β), phorbol 12-myristate 13-acetate, and serum transiently down-regulated PGHS-2 expression. Human PGHS-2 promoter constructs (−1840/+123 and −831/+123) fused to a luciferase reporter and transfected into untreated KAT-50 cells exhibited substantial activity. NS-398, a highly selective inhibitor of PGHS-2 could inhibit substantial basal prostaglandin E₂ production. Exogenous IL-1 receptor antagonist or IL-1α neutralizing antibodies could attenuate constitutive PGHS-2 expression in KAT-50 cells, suggesting that endogenous IL-1α synthesis was driving PGHS-2 expression. Our findings suggest that normal thyroid epithelium expresses high constitutive levels of PGHS-2 in situ and in vitro and this enzyme is active in the generation of prostaglandin E₂. Thus, unprovoked PGHS-2 expression might be considerably more widespread in healthy tissues than is currently believed.

The human thyroid is a frequent site for the occurrence of inflammatory and neoplastic disease. Much of the inflammation found in this gland appears to be autoimmune, and the common processes include Graves’ disease and Hashimoto’s thyroiditis (1). Thyroid immunity has been the subject of substantial investigation because of the high incidence of these autoimmune diseases in the general population. Despite the particular susceptibility exhibited by thyroid to inflammation, little insight currently exists into the molecular basis for the peculiarities associated with thyroidal immunity. The currently undefined role of arachidonate metabolites in the normal regulation of thyroid growth and function, and in the mediation of thyroid disease, is of potential importance.

Cyclooxygenases, also known as prostaglandin-endoperoxide H synthases (PGHSs) (EC 1.14.99.1) are a family of two bi-functional enzymes that catalyze the conversion of arachidonate to prostaglandins (2). PGHSs are heavily glycosylated and contain heme prosthetic groups. They are membrane-associated, rate-limiting enzymes, each with two discrete, active sites catalyzing both cyclooxygenase and peroxidase steps in the biosynthesis of prostaglandins. Prostaglandin G₂ is generated from arachidonate, and then it is converted to prostaglandin H₂. The two PGHS isoforms are encoded by distinct human genes and localize to different human chromosomes, and yet they have protein x-ray crystallographic structures that are remarkably similar (3). PGHS-1 is a constitutively expressed enzyme that is found at high levels of abundance in most tissues and cells in culture (4–6). The levels of PGHS-1 expression are relatively invariant with respect to tissue involvement in disease or cytokine and mitogen action. It is the enzymatic activity attributable to PGHS-1 that is currently thought to contribute predominantly to basal PGE₂ production in healthy tissues and to maintain the integrity of renal and enteric epithelium (2). In contrast, PGHS-2, the inflammatory cyclooxygenase, is ordinarily not expressed in most tissues in states of health but can be massively up-regulated by cytokines, growth factors, tumor promoters, and serum in many cell types (7–15). PGHS-2 has been demonstrated in situ in inflamed tissues (16). The pattern of cellular distribution of PGHS-1 and -2 differs (17), suggesting that the two enzymes might utilize discrete pools of arachidonate and participate in different metabolic pathways. The question of why two distinct but very similar enzymes might be co-expressed by the same cell has not been answered, but PGHS-1 and -2 are thought to function, at least in part, independently (2). PGE₂ production associated with the inflammatory response is currently believed to emanate primarily from the activity of PGHS-2. Moreover, the decrease observed in prostaglandin production in vivo and in vitro following glucocorticoid treatment is attributable to the down-regulation of PGHS-2 expression (9, 18).

Constitutive expression of PGHS-2 has been observed in a very few tissues and cell types. For instance, in rat brain, the greatest constitutive PGHS-2 expression is associated with the hippocampus, the pyramidal cells of the piriform cortex,
the amygdala, and neurons in the neocortex (19, 20). The macula densa of the kidney was found to express substantial levels of PGHS-2 under physiological conditions (21). Unprovoked PGHS-2 has also been detected in bronchial epithelium (22) and in granulosa (23), pancreatic islet (24), and hepatic stellate cells (25). The function of PGHS-2 expressed in non-pathological states is uncertain but opens the possibility that the biological role of this cyclooxygenase isofrom is not limited to the mediation of inflammatory responses. Thus, the current categorization of cyclooxygenases as representing a purely housekeeping enzyme (PGHS-1) on the one hand, and a protein expressed exclusively in disease (PGHS-2) on the other, may fail to embrace the apparent complexities of the prostanoid biosynthetic machinery.

Very little is currently known about the expression of PGHS isofroms or the production of prostanoids and other eicosanoids in the human thyroid. The biosynthesis and action of lipid mediators in thyroid tissue may be complex because multiple cell types normally reside or are recruited there in states of disease. We have reported recently that human thyroid fibroblasts can synthesize PGE2 and that the production of this prostanoid is up-regulated by cytokines such as IL-1β (26). Moreover, we have postulated that fibroblasts, by virtue of their diverse array of small molecule expression, represent potentially important orchestrators of the early events in tissue remodeling (27). With regard to thyroid epithelial cells, recent studies have suggested that the proliferation-promoting actions of thyroid-stimulating immunoglobulins, as assessed in the FRTL-5 rat thyrocyte line, are mediated through activation of phospholipase A2 and arachidonate release and can be attenuated with indomethacin, a nonselective cyclooxygenase inhibitor (28, 29). Although the results of these studies imply that cyclooxygenase products are involved in the growth-stimulating effects of thyroid-stimulating immunoglobulin on rat thyrocytes in vitro, the expression of specific PGHS isofroms has not been examined previously.

In the present study, we assessed the expression of both PGHS-1 and -2 proteins by immunohistochemical means in situ in thin tissue sections from a wide spectrum of thyroid disease. We report here the unexpectedly high level of PGHS-2 protein expression in thyroid follicular epithelium, both in normal tissue and in that involved in or adjacent to disease. In addition, PGHS expression and activity were assessed in an established, well differentiated human thyrocyte cell line, KAT-50 (30).

Surprisingly, KAT-50 cells express very high constitutive levels of PGHS-2 mRNA and protein in culture and produce PGE2. A substantial fraction of the PGE2 generating activity in untreated KAT-50 cells can be attenuated with dexamethasone or NS-398, a highly selective inhibitor of PGHS-2 (31). Thus, we present compelling evidence that thyroid epithelium expresses PGHS-2 at high levels both in vivo and in vitro and that this expression is constitutive and thus may play an important role in normal thyroid function and immunity, as well as in the pathogenesis of disease.

