Localization of \(N\)-Acetyltransferases NAT1 and NAT2 in Human Tissues

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Human acetyl coenzyme A-dependent \(N\)-acetyltransferase (EC 2.3.1.5) (NAT) catalyzes the biotransformation of a number of arylamine and hydrazine compounds. NAT isoforms are encoded at 2 loci; one encodes NAT1, formerly known as the monomorphic form of the enzyme, while the other encodes the polymorphic NAT2, which is responsible for individual differences in the ability to acetylate certain compounds. Human epidemiological studies have suggested an association between the “acetylator phenotype” and particular cancers such as those of the bladder and colon. In the present study, NAT1- and NAT2-specific riboprobes were used in hybridization histochemistry studies to localize NAT1 and NAT2 mRNA sequences in formalin-fixed, paraffin-embedded human tissue sections. Expression of both NAT1 and NAT2 mRNA was observed in liver, gastrointestinal tract tissues (esophagus, stomach, small intestine, and colon), urothelial cells, and the epithelial cells of the respiratory bronchioles. The observed heterogeneity of NAT1 and NAT2 mRNA expression between human tissue types may be of significance in assessing their contribution to known organ-specific toxicities of various arylamine drugs and carcinogens.

Key Words: \(N\)-acetyltransferase; carcinogenesis; hybridization histochemistry; liver; bladder.

Acetylation is an important phase-II pathway involved in the detoxification of a large number of arylamine and hydrazine drugs and chemical carcinogens. It is also implicated in the metabolic activation of DNA-binding electrophiles, of a range of occupational, food-derived and environmental chemicals that have been postulated to be causative agents in the development of certain forms of human cancer. The biotransformation of aromatic and heterocyclic amine procarcinogens to reactive forms is a complex procedure requiring at least two enzyme-mediated steps: \(N\)-hydroxylation, which is principally controlled by cytochrome P450 (CYP) 1A2 and subsequent \(O\)-acetylation of the \(N\)-hydroxyster metabolite by NAT to form \(N\)-acetoxyarylamines (McManus et al., 1990). Another route of carcinogen activation has been proposed whereby consecutive acetylation and oxidation is followed by intramolecular \(N\), O-acetyl transfer, resulting in the formation of \(N\)-acytoxyarylamines (Hein, 1988). Such acetoxy ester metabolites are highly unstable and spontaneously liberate aryl nitrenium ions that are capable of producing adducts by covalently binding to DNA. These DNA adducts may cause mutations in genes controlling cellular proliferation and consequently lead to tumor formation.

The human NAT isoforms are encoded at 3 separate loci, one of which (NATP) contains multiple premature termination codons and most likely represents a non-expressed pseudogene (Blum et al., 1990). The two expressed genes, NAT1 and NAT2, are both located on chromosome 8 and share 87% and 81% nucleotide and amino acid sequence identity, respectively. Although only 10% of the amino acid sequence differences between NAT1 and NAT2 were identified as non-conservative, functional studies have demonstrated significant differences between the two isoforms. NAT1 preferentially metabolizes \(p\)-aminobenzoate and \(p\)-aminosalicylate, and has traditionally been designated as the “monomorphic” form. Recent studies, however, have identified allelic variants of NAT1, one of which has been associated with elevated enzyme activity in preliminary studies (Bell et al., 1995). At least 15 and perhaps as many as 23 different allelic variants of NAT2 have been identified to date, and their frequency in the population provides a molecular explanation for the polymorphic metabolism of model substrates such as sulfamethazine and procainamide (Grant et al., 1991, 1997; Grant, 1993; Hein et al., 1997). The representation of these mutant alleles also differs widely between populations of different ethnic or geographical location (Grant et al., 1997), with slow acetylators accounting for 10% and 40–70% of Oriental and Caucasian populations, respectively (Deguchi et al., 1990; Hickman and Sim, 1991).

An ever-increasing number of studies have demonstrated an
association between the NAT2 phenotype/genotype and drug toxicities. One group recently proposed that discoid lupus erythematosus is responsive to sulfasalazine therapy only in rapid acetylators and that slow acetylators show increased toxicity to this drug (Sabbagh et al., 1997). Individual predisposition to certain forms of cancer has also been linked to the NAT2 phenotype/genotype. The slow NAT2 acetylator phenotype is associated with an increased risk of bladder cancer in smokers whereas the rapid acetylator phenotype is over-represented in patients with colorectal cancer (Brookmøller et al., 1996; Ilett et al., 1987). Another study has shown that carriers of the NAT2*4/*4 genotype, which results in a high acetylation capacity, are at a significantly increased risk of lung cancer (Cascorbi et al., 1996).

