Nonadrenal Epinephrine-forming Enzymes in Humans

Characteristics, Distribution, Regulation, and Relationship to Epinephrine Levels

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Abstract

Animal studies indicate that nonadrenal tissues may synthesize epinephrine (E). Here we demonstrate phenylethanolamine *N*-methyltransferase (PNMT) and/or nonspecific *N*-methyltransferase (NMT) enzymatic activity in human lung, kidney, heart, liver, spleen, and pancreas. There was a significant overall correlation (r = 0.34) between tissue PNMT and E. PNMT and NMT in human tissues differed in substrate and inhibitor specificity, thermal stability, and antigenicity. By these criteria, PNMT in human lung and in human bronchial epithelial cells were indistinguishable from adrenal PNMT. PNMT and/or NMT activity were present in red blood cells (RBCs), and cancer cell lines. Human kidney, lung, and pancreas showed immunohistochemical staining with an antibody to adrenal PNMT. RBC PNMT activity was lower in males than females and was increased in hyperthyroidism and decreased in hypothyroidism. PNMT activity in a human bronchial epithelial cell line was dramatically increased by incubation with dexamethasone. E and ^{3}H-E levels in plasma and urine during an intravenous infusion of ^{3}H-E into humans indicated that kidney may synthesize half of urinary E. We conclude that PNMT and NMT are widely distributed in human tissues, that they may synthesize E in vivo and are influenced by glucocorticoid and thyroid hormones. (*J. Clin. Invest.* 1995. 95:2896–2902.) Key words: PNMT • nonspecific *N*-methyltransferase • dexamethasone • lung • RBC

Introduction

Epinephrine (E, also called adrenaline)[1] was considered an exclusively adrenal hormone for many years. Subsequent investigations found evidence for local E synthesis in brain (1) and retina (2), but E synthesis is still considered to be limited to few anatomic loci. A variety of stressors can cause massive adrenal E release into the circulation. This blood-borne E plays an important role in regulating cardiovascular and pulmonary function as well as glucose metabolism during stress. E acts mainly by stimulating β_2 receptors (3), for which it has a much higher affinity than any other known endogenous compound.

It is unclear whether E plays a physiologic role in unstressed human subjects. Resting plasma E levels are usually < 80 pg/ml and are apparently below the threshold at which they influence heart rate, blood pressure, carbohydrate metabolism (4, 5), or bronchial tone (6). However, drugs that block β_2 receptors induce bronchoconstriction (7) and decrease heart rate (8, 9) and cardiac output (9) in resting humans.

One hypothesis to explain these observations is that peripheral tissues make and release enough E to tonically stimulate β_2 receptors. Rat kidney synthesizes E in vivo (10) and lung, heart, and several other peripheral tissues can synthesize E from norepinephrine in vitro (11–13). Recent studies in adrenalectomized rats demonstrate that extraadrenal E synthesis can play an important regulatory role in animal models of hypertension, diabetes, and glucocorticoid toxicity (14, 15).

Human heart releases E in vivo (16), and E is present in human plasma and urine after bilateral adrenalectomy (17, 18). The location and nature of the enzymes that catalyze this extra-adrenal E synthesis in man are unknown. E is formed by the *N*-methylation of norepinephrine, and several *N*-methylating enzymes are present in humans and animals. Of these, phenylethanolamine *N*-methyltransferase (PNMT) is the only one that has been documented to synthesize E in vivo. This enzyme has a high substrate specificity for phenylethanolamines such as norepinephrine (19). Drugs which selectively inhibit this enzyme reduce blood pressure in hypertensive rodents, apparently at an extra-adrenal site (14).

Histamine *N*-methyltransferase probably does not synthesize E in human tissues because norepinephrine is not a substrate for this enzyme (20). Nonspecific *N*-methyltransferase (NMT) (also referred to as indoleamine *N*-methyltransferase) can *N*-methylate many phenylethylamines such as dopamine and phenylethanolamines such as norepinephrine (21). Serotonin is apparently the most readily methylated substrate of this enzyme (21).

Few attempts have been made to characterize the E-forming enzymes present in human extra-adrenal peripheral tissues. Henderson et al. (22) concluded that human lung contains only NMT because lung sections did not bind their anti-PNMT antibody and because fairly high concentrations of PNMT-inhibiting drugs did not reduce the enzymatic activity of lung homogenates. Pendleton et al. (23) reached a similar conclusion based on their studies of substrate and inhibitor specificity of human lung homogenates. Axelrod (21) found low levels of serotonin *N*-methylating activity in a human lung obtained at autopsy, suggesting the presence of NMT. Vogel et al. (24) and Hobel et al. (25) reported PNMT activity in several human peripheral tissues. However, both studies used substrates that are also *N*-methylated by NMT, so the enzyme-mediating the activity they observed is uncertain. Hobel et al. (25) also reported that the

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1. *Abbreviations used in this paper:* E, epinephrine; NMT, nonspecific *N*-methyltransferase; PNMT, phenylethanolamine *N*-methyltransferase; RBC, red blood cell.

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amount of enzymatic activity present in uterus and red blood cells changed during pregnancy.