EXPERIMENTAL PROCEDURES

Materials—NS-398 as well as anti-PGHS-1 and PGHS-2 monoclonal antibodies were purchased from Cayman Chemical Co. (Ann Arbor, MI). IL-1β was obtained from BIOSOURCE (Camarillo, CA), and dexamethasone (1,4-pregnen-9-fluoro-16a-methyl-11β,17β,21-triol-3,20-dione), phorbol 12-myristate 13-acetate (PMA), and cycloheximide were from Sigma. Human PGHS-1 and PGHS-2 cDNA plasmids were gifts from Donald Young (University of Rochester, Rochester, NY). Plasmids containing fragments of the human PGHS-2 promoter were generously provided by Dr. Stephen M. Prescott (University of Utah, Salt Lake City, UT). Plasmid −1800pGL2 contained −1840/+123, and plasmid −800pGL2 contained the sequence −831/+123. pSV-b-galactosidase was a gift of Dr. Brian Wilcox (Albany Medical College) and was used as a transfection efficiency control. pGL2 Basic and pGL2 Promoter were purchased from Promega (Madison, WI). Anti-IL-1α and IL-1β antibodies were from R&D Systems (Minneapolis, MN), and IL-1 receptor antagonist (IL-1ra) was a gift from Amgen (Boulder, CO). Luciferase and b-galactosidase were measured with a Dual-Light kit from Tropix. Human thyroid, adrenal, and fetal connective tissues were obtained from the University of Rochester. These activities were approved by the Institutional Review Board of the Albany Medical College.

Immunohistochemistry—Thyroid tissue obtained from reactive and neoplastic diseases (37 total) was examined for the expression of PGHS-2 protein. These cases included nodular hyperplasia (8 cases), chronic thyroiditis (9 cases), diffuse goiter (9 cases), Graves’ disease (5 cases), follicular adenoma (8 cases), follicular carcinoma (3 cases), papillary carcinoma (3 cases), and medullary carcinoma (5 cases). In addition, at least one case from each of these categories was also stained for PGHS-1. Five-μm-thick sections were cut from formalin-fixed thyroid tissue that had been embedded in paraffin and were applied to glass slides. After routine deparaffinization and rehydration, tissue sections were incubated in APW wash solution (Ventana Medical Systems, Tuscon, AZ). Sections were placed in the reaction chamber of a Ventana ES automated immunohistochemistry system, which uses an indirect biotin-avidin detection method. Endogenous peroxidase activity was blocked with 1% H2O2. Sections were then incubated with primary anti-human PGHS-1 and PGHS-2 antibodies (Cayman) at a dilution of 1:200 for 2 h at room temperature. Following washing, sections were incubated with a biotinylated secondary anti-mouse antibody, supplied by Ventana, for 8 min at 41 °C. Isotype-matched antibodies were used as a negative control.

Cell Culture—KAT-50 cells were a generous gift from Dr. K. Ain, University of Kentucky (Lexington, KY) (30). They were maintained in a humidified, 5% CO2 incubator at 37 °C covered with Eagle’s medium (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS) and antibiotics. These cells have been characterized as expressing, among other thyroid markers, thyroglobulin mRNA and the sodium/iodine symporter, and were used between the 20th and 35th passage. Medium was changed every 3–4 days. Human orbital fibroblasts were obtained from explants of surgical waste emanating from individuals with severe thyroid-associated ophthalmopathy. Fibroblasts were cultivated as described previously (32) in Eagle’s medium supplemented with 10% FBS, glutamine, and antibiotics. They were used between the 2nd and 12th passages.

Western Blot Analysis of PGHS Protein Expression—Relative levels of PGHS-1 and -2 proteins in thyroid and control tissues, fibroblasts, and KAT-50 cells were determined by Western immunoblot analysis utilizing monoclonal antibodies generated against human PGHS-1 and PGHS-2 and obtained from Cayman. Confluent cultures, usually cultivated in 60-mm-diameter plates, were shifted to 1% FBS for 48 h. Monolayers were washed and harvested, and cellular protein was solubilized in an ice-cold buffer containing 1% CHAPS, 1 mM EDTA, 20 mM HEPES (pH 7.5), 10 μg/ml soybean trypsin inhibitor 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 polyvinylidene difluoride membrane (Bio-Rad). The primary antibodies (10 μg/ml) were incubated with the membranes for 2 h at room temperature and the membranes were then washed extensively and reincubated with secondary, peroxidase-labeled antibodies for 2 h. Following washes, the ECL (Amersham Pharmacia Biotech) detection system was used to generate the specific signals. Resulting bands were quantitated densitometrically with a BioImage (Millegen) scanner.

Northern Blot Analysis of PGHS-encoding mRNAs—Total cellular RNA was extracted using a method published by Chomczynski and Sacchi (33) from thyroid and control tissues or from near-confluent 100-mm-diameter plastic plates of KAT-50 cells that had been incubated without or with the test compounds indicated in the figure legends. Following extensive rinsing with PBS, monolayers were covered with a solution containing guanidium isothiocyanate (ULTRASPEC RNA isolation systems, Biotec Laboratories, Houston, TX), and RNA was precipitated from the aqueous phase by addition of isopropanol, washed with 75% ethanol, and solubilized in diethyl pyrocarbonate-treated water. Equal amounts of RNA (usually 10–20 μg) were electro-phoresed in 1% agarose formaldehyde gels and transferred to Zetaprobe (Bio-Rad). The integrity of the electrophoresed RNA was verified by UV inspection following ethidium bromide staining. [32P]dCTP random-primed (Bio-Rad) PGHS probes were hybridized in a buffer containing 5× SSC, 5× Denhardt’s solution, 50% formamide, 50 mM phosphate.
buffer (pH 7.2), 1% SDS, and 0.25 mg/ml sheared, denatured salmon sperm DNA at 42 °C overnight. Membranes were washed under high stringency conditions and exposed to X-OMAT AR film (Kodak, Rochester, NY) at ~70 °C. To normalize the amounts of RNA transferred, either membranes were stripped according to the manufacturer’s instructions. Plasmid DNA was rehybridized with a radiolabeled human GAPDH cDNA probe. Radioactive DNA/RNA hybrids were quantitated by subjecting autoradiographs to densitometric analysis.

