Effects of Sodium Diclofenac on the Concentration Function in Animals with Different Neurohypophyseal Status

V. A. Lavrinenko, A. V. Babina, L. V. Shestopalova, N. F. Beizel, and L. N. Ivanova

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The characteristics of the osmotic concentration system under conditions of sodium diclofenac treatment were studied in Wistar rats with normally functioning vasopressin gene and homozygotic Brattleboro rats completely lacking endogenous vasopressin. Blockade of prostaglandin synthesis in rats with different neurohypophyseal status stimulated urinary osmolality to a different degree. Different contribution of sodium cations and urea to osmotic concentration was revealed.

Key Words: *diclofenac; osmotic concentration; Brattleboro rats*

According to modern concepts, vasopressin (VP), a neurohypophyseal hormone, is the main hormone regulating the concentration function of the kidney. The hydrosmotic effect of VP is modulated by prostaglandin E_2 (PGE_2) by the negative feedback mechanism [1]. Various regulatory effects of PG in reabsorption of osmotically free water and electrolytes in the osmoregulated epithelium have been demonstrated [2,3].

Sodium diclofenac, a representative of a large class of nonsteroid anti-inflammatory drugs, causes reversible competitive inhibition of cycloxygenase [7], thus blocking PG synthesis and abolishing their modulator action on the antidiuretic effect of VP.

Since the efficiency of the concentrating mechanism functioning can be evaluated by the capacity of the countercurrent reversing multiplication system to create the sodium and urea corticomedullary gradient [6,10], we studied the levels of these substances in various renal tissue zones in animals differing by the neurohypophyseal status under conditions of PG synthesis blockade.

MATERIALS AND METHODS

The study was carried out on homozygotic Brattleboro rats with hereditary hypothalamic diabetes insipidus and Wistar rats with normal neurohypophyseal status. Experiments were carried out with consideration for the Helsinki Declaration regulations on humane attitude to animals. Adult animals (n=60) were kept on standard ration with free access to water. Animals of each strain were divided into 2 groups: controls and injected with sodium diclofenac (0.1 mg/100 g, two injections daily for 2 days).

In order to evaluate the efficiency of osmotic concentration during the experiment, urinary samples were collected twice a day and the concentrations of osmotically active substances were measured by the cryoscopic method on an OMT-5-01 osmometer (Burevestnik). Urea was measured by the diacetylmonoxime method (Vital Diagnostics). Sodium cations were measured by flame photometry on a Hitachi Z-8000 atomic adsorption spectrophotometer. The corticomedullary gradient was calculated as the proportion of the measured substance concentration in the papilla to its concentration in the renal cortex.

The significance of differences in the functional parameters between the experimental groups was evaluated by Student's t test for independent samples and two-way ANOVA with genotype and drug effect as the independent variables.

RESULTS

Comparative study of the concentration function was carried out in animals with different neurohypophyseal

Novosibirsk State University, Russia. ***Address for correspondence:*** igor@academ.org. V. A. Lavrinenko

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status with comparable values of the excreted urine osmolality. In Wistar rats low osmolality (195±29 mOsm/kg H_2O) was attained by diet. Control Brattleboro rats were characterized by polyuria and low osmolality of excreted urine (123±11 mOsm/kg H_2O) due to low reabsorption of osmotically free water in the renal tubules, a direct result of the absence of endogenous VP regulating cell membrane permeability in the collecting tubule epithelium. Injection of diclofenac (PG synthesis blocker) stimulated significantly the osmotic concentration in all animals in comparison with controls of the same strain (Fig. 1). In Wistar rats treated with diclofenac, urine osmolality increased to just 333±43 mOsm/kg H_2O, while in Brattleboro rats it reached 568±35 mOsm/kg H_2O. Two-way ANOVA detected significant differences ($F_{1,102}$=18.02, p<0.001) in response to diclofenac in animals of different genotypes differing by the neurohypophyseal status.