NAT1 expression is widely distributed throughout the human body, whereas NAT2 expression is considered to be more limited. Both forms have been identified by activity studies to be present not only in the liver but also in other tissues such as gut, uroepithelial cells, and lymphocytes (Coroneos and Sim, 1991; Cribb et al., 1991; Kirlin et al., 1991; Kloth et al., 1994; Pink et al., 1992). However, because of the overlapping substrate specificities and the unresolved functional significance of allelic variants of NAT1 and NAT2, one must use caution when interpreting tissue localization of these isozymes based on metabolic activities. Weber and Vatsis, in a recent review, also commented on the potential limitations of using data from heterologous expression systems to determine the relative importance of NAT1 versus NAT2 in the activation of aromatic and heterocyclic amines (Weber and Vatsis, 1995).

Despite the evidence of NAT1 and NAT2 activity in various human tissue types, little is known about their cellular localization. In this study, we have used hybridization histochemistry to characterize NAT1 and NAT2 expression at the mRNA level in both hepatic and extrahepatic human tissues. Mapping of the distribution of NAT mRNA in human tissues will not only provide information concerning their cellular localization, but also aid in the understanding of their relative roles in site-specific tumorigenesis.

**MATERIALS AND METHODS**

**Human tissues.** Human liver samples were obtained from renal transplant donors maintained on life-support systems until the kidneys could be removed, and livers were recovered within 30 min of death. Human stomach, jejunum, colon, lung, ureter, and bladder specimens were either surgical or biopsy specimens which had been reported as histologically normal. The use of human tissues in these studies had Flinders Medical Centre and Royal Brisbane Hospital Ethics Committee approval. The clinical details of the human liver samples used in these studies have been published previously (McKinnon et al., 1991, 1995). In the case of extrahepatic tissues, detailed case histories of patients were not available due to Ethics Committee restrictions. Tissues designated for hybridization histochemistry studies were fixed in 10% (v/v) formalin in sodium phosphate buffer (pH 7.4), processed routinely, and embedded in paraffin.

**Hybridization probes.** All enzymes and reagents required for the transcription of RNA probes were obtained from Stratagene (La Jolla, CA). RNA probes were transcribed from NAT1 and NAT2 genomic DNA sequences subcloned into the Bluescript SK transcription plasmid. The NAT1 insert was a 145-bp EcoRI-DdeI restriction fragment corresponding to bases −153 to −8 (relative to the translation start site) of the NAT1 transcript’s 5’ non-coding region. The NAT2 insert was a 313-bp BamHI-EcoRI fragment corresponding to bases +856 to +1169. The recombinant plasmids were linearized and subjected to proteinase-K digestion prior to RNA probe synthesis. Both antisense and sense probes were transcribed from each plasmid for use as test and control probes respectively. Riboprobes for hybridization histochemistry were labeled by incorporation of [α-35S]UTP (Du Pont, Australia).

To verify probe specificity, EcoRI digests of NAT1 and NAT2 DNA were prepared from Bluescript plasmids containing the protein-coding and flanking regions of the NAT1 (1.3 kb) and NAT2 (1.9 kb) inserts (Blum et al., 1990). The EcoRI digests spanning the NAT1 and NAT2 genes were also used as templates for polymerase chain reaction (PCR) experiments using primers designed to amplify the specific NAT1 (145 bp) or NAT2 (313 bp) regions. The EcoRI digests of human genomic DNA spanning the NAT1 and NAT2 genes and the PCR-amplified NAT1 and NAT2 DNA were tested against the NAT1 and NAT2 probes in a standard Southern blot procedure. NAT1- and NAT2-specific probes were labeled with 32P (Amersham, UK) using an Oligolabelling Kit (Pharmacia Biotech, USA). Probe hybridization was performed using standard protocols with 1 × 105 cpm of radiolabeled probe added to each blot and hybridization performed at 60°C overnight. Non-specific binding of the probe to the membrane was removed by sequential washes of increasing stringency to a final wash in 1 × SSC (150 M NaCl, 15 mM Na citrate) at 60°C. Autoradiographs were exposed for 80 min at room temperature.

