Isolation and characterization of the lower portion of the thin limb of Henle in primary culture

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Isolation and characterization of the lower portion of the thin limb of Henle in primary culture. Am. J. Physiol. 274 (Renal Physiol. 43): F775–F782, 1998.—To further characterize cells of the lower portion of the thin limb of Henle (TLHlp) under defined conditions in vitro, we developed a technique to enrich this cell population in suspension. TLHlp cells were isolated by enzymatic digestion of rat inner medulla, elimination of collecting ducts by lectin-coated beads, and differential centrifugation. Immunohistochemical staining of primary cultures of TLHlp cells with various markers revealed the preparations to be >90% pure. The hormonal stimulation pattern of PGE₂ and cAMP production by arginine vasopressin preparations to be inaccessible for investigation. This is possibly the reason why in some studies, these tubular segments were omitted (e.g., 1, 14).

We have now developed a technique to enrich cells of the lower, inner medullary portion of the long-looped thin limbs of Henle for the first time in suspension to a very high degree of purity, with the option to start primary cultures. To isolate this cell population, cells had to be purified from the other inner medullary cell types: the tubular cells of the inner medullary collecting duct (IMCD), the interstitial cells, and the vasculature, whereby each of these populations is composed of different subtypes, respectively.

The technique reported here permits studies both in directly isolated and in cultured cells, permitting, for example, the examination of long-term effects under defined conditions in vitro. The presented method will therefore allow new questions to be addressed regarding these tubular segments.

METHODS

Cell isolation. Male Wistar rats (Zentralinstitut für Versuchstierzucht, Hannover, Germany; body wt 200–250 g) were killed by cervical dislocation. Kidneys were immediately removed, and the inner medulla, defined as the white portion only, was excised. Tissue was placed in 290 mosmol/kgH₂O ice-cold HEPES-Ringer buffer (composition in mmol/l): NaCl, 164, H-HEPES, 16, Na-HEPES, 14, glucose, 3.2, CaCl₂, 1.8, MgSO₄, 1.8, KH₂PO₄, pH 7.4. It was minced with a razor blade and subsequently incubated at 37°C in the same buffer containing in addition 0.2% (wt/vol) collagenase (CLS II; Cooper, Frankfurt, Germany) and 0.2% hyaluronidase (Boehringer, Mannheim, Germany) for 75 min. After 30 min, we added 0.001% DNase to prevent cell clumping, and cells were dispersed by pipetting them through a fire-polished, large-bore Pasteur pipette.

After incubation procedure was completed, the cell suspension was centrifuged at a low-speed centrifugation at 28 g for 2 min to remove the bulk of the IMCD cells by enrichment of this cell type in the pellet. By repeating this step three times, IMCD cells, used for comparison in the biochemical studies, were enriched in the pellet (24). To obtain the cells of the TLH, supernatants of the first two centrifugations were saved and subsequently spun down (150 g for 10 min). The cells enriched in the pellet were resuspended in 1–2 ml HEPES-Ringer buffer. This suspension still contained a high amount (~30%) of collecting duct cells, which could be removed almost completely by an incubation with Dolichos biflorus agglutinin (DBA)-labeled beads. This lectin binds in the inner medulla exclusively to the collecting duct cells (24). The beads were prepared by incubating Tosyl-activated beads (10⁶ beads/ml; Dynal, Oslo, Norway) with avidin DX (10⁻⁴ M; Vector Laboratories, Burlingame, CA) in 150 mM NaCl and 50 mM NaHCO₃ (pH 8.5) for 12 h. After washing with PBS, they were subsequently incubated with 0.5 × 10⁻⁴ M biotinylated DBA (Vector Laboratories, Burlingame, CA) in 150 mM NaCl, 0.1
grown on collagen (20 µg/cm² collagen type VII, Sigma)–plated on three wells of a 96-well microtiter plate or equivalently completely removed by two purification steps in a magnetic field. The cell suspension was incubated with the DBA-coated biotinylated DBA was removed by an additional washing step. The cell suspension was incubated with the DBA-coated supports in a 1:1 mixture of DMEM and Nutrient Mix Ham’s F-12 (1:1), supplemented with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, nonessential amino acids (1% (wt/vol) Nycodenz [5-n-(2,3-dihydroxypropyl)amid]-isopthalmid (Nycomed, Oslo, Norway)) in solution A (0.3 mM CaNa₂-EDTA, 5 mM Tris-HCl, 3 mM KCl; osmolality, 284 mosM, pH 7.4). Twenty and eight percent Nycodenz solutions were obtained by appropriate dilution with 7.45% (wt/vol) sucrose dissolved in solution A. Two milliliters of the 20% Nycodenz solution were overlayered with 3 ml of the 8% Nycodenz solution in a 15-ml plastic tube. A continuous gradient was obtained by cautiously putting the tube into a horizontal position for ~60 min.

