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PHENOL SULFOTRANSFERASE ACTIVITIES AND LOCALIZATION IN HUMAN NASAL POLYP EPITHELIUM

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SUMMARY: Nasal polyp epithelia, which exhibit a wide variation in epithelial cell morphologies, were tested for phenol sulfotransferase (PST) enzymes. Immunohistology revealed little or no detectable PST antigen in normal ciliated pseudostratified epithelia or in simple squamous metaplastic epithelia; however, intense expression was observed in regions of non-ciliated epithelial cell hyperplasia and in squamous epithelial cells overlying such hyperplastic sites. Western blots confirmed the presence of both P-PST (32 kDa) and M-PST (34 kDa) in the tissue extracts. Bimodal distribution of PST activity as a function of 4-nitrophenol concentration was consistent with expression of these two PST isoforms. These results indicate dynamic and epithelial differentiation-dependent expression of human PSTs in the nasal mucosa and suggest that these sulfotransferases can be modulated within human airways *in vivo*.

The sulfotransferases are a family of conjugative enzymes that catalyze the transfer of a sulfonyl group, SO_3, from the donor substrate 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to drugs, xenobiotics, steroids, and other endogenous compounds (1). The phenol or aryl sulfotransferases (PSTs, EC 2.8.2.1) exhibit differential specificities for varied hydroxylated aromatic compounds and form the corresponding sulfate esters (2, 3). Products of this reaction are generally more water soluble than the parent compound, and it is thought that this conjugation thereby aids in detoxification and excretion. Some compounds, however, are activated to ultimate carcinogens by sulfonation (1). Therefore, there is currently considerable interest in how these enzymes are regulated or differentially expressed in normal and pathological conditions.

Two distinct human PSTs have been classified based on both substrate specificities and physical characteristics. P-PST, which is most reactive with simple phenolic substrates (4), is fairly resistant to heat inactivation and is frequently referred to as the thermostable or TS-PST (5, 6). M-PST is very reactive with catechol monoamines (7), yet comparably more sensitive to heat inactivation and thus termed the thermolabile or TL-PST. Characterization of the cDNAs encoding these two enzymes revealed 93% identity of their the amino acid sequences (8-11).

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It has been known for some time that human and bovine bronchial tissues are able to sulfonate xenobiotics (12). We have examined the bovine respiratory system both enzymologically and immunohistologically, and have established that the airway epithelium in that species contains phenol sulfotransferase (13). Therefore, lumenal cells with immediate exposures to inhaled xenobiotics contain the metabolic machinery for detoxification via the sulfonation pathway. Little is known, however, about the human respiratory epithelium. Total human lung parenchymal homogenates reportedly contain both P-PST and M-PST activities (14), although the cellular distribution of these enzymes is currently unknown. Also not investigated for sulfotransferase expression is the the uppermost portion of the respiratory system, the nasal cavity (15). It has been reported that the epithelium at this site contains several phase I and II activities (15-18), and the presence of PST antigen has been reported in rat nasal mucosa (19). In this investigation we have examined human nasal polyps using both immunological and enzymological methods. The results indicate expression of both P-PST and M-PST enzyme activities and proteins in this tissue. Interestingly, immunohistological examination reveals expression to be dependent on the state of epithelial differentiation, with greatest immunopositivity in regions showing simultaneous epithelial hyperplasia and squamous metaplasia. This is the first report indicating dynamic and differentiation-dependent expression of phenol sulfotransferases in a human tissue *in vivo*.

MATERIALS AND METHODS

Reagents. [^{35}S]-PAPS (2 Ci/mmole) was from New England Nuclear (Boston, MA). 4-Nitrophenol was from Sigma (St. Louis, MO). Other reagents were of the highest purities available. Bovine lung phenol sulfotransferase was prepared from lung parenchyma as previously described (20). Human liver thermostable P-PST (700-fold purified) and thermolabile M-PST (300-fold purified) were provided by Diane Otterness and Dr. Richard Weinshilboum (Dept. of Pharmacology, Mayo Clinic, Rochester, MN). Both samples were obtained by chromatography over DEAE-Sepharose, Affigel Blue, and heparin-Sepharose (6), with final estimated purities based on Coomassie R-250 stained polyacrylamide gels of 20% (TS-PST) and 10% (TL-PST). These samples were used to determine the crossreactivity of mouse anti-bovine PST (13) with the human phenol sulfotransferases (not shown). Crossreactivity is consistent with the 83% amino acid sequence identity between the bovine PST and human P-PST (21).