Recently developed techniques now permit investigation into whether human extra-adrenal tissues synthesize E. These include a radioenzymatic assay for E-synthesizing enzymes that uses norepinephrine as a substrate (26), a more sensitive assay for E (27), and a high-affinity antibody for PNMT. In this study we tried to answer three main questions: (*1*) Which E-forming enzymes are present in human tissues? (*2*) Does their activity contribute to tissue and urine E levels? (*3*) What are some of the factors that influence their enzymatic activity?

Methods

Human tissue samples. 62 samples were collected from 10 different organs or organ components either shortly after death by the University of California, San Diego (UCSD), Organ and Tissue Acquisition Center, or at autopsy, usually within 12 h of death. The organs sampled included cardiac atria, ventricle, kidney, liver, pancreas, spleen, lung, primary bronchus, trachea, and adrenal. Samples were collected from five males and seven females. Their mean age was 46±5 yr. Cause of death included intracranial bleeding (3 subjects), head trauma (3 subjects), liver cirrhosis (2 subjects), stroke, myocardial infarction, arteriosclerosis, and pneumonia. Upon collection, organ samples were frozen at −70°C. Two separate 200-mg tissue samples were taken from each organ and were homogenized in 1 ml of 0.1 M Tris, pH 7. The homogenates were centrifuged for 10 min at 6,000 *g* at 4°C, and the resulting supernates were assayed for PNMT and NMT activities and catecholamine levels.

Human cell lines. Cell pellets from six immortalized human cell lines, unused culture media, and spent media in which cells had been grown were provided to us as the generous gift of Dr. Leonard Deftos. These cell lines included a transformed SV-40 prostate epithelial cell line (PNT1B), a medullary thyroid carcinoma (TT), a metastatic prostatic adenocarcinoma (LnCAp), a lung small cell carcinoma (H727), a prostate adenocarcinoma (PC3), and a lung squamous cell line (M103). All cells were grown in RPMI except PC3, which were grown in HF12 media. Fetal calf serum concentrations varied from 5 to 10%. Just before harvesting, spent media from each cell line was collected, and the cells were trypsinized for 1–2 min. 8 ml of fresh media was then added to the cells, and they were transferred to a conical tube. After cells were removed from culture dishes, they were centrifuged for 5 min at 200 *g*. The cell pellets and spent media from each cell line were stored at −70°C. Cell pellets were later suspended in 1 ml of ice cold 0.1 M Tris, pH 7, and were homogenized for ~ 5 s with a Polytron (Brinkman Instruments, Inc., Westbury, NY). The cell homogenates were then centrifuged for 10 min at 6,000 *g*. The resulting supernates were assayed for PNMT and NMT activities and catecholamine levels. Protein concentration was determined by the Lowry method. Samples of virgin and spent media from each cell line were also assayed for catecholamines.

Immunohistochemistry. Samples of human lung, kidney, and pancreas were obtained from the UCSD human tissue bank. Several thin sections were cut from each tissue using a freezing microtome. Duplicate sections from each tissue were fixed by submerging them in cold acetone for 15 min and then air-drying them. Sections were overlaid with 0.03% hydrogen peroxide for 10 min, then washed for three 10-min periods with phosphate buffered saline (PBS). One slide from each tissue was overlaid with a 1:700 dilution of anti-bovine PNMT antiserum for 30 min in a humid chamber. This antiserum was generated in rabbits against *S*-adenosylhomocysteine-AH-Sepharose 4B purified bovine PNMT and was the generous gift of Dr. Dona L. Wong. The duplicate slide from each tissue was incubated with normal rabbit serum instead of the anti-PNMT antiserum. The slides were then washed three times for 5 min with PBS. Next the slides were overlaid with a solution containing goat anti-rabbit antibody for 30 min. This secondary antibody solution was then drained off, and the slides were washed three times for 5 min with PBS. Slides were developed by overlaying with a substrate solution containing 3-amino-9-ethylcarbazol for 15 min. The substrate solution was then drained, and the slides were again washed three times for 5 min with PBS. Slides were counterstained with hematoxylin, washed, and mounted with glycerol gelatin. The slides were photographed in black and white on panchromatic film (Eastman Kodak Co., Rochester, NY) and were printed on Polimax paper (Eastman Kodak Co.).

Inhibition by anti-PNMT antibody. Four 75-μl aliquots of dilute (1:50) homogenate of adrenal tissue from three different cadavers were incubated with 0, 40, 80, or 160 nl of anti-bovine PNMT antibody for 1 h at 37°C and then overnight at 4°C. The antibody was the same as that used for immunohistochemistry studies. After incubation, the samples were centrifuged for 30 min at 10,000 *g* and 50-μl supernates were assayed for PNMT activity. In a similar experiment, 10 75-μl aliquots of lung homogenate from three cadavers were also incubated with 0, 0.6, 2.5, 10, or 40 nl of anti-PNMT antibody and were centrifuged as above. The resulting supernates were assayed for both PNMT and NMT activity.