The self-formed gradient had a density from 1.052 to 1.093 g/cm³. The cell suspension was placed on the top of the gradient and spun at 1,500 g for 45 min. The gradient was then separated into three fractions, comprising the following densities: I, 1.052–1.069 g/cm³; II, 1.070–1.080 g/cm³; and III, 1.081–1.093 g/cm³. As outlined in the following, TLH cells were predominately enriched in fraction I, to a lesser extent in fraction II, and least enriched in fraction III. The Nycodenz was removed by two centrifugations (430 g, 10 min) through culture medium (see below), and the cells were either directly examined in suspension or plated in culture wells. If cells were subjected to hypotonic lysis, this step was performed following the density gradient centrifugation. The hypotonic shock was induced by diluting the incubation buffer with double distilled H₂O 1:2 or 1:3. Cells were exposed to hypotonicity for periods between 60 s and 45 min, after which an equal volume of culture medium was added again. Cells were then immediately centrifuged at 150 g for 10 min and resuspended in culture medium.

IMCD cells and inner medullary fibroblasts (IMF) used for the comparative biochemical studies were prepared as reported previously (8, 24). For one experiment, cells were derived from the same pool of animals.

Cell culture. At least five but usually 10 rats were killed per experiment. Isolated cells were either examined directly, or primary cultures were started. TLH cells obtained from one rat (corresponding to ~55±3±4 µg protein, n = 30) were plated on three wells of a 96-well microtiter plate or equivalent area. We omitted cell counting, because the cell suspension consisted predominantly of tubular fragments of variable size and only to a minor extent of single cells. Cells were grown on collagen (20 µg/cm² collagen type VII, Sigma)-coated supports in a 1:1 mixture of DMEM and Nutrient Mix Ham’s F-12 (1:1), supplemented with 10% FCS, 2 mM glutamine, 1 mM sodium pyruvate, nonessential amino acids (1% (wt/vol) Nycodenz), 50 U/ml penicillin, and 50 µg/ml streptomycin (all from Gibco-BRL, Life Technologies). Medium was usually changed every 2nd day.

Cryosections. Kidneys were dissected into cortex and medulla. They were immediately frozen in isopentane cooled by liquid nitrogen. Frozen sections with a thickness of 6–12 µm were prepared with a cryostat (Reichert-Jung, Nussloch, Germany).

Immunohistochemistry. Frozen sections as well as cells cultured on glass coverslips were washed in PBS, fixed either with acetic acid (–20°C, 15 min) or methanol (4°C, 15 min, factor VIII-related antigen staining), washed with PBS, and incubated with the respective antibodies under the conditions given below. Substitution of the primary antibody with an irrelevant antibody of the same immunoglobulin type served as negative control. Specimens were washed thoroughly with PBS and incubated with an appropriate FITC- or rhodamine-labeled second antibody for 60 min at 37°C. Incubations with the human isoagglutinin anti-I were performed at 4°C overnight for 12 h, and the subsequent incubations at room temperature (20°C). Ice-cold PBS was used in the washing steps. For lectin staining, specimens were incubated with the FITC- or rhodamine-labeled lectin dissolved in PBS at a concentration of 30 µg/ml for 30 min at room temperature in the dark. Nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; Sigma, 5 µg/ml stock in methanol, 1:100 diluted with PBS before use) for 15 min at 37°C. To prevent bleaching, specimens were finally embedded with Mowial (Calbiochem, La Jolla, CA; 10 wt/vol% in 25% glycerol, pH 8.5, with Tris-HCl) and evaluated with a Zeiss fluorescence-microscope (Zeiss, Oberkochen, Germany) equipped with filters for FITC, rhodamine, and ultraviolet (DAPI).