Tissues and extraction. Human nasal polyps were placed in cold medium MEM and transported to the laboratory within 1 h of surgical resection. One half of each tissue was either placed in PBS/10% formalin at 5°C (see below), or placed in cold 20 mM Hepes/20% sucrose/5 mM ß-mercaptoethanol/1 mM EDTA/pH 7.4 (Buffer A) and homogenized for 30 s by Polytron (Brinkman). Further disruption was achieved by 2 x 30 s sonication bursts (Heat Systems Model W-220F, setting on 4) with intermittent cooling of the microtip. The homogenates were then centrifuged (20,000 xg, 30 min) to prepare cytosolic fractions, which were then stored at -80°C. Total protein concentrations were determined by a Coomassie G-250 binding assay (BioRad, Richmond, CA), using bovine serum albumin as the standard. Three patients were male (10, 53, 64 y), one was female (42 y), and one unidentified.

Immunohistology. Mouse anti-bovine PST anti-serum was employed for immunohistology as follows. Nasal polyps were fixed overnight in PBS containing 10% formalin at 5°C. Standard methods were used to prepare serial 8 µm sections of paraffin embedded tissues, which were mounted on poly-lysine coated glass slides. These were cleared in two xylene washes, followed by two washes in absolute ethanol. Endogenous peroxidase was inactivated by 30 minute incubation in methanol/0.3% H_2O_2, followed by rehydration in a series of graded ethanol/water washes. Duplicate sections were overlayed with calcium-free PBS/10% normal rabbit serum for 15-30 min, rinsed with PBS, and then incubated 1-2 h with PBS/1% normal (negative control) or immune mouse sera. After several PBS washes, sections were decorated with peroxidase

conjugated rabbit anti-mouse IgG (ICN, 1:500 in PBS) for 1 h, washed five times with PBS, and developed for 5 min with PBS/0.03% H_2O_2/0.04 mg/ml DAB/1.6 mg/ml NiCl.

For immunofluorescence decoration, tissue sections were prepared, blocked and probed as above except for the omission of peroxidase inactivation. After normal mouse or anti-PST sera and subsequent PBS washes, sections were incubated in the dark with FITC-conjugated rabbit anti-mouse IgG (ICN, 1:100 in PBS). Photomicrography was achieved using a Zeiss photomicroscope III equipped with an epifluorescence condensor.

Immunoblot analyses. Due to detection limits, crude nasal polyp extracts required enrichment prior to immunoblot analyses. This was accomplished by passing 2 ml of crude cytosolic extract over a 1 ml bed volume of Reactive Green agarose (Sigma) equilibrated with Buffer A. After a 10 ml wash with Buffer A, adsorbed proteins were eluted with Buffer A plus 2 M NaCl and 1 ml fractions were collected. Enzymatic assays using 10 μM and 1 mM 4-nitrophenol confirmed the binding and elution of phenol sulfotransferase activity (not shown). To an aliquot of an eluted fraction (#3) containing the greatest 280 nm absorbance was added 0.1 volume of 10% sodium dodecyl sulfate then 2 volumes ethanol. Denatured protein was collected by centrifugation, washed with 80% ethanol in water, dried, and redissolved in gel loading buffer. Enriched polyp extracts and phenol sulfotransferases were subjected to polyacrylamide gel electrophoresis (22) through 10% gels, which were then electroblotted (23) to Immobilon membranes (Millipore, Bedford, MA). Blots were blocked in 20 mM Tris-Cl/100 mM NaCl/pH 7.5 (TBS) containing 5% (w/v) Carnation Instant milk, and subsequently incubated 18 h in TBS/milk containing 1:1000 dilutions of either non-immune or mouse anti-bovine PST mouse sera. Anti-bovine PST was previously described (13). After 5 TBS washes, a 1 h incubation with 1:2000 peroxidase conjugated rabbit anti-mouse IgG in TBS, and 5 final TBS washes, antigen detection was achieved using chemiluminescence (ECL kit, Amersham).

Enzyme analyses. Phenol sulfotransferase activity was measured at 23°C using a modified (24) radioisotopic procedure in which [^{35}S]-labeled 4-nitrophenyl sulfate is separated from [^{35}S]-PAPS by ascending thin layer chromatography (25, 26). The assay buffer consisted of 50 mM potassium phosphate, 2 mM 2-mercaptoethanol, pH 7.4 (13). For single time-point measurements, the extent of transfer of sulfate from PAPS to labeled product was 10% or less, which fell within time- and protein-linearity of the assay.