In a third experiment human bronchial epithelial cells (16HBE14o−) were grown to confluence in three wells of a six-well plate in minimal essential medium with 10% fetal calf serum. The wells then received fresh media containing 1 μM dexamethasone and were incubated for an additional 7 d. The media was then removed, the cells were washed twice with calcium and magnesium-free Hanks' buffered saline solution, and cells were then scraped into a buffer consisting of 20 mM KPO_4, 0.15 M NaCl, 5 mM EDTA, 50 mM Tris, pH 7.8. The cells were sonicated on ice using a sonifier (Branson Ultrasonics, Danbury, CT). After sonication, freshly prepared phenylmethylsulfonylfluoride dissolved in ethanol was added to a final concentration of 1 mM. The cells were then frozen at −70°C. The cell homogenates were later thawed and centrifuged for 10 min at 6,000 *g*. The supernate from all cells was pooled and then allocated into 10 50-μl aliquots. The aliquots were incubated with 0, 0.6, 2.5, 10, or 40 nl of anti-PNMT antibody and were centrifuged as above. The resulting supernates were assayed for both PNMT and NMT activity.

Thermal stability. 14 50-μl aliquots of dilute (1:50) homogenates of adrenal tissue obtained from three different autopsies were assayed for PNMT and NMT activities with the modification that two aliquots were incubated at each of the following temperatures for 1 h: 4, 25, 37, 45, 55, 65, 75°C. At each temperature one aliquot was assayed for PNMT activity and the other was assayed for NMT activity.

In a related experiment, 14 50-μl aliquots of lung homogenate derived from each of six cadavers were assayed for both PNMT and NMT activity at various incubation temperatures as described above.

In a third experiment human bronchial epithelial cells (16HBE14o−) were grown, treated, and pooled in a manner similar to that described above. The pooled supernate was then allocated into 14 50-μl aliquots. The aliquots were then assayed for PNMT and NMT at the seven incubation temperatures described above.

Dialysis. Two 50-μl aliquots of lung homogenate supernate from five different autopsy samples were pipetted into polypropylene tubes. The tubes were then capped and stored for 18 h at −70°C. 200 μl of lung homogenate supernate from the same samples were also pipetted into Spectra/Por dialysis tubing (Spectrum Medical Industries Inc., Houston, TX) with a 12–14 kD cutoff. Dialysis tubing containing the lung homogenates was then placed in a flask containing 1 liter of 0.1 M Tris, pH 7, at 4°C for 18 h. 50-μl aliquots of the dialyzed lung homogenates were then assayed for PNMT and NMT activity along with the undialyzed lung homogenate aliquots.

Bronchial epithelial cells were grown in all wells of a six-well plate and were treated with dexamethasone in a manner similar to that previously described. The cells from each well were harvested into separate tubes, sonicated, and centrifuged, and the supernate was frozen at −70°C. The supernate from each of the six tubes was later thawed and divided into two 150-μl aliquots. One of each pair of aliquots was pipetted into dialysis tubing and was dialyzed as described above. The other aliquot was refrozen and stored at −70°C for 18 h. 50-μl aliquots

from the six sets of dialyzed and undialyzed cell supernates were then assayed for PNMT and NMT activity.

Distribution of PNMT and NMT activity in RBCs of humans. 7 ml of blood was collected by venipuncture from 103 randomly selected blood donors. 57 were males with a mean age (±SEM) of 39.9±1.8 yr, ranging from 18 to 68 yr and 46 females 40.7±2.0 yr, ranging from 19 to 74 yr. After collection, blood was immediately placed on ice and was centrifuged for 10 min at 3,000 *g* within 3 h of collection. After the plasma and buffy coats were removed, 2 ml of packed RBCs were transferred to a 13 × 100 mm polypropylene tube and 2 ml of ice-cold distilled water was added to lyse the cells. The tubes were then vortexed and frozen at −70°C until 50-μl aliquots were assayed for PNMT and NMT activity.

Effect of dexamethasone on PNMT in bronchial epithelial cells. Human bronchial epithelial cells (16HBE14o−) were grown to confluence in all wells of a six-well plate in minimal essential medium with 10% fetal calf serum. Three of the wells then received fresh media containing 1 μM dexamethasone, and the remaining three wells received the same media without dexamethasone. The cells were incubated for an additional 7 d. Supernates of the cell homogenates were then prepared from each well in the same manner as described above. 50 μl of supernate from each well of cells was assayed for PNMT and NMT activity. Protein concentrations were determined by the Bradford method.

Effect of thyroid function on RBC PNMT activity. 7 ml of heparinized blood was collected from 8 normal healthy subjects (age 42±4 yr, mean±SEM), 4 hyperthyroid subjects (40±16 yr), and 11 hypothyroid subjects (47±5 yr) before and after thyroid replacement therapy for 4±0.5 mo. The blood was immediately placed on ice and within 1 h was centrifuged at 3,000 *g* for 10 min at 4°C. The plasma and buffy coat were then removed and 2 ml of packed RBCs were transferred to a polypropylene tube. 2 ml of ice-cold distilled water was added to the RBCs, and they were then mixed with a vortex and frozen at −70°C until assayed for PNMT and NMT activity.