The following antibodies/lectins were used: anti-cytokeratin (1:20; Dakopatts, Glostrup, Denmark), human cold isoagglutinin anti-I serum (1:10; generous gift of Prof. D. Roelcke, Institute of Immunology, Heidelberg, Germany), anti-α-actinin-1 (1:200; generous gift of Prof. I. Sabolic, University of Zadar, Zadar, Croatia), anti-von Willebrand-associated antigen (1:20, “Clothimunní,” Behringwerke, Marburg, Germany), DYA (Vector Laboratories), and Bandeiraea simplicifolia I lectin (BSL-1, Sigma).

Electron microscopy. Cells were grown on Millipore filters (0.4 µm pore size) for 6 days and were conventionally processed for transmission electron microscopy after fixation in 3.5% glutaraldehyde in 0.1 M PBS (30 min, pH 7.4) and postfixation in 1% OsO₄ for 30 min, cells were dehydrated and embedded in Epon. Ultrathin sections were stained by standard procedures with uranyl acetate and lead citrate. Specimens were viewed with a Zeiss EM 10 (Oberkochen, Germany) at an accelerating voltage of 80 KV.

Measurement of PGE₂ production. Cells were preincubated for 30 min at 37°C in 290 mosM HEPES-Ringer buffer and subsequently in the same buffer containing the respective hormone or solvent for 30 min. PGE₂ production was measured as the amount of prostaglandin released into the incubation buffer by removing this buffer completely at the end of the incubation period and at once diluting 1:1 with ice-cold HEPES-Ringer buffer containing 20 µg/ml indomethacin (Sigma) and 1 mg/ml centrophorosine (Sigma). Preincubation and incubation of samples with these cyclooxygenase inhibitors reduced PGE₂ production to <10% of the respective control value. PGE₂ was determined with an 125I radioimmunoassay (NEN, Dreieich, Germany). Cross reactivities of the applied antibody for PGE₂, PGE₃, and arachidonic acid were 0.4, <0.01, and <0.0001%, respectively. Arginine vasopressin (AVP), angiotensin II, and dopamine were obtained from Sigma Chemical.

CAMP determination. Preincubation and incubation of cells for determination of intracellular cAMP was performed under similar conditions as for the PGE₂ measurements, with the exception that all incubation buffers contained 1 mM IBMX (Sigma). Incubation was stopped by the complete removal of the incubation medium and the addition of 500 µl ice-cold 3% TCA to the cells. TCA was extracted four times with water-saturated diethyl ether (5:1), and the cAMP was determined by 125I radioimmunoassay (NEN). Recovery of cAMP was ~95%. The titration curve was linear from 1–20 pmol cAMP.

Protein determination. PGE₂ and cAMP measurements were adjusted for protein. Protein was determined by the
Lowry method (16), with bovine serum albumin being used as standard.

Statistical analysis. Unless otherwise specified, the results are expressed as means ± SE. Depending on the experimental design, statistical analysis was performed using the Student’s t-test for paired or unpaired observations or ANOVA. Groups found to be different were further analyzed by the Newman–Keuls test. Statistical significance was considered as P < 0.05.

RESULTS

Cell isolation and immunohistochemical characterization of cell population. To obtain a pure suspension of the lower TLH cells, these had to be purified from the other inner medullary cell types: the collecting ducts (IMCD), the interstitial cells, and the cells of the vasculature. The bulk of cells obtained after enzymatic digestion of rat inner medulla were the IMCD cells. If these cells were not removed, they overgrew the other cell types in primary culture. We achieved a practically complete elimination of this cell type by two purification steps: the majority of IMCD cells were pelleted in a low-speed differential centrifugation step, and the remaining cells were then removed from the supernatant by use of DBA-coated beads. The resulting cell suspension was practically free of IMCD cells as evaluated by the specific staining of this cell type with the lectin DBA. We only occasionally observed IMCD cells in our preparations of TLH cells, and their amount never exceeded 1%.