**Hybridization histochemistry.** The hybridization histochemistry procedure was performed on formalin-fixed, paraffin-embedded sections as described previously (McKinnon et al., 1991). Two serial sections were included on each slide for probing with test (antisense) and control (sense) probes, respectively. For the hybridization histochemistry studies, at least four samples were tested for each tissue sample. A high stringency wash was necessary to reduce background with the riboprobes and was performed in 0.1 × SSC (15 M NaCl, 1.5 mM Na citrate) containing 30% formamide at 53°C and 55°C for NAT1 and NAT2 probes, respectively. All test sections were evaluated by direct comparison with a control section present on the same slide that had been hybridized with the sense probe. Control sections demonstrated few, or no, autoradiographic grains that were uniformly distributed across the section and these were interpreted as background. By comparison, test sections hybridized with antisense probe exhibited autoradiographic signal at higher levels than those of the control section and this was documented as a positive result.

**FIG. 1.** Determination of NAT probe specificity. Duplicate blots were hybridized with: A, NAT1-selective probe or B, NAT2-selective probe (right panel). Lanes 1–2: 2 independent PCR reactions using NAT1-specific primers and NAT1-containing plasmid (see lane 5) as template; lanes 3–4: 2 independent PCR reactions using NAT2-specific primers and NAT2-containing plasmid (see lane 6) as template; lane 5: EcoRI-digested pBluescript plasmid containing a 1.3 kb subcloned EcoRI fragment of human genomic DNA spanning the NAT1 gene; lane 6: EcoRI-digested pBluescript plasmid containing a 1.9 kb subcloned EcoRI fragment of human genomic DNA spanning the NAT2 gene.
RESULTS

Probe Specificity

The protein-coding regions of the *NAT1* and *NAT2* genes share 87% nucleotide sequence identity. To design riboprobes specific for NAT1 and NAT2, we chose the sequences from –153 to –8 of the 5′ non-coding region of NAT1, and from +1856 to +1169 of the 3′ region of NAT2 (protein coding terminates at +870). NAT1 in the region of –153 to –8 shares 66% homology with the corresponding region 5′ to the NAT2 gene’s protein-coding sequence and has no detectable homology with its non-coding exon 1, located 8–9 kb upstream. NAT2 in the region of +1856 to +1169 shares 76% homology with NAT1. To demonstrate the specificity of the two probes, Southern blotting of DNA fragments containing the sequences homologous to the NAT1 and NAT2 probes was performed (Fig. 1). The NAT1 (145 bp) probe recognized both the NAT1-specific (145 bp) PCR product and the pBluescript-NAT1 (1.3 kb) *Eco*RI digest, but gave no signal when hybridized against the NAT2 (313 bp) PCR product or the pBluescript NAT2 (1.9 kb) *Eco*RI digest (Fig. 1A). Similarly, the NAT2 (313 bp) probe hybridized to both the NAT2-specific (313 bp) PCR product and the pBluescript-NAT2 (1.9 kb) *Eco*RI digest but did not recognize the NAT1 (145 bp) PCR product or the pBluescript NAT1 (1.3 kb) *Eco*RI digest (Fig. 1B).

*NAT* mRNA Expression in Human Tissues

Hepatocytes in all zones of the liver acinus displayed positive signals for NAT1 (Fig. 2A), with maximal intensity in hepatocytes adjacent to the portal tracts (zone 1). Similarly,
NAT2 was detected throughout all liver hepatocytes (Fig. 2C), with an increase in signal at the zone 1 hepatocytes surrounding portal tract regions.

The stratified squamous epithelium of the esophagus displayed positive signals for both NAT1 and NAT2 mRNA that appeared to have greatest intensity in the basal proliferating cells (Figs. 3A and 3C). In the stomach, the surface columnar epithelial cells exhibited a NAT1 signal (Fig. 4A). In contrast, the NAT2 mRNA signal in the stomach was very low, being only marginally greater than, or comparable to, that of control sections (data not shown). In the small intestine, the more differentiated epithelial cells of the villi were positive for NAT1 and NAT2 mRNA (Figs. 5A and 5C), as were epithelial cells within the crypts of Lieberkuhn. In the colon, low levels of expression of NAT1 and NAT2 was observed in the columnar epithelial cells and epithelial cells of the crypts of Lieberkuhn (Figs. 6A and 6C).

NAT1 and NAT2 mRNA expression in the bladder was predominantly in the transitional epithelial cells lining the lumen (Figs. 7A and 7C). In the ureter, low level expression of NAT1 mRNA could be determined throughout the transitional epithelial cell layer (Fig. 8A). Similarly, NAT2 mRNA signal in the ureter was located within the transitional epithelium (Fig. 8C). In contrast, the other urinary tissue examined in the study, the renal pelvis and tubules in the kidney, demonstrated very low levels of NAT1 and NAT2 mRNA expression, being only slightly higher than, or comparable to, background levels (data not shown).