In vivo E synthesis by human kidney. Three healthy males aged 35±4 (SEM) yr were placed in a prone position and were catheterized in a radial artery and in an antecubital vein of the opposite arm. Shortly thereafter ^{3}H-E (New England Nuclear, Boston, MA) was infused intravenously at a rate of 1.46 μCi/min for 10 min and then at half this rate for 2 h and 50 min. To enhance the urine formation rate, subjects received a standard water load. At the start of the final hour of the ^{3}H-E infusion subjects urinated as completely as possible. During the final hour of the infusion 7 ml blood was drawn four times at 15-min intervals. The resulting plasma was stored at −70°C and later was assayed for ^{3}H-E and E levels. Just before the end of the ^{3}H-E infusion, as much urine as possible was again collected from subjects for determination of urine formation rate and urinary ^{3}H-E and E levels. Urine was frozen at −70°C until assay. Plasma and urinary E levels were assayed by our radioenzymatic method (27). ^{3}H-E levels in urine and plasma were determined by alumina extraction (26).

Urine ^{3}H-E clearance and expected and actual rates of E excretion into urine were calculated according to the following formulas:

Urine ^{3}H-E clearance (ml/min) = urine formation rate (ml/min) × ^{3}H-E in urine (dpm/ml)/^{3}H-E in plasma (dpm/ml)

Expected urine E excretion (ng/min) = urine ^{3}H-E clearance (ml/min) × mean arterial E level (ng/ml)

Actual urine E excretion rate (ng/ml) = urine formation rate (ml/min) × mean urine E level (ng/ml)

Assays. PNMT and NMT activity was assayed by the radioenzymatic method of Ziegler et al. (26). Briefly, 50-μl samples of tissue homogenate were incubated for 2 h at 25°C with 50 μl of a solution containing 10^{-3} M norepinephrine as substrate, ^{3}H-S-adenosylmethione, EDTA, dithiothreitol, and Tris buffer at pH 8.5. The PNMT inhibitor SKF 29661 (10^{-3} M) (28) was also added to a duplicate aliquot of each homogenate sample to determine NMT activity. After incubation, the samples were shaken with alumina and then repeatedly washed with cold water. Catecholamines were then eluted from the alumina with 0.1 M perchloric acid. After a precipitation step, the samples were transferred to counting vials. Phosphate buffer, scintillant, and 1% di-ethylhexylphosphoric acid in toluene were then added, and the samples were counted in a liquid scintillation counter. NMT activity was calculated by subtracting the dpm present in the buffer blank tubes from the dpm of tritiated product present in the sample tubes which contained 10^{-3} M SKF 29661. PNMT activity was calculated by subtracting the dpm present in the sample tubes containing SKF 29661 from the dpm present in duplicate sample tubes in the absence of SKF 29661. This was done because both PNMT and NMT can *N*-methylate NE, but only PNMT activity is inhibited by the PNMT inhibitor SKF 29661. The assay was run under conditions designed to optimize product formation. A detailed description of the method and information regarding its sensitivity and specificity are given in reference 26.

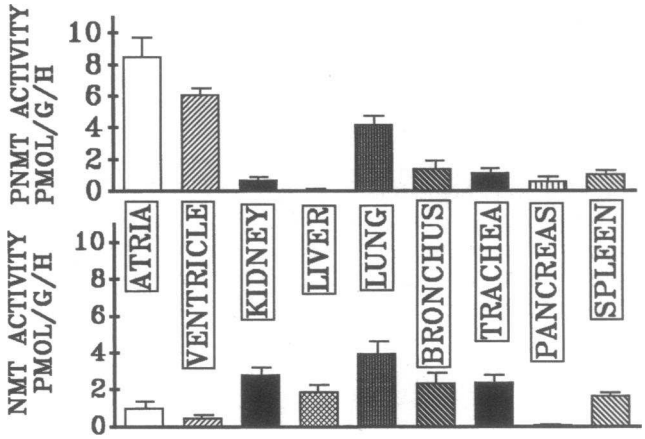

Figure 1. PNMT and NMT activities in nine human tissues obtained at autopsy. Each value is the mean±SEM of determinations made on 3–11 autopsy samples and is expressed in picomoles ^{3}H-E formed per gram wet weight tissue per hour.

E levels were determined in tissues and fluids by the radioenzymatic method of Kennedy and Ziegler (27). Briefly, the catecholamines in 50–100-μl homogenate samples were extracted into a solvent and then into dilute acetic acid. The catecholamines in this solution were then *O*-methylated by incubation in the presence of excess ^{3}H-S adenosylmethionine and catechol-*O*-methyltransferase. The *O*-methylated catecholamine metabolites were then separated by thin-layer chromatography, and the metanephrine band was scraped into a scintillation vial. Metanephrine was converted to vanillin, scintillant was added, and the sample was counted on a liquid scintillation counter.

