Antidiuretic hormone reduces the high PGE₂ synthesis in papillary collecting duct of DI rats

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Abstract. PGE₂ synthesis was measured along the nephron of Brattleboro (DI) rats, lacking ADH, and control LE rats, using an enzyme immunoassay. Experiments were performed in vitro, in the absence of exogenous arachidonic acid, using microdissected tubular segments. The effect of a chronic treatment by dDAVP was tested on three ADH sensitive tubular segments, medullary thick ascending limb (MTAL), medullary collecting tubule (OMCD) and papillary collecting duct (IMCD).

No difference in PGE₂ synthesis was present between LE and DI in glomerulus and tubular segments up to OMCD. In both strains, values were low in the proximal tubule and the loop of Henle, and gradually increased along the collecting tubule. In IMCD, PGE₂ synthesis was much higher in DI (12.8 ± 2.0 pg per 30 min per mm tubular length) than in LE (3.8 ± 0.5, LE vs. DI p < 0.001). In MTAL and OMCD, dDAVP treatment did not affect PGE₂ synthesis. In IMCD, dDAVP reduced PGE₂ synthesis to values (5.3 ± 0.8 pg per 30 min per mm tubular length), which were not significantly different from those of LE. Neither oxytocin, which has been shown to be elevated in DI rats, nor furosemide, that reduced papillary osmolarity to values comparable to those of DI rats, were able to increase PGE₂ synthesis in IMCD of LE rats. The mechanism of the increase in PGE₂ synthesis in IMCD of DI rats, and of the inhibitory effect of dDAVP is yet unknown; it may participate to compensate for the lack of ADH in the Brattleboro rat.

Key words: Enzyme immunoassay – Microdissected tubules – Oxytocin – Furosemide – Brattleboro rat

Introduction

A bulk of evidence indicates that antidiuretic hormone (ADH) increases prostaglandin (PG) synthesis in the kidney (reviewed in [4, 26]). However, the precise effects of this hormone on a typical target nephron segment for ADH, such as the cortical collecting tubule, remain controversial, and few studies have been addressed to other tubular segments [4, 26]. Brattleboro rats (DI), genetically lacking ADH, represent an interesting model to evaluate the effects of ADH on PG synthesis. In the present study, we measured PGE₂ synthesis, using an enzyme immunoassay, in isolated ADH sensitive medullary tubular segments of DI rats, treated or not with 1 d-amino-8-D-arginine vasopressin (dDAVP), a non pressor vasopressin analog [25], and compared it to that of control Long Evans rats (LE).

The stimulatory effect of ADH on PGE₂ synthesis is likely to occur mainly through a mobilization of phospholipids cellular stores [1, 29], leading to an increased availability of intracellular arachidonic acid, the substrate for cyclooxygenase activity, responsible for PG synthesis [1, 3]. We thus performed our experiments in the absence of exogenous arachidonic acid.

In the collecting tubule, Kirschenbaum et al. [18] and Schlondorff et al. [28] reported a stimulatory effect of AVP on PGE₂ synthesis. Surprisingly the main result of the present study was to evidence an unexpected effect of ADH on PGE₂ synthesis in the papillary collecting duct, which consists in an inhibition of the high level of synthesis observed in DI, as compared to LE rats.