NAT1 mRNA expression in the lung was at very low levels, being only marginally greater than that of control sections (data not shown).

FIG. 3. Identification of NAT1 and NAT2 expression in human esophagus. Histological sections were hybridized with 35S-labelled NAT1 antisense (A), NAT1 sense (B), NAT2 antisense (C), or NAT2 sense (D) riboprobes. The antisense NAT1 and NAT2 probes demonstrate an increase in autoradiographic grains at the base of the stratified epithelial layer (hematoxylin and eosin, original magnification ×100).
Bronchial epithelial cells and alveolar lining cells all displayed low levels of NAT2 mRNA signal (Fig. 9A).

**DISCUSSION**

Most of our current knowledge concerning the tissue localization of human NAT1 and NAT2 comes from metabolic studies utilizing cytosols isolated from hepatic and extrahepatic human tissues. Although such studies have provided valuable insights into the whole-organ expression of the NAT enzymes, they provide no information about the specific cellular localization of these enzymes in discrete cell types within these tissues. In the present study, we have used hybridization histochemistry to report the distribution of mRNA transcripts encoding NAT1 and NAT2 in human liver, gastrointestinal tract, ureter, bladder, and lung. In human liver, NAT1 and NAT2 grain intensity appeared to be highest in the periportal regions (zone 1). This is in contrast to phase-I enzymes such as CYP1A1/1A2, CYP3A, and NADPH-cytochrome P450 reductase, which are predominantly localized around central veins (zone 3 hepatocytes) (Hall *et al.*, 1989; McKinnon *et al.*, 1991, 1995). In the rabbit however, we have shown that CYP4B1 mRNA and protein are expressed in zone 1 hepatocytes (McKinnon *et al.*, 1994). Preliminary results in our laboratory have demonstrated aryl sulfotransferases to be expressed equally in all zones of the acinus (data not shown). The zone 1 hepatocytes surrounding the portal tracts are also considered to be proliferative in nature and this may indicate an as yet unidentified role of NAT isozymes in cellular proliferation. Few studies have indicated the presence of NAT in esophageal tissue despite it being the entrance to the gastrointestinal tract and an early contact point for dietary carcinogens. In fact, there is only limited data on the presence and localization of xenobiotic metabolizing enzymes within esophageal tissue. In one study, CYP1A, epoxide hydrolase and glutathione S-transferase, but not CYP3A and CYP2C, were identified in the stratified squamous epithelium of human esophagus (Murray *et al.*, 1994). It should be noted, however, that the authors reported a low frequency of protein expression for these drug-metabolizing enzymes, with not all esophageal samples being immunopositive. In contrast, another laboratory reported the presence of CYP3A4 and CYP3A5 mRNA and the localization of CYP3A protein to the squamous epithelium of the esophagus (Kolars *et al.*, 1994). The evaluation of NAT activity in human gastrointestinal tissues has been primarily concerned with the colon, due to the suggestion that rapid acetylators may possess a higher incidence of colon cancer (Ilett *et al.*, 1987). Our work has indicated the presence of NAT1 and NAT2 mRNA in small intestine and colon. In addition to the presence of NAT1 and NAT2 mRNA expression in the surface epithelial cells of both the small intestine and colon, NAT1 and NAT2 activity studies in human and animal models (Bell *et al.*, 1995; Hein *et al.*, 1991; Ilett *et al.*, 1991; Kirlin *et al.*, 1991; Land *et al.*, 1994). Our study demonstrates that NAT1 and NAT2 mRNA was present in the stratified squamous epithelial component of the esophagus, particularly in the proliferative cells lying above the lamina propria. Interestingly, the zone 1 hepatocytes surrounding the portal tracts are also considered to be proliferative in nature and this may indicate an as yet unidentified role of NAT isozymes in cellular proliferation.
expression was also detected in the epithelial cells of the crypts of Lieberkuhn. These cells are proliferative in nature and are responsible for the replenishment of the surface epithelial cells of the villi. In the rat, NAT mRNA has been shown to be predominantly localized to the basal areas of gastric glands in glandular stomach and the differentiated surface epithelial cells of the small intestine and colon (Debiec-Rychter et al., 1996). Similarly, expression of murine NAT protein is predominant in the tips of the small intestine villus (Stanley et al., 1997).