Results

PNMT and NMT activity were both detectable in most human tissues (Fig. 1). In this assay, blanks averaged 380±66 cpm (95% confidence interval), and were not effected by the presence of 10^{-3} M SKF 29661. In contrast, cardiac atria on average generated ~ 8,700 cpm in the absence of SKF 29661 and 2,700 cpm in the presence of this PNMT inhibitor. Lung homogenates yielded on average roughly 5,600 cpm, and this was reduced to ~ 3,500 cpm in the presence of PNMT inhibitor. PNMT activity predominated in heart and lung, whereas NMT was more prevalent in kidney, liver, bronchus, and trachea. Overall, tissue E levels correlated with PNMT activity (r = 0.34 P = 0.025) but not with NMT (r = 0.11) Because circulating E can be taken up by sympathetic nerves, it was of interest to

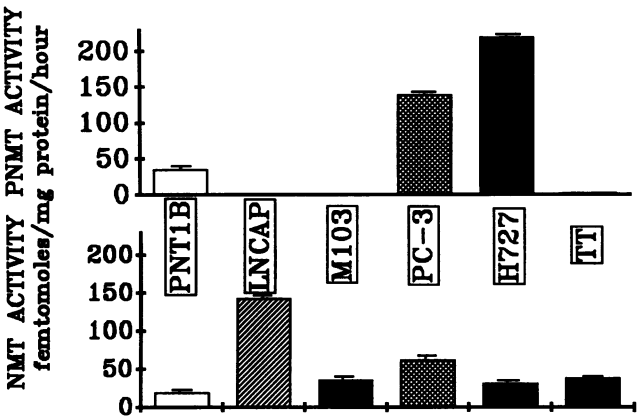

Figure 2. PNMT and NMT activity in homogenates of six human cell lines. Each value is the mean±SEM of three determinations.

inspect E levels in tissue with relatively poor sympathetic innervation such as lung. In bronchus the correlation coefficient (r) between E and PNMT activity was 0.968 (P = 0.007) and between E and NMT was 0.636. In trachea the correlation between E and PNMT was 0.825 and between E and NMT was 0.864. The correlation between E and lung NMT was also highly significant (r = 0.976, $P < 0.001$).

PNMT and/or NMT activity are present in human cell lines derived from a variety of tissues (Fig. 2). PNMT activity was readily detectable in the PNT1B, PC3, and H727 cell lines. NMT activity was present in all cell lines. E was present in homogenates of PNT1B, M103, and H727 cells at concentrations of 37, 4.7, and 24.5 pg/mg protein, respectively. E was not detectable in spent media from these cells.

PNMT was visualized in sections of human kidney, lung, and pancreas with immunoperoxidase staining. In all three tissues, dark brown peroxidase staining indicative of binding of anti-PNMT antibody was widely but not uniformly distributed. In the kidney, staining was most evident in glomeruli (Fig. 3 *A*), distal tubule, and in endothelial cells. In the lung, PNMT staining was most prominent in pulmonary alveolar cells. Staining of both parenchymal cells and islet cells was observed in the pancreas. Control slides of kidney (Fig. 3 *B*), lung, and pancreas that were incubated with normal rabbit serum instead of anti-PNMT antiserum did not exhibit peroxidase staining.

Extra-adrenal PNMT resembled adrenal PNMT (Fig. 4). PNMT activity in human adrenal and lung homogenates were both inhibited by anti-PNMT antibody but NMT was not. Human lung and adrenal PNMT also had similar changes in activity when incubated over a range of temperatures (Fig. 5). NMT in lung and adrenal homogenates remained more active at higher incubation temperatures than PNMT. Lung PNMT activity was more than tripled by dialysis, but NMT activity was unchanged (Fig. 6). Similar experiments were performed in homogenates of the 16HBE14o− human bronchial epithelial cell line. PNMT activity in these cells was inhibited by anti-PNMT antibody. PNMT thermal stability in these cells was similar to that of adrenal PNMT, but dialysis did not enhance *N*-methylating activity in these cells.

PNMT, and to a somewhat lesser extent, NMT were present in human RBC. RBC PNMT activity in a population of healthy females was normally distributed and was ~ 7% higher than in males (P = 0.014 by Wilcoxon rank sum test) (Fig. 7). The