RESULTS AND DISCUSSION

Epithelial differentiation is remarkably variable in nasal polyp tissues (27), thus providing an interesting opportunity to investigate the expression of PSTs within a dynamic cellular environment. Histologies include normal pseudostratified ciliated epithelia, non-ciliated hyperplastic epithelia, and squamous metaplasia. Epithelial cells are frequently destroyed, however, due to the effects of toxic eosinophilic products (27). Immunoperoxidase detection of phenol sulfotransferase antigen revealed little if any positivity in normal pseudostratified columnar epithelium (Figure 1 A-C). In sharp contrast, regions of the tissues with cuboidal epithelial hyperplasia were immunopositive, especially in accompanying lumenal squamous metaplastic cells (Figure 1 D-F). Simple stratified squamous epithelium appeared devoid of PST antigen (not shown). Regions with very elongated columnar epithelial cells appeared negative by the immunoperoxidase method, although overlaying stratified squamous epithelial cells showed positive staining (not shown). The immunoperoxidase results were essentially confirmed using immunofluorescence detection (Figure 2, second patient), which indicated intermittent PST positivity in pseudostratified columnar epithelia (Fig. 2A) in contrast to nearly unifiorm and strong positivity in cuboidal hyperplastic epithelia (Fig. 2C).

Immunoblot analyses were performed to examine the phenol sulfotransferase isoforms in the nasal polyp extract. Immunoreactive bands at 32 kDa and 34 kDa were observed (Figure 3),

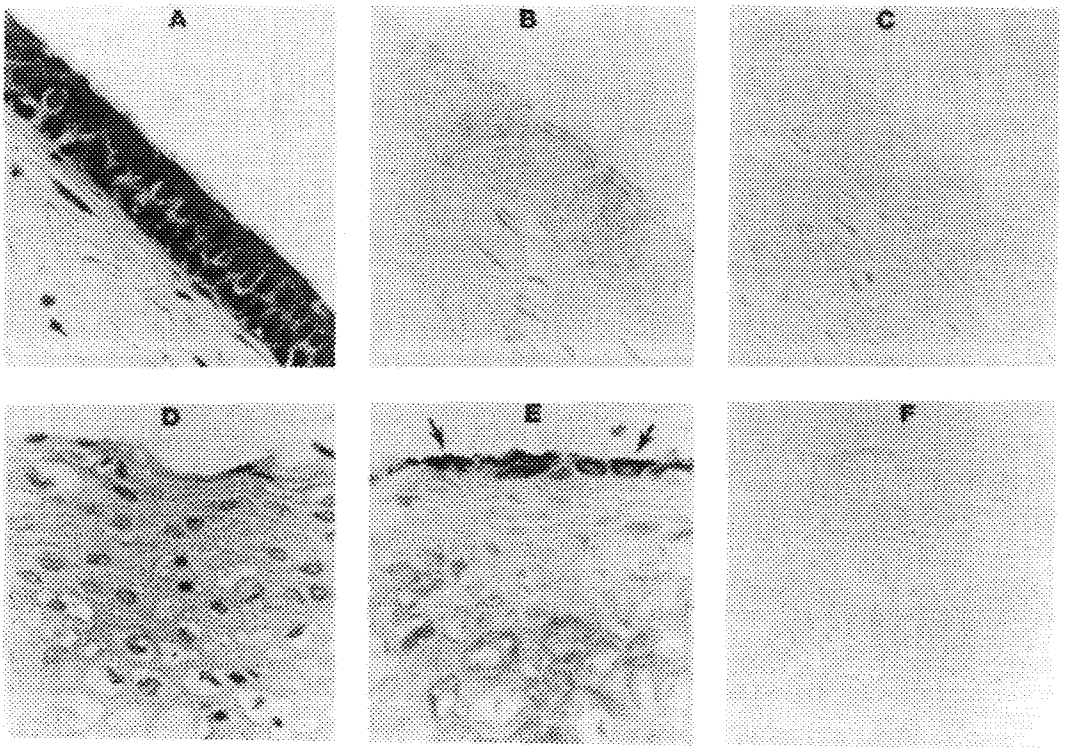


Figure 1. Immunoperoxidase localization of phenol sulfotransferase antigen in nasal polyp epithelia. Both rows of tissue sections were from different regions of the same polyp (patient unidentified) and were therefore simultaneously processed. An area with normal ciliated cell morphology (Panels **A-C**) is contrasted with a region with cuboidal hyperplasia and squamous differentiation (**D-F**). H&E stained (**A,D**), anti-PST anti-serum (**B,E**), control serum (**C,F**). Note that the normal airway epithelium appears essentially negative, whereas the hyperplastic and squamous regions (**E**) are positive (arrows).

which is consistent with previous reports on the migration of P-PST and M-PST proteins during polyacrylamide gel electrophoresis in the presence of dodecyl sulfate (1). This analysis also confirms the specificity of the anti-serum used for the immunohistological tests (Figs. 1 and 2). Given the cross-reactivity, however, of the antiserum with both human P-PST and M-PST proteins (Figure 3), it is not possible to say with certainty if the two phenol sulfotransferase isoforms are differentially expressed between different cell types. Future investigations will require the development of PST isoform-specific reagents.