Many studies have documented the presence of phase-I enzymes, CYP3A4 and CYP4B1, and the flavoprotein NADPH-cytochrome P450 reductase in the human gastrointestinal tract (Hall et al., 1989; Kolars et al., 1994; McKinnon et al., 1994, 1995; Murray et al., 1988). CYP3A4 mRNA was observed throughout the tract and was localized to mature surface epithelial cells whereas CYP4B1 mRNA expression was low and restricted to the surface epithelial cells of the colon (McKinnon et al., 1994, 1995). CYP3A and NADPH-cytochrome P450 reductase proteins have been identified in mature epithelial cells of a range of human gastrointestinal tissues including small intestine, colon and stomach (Hall et al., 1989; Kolars et al., 1994; Murray et al., 1988). In contrast, using a range of techniques, we have been unable to demonstrate the presence of CYP1A expression in the human gastrointestinal tract (McKinnon et al., 1992). Given that the majority of food-derived chemicals require metabolism to exert their mutagenic/carcinogenic effect, this observation would suggest that N-hydroxylation of heterocyclic amines occurs in the liver, and the metabolite is then transported to the gastrointestinal tract where it undergoes further activation by the phase-II enzymes.

FIG. 5. Identification of NAT1 and NAT2 expression in human small intestine. Histological sections were hybridized with 35S-labelled NAT1 antisense (A), NAT1 sense (B), NAT2 antisense (C), or NAT2 sense (D) riboprobes. The NAT1 and NAT2 antisense probes demonstrate increases in autoradiographic signal in the surface epithelial cells of the villi and epithelial cells of the crypts of Lieberkuhn (hematoxylin and eosin, original magnification ×200).
enzymes. The hydroxylamine of heterocyclic amines may undergo acetylation and/or sulfonation to yield highly reactive acetoxy or sulfoxyl derivatives. Clearly, the current study illustrates that NAT transcripts are expressed in a range of gastrointestinal tissues, and indeed, preliminary studies in our laboratory have indicated the additional presence of aryl sulfotransferases in stomach, small intestine, and colon (Windmill et al., 1997). Other drug-metabolizing enzymes such as glutathione S-transferase and UDP-glucuronosyl-transferase have also been identified throughout surface epithelial cells of the mucosa within the human gastrointestinal tract (Peters et al., 1987; Terrier et al., 1990).

In humans, detection of NAT expression and activity in urinary tissues has focused on the bladder, due to the suggested predominance of bladder cancer patients bearing the NAT2 slow acetylator genotype (Kloth et al., 1994; Mommsen and Aagaard, 1986). N-acetylation is generally considered a detoxification step, since it competes for the amine with the activating, CYP1A2-dependent N-hydroxylation pathway. Individuals producing large quantities of N-hydroxyarylamines are potentially at risk of developing bladder cancer, since NAT is capable of converting these metabolites to unstable acetoxy esters (Frederickson et al., 1992). The present study demonstrates the presence of both NAT1 and NAT2 mRNA in the urothelium, particularly in the bladder. This is in agreement with human functional and localization studies that have indicated the presence of NAT in urothelium and bladder cell lines (Coroneos and Sim, 1991; Pink et al., 1992; Stanley et al., 1996). Although a recent immunohistochemical study could detect only NAT1 and not NAT2 in bladder epithelium, the authors suggested that their antibody was not of high enough affinity to detect any low levels of NAT2 that could be present.

**FIG. 6.** Identification of NAT1 and NAT2 expression in human colon. Histological sections were hybridized with 35S-labelled NAT1 antisense (A), NAT1 sense (B), NAT2 antisense (C), or NAT2 sense (D) riboprobes. The antisense NAT1 and NAT2 probes exhibit low autoradiographic signal in the surface epithelial cells and the crypts of Lieberkuhn (hematoxylin and eosin, original magnification ×200).
NAT mRNA localization has also been demonstrated in rat bladder epithelium and distal tubules of the rat kidney (Debiec-Rychter et al., 1996). Transitional epithelium of the murine bladder and the epithelium lining proximal convoluted tubules both demonstrated the presence of NAT protein, suggesting that NAT could metabolize carcinogens present in the urine (Stanley et al., 1997). Activity assays using renal cytosols from a range of animals have demonstrated NAT activity, including the capacity to activate mutagenic heterocyclic amines (Hearse and Weber, 1973; Reeves et al., 1991). In contrast, we were unable to illustrate conclusively the presence of NAT1 or NAT2 mRNA in human kidney. As well as NAT, human urinary tissues express NADPH-cytochrome P450 reductase, CYP3A and glutathione S-transferase (Hall et al., 1989; Murray et al., 1988; Singh et al., 1991; Terrier et al., 1990).