We tested several procedures to separate TLH cells from the remaining interstitial and vascular cells. The best enrichments were obtained by centrifugation of the remaining cells over a continuous Nycodenz gradient. TLHlp cells were enriched at a relative low density of 1.052–1.069 g/cm³, as evaluated by the specific staining of this cell population. Two protocols for the specific recognition of TLHlp cells were employed: protocol I, the human cold isoagglutinin anti-I exclusively stains in rat inner medulla the descending and ascending TLH (Fig. 1, A and B; Ref. 21). This binding pattern was preserved in primary culture, as shown in Fig. 1C. For protocol II, TLH cells were unambiguously characterized by their positivity for anti-cytokeratin and negativity for the lectin DBA. Both staining protocols consistently confirmed the high purity of the TLH cell preparations. To detect contaminations with cells of the vasculature and the interstitium, preparations were stained with the lectin BSL-1. This lectin exclusively reacts with these cell types in rat inner medulla (8), whereas the tubulooepithelial cells of the TLH and the collecting duct were completely negative (Fig. 1, D and E). During the first 3 days of culture, some of the tubulooepithelial cells appeared also to be positive for BSL-1. This was due to the fact that BSL-1-positive intracellular substance adhered to these cells during the first days of primary culture.

To estimate total contamination by BSL-1-positive cells, the number of BSL-1-positive cells was divided by the number of all cells counted per microscopic observation area (100 × 100 µm). For example, on day 4 of primary culture, contamination by BSL-1-positive cells was ~5.5% (10.7 ± 1.26 BSL-1-positive cells/196 ± 16.7 DAPI positive nuclei; n = 76). Considering, in addition, the contamination by collecting duct cells, which was <1%, the purity of the preparation of TLH cells was clearly >90%. The criteria to distinguish between the respective inner medullary cell types are summarized in Table 1.

The deep TLH consists of the descending and ascending limb, which differ significantly with respect to some of their physiological functions. To evaluate the distribution between these parts in our preparation, we stained the cell population with an antibody against aquaporin-1, a water channel protein present only in the descending but not ascending TLH loop (18). About 50% of the TLHlp cells were positive for this antibody, suggesting an equal distribution between descending and ascending limb (Fig. 2). This percentage distribution did not change significantly during the first 7 days in culture. We examined several procedures to separate cells of the descending and ascending limb populations, such as various protocols of hypotonic lysis (variation of the hypotonic shock by acute reduction of osmolality down to 70 mosM and extension of the exposure time up to 45 min) or differential centrifugation. However, we could not obtain any satisfactory separation of the two cell populations using these protocols.

Electron microscopy. Primary cultures of the TLH were mixed in comparison to IMCD duct cells as a flat, simple epithelium (Fig. 3, A and C). The cytoplasm contained only a few organelles. The apical surface was covered with scattered microvilli, whereas the basal lateral side exhibited no significant infoldings. Zonulae occludentes consisting of tight junctions composed of a single fibril close to the apical surface were observed (Fig. 3C).

Biochemical characterization of the isolated TLH cells. Freshly isolated TLH cells excluded to >98% trypan blue. To biochemically characterize the isolated cell population of TLHlp cells, we compared both freshly isolated and in primary culture in the responsiveness of PGE2 and cAMP by stimulation of various hormones in isolated TLH cells with that in IMCD and a cell population, in which IMFs were enriched.

PGE2 production. Both freshly isolated and day 4 primary cultures of TLHlp cells produced substantial amounts of PGE2 (12.8 ± 1.8 and 17.0 ± 1.7 ng PGE2/mg protein−1·h−1, respectively). As shown in Fig. 4, neither AVP (1 µM) nor angiotensin II (50 nm) had any significant effect on PGE2 production in the fraction of TLH cells. In contrast, both of these hormones stimulated PGE2 production significantly in a day 4 culture of a fraction, where IMFs were enriched. Hormonal response of freshly isolated IMF appeared impaired, probably due to the fact that this cell type with its long cytoplasmic processes was damaged during the isolation procedure and required a period of recovery. AVP had a significant effect on PGE2 production of both freshly isolated and day 4 cultures of IMCD cells. In the presence of dopamine (100 µM), PGE2 production in the TLHlp cell fraction strongly increased by +250 ± 46%...
Fig. 1. Binding pattern of the isoagglutinin anti-I in cryosections and primary cultures of rat inner medulla. 