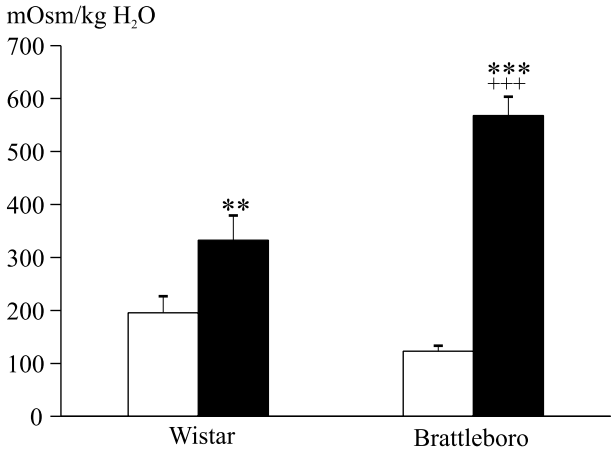

Fig. 1. Urine osmolality. Light bars: control; dark bars: diclofenac treatment. **p<0.01, ***p<0.001 in comparison with the corresponding controls; ^{+++}p<0.001 in comparison with the corresponding group of the other strain.

The sodium cation and urea corticomedullary gradients created by the counter-current multiplication system and promoting the water osmotic flow are essential for evaluation of the efficiency of the renal concentration function. Studies of these substances distribution in the renal zones with different functions (cortex, outer medullary layer, inner medulla) showed its regularities. The concentrations of sodium cations and urea increased in the direction from the cortical matter to the inner medullary zone in all animals (Table 1). No differences in sodium cation and urea levels in the renal tissue were detected in Wistar and Brattleboro controls with the same osmotic concentration levels (by the experiment conditions), presumably due to low secretion of endogenous VP in albino rats [9]. Significant differences were detected in the sodium and urea concentrations in different renal tissue zones under conditions of PG synthesis blockade (Table 1). A significant increase of sodium concentrations in all renal zones of albino rats was presumably caused by reduced bloodflow and accumulation of osmotically active substances in renal tissue. This was in line with a previous report [11] according to which PGE_2 in the kidney reduced the vasoconstrictive effects of angiotensin-II, catecholamines, and VP. In Brattleboro rats, sodium concentration increased only in the outer and inner medullary zones containing the counter-current reversing multiplication system, but not in the cortex. As a result of these changes, the corticomedullary sodium gradient in albino rats did not change in comparison with the control, while in VP-deficient rats this parameter increased significantly (p<0.001; Fig. 2). The degree of sodium gradient changes in response to PG synthesis blockade depended on the neurohypophyseal status of animals ($F_{1.49}$=10.41, p<0.01).

Analysis of the urea levels showed similar changes (Table 1). In Wistar rats, urea concentrations increased in all renal tissue zones, while in Brattleboro rats the level of this osmotically active substance in-

TABLE 1. Levels of Osmotically Active Substances in Renal Tissue (M±m)

Parameter		Wistar rats		Brattleboro rats	
		control	diclofenac	control	diclofenac
Content of sodium cations, μg/mg tissue	cortex	7.11±0.22	12.21±0.56***	7.27±0.22	7.94±0.44
	outer medulla	11.87±0.44	30.45±1.18***	15.54±0.70	19.01±1.40*
	inner medulla	25.98±1.21	45.00±3.14***	25.72±0.90	42.36±4.49***
Urea content, mmol/g tissue	cortex	1.94±0.22	4.70±0.36***	1.55±0.18	1.97±0.09
	outer medulla	4.06±0.45	14.59±1.56***	3.91±0.45	6.05±0.56
	inner medulla	6.01±0.51	20.59±2.05***	5.68±0.52	10.22±1.31*

Note. *p<0.05, **p<0.01, ***p<0.001 in comparison with the corresponding controls.