Material and methods

Studies were performed on female rats (160 – 180 g BW). All animals were fed a standard diet and had free access to tap water up to the beginning of experiments. We first established the profile of PGE₂ synthesis along the entire length of the nephron, in order to evaluate if major differences appeared between Long Evans (LE: n = 6) and Brattleboro (DI: n = 6) rats. Then, we examined the effects of hormone replacement on PGE₂ synthesis of DI rats, in three ADH-sensitive tubular segments, medullary thick ascending limb of the loop of Henle (MTAL), medullary collecting tubule from the inner stripe of outer medulla (OMCD), and papillary collecting duct (IMCD). DI rats (n = 4) received 1 d-amino-8-D-arginine vasopressin (dDAVP) from 10 days of age to adulthood (about 10 weeks). We chose to administrate dDAVP because this analog is totally devoided of pressor action [25]. In the first 3 weeks of treatment, an aqueous solution of dDAVP, emulsified in peanut oil, was injected subcutaneously, once a day, with doses increasing with age from 150 to 300 ng per day. In the subsequent weeks, aqueous dDAVP (200 ng per day) was infused by Alzet minipumps (model 2002, Alzacorp, Palo Alto, CA, USA) placed intraperitoneally and changed every other week under ether anesthesia. Since a major difference in PGE₂ synthesis between LE and DI rats was present in IMCD, complementary experiments were performed on this tubular segment, in order to address some possible causes for this difference. DI rats have been re-
ported to have elevated oxytocin levels [10, 21]. In order to examine whether high oxytocin levels could induce high PGE2 synthesis, PGE2 synthesis was compared in IMCD of control LE rats, and LE rats treated with high doses of oxytocin (Oxy LE, n = 5). For this purpose, LE rats received 2 μg per day of oxytocin (Sigma St. Louis, MO, USA) for 1 week. This dose can be estimated to be 10-fold higher than the oxytocin release per day in female DI rats [21]. Oxytocin was infused by Alzet minipumps placed intraperitoneally. Animals from all these experimental series were placed in metabolic cages during the week before experiments in order to measure urinary flow rate and osmolarity.

Since papillary osmolarity was much lower in DI than LE rats, and since papillary osmolarity has been shown to modulate PGE2 synthesis [6-8, 17, 24, 33], we examined if its reduction in LE rats could influence PGE2 synthesis. LE rats (n = 3) were treated acutely with furosemide (5 mg/kg BW) injected peritoneally. Urinary flow rate and osmolarity were measured using metabolic cages for 45 min following the injection. Microdissection of tubular segments was performed immediately after.

### Preparation of isolated tubular segments

The kidney was prepared as follows. After ether anesthesia, the kidneys were perfused with 10 ml ice-cold saline solution (in mM: NaCl 137, KCl 5, MgSO4 0.8, Na2HPO4 0.33, KH2PO4 0.44, MgCl2 1, CaCl2 1, tris(hydroxymethyl) aminomethane (Tris)-HCl 10, glucose 5, pH 7.4) through the aorta, after clamping above and under renal arteries. Then, 10 ml collagenase solution were perfused (identical to the preceding saline solution, plus 0.1% collagenase, Cooper, CLS II, 170 U/mg). Afterwards, thin pyramid pieces were cut, and incubated for 1 h at 30°C in the presence of the collagenase solution. Microdissection was performed at 4°C in a collagenase-free solution (identical to collagenase solution, except for the absence of collagenase, 0.25 mM CaCl2 instead of 1 mM, and the addition of 0.1% bovine serum albumin). The following nephron segments were microdissected: glomerulus (GLOM), proximal convoluted tubule (PCT), thin limb of Henle’s loop, either descending or ascending (TL), thick medullary ascending limb (MTAL) and the ascending limb (ASC), cortical collecting tubule (CCD), and several measurements of external diameter were performed on each tubule. Tubular volume per mm length was then calculated.

### Tubular volume

Tubular volume was determined on tubular segments from each of the three groups of rats, in separate animals: microdissected tubules were photographed (x 40) and several measurements of external diameter were performed on each tubule. Tubular volume per mm length was then calculated.

### Statistical analysis

Variance analysis was used to compare results of PGE2 synthesis within segments of the same group; Students t-test was used to compare results between groups for one given segment.

### Results

The profiles of PGE2 synthesis along the nephron of both LE and DI strain are illustrated in Fig. 1. Results are expressed per mm tubular length. In the two strains, the PGE2 synthesis was of low magnitude in the initial parts of the nephron, and gradually increased along the distal nephron, to reach maximal values in the papillary collecting duct. In each strain, variance analysis indicated that PGE2 synthesis was significantly different from one nephron segment to another (LE: F = 21.10, p < 0.001; DI: F = 20.15, p < 0.001). On the whole, values of PGE2 synthesis were slightly lower in DI than in LE; however differences did not