A and B: double staining of a cryosection of rat inner medulla with isoagglutinin anti-I and lectin, Dolichos biflorus agglutinin (DBA). Only the thin limbs of Henle were positive for anti-I (some are marked by arrows, FITC fluorescence; A), whereas the DBA-positive collecting ducts (rhodamine fluorescence, B) were completely negative for this parameter. Bar, 20 μm.

C: low-power magnification of a day 5 primary culture of thin limb of Henle stained with anti-I, demonstrating the high purity of the preparation. Cells exhibited an inhomogeneous staining pattern, whereby the intensity of the weak positive cells was clearly above background. Bar, 50 μm.

D and E: day 4 primary culture of thin limb of Henle double labeled with anti-I and the Bandeiraea simplifolia 1 lectin (BSL-1) to detect contamination by interstitial and endothelial cells. This lectin is a selective marker of these cell types in rat inner medulla. Anti-I staining (FITC fluorescence, D) suggests a pure lower portion of the thin limb of Henle (TLHlp) preparation. However, evaluation of the slide with the filter for rhodamine fluorescence reveals contaminations with BSL-1-positive cells (E), overgrowing the TLHlp cells. It was impossible to detect these contaminations in unstained cultures by only light microscopy. Example demonstrated here was not representative for our preparations. Screening of the preparations with BSL-1 showed that the percentage of BSL-1-positive cells was not >5% (see text for details). Bar, 20 μm.
Intracellular cAMP. We also examined the response of intracellular cAMP to hormonal stimulation. As shown in Fig. 5, AVP (1 µM) had no significant effect in TLHlp (freshly isolated, 126.6 ± 22.0%, not significant, day 4; 19.5 ± 4.9%, not significant) nor in IMF but significantly stimulated intracellular cAMP in IMCD cells (freshly isolated, 1,032 ± 94%, day 4; 559 ± 34.7%, P < 0.001). However, forskolin markedly increased intracellular cAMP in all fractions (in TLHlp freshly isolated, 607 ± 68%, day 4; 883 ± 181%, P < 0.001), with the exception of freshly isolated IMF, demonstrating the functional integrity of this pathway in these cells.

Therefore, the isolated TLHlp population exhibited a hormonal stimulation profile different from that of IMCD cells and IMF. In particular, these experiments provided no indication of a significant contamination of the isolated TLH cells by other inner medullary cell types. In accordance with the immunohistological studies, they confirm the high purity of the isolated TLH cells.

Beginning from day 10, in primary culture, we observed an increasing number of dying TLH cells detaching from the support. The examination interval was therefore limited under the applied culture conditions to ~1 wk.

**DISCUSSION**

The inner medulla is the site where final adjustment of urine osmolality and electrolyte composition occurs. Its important regulatory function is emphasized by the fact that a variety of hormones, which are involved in the regulation of water and electrolyte homeostasis, possess target sites in this area, such as AVP (19, 25), angiotensin II (20, 28, 29), dopamine (10), the atrial natriuretic peptide (4), or bradykinin (5, 7, 29). The actions of these hormones are usually not confined to one site, but they exert their effects on various inner medullary cell types, suggesting that the final adjustment of urine composition represents the result of an interaction between these cells. The role of the lower portion of the TLH in these regulatory processes is so far poorly elucidated. We now report a new technique to isolate the lower TLH cells. This procedure permits for the first time the isolation of this cell population in suspension, thereby allowing examination of these cells under defined culture conditions in vitro. The isolation procedure is relatively easy and requires no expensive equipment.

Essential for the enrichment of TLH cells was the complete removal of IMCD cells, which otherwise would have overgrown the other cell types in primary culture. This was achieved by the use of magnetic beads coated with the lectin DBA, which binds in the inner medulla.
exclusively to the collecting duct cells (24). The IMF, characterized by its positivity for the lectin BSL-1 and negativity for the factor VIII-related antigen (8), proved to be the major contaminating cell type of the TLH cell preparation. This may be explained by specialized junctions between TLH cells and IMF but not between fibroblasts and collecting duct cells (15). An incomplete disintegration of these junctions, whose physiological significance is so far unclear, may contribute to the difficult separation of these two cell types. Indeed, we occasionally observed fibroblasts adhering to TLH tubules in freshly isolated suspensions. Nevertheless, the percentage of contaminating fibroblasts did not exceed 5% in the TLH preparations.