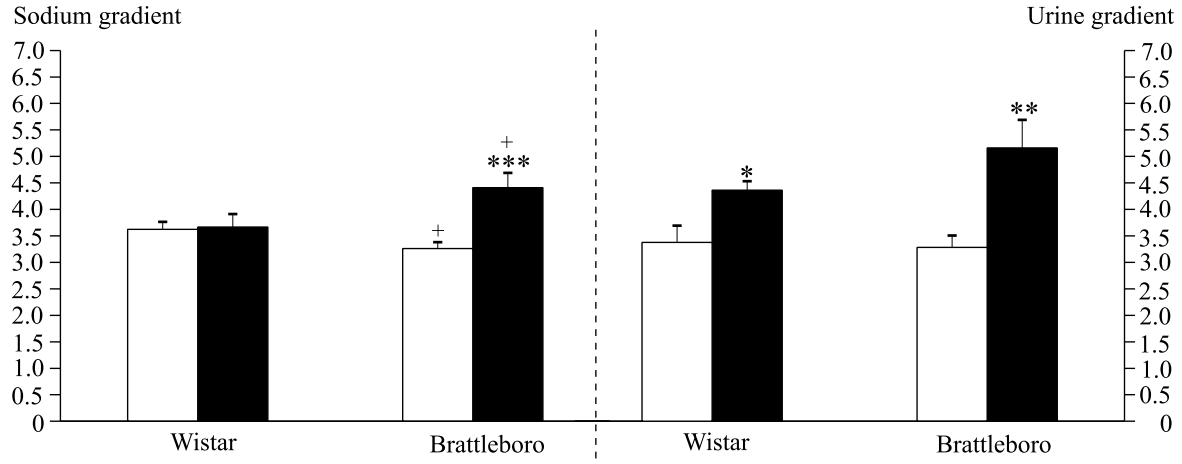

Fig. 2. Sodium cation and urea corticomedullary gradients. Light bars: control; dark bars: diclofenac. **$p<0.05$, ***$p<0.001$ in comparison with the corresponding control; $^{+}p<0.05$ in comparison with the corresponding group of the other strain.

creased only in the medullary zone ($p<0.01$). These changes in the urea levels in different zones of the kidney led to significant differences in the corticomedullary gradient in all diclofenac treated animals in comparison with the controls ($p<0.05$ for Wistar rats, $p<0.01$ for Brattleboro rats; Fig. 2).

The study showed that blockade of PG synthesis in animals with different neurohypophyseal status led to different degree of elevation of the osmotic concentration of the urine in animals with comparable levels of initial osmolality of the urine in the control. In VP-deficient Brattleboro rats the corticomedullary sodium and urea gradients increased, this leading to a greater increase of the urine osmolality in comparison with Wistar rats in which it was associated with elevation of only the urea gradient.

Prostaglandins modulate the efficiency of osmotic concentration by reducing the corticomedullary gradient. Prostaglandin E_2 reduces sodium and chlorine reabsorption in the thick ascending segment of the loop of Henle in the medulla and reduces urea and sodium reabsorption from the collecting tubules of the inner medulla [5,8]. These effects of PGE_2 lead to reduction of the concentrations of osmotically active substances in the interstitial fluid and stimulate blood circulation in the medulla, thus washing out the osmotically active substances from the renal medulla and reducing the efficiency of the concentration mechanism. More effective osmotic concentration under conditions of diclofenac treatment can be due to the absence of the diuretic effect of PGE_2 on the kidney. This is in line with the results of previous studies [4] demonstrating stimulation of sodium and urea accumulation in the renal papilla interstitium under the effect of PG synthesis inhibitors *in vivo*.

Hence, injections of sodium diclofenac inhibiting the cycloxygenase pathway of PG synthesis, to animals with normally functioning VP genes (Wistar) and animals completely lacking endogenous VP (homozygotic Brattleboro) differently modulated the work of the osmotic concentration system components.

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