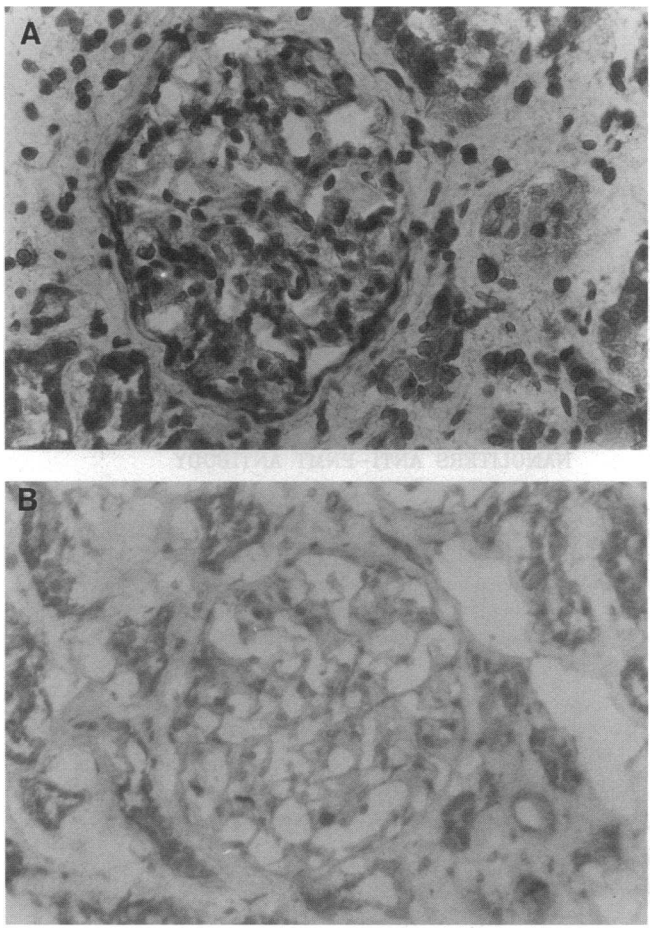

Figure 3. Immunoperoxidase staining of sections of human kidney (×20) incubated with anti-PNMT antiserum (*A*), or normal rabbit serum (*B*). Marked glomerular immunoperoxidase staining was observed in the section incubated with anti-PNMT antiserum but not in the slide incubated with normal rabbit serum. The dark brown immunoperoxidase staining that was present with anti-PNMT antiserum appears as very dark areas in this black and white photograph.

distribution of PNMT activities in a population of healthy males was skewed ($P < 0.1$). RBC NMT activity was similar in both sexes and was normally distributed. The activity of both *N*-methylating enzymes in RBCs was unrelated to age.

Both PNMT and NMT activities are increased in some rat extra-adrenal tissues after glucocorticoid treatment. Incubation of a PNMT containing human bronchiolar cell line with 1 μM dexamethasone for 7 d dramatically increased its PNMT activity (Fig. 8) but did not significantly alter NMT activity.

Human PNMT also appears to be responsive to thyroid hormones. RBC PNMT activity was reduced below normal levels in hypothyroid patients and returned to normal levels when patients became euthyroid after chronic thyroxine treatment (Fig. 9). Hyperthyroid subjects had elevated RBC PNMT activity. NMT activity was unaltered by either hypothyroidism or hyperthyroidism.

During the final hour of a ^{3}H-E infusion, three normal subjects excreted urine at a rate of 7.6±8 ml/min. Their rate of ^{3}H-E clearance during this period was 154±30 ml/min, which is close to the rate of normal human glomerular filtration. During this interval the subjects had a mean arterial plasma E level of

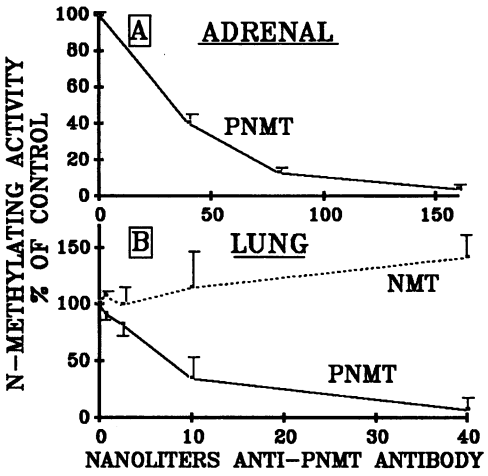

Figure 4. PNMT activity in adrenal (*A*) and PNMT and NMT activity in lung homogenates (*B*) in the presence of anti-PNMT antibody. Each value is the mean±SEM of determinations from three different autopsy specimens. Adrenal homogenates had substantially higher basal PNMT activity than lung homogenates. Inhibition of PNMT activity in both tissues was significant by ANOVA, $P < 0.015$.

45±7 pg/ml and a urine E level of 1802±491 pg/ml. They should have excreted 6.9±2.0 ng E/min into their urine. They actually excreted 13.0±2.1 ng E/min into their urine, significantly ($P < 0.003$ by t test) more than expected.

Discussion

The results suggest that most human peripheral tissues contain both PNMT and NMT, and that these enzymes may contribute substantially to E levels in some tissues and urine. This nonadrenal PNMT may be influenced by endogenous inhibitors, gender, and glucocorticoid and thyroid hormones.

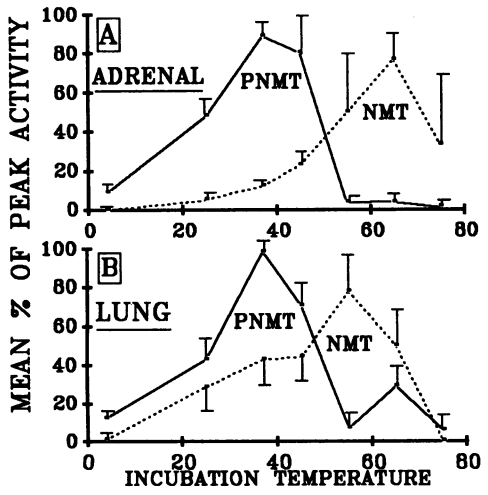

Figure 5. PNMT and NMT activity in homogenates of human adrenals (*A*) and lung (*B*) incubated at various temperatures. Each value is the mean±SEM of determinations made from three to six autopsy specimens. In both adrenal and lung the temperature sensitivity of PNMT differed significantly from NMT by two-way ANOVA mixed model, $P < 0.0001$.