Immunohistochemical markers. The purity of the TLHp preparation was assessed immunohistochemi-

Fig. 3. Transmission electron micrograph of a cross section of a day 6 primary culture of TLHp cells. A and B: TLHp cells present as a flat epithelium in comparison to inner medullary collecting duct cells cultured under similar conditions (B). They exhibited scattered microvilli at the apical surface. Bar, 2 µm. C: cells contained only few cytoplasmic organelles. Basolateral membrane was without infoldings. Zonulae occludentes consisting of tight junctions composed of a single fibril close to the apical surface were observed (arrow). Bar, 0.5 µm.

Fig. 4. Effect of 1 µM arginine vasopressin (AVP), 50 nM angiotensin II (ANG II), and 100 µM dopamine (Dop.) on PGE2-production of freshly isolated and day 4 primary cultures of TLHp cells (TLH), compared with inner medullary collecting duct cells (IMCD) and a cell fraction, where inner medullary fibroblasts (IMF) were enriched. Stimulation pattern in TLHp differed from that of the other inner medullary cell fractions. Values are given as percent stimulation by the respective hormone vs. control. Basal PGE2 production varied among the inner medullary cell fractions (freshly isolated/day 4 culture): TLHp, 12.8 ± 1.8/17 ± 1.7; IMCD, 119 ± 20.9/78.4 ± 18.8; IMF, 2.3 ± 0.52/4.9 ± 0.34 ng PGE2·mg protein−1·30 min−1. *P < 0.05 vs. basal. Each column is average ± SE of >15 experiments. Open bars, day 0; solid bars, day 4.

Fig. 5. Effect of 1 µM AVP compared with that of 1 µM forskolin (For.) on intracellular cAMP content of freshly isolated and day 4 cultured cells of TLH, IMCD, and IMF. We observed a significant stimulation by AVP only in the cell fraction of IMCD. Values are given as percent stimulation by the respective hormone vs. control. *P < 0.05 vs basal. Each column is average ± SE of >6 experiments.
physically by three methods: 1) specific staining of TLH cells by the human isoagglutinin anti-I, which binds in the inner medulla exclusively to the cells of the descending and ascending limb both in humans and rodents (21). Although the binding pattern of anti-I in cultured cells was not homogeneous, these cells could be clearly discriminated from the other inner medullary cell types by this staining. The reason for this inhomogeneity is unclear: a weak staining appeared to be at least in part due to an increased cell surface area, as these cells spread in culture. 2) TLHlp cells could also be specifically identified by their positivity for cytokeratin and negativity for the lectin DBA (24), confirming the results of the anti-I staining. 3) Because contamination by other inner medullary cell types was virtually undetectable by morphological examination of unstained cells, using light microscopy, we looked for specific markers of these cell types. This was possible by staining collecting duct cells with the lectin DBA and the interstitial cell fraction containing IMF and endothelial cells with the lectin BSL-1. All potentially contaminating cell types of the inner medulla should therefore be excluded, since we never observed cells that were negative both for cytokeratin and the lectin BSL-1 in cell preparations derived from the inner medulla (8).

Immunohistochemical staining of the preparation with an antibody against aquaporin-1 demonstrated that ~50% of the cells were positive for this parameter. This suggests that these represent cells of the descending limbs, since aquaporin-1 was only demonstrated in this tubular segment (18), and the cells negative for aquaporin-1 are those of the ascending limbs. The relatively constant ratio of aquaporin-1 positive to aquaporin-1-negative cells during the first 7 days in culture argued against a rapid loss of phenotypic aquaporin-1 expression in culture and an overestimation of the portion of ascending limbs. A rapid dissipation of aquaporin-2 mRNA expression within a few days of primary culture has been reported in IMCD cells (6). Because ascending and descending thin limbs significantly differ not only with respect to their water but also sodium, chloride, and urea permeability (12, 22), we were also interested in their separation. Based on the distinct distribution of the aquaporin-1 water channel, we examined procedures to separate descending and ascending limb cells according to their osmotic resistance, a technique reported for example to isolate IMCD cells (7). However, we observed no selective lysis using different protocols of hypotonic shock, suggesting that this water channel is closed under the applied isolation conditions or is of minor significance for the lysis of cells.