For the PGHS-2 mRNA stability studies, 5,6-dichlorobenzimidazole (DRB) (20 μg/ml), an inhibitor of gene transcription, was added to the culture medium in the time intervals marked in Fig. 8, and the abundance of steady-state PGHS-2 mRNA was quantitated with Northern blot hybridization. The PGHS-2 mRNA signals were normalized to their respective GAPDH signals. Data from two separate, identical experiments were combined, and the normalized results were subjected to a polynomial curve fit program (Delta Graph) using the least-squares method to generate the curve shown in Fig. 8. To calculate the $t_1/2$ for PGHS-2 mRNA, the densitometric values were modeled assuming first order decline employing the ADAPT II program described by D’Argenio and Schumitzky (34). Those values that were undetectable were coded as midway between the limit of detection and zero in the analysis.

Transfection of PGHS-2 Promoter Plasmid Constructs into KAT-50 Cells—KAT-50 cells were allowed to proliferate to a state of 60–80% confluence, and then monolayers were incubated with 1% FBS-enriched medium for 24 h. Transfections were performed using Cellfectin (Life Technologies) following the manufacturer’s instructions. Plasmids utilized for these studies included −1800pGL2, containing −1840/−123 and −800pGL2 containing −831/−123, and are thus 5 base pairs upstream from the ATG of the human PGHS-2 promoter (35, 36). These were fused upstream of the firefly luciferase reporter gene. Control plasmid pGL2 was used to determine the basal level of luciferase activity exhibited by these cells and pGL2 Promoter to compare the strength of the PGHS-2 promoter to that of the SV40 promoter. pSV40-β-galactosidase was used as a transfection efficiency control. 10 μl of Cellfectin was added to 100 μl of serum-free medium without antibiotics, and the suspension was mixed gently with the DNA, either the test plasmid (0.36 μg) or pGL2 (0.36 μg) together with pSV-β-galactosidase (0.4 μg). Complexes were allowed to form over 15 min at room temperature before being added to the washed monolayers. Cultures were incubated for 5 h at 37 °C in a 5% CO2 atmosphere, and then the transfection mixture was removed and replaced with medium supplemented with 1% FBS. Cultures were incubated for 24 h, cell monolayers were washed in PBS, and the reporter lysis buffer (Tropix) was added for 15 min at room temperature. The extract was vortexed and frozen at −80 °C. Samples were thawed and assessed for luciferase and β-galactosidase activity in a LUMI-VETTE luminometer (CHRONO-LOG). The luciferase activities were corrected for their respective β-galactosidase levels and therefore reflect relative transfection efficiency.

PGE$_2$ Assay—PGE$_2$ levels were determined as described previously (14) using a radioimmunoassay (Amersham Pharmacia Biotech). Briefly, KAT-50 cells were inoculated in 24-well plates covered with medium supplemented with 10% FBS. One day prior to experimental manipulation, culture wells were shifted to medium with 1% FBS, and the following day, the test compounds indicated in the legend to the figure were added. Thirty min prior to monolayer harvest, medium was removed and replaced with 150 μl of PBS with the respective additives. Following the incubation, the PBS was removed quantitatively and subjected to the assay for PGE$_2$ following the manufacturer’s instructions. These studies were conducted with three separate plates per treatment group. Data are expressed as the mean ± S.E. of triplicate cultures from representative experiments.

IL-1α and IL-1β Assays—Details concerning these studies have been published previously (15). Briefly, KAT-50 cells were allowed to proliferate to near confluence in 24-well plastic culture plates covered with medium containing 10% FBS. Monolayers were then shifted to medium with 1% FBS for 16–24 h, and then test compounds were added at the times indicated in the figure legends. Medium was removed, monolayers were washed with PBS, and cellular material was harvested in a buffer containing 15 μM CHAPS, 1 mM EDTA, 20 μM Tris-HCl (pH 7.5), 10 μg/ml leupeptin, 10 μg/ml phenylmethylsulfonyl fluoride, 3 μg/ml aprotinin, and 0.5% Nonidet P-40. Cellular proteins (5 or 10 μg) were subjected to specific ELISAs for IL-1α and IL-1β using kits from Immunotech (Westbrook, ME). These assays were conducted according to the manufacturer’s instructions. Data are expressed as the mean ± S.E. of triplicate culture wells.

Statistics—Data are usually expressed as the mean ± S.E. of replicate determinations unless indicated otherwise. Statistical significance was determined by Student’s t-test.

RESULTS

PGHS-1 and PGHS-2 Protein Expression by Thyroid Follicular Epithelium in Situ—Sections of thyroid tissue, obtained from surgical thyroidecomies performed as therapy for a wide variety of diseases, were examined for PGHS protein expression by immunohistochemical staining using isoform-discriminatory monoclonal antibodies directed at either PGHS-1 or PGHS-2. As the photomicrographs in Fig. 1 demonstrate, staining with either PGHS-1 or PGHS-2 antibodies yielded very similar patterns. Both enzymes are expressed abundantly in most follicular epithelial cells in each of the cases examined. In addition, both antibodies labeled vascular media, peripheral nerve, and some lymphoid cells. In contrast, other stromal elements failed to stain with either antibody. The epithelium exhibited diffuse, largely cytoplasmic immunoreactivity, in lesional cells and normal elements adjacent to the lesions or in separate blocks of nonlesional tissue. The intensity of staining was generally moderate to strong and correlated with the cytoplasmic volume rather than the presence or absence of disease. Little or no nonspecific background staining was observed in sections stained with isotype-match controls (Fig. 1, middle left panel).