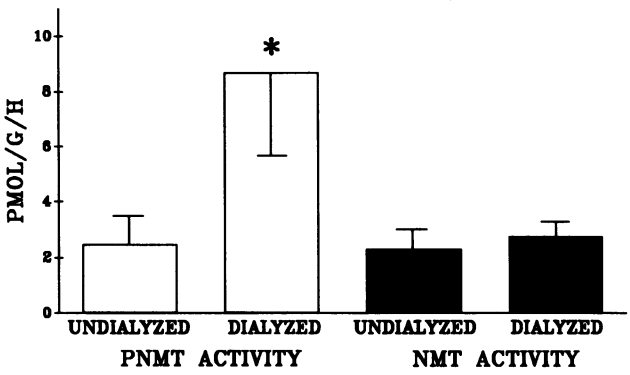

Figure 6. PNMT and NMT activity in lung homogenates with and without dialysis against 0.1 M Tris, pH 7, for 18 h at 4°C. Values are mean±SEM of determinations made from five different autopsy specimens and are expressed in picomoles of ^{3}H-E formed per gram wet weight tissue per hour. $*P < 0.05$ vs. PNMT activity of undialyzed samples by paired t test.

Rodent studies indicate that both PNMT (24) and NMT (22) activity are stable for as long as 30 h after death when tissues are treated in a manner simulating human postmortem conditions. Inasmuch as most of our tissue samples were collected and frozen within a few hours of death, it is likely that little degradation of enzyme activity occurred.

There was marked variability in PNMT and NMT activity in human tissues. PNMT activity was highest in heart and lung, NMT activity was highest in lung and kidney. E levels also varied markedly in the different tissues, and we found a modest, but statistically significant overall correlation with PNMT activity ($r = 0.34$). In the lung and bronchus the relationship between E levels and E-forming enzyme activity was exceptionally strong ($r > 0.95$). This may be due to the relatively high *N*-methylating activity in these tissues combined with comparatively sparse sympathetic innervation (29). Sympathetic nerves

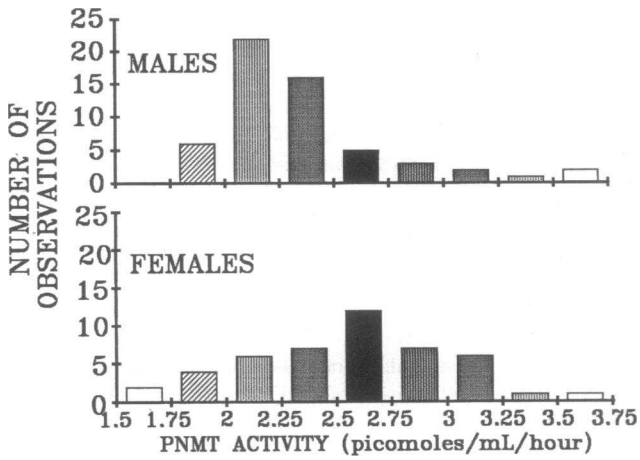

Figure 7. Histograms displaying distribution of PNMT activities found in RBC of 57 male and 46 female blood donors. There is less than 1 chance in 10 that PNMT activity in RBCs of males is normally distributed. RBC PNMT in males was significantly lower than in females $P = 0.014$ by Wilcoxon Rank Sum test.

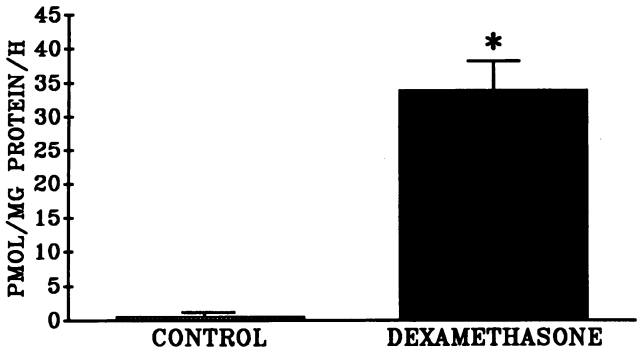

Figure 8. PNMT activity in immortalized human bronchial epithelial cells incubated in the presence or absence of 1 μM dexamethasone for 7 d. Values are the mean±SEM of determinations made from three separate wells of cells. $*P = 0.002$ by Student's t test.

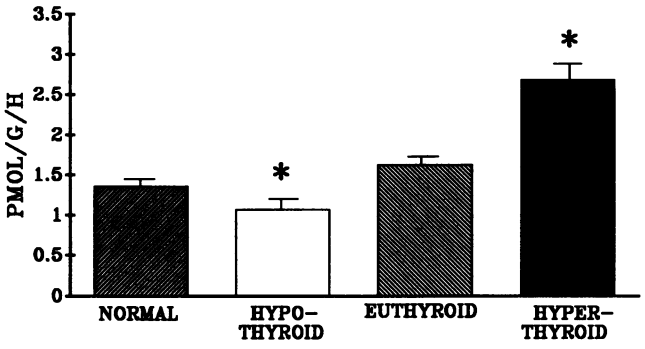

Figure 9. PNMT activity in RBC lysate from normal subjects, hypothyroid patients before and after chronic treatment with thyroxine, and hyperthyroid subjects. Each value is the mean±SEM of determinations made from 4–15 subjects. Overall intergroup differences were significant by one-way ANOVA, $P < 0.001$. $*P < 0.05$ vs. euthyroid by Newman-Keuls test.

can take up E from blood, so sparse innervation lessens the contribution of circulating E to tissue E levels.