Morphology of cells. Transmission electron microscopic studies of the TLH revealed that the lower portion of the descending limb of the long loops and the ascending limb are with respect to their cellular morphology similar: simple, flat epithelia with scattered, short microvilli at the apical surface and lack of basolateral infoldings. Their cytoplasm contains only few organelles (12, 23). The morphology of cultured TLHlp resembled very closely these morphological criteria, suggesting that the epithelium developed properly under the applied culture conditions. In contrast, the upper portion of the long descending limb is characterized by taller cells with numerous microvilli at the apical surface, extensive basal infoldings and many cytoplasmic organelles such as mitochondria (12, 23). We did not observe cells exhibiting this morphology in the cultures.

Biochemical characterization. Studies on microdissected tubular segments of the TLH suggested that this part of the tubular system is also an active site for PGE2 formation. However, in these studies, the upper parts of the TLH were examined (1, 14), whereas the cells studied here were derived from its lower parts. This difference in localization, besides differences in species or in the experimental setup, may be responsible for the approximately one potency higher basal PGE2 production, suggesting an increased PGE2 production toward the papillary tip. Another reason for the relative high PGE2 production by cultured cells may be due to a recovery in PGE2 production from impairment during the isolation procedure.

APV and angiotensin II had no significant effect on PGE2 production of TLHlp cells. These hormones exhibited effects in the other cell fractions derived from the inner medulla: in IMF, they stimulated PGE2 production significantly, in accordance with observations of Zusman and Keiser (29) in subcultured IMF. The diminished or lack of effects of these hormones in freshly isolated IMF may be caused by their susceptibility to cellular injury during the isolation procedure because of their long cytoplasmic processes. This assumption is supported by the finding that even the action of forskolin on intracellular cAMP was severely disturbed in IMF. In freshly isolated TLH cells, we did not observe any major impairment of cellular functions due to the isolation procedure.

APV stimulated in IMCD cells both PGE2 and intracellular cAMP as reported by others (17, 19, 24, 25). We detected no significant effect of AVP on intracellular cAMP in TLHlp, although Imai and Kusano (11) reported an increase of intracellular cAMP in response to AVP in the upper parts of the thin ascending limb of Henle close to the thick ascending limb and the thick ascending limb itself. The explanation for this discrepancy may be that our preparations also comprised the deeper portions of the thin ascending limb and the descending limbs, suggesting that these lack an APV-sensitive adenylate cyclase.

With respect to dopamine, we report now for the first time a highly significant increase of PGE2 production in TLHlp, clearly exceeding that in collecting duct cells in terms of percent production, which was abolished in the presence of cyclooxygenase inhibitors. This result demonstrates that the nonresponse to AVP, ANG II, and bradykinin was not due to a disturbed PGE2 production in these cells. It was not our aim to elucidate the mechanisms underlying this stimulation. However, our results strongly suggest that at least in a part of TLH cells, PGE2 production could be stimulated by dopamine, probably via DA2K dopamine receptors, a mechanism reported for IMCD cells (9). An enrichment of this
kidney-specific dopamine receptor was demonstrated in the inner medulla by autoradiographic techniques (10).

Because our experiments demonstrate that the cells of the deep TLH exhibit a hormonal stimulation profile distinct from the other inner medullary cell types, the method reported here should be suitable for studying the mechanisms underlying the action of hormones or other questions regarding this cell population under defined conditions in vitro. The period of study of these cells in culture was limited to ~1 wk under the applied culture conditions, since from day 10 in culture, an increasing number of cells died. We observed a similar life span in cultured IMCD cells (unpublished observations). However, this interval should be sufficient to adequately address most questions. It should therefore prove an additional powerful tool for the examination of the functions of these cells that are so difficultly accessible by other techniques.

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Portions of this study have been published in abstract form: 


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