Preliminary assays of phenol sulfotransferase activity in nasal polyp cytosolic extracts indicated dependence on the concentration of 4-nitrophenol in the assay. Systematic variation of the acceptor substrate concentration revealed a bimodal distribution of activity (Figure 4). This behavior has been shown to derive from the two phenol sulfotransferases, in which the P-PST sulfonates μM concentrations of phenol followed by substrate inhibition; the M-PST then shows increasing activity from 50-500 μM 4-nitrophenol, followed by a second substrate inhibitory phase (28). Maximal activities in the polyp extracts were observed at 0.1 μM and 500 μM 4-nitrophenol. This latter value compares well with previous reports for the optimal substrate concentration for the

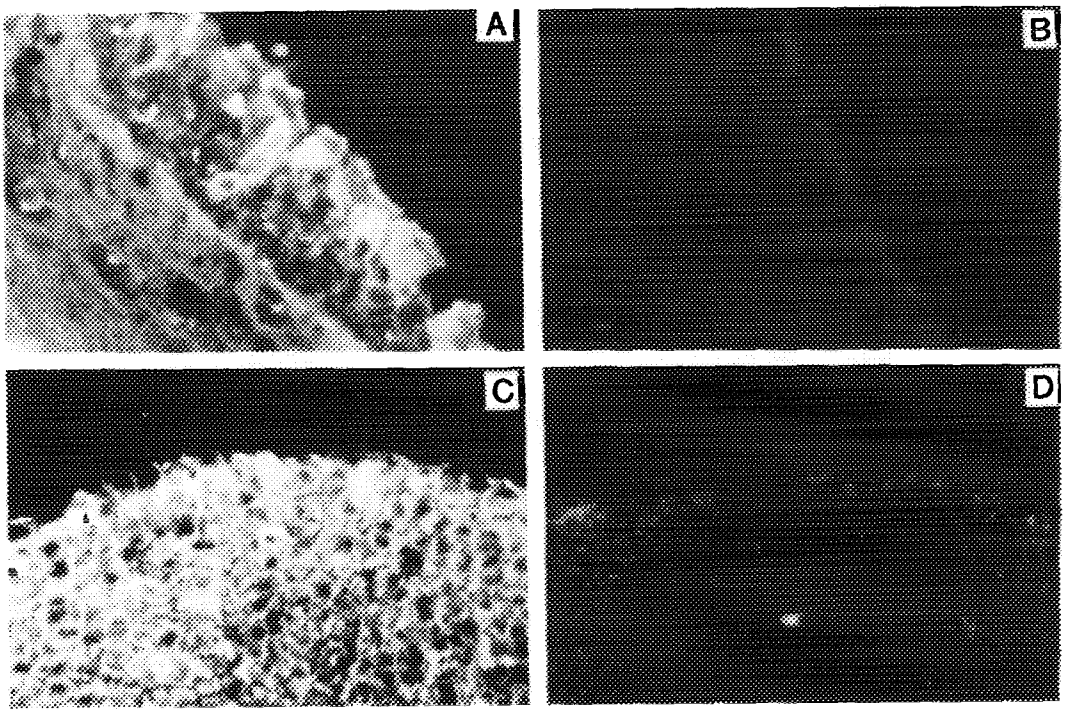
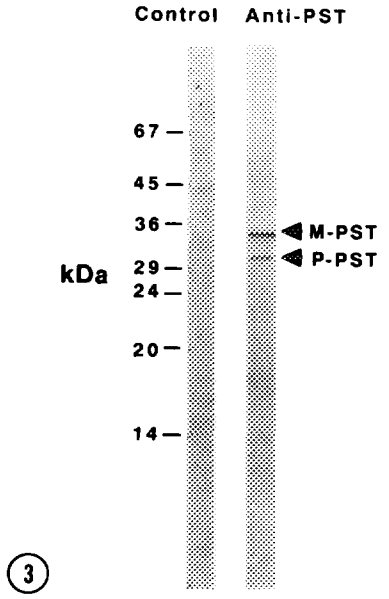