We next determined whether PGHS-2 mRNA could be detected in thyroid tissue. As the Northern blot in Fig. 24 indicates, a predominant 4.8-kb transcript is easily detected in total cellular RNA samples from normal thyroid tissue, remote from any disease process. PGHS-1 mRNA is also found in high abundance in these same samples as a single 5.2-kb band. Using Western blotting, PGHS-1 and -2 protein were also detected in thyroid tissue from three different cases. (Fig. 2B). As the figure demonstrates, normal tissue from multinodular goiter (patients 1 and 3) and Graves’ disease (patient 2) all express easily detectable levels of both isoenzymes. The levels of the enzyme in the sample from the patient with Graves’ disease are somewhat lower than that found in the normal tissue from the two multinodular glands. PGHS-1 appears as a 68-kDa protein band, whereas PGHS-2 migrates to 72 kDa, consistent with our findings in human fibroblasts (14). In contrast to the abundant PGHS-2 mRNA and protein signals observed in thyroid tissue, normal intestinal fat failed to express PGHS-2 mRNA on Northern analysis (Fig. 2C), and fat and normal striated muscle failed to exhibit PGHS-2 protein on Western blots (Fig. 2D). Thus, it would appear that both PGHS-1 and -2 mRNA and protein are abundantly expressed in normal and pathological thyroid tissue, and this expression localizes to epithelial cells.

Untreated KAT-50 Cells Express Constitutive PGHS-1 and PGHS-2 mRNA in Culture—The recent reports of human thyroid epithelial cell lines being isolated and characterized should facilitate studies concerning thyroid growth and function. One of these, KAT-50, derived from the non-neoplastic goiter of a young child, expresses thyroglobulin and the sodium-iodine symporter (30). Moreover, we have determined that these cells express high levels of thyrotropin receptor mRNA (T. J. Smith, unpublished observations). We therefore determined whether the observations made concerning PGHS-2 expression in situ (Figs. 1 and 2) could be verified in these cells. Near-confluent cultures of KAT-50 cell monolayers were analyzed by Northern blot analysis for the expression of PGHS-1 and PGHS-2 mRNAs. Cultures were shifted to medium supplemented with 1% FBS overnight prior to any experimental manipulations. As the blot pictured in Fig. 3A indicates, PGHS-1 mRNA is expressed at a high steady-state level by these cells under basal culture conditions. The
size of the PGHS-1 transcript expressed by KAT-50 cells is 5.2 kb, and thus it resembles the mRNA expressed in orbital and dermal fibroblasts (14), endothelial cells (6), monocytes (37) and thyroid tissue (Fig. 2A). However, it differs from the predominant 2.8-kb species that has been demonstrated in some other human cell types (5). KAT-50 cells also appear to express high levels of PGHS-2 mRNA under untreated (basal) culture conditions. The PGHS-2 transcript appears as a single predominant band of 4.8 kb, similar to that observed in several other cell types (11–15) and in freshly obtained thyroid tissue. Because this study was conducted by shifting the KAT-50 cultures to medium with a reduced serum concentration (1% FBS), the readily detectable PGHS-2 mRNA levels were not a consequence of serum induction. Addition of dexamethasone (10 nM) to the culture medium for 6 h resulted in a substantial down-regulation in the levels of PGHS-2 mRNA but had no effect on the abundance of the transcript in KAT-50 cells incubated in medium with 1% serum. Furthermore, addition of the tumor promoter, PMA (100 ng/ml), a potent inducer of PGHS-2 in many cultured cell types, also dramatically down-regulated PGHS-2 mRNA levels to the limits of detection on Northern analysis at 6 h (Fig. 3A). Similar to that of dexamethasone, the effects of IL-1β and PMA were transient so that at 24 h, levels of the mRNA had returned to those observed in controls. We next examined whether the presence of 10% FBS in the medium would alter the effects of IL-1β, PMA, and dexamethasone on PGHS-2 mRNA expression. As the Northern blot in Fig. 3B suggests, the higher serum concentration lowered PGHS-2 mRNA levels and addition of any of these compounds failed to influence mRNA levels further.

To determine whether the high levels of PGHS-2 mRNA found to be expressed by untreated KAT-50 cells were dependent on the synthesis of an undefined protein, we assessed the impact that inhibition of ongoing protein synthesis might have on cyclooxygenase expression. Treatment of the cultures with cycloheximide (10 μg/ml), a concentration associated with a
greater than 90% inhibition of protein synthesis in human cells (38), resulted in a rapid decrease in the levels of PGHS-2 mRNA (Fig. 3A). This is evident at both 2 and 4 h after the inhibitor was added to the culture medium containing 1% FBS. Thus, it would appear that ongoing protein synthesis is necessary for the constitutive expression of PGHS-2 in KAT-50 cells. These results are at marked variance with those in cells ordinarily not expressing PGHS-2, such as fibroblasts and endothelial cells (11, 12). In earlier studies, PGHS-2 mRNA was found to be rapidly and transiently up-regulated by cycloheximide (11, 12). Thus, it would appear that ongoing protein synthesis is necessary for the constitutive expression of PGHS-2 in KAT-50 cells, whether untreated or following incubation with IL-1β. On the other hand, Fig. 4A also demonstrates that PGHS-1 protein was undetectable in the KAT-50 cells, whether untreated or following incubation with IL-1β (10 ng/ml) for 16 h, despite the very high levels of PGHS-1 mRNA expressed by these cells (Fig. 3). This isozyme can be easily detected in orbital fibroblasts as a 68-kDa band on these blots and is invariant with regard to IL-1β treatment. The absence of PGHS-1 protein expression in KAT-50 cells in vitro contrasts to the expression of this enzyme in situ in thyroid tissue (Fig. 1). Thus, these cultured cells may have escaped from factor(s) supporting PGHS-1 protein expression observed in the intact gland. When IL-1β (10 ng/ml) was added to the culture medium of nearly confluent KAT-50 cells, PGHS-2 protein expression was rapidly
IL-1 is a critical mediator of the up-regulation of PGHS-2 by CD40/monolayer harvest, some of the cultures received IL-1 shifted to medium enriched with 1% FBS for 24 h. Sixteen h before an individual with severe thyroid-associated ophthalmopathy, were of KAT-50 cells and human orbital fibroblasts, in this case from KAT-50 cells and orbital fibroblasts. A, PGHS-2 mRNA levels (Fig. 3), IL-1 with dexamethasone (10 nM) under conditions that attenuate thyroid tissue. B, PGHS-2 protein expression was compared in IL-1β (10 ng/ml)-treated orbital fibroblasts, untreated KAT-50 cells, and normal thyroid tissue.

and dramatically down-regulated (Fig. 5A). The levels were 11% compared with basal values after 1 h of IL-1β exposure and had begun to increase rapidly to 36 and 68% of control at 4 and 8 h, respectively. Serum also appears to exert a substantial inhibitory effect on PGHS-2 protein expression in KAT-50 cells (Fig. 5B), consistent with its effects on PGHS-2 mRNA levels. In a study in which FBS concentrations were varied and cultures were exposed to serum for 24 h, the highest levels of PGHS-2 were observed in cells maintained in the absence of serum. Addition of 1% serum lowered levels by 36%, and PGHS-2 expression was undetectable in the presence of 10% FBS. This finding is in striking contrast to that observed in cells of PGHS-2, where serum induces strongly the expression of PGHS-2 (11).