The view that human lung can synthesize E is further supported by the finding that some lung cell lines can synthesize E in vitro in the absence of exogenous dopamine or norepinephrine. Homogenates of H727 and M103 cells had *N*-methylating activity, E, norepinephrine, and dopamine despite the absence of detectable levels of these compounds in either unused or spent culture media. The lack of detectable E in spent media may be due to the small cell mass, instability of E in culture media, or a low spontaneous excretion rate in the absence of a specific secretogogue. These two cells lines are derived from neuroendocrine cells (30, 31), which comprise only a small fraction of lung tissue. However, we found considerable PNMT activity in bronchial epithelial cells and widespread binding of anti-PNMT antibody to lung tissue. Thus, the potential for E synthesis in human lung is substantial. The assay we used measures conversion of norepinephrine to E in vitro, unlike most prior assays that use a substrate such as phenylethanolamine to infer E-forming activity. An enzyme present in lung is indistinguishable from adrenal PNMT with respect to antibody binding, temperature sensitivity, and inhibition of enzymatic activity by the drug SKF 29661 or antibody. In addition, the enzyme in cell culture is glucocorticoid inducible, as is adrenal PNMT.

The location of PNMT in the renal glomeruli and tubules suggests that the kidney may synthesize E at sites predisposing it to urinary excretion. The rat kidney synthesizes E and adrenalectomized rats excrete E (10). E is also present in the urine of adrenalectomized humans (18). Our subjects excreted much more E into urine than they would have filtered from blood. Renal E production could play a role in kidney function because tubular α receptors increase sodium reabsorption and renal β receptors stimulate renin release.

Paraganglion cells, some of which contain PNMT, are present in many peripheral organs and could contribute to tissue E. However, our immunohistochemistry results suggest that these cells comprise only a small fraction of the PNMT containing cells in most organs, so their contribution to extra-adrenal E production in normal humans is likely to be small.

Studies in the rat shed some light on whether extra-adrenal tissues might synthesize enough E to be biologically active. Adrenalectomized rats treated with a glucocorticoid develop high levels of PNMT in lung (11), and lung E levels fall when rats are given a PNMT inhibitor (32). Glucocorticoid-treated rats also develop high blood pressure and insulin resistance, which normalize after treatment with a PNMT inhibitor (14, 15). These studies demonstrate a potential physiologic role for human extra-adrenal PNMT.

Dialyzable inhibitors of PNMT are present in lung tissue. These dialyzable inhibitors are not present in all cells with PNMT because dialysis of homogenates of cultured bronchial epithelial cells did not increase their PNMT or NMT activity. Tissue assays of PNMT activity probably underestimate actual PNMT levels because of these inhibitors.

Our finding of PNMT and NMT activity in human lung differs from the results of previous authors (22, 23) who found only NMT. These prior studies found no inhibition of lung *N*-methylating activity by the competitive PNMT inhibitor SKF 64139. The lack of effect of PNMT inhibiting drugs on the activity of their lung homogenates may be because these investigators had a much higher substrate:inhibitor ratio in their assays than we did. Furthermore their substrate (phenylethanolamine) is three times more readily *N*-methylated than ours (norepinephrine) (19). Thus, it is possible that their inhibitor was displaced by excess substrate. Henderson et al. (22) found no binding of their anti–bovine PNMT antibody, whereas our anti–rat PNMT antibody bound to lung. Human and bovine PNMT are slightly different structurally from each other (33, 34), and the reactivity of polyclonal antibodies from different sources is likely to be variable.

We studied PNMT and NMT activity in RBCs to determine the potential contribution of these cells to tissue enzyme levels, and to gain some insight into the distribution of these enzymes in a population of healthy humans. It seems unlikely that RBCs greatly influence tissue enzyme activity levels inasmuch as RBC lysate resulted in PNMT and NMT activity levels comparable to those we found in most tissues, yet RBCs comprise only a small fraction of the total volume of the tissues that we examined. RBC PNMT activity was normally distributed in females. Male RBC PNMT activity was significantly lower than in females because of a skewing of the distribution of PNMT activities. PNMT activity was elevated in hyperthyroid patients. If hyperthyroidism also increases PNMT activity in organs such as heart and skeletal muscle, then local E production could

account for the increased heart rate and tremor seen in this disease.

In conclusion, human tissues outside the adrenal contain E-synthesizing enzymes. One of these is indistinguishable from adrenal PNMT and levels of extra-adrenal PNMT correlate with tissue E levels. PNMT in a human cell line was induced by a glucocorticoid. Radiotracer studies indicate that nearly half of urine E may be made by the kidney. Prior studies show that extra-adrenal PNMT plays an important role in glucocorticoid hypertension and insulin resistance in rats. Extra-adrenal PNMT may play a physiologic and pathologic role in humans.

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