Endothelin Production by Human Inner Medullary Collecting Duct Cells[1]

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The inner medullary collecting duct (IMCD) is the major nephron site of endothelin-1 (ET-1) production (1) in experimental animals. Subsequent studies have determined that ET-1 binds to high-density, high-affinity receptors on IMCD cells (2), resulting in potent inhibition of water transport (3) and Na/K ATPase activity (4). Hence, this peptide may function as an autocrine regulator of IMCD sodium and fluid reabsorption. Whether such an autocrine system exists in human kidneys is unknown. Recent studies suggest, however, that the human renal inner medulla does contain endothelin. For example, homogenated inner medulla contains the greatest concentration of endothelin found in the human kidney (5). Additionally, autoradiographic studies have localized the greatest binding of endothelins to the human medulla (6). It remains unclear, however, what cell types are synthesizing endothelin in the human inner medulla. This study was undertaken, therefore, to determine if human IMCD cells are capable of endothelin production.

METHODS

Cell Culture

Four human kidneys were obtained at the time of nephrectomy for renal cell carcinoma. All tumors involved only the renal cortex. IMCD cells were isolated and cultured by a modification of the technique previously described for human papillary collecting duct cells (7). Briefly, the inner medulla was minced and incubated in 0.1% collagenase (type II; Worthington Diagnostics Systems, Freehold, NJ) for 1 h at 37°C. The osmolality was adjusted with distilled water to 120 mosM in order to disrupt all cells except those of the collecting duct (1). Cells were suspended in 50:50 Dulbecco modified Eagle medium:F12 containing 5 μg/ml of insulin, 5 μg/ml of transferrin, 5 ng/mL of selenium, 50 nM hydrocortisone, and 25 ng/mL of prostaglandin E_1. All studies were performed at confluence, approximately 6 to 8 days after plating. All cultures were free of endothelial cells, as determined by negative immunofluorescence for factor VIII–related antigen and lack of uptake of dilacetylated low-density lipoprotein (99% of human umbilical vein endothelial cells were positive by these criteria).

Measurement of Endothelin Release

Confluent human IMCD cells were incubated in the above culture media without phenol red for 24 h at 37°C in a 5% CO_2 incubator. Endothelin was extracted from supernatants with a C_8 cartridge (Analytichem Int., Harbor City, CA) as previously described (1). This procedure yielded 85% recovery of ET-1 and ET-3. Eluates were dried and suspended in RIA buffer. ET-1 and ET-3 were measured by the use of commercial kits (Peninsula Laboratories, Belmont, CA) (an ET-2 RIA is not currently available). These antibodies have less than 7% cross-reactivity with other endothelin isoforms. Cell monolayer protein was solubilized with 0.1 N NaOH, an aliquot was mixed with Bradford reagent (Bio-Rad, Richmond, CA), and absorbance at 590 nm was determined (8). All data are expressed as mean ± SE.

Detection of Endothelin mRNA

The presence of mRNA for human ET-1 and ET-3 was detected by reverse transcription and polymerase chain reaction (PCR) as previously described by this laboratory (2). Freshly isolated confluent monolayers of human IMCD cells were homogenized in guanidinium thiocyanate and RNA isolated by cesium chloride density gradient centrifugation (2). Total RNA was washed, ethanol precipitated, quantified spectrophotometrically, and reverse transcribed with random primers. cDNA was amplified by 35 cycles of PCR with specific primers spanning positions 375 to 547 in human ET-1 cDNA (9) and 811 to 1,042 in human ET-3 cDNA (10). Products were electrophoresed through 8% polyacrylamide containing ethidium bromide, and photographs were taken of the gel.

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RESULTS AND DISCUSSION

Confluent cultures of human IMCD cells released 119.4 ± 12.2 pg of ET-1/mg of total cell protein/day into the bathing media (N = 9). In contrast, these cells released only 2.8 ± 2.6 pg of ET-3/mg of total cell protein/day into the bathing media (N = 9). In order to confirm that IMCD cells were synthesizing ET-1 and ET-3, RNA from confluent cultures of human IMCD cells was reverse transcribed and the resultant cDNA was amplified by PCR with primers specific for human ET-1 and ET-3. As shown in Figure 1, mRNA for both ET-1 (172 base pairs [bp]) and ET-3 (231 bp) was present in these cells. To rule out phenotypic changes in culture, freshly isolated human IMCD cell mRNA was analyzed. Again, both ET-1 and ET-3 mRNA were present (Figure 1). It should be noted that the PCR was not quantitative, hence, the relative amounts of ET-1 and ET-3 mRNA cannot be determined from these data.

These studies provide the first direct evidence that human IMCD cells produce endothelins. The amount of ET-1 produced by human IMCD cells (119 pg/mg of protein/day) was less than that produced by rabbit (1,300 pg/mg of protein/day) (1) or rat (1,016 pg/mg of protein/day) (8) IMCD cells. This does not, however, preclude a physiologic role for human IMCD cell-derived ET-1. For example, 25 pg/mL (10 pM) of ET-1 inhibits Na/K ATPase activity in rabbit IMCD (4), a concentration at the lower end of the range of those seen in our cultures (20 to 100 pg/mL). It is also possible that ET-1 production by these cells is further augmented under certain conditions. Urinary ET-1 clearance is 50% of GFR in normal patients but rises to 300% of GFR in patients with renal disease (with a 50% reduction in GFR) (11). This suggests that tubule-derived (probably including IMCD) ET-1 is augmented in renal disease.

The significance of alterations in ET-1 production by the IMCD is beginning to be elucidated. ET-1 binds to and activates, endothelin receptors on IMCD cells in an autocrine manner (12). Because ET-1 inhibits sodium and water transport systems in the IMCD (3,4), the potential exists for variations in IMCD ET-1 production to directly modulate IMCD sodium and water reabsorption. Recent studies suggest that increases in extracellular sodium concentration may decrease IMCD ET-1 production both *in vitro* and *in vivo* (13). Increasing inner medullary sodium concentration, as occurs with dehydration, would cause decreased IMCD ET-1 production, thereby decreasing ET-1–induced tonic inhibition of sodium and water reabsorption by the IMCD. Hence, IMCD-derived ET-1 may serve as a link between body volume and tubule sodium and water transport. Further studies are needed to determine if this system is operative and important in the regulation of volume homeostasis.

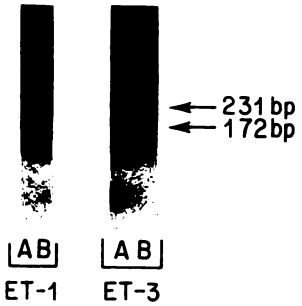

Figure 1. Reverse PCR detection of ET-1 (172 bp) and ET-3 (231 bp) mRNA in cultured (A) and acutely isolated (B) human IMCD cells. Each lane was loaded with 20 μL from the PCR involving 250 ng of cDNA. A representative gel is shown of three separate experiments.

In contrast to ET-1, the amount of ET-3 release was so low as to raise the question of whether this endothelin isoform modulates IMCD function. Previous studies have also detected very little ET-2 or ET-3 in homogenated human inner medulla (5). Similarly, relatively little ET-3 is released by rabbit IMCD cells (1). Whether IMCD-derived ET-3 can affect the function of this cell type remains to be determined. It is conceivable that conditions, such as those discussed above, exist *in vivo* in which IMCD are stimulated to produce greater amounts of ET-3 than were detected in these studies.

In summary, human IMCD cells produce predominantly ET-1. The factors regulating human IMCD ET-1 production, as well as the effect of ET-1 on this cell type, are areas for further investigation.

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