Constitutive PGHS-2 Expression in KAT-50 Cells Is Dependent upon the Synthesis of IL-α—We have begun to examine the factors expressed by KAT-50 that might underlie the constitutive expression of PGHS-2. Because IL-1α and IL-1β are actively synthesized widely in many cell types and are important inducers of PGHS-2 (12, 13), we determined whether endogenous IL-1 production could be related to PGHS-2 expression in KAT-50 cells. We have found very recently that IL-1α but not IL-1β expression and inducibility in human orbital fibroblasts is a critical mediator of the up-regulation of PGHS-2 by CD40/CD40 ligand engagement (15). To determine whether IL-1α and/or IL-1β are expressed in KAT-50 cells, we performed specific ELISAs for these cytokines. As the data in Fig. 6A indicate, we could readily detect IL-1α under basal culture conditions. The level in cell layers was 16.5 ± 1 pg/10 μg of protein (mean ± S.E., n = 3). When the cultures were treated with dexamethasone (10 nM) under conditions that attenuate PGHS-2 mRNA levels (Fig. 3), IL-1α was down-regulated to undetectable levels. In contrast, IL-1β could not be detected in untreated KAT-50 cells or those receiving dexamethasone (Fig. 6A). Thus, it would appear that the glucocorticoid might be influencing the levels of PGHS-2 expression through actions on IL-1α synthesis. To test further the central role of IL-1α expression in supporting constitutive PGHS-2, the effects of 10% FBS on the levels of the cytokine were examined. This concentration of FBS elicited a time-dependent and dramatic decrease in IL-1α levels so that within 1 and 2 h of serum addition to the medium, the cytokine concentration in KAT-50 cell-conditioned medium was reduced by 59 and 93%, respectively (Fig. 6A). To determine directly whether the expression of IL-1α was proximately related to the constitutive expression by KAT-50 cells of PGHS-2, neutralizing antibodies against IL-1α (1 μg/ml), IL-1β (1 μg/ml), or exogenous IL-1ra (500 ng/ml) were added to the culture medium of near-confluent KAT-50 cultures. The cells were then assessed for PGHS-2 protein expression by Western blot analysis. Anti-IL-1α antibody and IL-1ra, but not anti-IL-1β antibody, could substantially down-regulate PGHS-2 protein levels when added to the culture medium for 16 h (Fig. 6B). The inhibition with anti-IL-1α was 45%, whereas IL-1ra inhibited PGHS-2 protein expression by 97% (Fig. 6B). As the Northern blot in Fig. 6C indicates, addition of exogenous IL-1ra also dramatically attenuated the PGHS-2 mRNA expression, suggesting that IL-1α is exerting a pre-
cells treated with nothing (control) or with neutralizing antibodies to PGHS-2 signals are shown.

Densities of the resulting bands were determined, and the corrected for background binding with a PGHS-2 cDNA probe and then with a GAPDH probe. The gels were autoradiographed and analyzed using a BioImage densitometer.

 iets were then analyzed for IL-1 expression in these cells under basal culture conditions that was more than 62-fold higher than the activity of pGL2 Basic (control) plasmid. Moreover, the PGHS-2 promoter strength was 13-fold greater than that manifested by the SV40 promoter. A smaller PGHS-2 promoter construct representing −831/+123 base pairs yielded considerably less activity but was still approximately 35-fold above the control plasmid. These results are entirely consistent with the substantial PGHS-2 expression found in untreated KAT-50 cells and provide insight into the extreme activity exhibited by this promoter. It is of considerable mechanistic relevance that both constructs demonstrating activity in the transfected KAT-50 cells contain several regulatory elements including two putative NF-κB sites present in the human PGHS-2 promoter. These are found at −214/−204 and −447/−437 nt (35).

Assessment of PGHS-2 mRNA Stability in Cultured KAT-50 Cells—The 3′ untranslated region of the human PGHS-2 mRNA contains at least 22 AUUUA instability determinants (10, 36) and therefore exhibits the potential for behaving as a rapidly turning over transcript. We therefore set out to determine whether the PGHS-2 mRNA was particularly long-lived in KAT-50 cells and whether this relative stability could underlie the high constitutive levels of PGHS-2. The rate of mRNA turnover in KAT-50 cells was assessed by blocking transcriptional activity. Cultures were treated with DRB (20 μg/ml), an inhibitor of gene transcription, for the intervals of time indicated along the abscissa in Fig. 8. As the data in the figure suggest, there is a slow decrease in the steady-state levels of PGHS-2 mRNA over the first 2 h of DRB addition to the culture medium. The transcript level decreases more rapidly over the next 2 h so that PGHS-2 mRNA levels are undetectable by 4–5 h. The calculated t1/2 of the PGHS-2 mRNA in KAT-50 cells, based on the data from two independent studies displayed in Fig. 8, is 3.22 h. Interestingly, the degradation of PGHS-2 mRNA in these thyroid epithelial cells is somewhat delayed compared with that observed in ECV304, an immortalized human endothelial cell line, where the t1/2 was approximately 1 h in DRB-treated cultures not exposed to any cytokine (12). Unlike the studies we performed in the KAT-50 cells, those earlier studies in ECV304 cells involved treating the cultures initially with cycloheximide, and this inhibitor can influence mRNA stability. Thus, although of interest, direct comparisons between the studies in KAT-50 and ECV304 cells

translational effect on PGHS-2 expression. These results are consistent with endogenously generated IL-1α down-regulates constitutive PGHS-2 expression. A (left), cell layers from a 24-well plate were allowed to proliferate to near confluence and shifted to medium containing 1% FBS, and some were then treated with dexamethasone (10 μM) for 16 h. Monolayers were solubilized and subjected to an ELISA specific for IL-1β. A (right). B, Western analysis of PGHS-2 protein expression in KAT-50 cells treated with nothing (control) or with neutralizing antibodies against IL-1α (1 μg/ml), IL-1β (1 μg/ml), or with IL-1ra (500 ng/ml) for 16 h. Cell layers were solubilized, 30 μg of each sample was subjected to PAGE, and the separated proteins were transferred to membranes and probed with monoclonal antibodies against PGHS-2. The signals generated with ECL detection were scanned with a BioImage densitometer. C, Northern analysis of PGHS-2 transcript levels in KAT-50 cells treated with IL-1ra (500 ng/ml) for the duration indicated in the figure. Total cellular RNA (30 μg) was electrophoresed, transferred, and hybridized with a PGHS-2 cDNA probe and then with a GAPDH probe. Densities of the resulting bands were determined, and the corrected PGHS-2 signals are shown.