Figure 2. Immunofluorescence localization of phenol sulfotransferase antigen in nasal polyp epithelia. Both rows of tissue sections were from different regions of the same polyp (53 y old male) and were therefore simultaneously processed. Panels **A** and **C** were probed with anti-PST anti-serum, whereas panels **B** and **D** were probed with control serum (Methods). Note the intermittent epithelial positivity in the pseudostratified columnar epithelial cells (**A**) contrasted to uniform and intense decoration of the non-differentiated hyperplastic cuboidal epithelial cells (**C**).

human M-PST (28). Our peak activity for the P-PST occurs, however, at a substrate concentration 10-fold less than previously reported. At this time it is unclear whether this discrepancy is due to a difference in the enzyme, or perhaps due to subtle differences in assay protocol such as buffer composition. Regardless, these results, in conjunction with the immunoblot data (Fig. 3) provide strong evidence for the expression of the two human phenol sulfotransferases in the nasal polyp tissues. The specific activities of 2-2.5 pmol/mg/min at both optimal substrate concentrations fall within the range previously reported for human lung (29).

These results provide the first evidence for the dynamic expression of phenol sulfotransferases in the human respiratory epithelium. The observation of greatly increased levels of antigen in the abnormal hyperplastic/squamous metaplastic lumenal cells relative to normal pseudostratified ciliated epithelium indicates that the expression of one or both of the human phenol sulfotransferases can be controlled, rather than being constitutively expressed. The observations of both P-PST and M-PST proteins (Fig. 3) and activities (Fig. 4) in the polyp extracts suggest that both enzymes will be subject to controlled expression. What factors may play a role in modulating this expression is the subject of ongoing investigations in this laboratory (30).



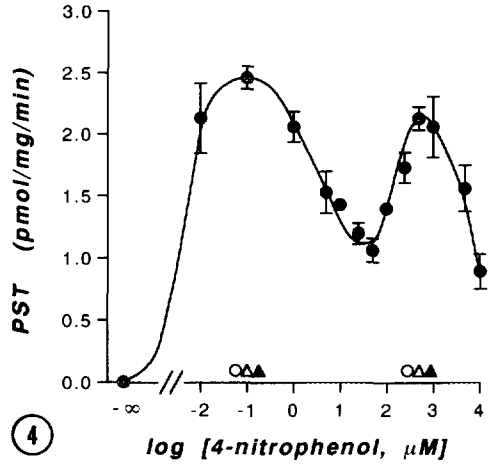


Figure 3. Immunoblot analysis of nasal polyp extract for phenol sulfotransferases. Crude extract (same patient as in Fig. 2) was partially purified over Reactive Green agarose (see Methods) prior to electrophoresis of 20 μg protein loads. Subsequent blots were probed using either normal mouse serum (Control) or mouse anti-bovine phenol sulfotransferase (Anti-PST). M-PST is the 34 kDa thermolabile enzyme, whereas P-PST is the 32 kDa thermostable enzyme.

Figure 4. Effect of 4-nitrophenol concentration on phenol sulfotransferase activities in nasal polyp extracts. Cytosolic extracts (7-10 μg protein/assay) were assayed directly at pH 7.4 using 60 minute incubations at 23°C (Methods). Symbols are averages ± SD of triplicate measurements, having been corrected for blank assays in which phenol substrate was omitted. *Solid circles* are from the same patient as in Figs. 2-3. Other symbols indicate extracts from three additional patients, in which the epithelia were essentially desquamated and immunopositivity was not observed (not shown).

The sulfonation of phenolic compounds is likely an important conjugative pathway in human airways (12, 13, 29) for drug and pollutant metabolism. The observation of increased expression of these enzymes in abnormally differentiated airway epithelium could provide an enhanced protective metabolic barrier to inhaled cytotoxic xenobiotics. On the other hand, sulfonation of N-hydroxy arylamines or hydroxymethylated compounds could result in increased toxification (31). It will therefore be interesting to examine these possibilities using cell culture models, and to extend the observations made in this investigation to other regions of the respiratory system. In addition, increased phenol sulfotransferase expression in nasal polyp hyperplastic epithelium suggests an interesting hypothesis. Chronic hyperplastic sinusitis, which is characterized by formation of nasal polyps, commonly occurs in asthmatic patients (32). Also, recent observations on tracheobronchial ß-adrenergic receptor expression in normal vs. asthmatic patients has indicated increased (rather than decreased) receptor numbers and increased ligand binding affinity at this crucial site (33). It seems reasonable to speculate, therefore, that if ligand binding *per se* is not problematic, then perhaps an increased rate of metabolism of the ß-agonist to an inactive form

may contribute to the disease state. Further experiments will be required, however, in order to determine if the phenol sulfotransferases have a role in the development or treatment of asthma.

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