The presence of NAT2 mRNA in the epithelial cells lining respiratory bronchioles, albeit at low levels, suggests that the enzyme may be important in metabolizing inhaled pollutants. In contrast to these results, NAT1 enzyme activity, but not NAT2, has been detected in laryngeal mucosal tissue from smokers and non-smokers (Stern et al., 1993) and in human lung cytosols (D. M. Grant, unpublished observations). Localization of NAT mRNA to the epithelial lining of rat and mouse bronchi supports our observations in humans; in the rat NAT1 was more predominant than NAT2 (Debiec-Rychter et al., 1996; Stanley et al., 1997). Several carcinogenic arylamines, such as 4-aminobiphenyl, β-naphthylamine and 2-amino-3-methylimidazo[4,5-f]quinoline have been detected in cigarette smoke, leading to the observation that smokers have a higher risk of bladder cancer (Brockmöller et al., 1996; Talaska et al., 1991). In contrast to bladder cancer, where slow acetylators

FIG. 7. Identification of NAT1 and NAT2 expression in human bladder. Histological sections were hybridized with 35S-labelled NAT1 antisense (A), NAT1 sense (B), NAT2 antisense (C), or NAT2 sense (D) riboprobes. The NAT1 and NAT2 antisense probes demonstrate increased autoradiographic grains in the urothelium (hematoxylin and eosin, original magnification ×200).
appear to be over-represented, carriers of the fast acetylator NAT2*4/*4 genotype may be at greater risk of lung cancer (Cascorbi et al., 1996). Human bronchial epithelial cells play an important role in the metabolism of inhaled foreign compounds and several studies have indicated the presence of drug-metabolizing enzymes in the lung, including CYP1A1, CYP1A2, CYP1B1, CYP2B7, CYP2E1, CYP3A, CYP4B1, NADPH cytochrome P450 reductase, aryl sulfotransferase, and glutathione S-transferase (Hall et al., 1989; Kivistö et al., 1995; McKinnon et al., 1994; Toussaint et al., 1993; Willey et al., 1996).

The ability of a particular arylamine to be activated to a carcinogenic end-product is likely to be strongly dependent upon the relative tissue levels of NAT1, NAT2 and CYP1A2, as well as of other enzymes capable of detoxifying or activating this class of chemical agent. Many of these enzymes are subject to environmental and genetic controls which, in turn, produce interindividual variations in enzyme activity (Grant et al., 1991). In the present study, we have demonstrated the localization of NAT1 and NAT2 mRNA in liver and in a range of extrahepatic tissues, notably the bladder and intestinal tissues. An interesting finding of this study is it demonstrates that NAT2 mRNA expression has a wide tissue distribution similar to NAT1. The presence of NAT in extrahepatic tissues suggests that the \( N \)-hydroxylated metabolite might be transported from the liver, where CYP1A2 is expressed, to its target tissue where NAT is capable of activating it to a reactive electrophile. Epidemiological studies have suggested roles for NAT1 and NAT2 in colon, bladder, and lung cancers (Bell et al., 1995; Brockmöller et al., 1996; Cascorbi et al., 1996; Ilett et al., 1987), and thus an understanding of NAT expression and distribution in human tissues is of considerable importance. In

FIG. 8. Identification of NAT1 and NAT2 expression in human ureter. Histological sections were hybridized with \(^{35}\)S-labelled NAT1 antisense (A), NAT1 sense (B), NAT2 antisense (C), or NAT2 sense (D) riboprobes. The NAT1 and NAT2 antisense probes demonstrate increases in autoradiographic signal in the urothelium (hematoxylin and eosin, original magnification \( \times 200 \)).
addition, the determination of profiles of other enzymes (e.g., sulfotransferases) involved in carcinogen bioactivation and detoxification will enhance our understanding of any possible role/s they may play in producing or protecting against site-specific carcinogenesis. A logical progression from the current study would be to examine the localization and expression of NAT protein using immunocytochemistry and metabolic activities. Clearly more work is needed to determine the influence that NAT genetic variations may have on levels of expression of mRNA and protein within human tissues, and to assess what role allelic variation may play in interindividual toxic responses to aromatic amines and various disease states.

REFERENCES