**FIG. 6.** KAT-50 cells express constitutive IL-1α protein that can be down-regulated by dexamethasone and serum. Neutralization of IL-1α down-regulates constitutive PGHS-2 expression. A (left), cell layers from a 24-well plate were allowed to proliferate to near confluence and shifted to medium containing 1% FBS, and some were then treated with dexamethasone (10 μM) for 16 h. Monolayers were solubilized and subjected to an ELISA specific for IL-1α and IL-1β. Data are expressed as the mean ± S.E. of triplicate cultures from a representative experiment. A (right), cell layers of KAT-50 cells were shifted from medium containing 1% FBS to that with 10% serum for the times indicated along the abscissa. The cells were then analyzed for IL-1β levels. B, Western analysis of PGHS-2 protein expression in KAT-50 cells treated with nothing (control) or with neutralizing antibodies against IL-1α (1 μg/ml), IL-1β (1 μg/ml), or with IL-1ra (500 ng/ml) for 16 h. Cell layers were solubilized, 30 μg of each sample was subjected to PAGE, and the separated proteins were transferred to membranes and probed with monoclonal antibodies against PGHS-2. The signals generated with ECL detection were scanned with a BioImage densitometer. C, Northern analysis of PGHS-2 transcript levels in KAT-50 cells treated with IL-1ra (500 ng/ml) for the duration indicated in the figure. Total cellular RNA (30 μg) was electrophoresed, transferred, and hybridized with a PGHS-2 cDNA probe and then with a GAPDH probe. Densities of the resulting bands were determined, and the corrected PGHS-2 signals are shown.

**FIG. 7.** Untreated KAT-50 cells in culture exhibit substantial PGHS-2 promoter activity. Near-confluent KAT-50 cells were transfected with the −1840/+123 or −831/+123 PGHS-2 promoter constructs fused to a luciferase reporter or the luciferase reporter without (pGL2 Basic) or with the SV40 promoter (pGL2 Promoter) as described under “Experimental Procedures.” Cultures were co-transfected with β-galactosidase reporter plasmids, the activity of which was assayed and used to correct each culture for transfection efficiency. The data are expressed as the mean ± S.E. of triplicate culture determinations from a single experiment that is representative of three independent studies.
PGHS-2 Expression in Human Thyroid Epithelium

Untreated KAT-50 Cells Synthesize PGE₂ with Activity That Exhibits Sensitivity to PGHS-2 Selective Inhibition—The constitutive expression of PGHS-2 mRNA and protein in KAT-50 cells implies that prostanooids such as PGE₂ might be generated under basal culture conditions and be attributable to the activity of this cyclooxygenase isoenzyme. We therefore began to assess the production of PGE₂ by quantitating the prostanooid released from these cells into the culture medium. As Fig. 9A indicates, levels of PGE₂ under unstimulated culture conditions were low. When graded concentrations of exogenous arachidonate (0–10 μM) were added to the culture medium, PGE₂ production was increased substantially (Fig. 9A). Synthesis of the prostanooid had nearly doubled (1.9-fold increase) with an arachidonate concentration of 5 μM, and the PGE₂ levels achieved with 10 μM were 6.0-fold above those observed in cultures without exogenous arachidonate. The nonselective compound, indomethacin, competitively inhibits both PGHS isoforms (40). NS-398, in contrast, is a highly selective PGHS-2 inhibitor that exhibits a substantial preference for that isoform (31, 41). Addition of NS-398 (10 μM) to the medium of KAT-50 cells blocks PGE₂ formation in arachidonate-treated KAT-50 cells by 57% (control, 1580 ± 195 pg/ml; NS-398, 680 ± 35, p < 0.002) (Fig. 9B). The inhibition achieved was similar to that observed previously in an endothelial cell line (12). The differences in mRNA turnover could result from differences concerning the currently held view that the sole role of PGHS-2 involves mediation of inflammation-related prostanooid production. Uncertainty over this concept emerged several years ago from studies involving mice with interruptions in the expression of PGHS-1 and -2. The resulting phenotypes suggested a broader role for PGHS-2 (42–44). PGHS-2 expression observed on immunohistochemical staining in normal thyroid tissue was primarily epithelial in location but also was found in vascular cells. This finding in thyroid was unexpected because precedent for such unprovoked PGHS-2 expression is limited to a very few anatomic sites and cell types. Indeed, the kidney and central nervous system have been shown to express PGHS-2 consistently under basal, nonpathological conditions (19–21). Confirming our findings in thyroid epithelium in situ is the strong corroborating evidence generated in untreated KAT-50 cells in culture in which PGHS-2 mRNA, protein, and PGHS-2-dependent PGE₂ production were also observed.

The molecular basis for the high level PGHS-2 expression in KAT-50 cells under basal culture conditions is not yet known. It does appear to derive from unprovoked PGHS-2 promoter activity (Fig. 7) and from relatively delayed mRNA degradation compared with that observed previously in an endothelial cell line (12). The differences in mRNA turnover could result from cell-specific expression of factors interacting with the 3′ untranslated region or from differences in the PGHS-2 transcript.
itself. The finding of constitutive PGHS-2 in KAT-50 cells implies that the expression of this cyclooxygenase in thyroid epithelium is driven by intrinsic phenotypic attributes of the epithelium rather than by a factor(s) emanating from neighboring cells, either those ordinarily resident in the gland or recruited in the setting of disease. It suggests further that the PGHS-2 expressed in situ in the thyroid is not a consequence of extrinsic humoral or neurological influences. Indeed, KAT-50 cells under basal conditions express substantial levels of IL-1α (Fig. 6) and blocking the cellular actions of this endogenously generated cytokine with either a neutralizing antibody or exogenous IL-1ra can markedly attenuate PGHS-2 expression. Thus in these cells, IL-1α is functioning as an autocrine factor. In contrast, KAT-50 cells do not appear to express high levels of IL-1β or to utilize that cytokine in the regulation of PGHS-2. Our finding that cyclooxygenase can block constitutive PGHS-2 mRNA expression indicates the importance of ongoing protein synthesis in driving the synthesis of this cyclooxygenase.

We have found that PGHS-1 mRNA, but not PGHS-1 protein, is expressed by cultured KAT-50 cells. This is somewhat surprising in light of the detectable PGHS-1 protein found by immunohistochemical means in situ in normal and disease thyroid tissue (Fig. 1). Thus, it would appear that an important alteration in thyroid epithelial cell phenotype associated with cultivation in culture may be the loss of PGHS-1 translation. Interestingly, the PGHS mRNAs in KAT-50 cells do not exhibit obvious differences from the transcripts expressed by fibroblasts with regard to electrophoretic mobility. Our findings suggest that the PGHS-2 expressed in KAT-50 cells may have assumed some of the metabolic functions ordinarily served by the PGHS-1 isomorph in thyrocytes in situ. Obviously, it would be of great interest to characterize the potentially different activities, substrate utilization, and physiological functions of the two isoforms in intact thyroid tissue. Particularly enlightening might be a comparison between the subcellular localization of the PGHS isoforms in thyroid tissue with that in tissues in which PGHS-2 expression is confined to induced states.

The potent, synthetic glucocorticoid, dexamethasone, inhibited PGHS-2 expression when added to the culture medium of KAT-50 cells (Fig. 3). Glucocorticoids have been shown to block the induction of PGHS-2 by cytokines and growth factors and, in so doing, the production of PGE₂ in several cell types (2). Moreover, endogenous glucocorticoids exert negative tonic effects on the expression of this enzyme in the intact animal, and when adrenal function is disrupted in the mouse, exaggerated induction of PGHS-2 has been observed (18). The finding that constitutive PGHS-2 is also attenuated by glucocorticoids, as is the case in KAT-50 cells, suggests that under nonpathological conditions, adrenal function may govern levels of cyclooxygenase expression in the thyroid. To date, no glucocorticoid response elements have been identified in the human PGHS-2 promoter (35), and it is currently believed that the action of these steriods on the NF-κB/IκB complex represents, at least in part, the molecular basis for their down-regulation of cyclooxygenase expression and PGE₂ production. At least two NF-κB sites have thus far been found upstream of the transcriptional start site in the PGHS-2 gene. These are located at −214/−204 and −447/−437 of the human promoter (35, 36). Thus both elements are present in the two reporter constructs utilized in our studies, both demonstrating substantial basal activity, utilized in the transfection studies reported here. Although they do not define the precise element(s) driving constitutive PGHS-2 gene transcription in the KAT-50 cells, our findings document substantial basal activity of this promoter in these cells. Future studies will be directed at generating a series of deletions by site-directed mutagenesis along the human PGHS-2 promoter and defining those elements that are relevant to the support of unprovoked PGHS-2 expression in KAT-50 cells.

PGHS-2 either is unexpressed or is found at extremely low levels in most nondiseased tissues. With regard to isolated cells, the majority fail to express detectable PGHS-2 mRNA or protein unless they are treated with proinflammatory cytokines, serum, or growth factors. The largest component of basal PGE₂ production found in these cells can be attributed to the activity of PGHS-1. With regard to human fibroblasts from the synovium (13) or orbit (14), PGHS-2 is not detectable under basal culture conditions. When the cells are treated with IL-1β or leukoregulin, two inducers of PGHS-2, they express high levels of the cyclooxygenase. Moreover, the increase in PGE₂ production observed in these fibroblasts following cytokine treatment can be blocked with PGHS-2-selective inhibitors, such as SC 58125 (46). The PGHS-2 up-regulation in fibroblasts and endothelial cells appears to be a function of changes in both gene transcription and mRNA stability (12, 14), whereas in other models, an enhancement of gene transcription appears to predominate. Thus, the regulation of PGHS-2 gene expression is complex and may be cell-specific. Moreover, the anatomic region and state of health of the tissue from which fibroblasts derive appear to determine the magnitude of PGHS-2 induction (14, 47).

Overexpression of PGHS-2 has been linked to neoplasia of the colonic epithelium. A number of reports have appeared recently suggesting that colon carcinomas exhibit high levels of PGHS-2 (48, 49), and some of the cell lines derived from these lesions have been found to retain this overexpression in vitro (50). Moreover, the high levels of PGHS-2 are linked to changes in cell adhesion, metastatic potential, and apoptosis (51, 52). The molecular basis for constitutive PGHS-2 expression in neoplastic cells is, in some cases, an increased rate of gene transcription (53). Breast carcinoma and several breast cancer cell lines and fibroblasts from these lesions exhibit particularly robust PGHS-2 expression (54, 55). PGHS-1 overexpression may also be related to neoplastic transformation. One report has demonstrated that PGHS-1 expression in immortalized ECV endothelial cells can induce neoplastic change (56). The chronic ingestion of nonsteroidal anti-inflammatory drugs is associated with a diminished incidence of colon cancer (57). In animals, PGHS-2 inhibition with highly selective agents has been found to decrease the numbers of colonic polyps in mice with genetic mutations and enhanced susceptibility to intestinal neoplasia (58, 59). Thus, intestinal expression of PGHS-2 may have important pathogenic roles in the development of neoplastic disease of the bowel. Our finding that normal thyroid epithelial cells in situ express high levels of PGHS-2 raises questions concerning a possible link between prostanoid biosynthesis and thyroid neoplasia. We have found high levels of PGHS-2 in malignant thyroid epithelium, but our studies have not employed quantitative measurements of the relative levels of cyclooxygenase in normal and diseased thyroid. Clearly, such studies should now be undertaken to the extent that current technology will allow.

One unanswered question that emerges from our observations relates to the role of PGHS-2 in normal thyroid function implied by constitutive expression. Thyroid tissue is extremely vascular, and the factors governing intrathyroidal blood flow/perfusion have not been fully investigated. Prostanoids have been recognized for their regulatory effects on blood flow in a variety of tissues (60) and thus locally generated prostanoids in thyroid may prove important determinants of thyroidal blood flow. We have demonstrated previously that PGE₂ is an impor-
tant determinant of orbital fibroblast shape (61, 62), and the induction of PGHS-2 in those cells results in a dramatic alteration of cellular morphology (14). The PGE₂ effects are mediated through an EP₂ type prostanoid receptor and result in a dramatic increase in cAMP levels (62). Rapoport and Jones (63) reported that thyroid-stimulating hormone induces a dramatic alteration in dog thyroid epithelial cell morphology in vitro. Although their report failed to link the thyroid-stimulating hormone-dependent effects on morphology to the activation of a particular signal transduction pathway, it is possible that prostanoid generation, already associated with thyroid-stimulating immunoglobulin action, might have been involved in the shape change they observed. In preliminary studies, we have found that thyroid-stimulating hormone can modulate PGHS-2 mRNA levels in KAT-50 cells, raising the possibility that the activated thyroid-stimulating hormone receptor might serve to modulate intrathyroid cyclooxygenase expression. Another possible role for PGE₂ generated under both basal and cytokine/growth factor-activated conditions might concern the modulation of thyrocyte proliferation. In a very recent study, SC 58125 and dexamethasone could attenuate the growth inhibitory effects of endothelin-1 and tumor necrosis factor α in human myofibroblastic hepatic stellate cells (25). The influences of these cytokines were shown to be mediated through the induction of PGHS-2, which is constitutively expressed in these cells (25). With regard to thyrocytes, immunoglobulins from patients with Graves’ disease have been shown to stimulate arachidonate release through an activation of the phospholipase A₂ system and inositol 1,4,5-trisphosphate production in FRTL-5 cells and human thyrocytes (28, 64). These IgGs elicit an enhanced rate of FRTL-5 proliferation, an action that can be blocked by indomethacin (29). Although the cyclooxygenase products were not analyzed, nor was any attempt made to identify the PGHS isoform involved, these findings do suggest that cyclooxygenases might be crucial participants in thyrocyte signaling related to cell proliferation. Moreover, Tahara et al. (65) have demonstrated that in FRTL-5 rat thyroid cells, thyrotropin can enhance cyclooxygenase-like activity and the formation of prostaglandins. Our finding that a substantial fraction of the PGE₂ production in untreated KAT-50 cells could be inhibited by NS-398, coupled with an absence of detectable PGHS-1 protein, implies that PGHS-2 represents the major functional cyclooxygenase in these cells. We are currently conducting studies to assess the effect of PGHS-2 interruption on the KAT-50 cell phenotype.

Another issue raised by our current findings concerns the potential impact of cyclooxygenase inhibition on thyroid hormone biosynthesis. The role of constitutive PGHS-2 expression in thyroid, as well as in the other tissues where it has been found thus far, is uncertain. However, the consequences of acute and chronic ingestion of nonsteroidal anti-inflammatory drugs on thyroid function have never been addressed. The effects of the currently available nonsteroidal anti-inflammatory drugs on basal PGHS activity may be subtle. The recent availability of new PGHS-2-selective drugs, with their greatly enhanced side effect profile, could allow more complete and sustained inhibition of thyroid PGHS-2 activity than achieved with currently available drugs. Thus, it will be of great epidemiologic importance to carefully monitor patients ingesting these newer drugs for any signs of thyroid dysfunction. The putative involvement of arachidonate metabolites in mediating growth-stimulating actions of thyroid-stimulating immunoglobulin suggests the possibility that PGHS-2 and its products may regulate thyrocyte cell division and apoptosis in these cells.

PGE₂ has been shown to influence several aspects of immune function. For instance, the prostanoid can bias the commitment of naive T lymphocytes (Tₐₐ₃) away from the Tₜ₃₁ phenotype and toward that of Tₜ₃₂ (66, 67). PGHS alters B cell behavior (68) and participates in the activation of mast cells (69). Prostanoids can modulate the expression of cytokines in lymphocyte subsets (67) and thus can directly determine the molecular environment present at sites of inflammation and wound repair. Because the lymphocyte phenotype profile appears to depend, at least in part, the character of intrathyroidal immune responses, the products of cyclooxygenases could condition immune responses occurring in that tissue.

A very recent paper by Sorli et al. (70) demonstrated that PGHS-2 is the predominant cyclooxygenase isoform expressed in the Syrian hamster islet cell line, HIT-T15, as well as in human and Syrian hamster islets. Moreover, PGHS-2 is expressed constitutively in HIT-T15 cells. Treatment of these cells with exogenous IL-1 increased the levels of PGHS-2 transiently. Unlike the KAT-50 cells and in situ staining in thyroid, where PGHS-1 mRNA and protein, respectively, were detectable, the islet cells failed to exhibit any evidence of PGHS-1 expression on either the mRNA or protein levels. Those earlier studies also demonstrated surprisingly high levels of NF-IL-6 and more modest levels of NF-kB in untreated cells expressing constitutive PGHS-2. Another recent report by Kwon et al. (45) has further characterized the expression of PGHS-2 in islet cells and implicates the proteasome complex and NF-kB in this expression. The potential implications of these findings in islet cells to the pathogenesis of diabetes mellitus are considerable, given the proposed modulatory role of prostaglandins on insulin release (39). Coupled with our own observations reported here, we raise the possibility that constitutive PGHS-2 expression might be considerably more widespread than currently believed and could involve tissues and cell types that have thus far not been inspected for cyclooxygenase expression. In particular, other endocrine organs might represent sites where PGHS-2 is expressed under normal circumstances. Thus, the profile of cyclooxygenase expression should be assessed in a wide array of these tissues before any conclusions concerning the biological function of these enzymes can be drawn. Further studies, including perhaps those involving animals in which the expression of the PGHS-2 gene is conditionally disrupted, either alone or in concert with that of PGHS-1, will be necessary to define the precise role of this cyclooxygenase in healthy and diseased thyroid tissue.

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Prostaglandin-endoperoxide H Synthase-2 Expression in Human Thyroid Epithelium: EVIDENCE FOR CONSTITUTIVE EXPRESSION IN VIVO AND IN CULTURED KAT-50 CELLS
Terry J. Smith, Timothy A. Jennings, Daniela Sciaky and H. James Cao

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