Antidiuretic Hormone—V_2-Receptor—Aquaporin-2 System in Rat Kidneys during Acute Inflammation

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Expression of antidiuretic hormone V_2-receptor, water channel protein aquaporin-2, and cytokines interleukin-1β and interleukin-6 was studied in the kidneys of rats with acute inflammation produced by intraperitoneal injection of lipopolysaccharide in a dose of 250 μg/100 g. Reduced expression of aquaporin-2 and V_2-receptor led to impairment of concentration capacity in the kidneys and decrease in urine osmolarity.

Key Words: *aquaporin-2; interleukin-1β; interleukin-6; lipopolysaccharide; vasopressin V_2-receptor*

Antidiuretic hormone (ADH) plays a major role in the maintenance of water balance in the organism. This neurohormone regulates the final stage of urine concentration and stimulates water reabsorption in the collecting ducts, thus preventing water loss under conditions of low water supply [3].

The mechanism underlying the action of ADH on collecting ducts involves activation of type-2 vasopressin receptors (V_2-receptors) on the basolateral epithelial membrane. V_2-Receptors trigger traffic of water channel protein aquaporin-2 to the apical membrane of cells in the collecting ducts. These changes are followed by a multifold increase in apical membrane permeability for water, which contributes to osmotic water flow from the lumen of collecting ducts into the renal interstitium. Activation of V_2-receptors not only triggers aquaporin-2 traffic to the membrane, but also stimulates expression of the corresponding gene [6].

Changes in water metabolism during inflammation are poorly understood. Our studies showed that inflammation has no effect on ADH gene expression in gigantocellular hypothalamic neurons and plasma ADH concentration [1]. The kidneys serve as the target organ for ADH. Here we studied the system of ADH, V_2-receptor, and aquaporin-2 in the renal collecting ducts.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 200-250 g. The animals were kept in a vivarium and had free access to water and food.

Control animals (n=7) intraperitoneally received sterile physiological saline (0.9% NaCl) in a single dose of 300 μl. Bacterial endotoxin (*E. coli* O111:B4 lipopolysaccharide, LPS; L-3012; Sigma) was injected intraperitoneally to experimental rats in a dose of 250 μg/100 g in 300 μl sterile physiological saline. LPS in the specified dose produces severe inflammation [8]. Experimental animals were decapitated 3 (n=4), 6 (n=9), 12 (n=7), and 24 h after LPS injection (n=7).

The blood was placed in cold tubes with heparin, centrifuged, and frozen at -20°C until measurement of sodium concentration. Urine samples from the urinary bladder were obtained by aspiration with a syringe after decapitation. The kidneys or their parts were excised immediately after decapitation, frozen on dry ice, and stored at -70°C for *in situ* hybridization, semi-

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quantitative reverse transcription-polymerase chain reaction (PCR), and Western blotting for aquaporin-2 content. For radioactive ligand—V_2-receptors binding assay the kidneys were excised immediately after decapitation and put in cold phosphate buffer; for immunohistochemical study the kidneys were placed in 4% paraformaldehyde.

Plasma sodium concentration was measured on a Beckman Elise Na/K/Cl Analyzer. Urine osmolarity was estimated using an Osmomat 010 analyzer. The amount of V_2-receptor protein on membranes of collecting ducts was determined by binding of radioactive ligand ^{3}H-vasopressin to the membrane fraction from the inner renal medulla and papillae [2].

Sections (12 μ) were prepared on a microtome and hybridized with labeled RNA probes to study expression of V_2-receptor mRNA, interleukin-1β (IL-1β) mRNA, and IL-6 mRNA [4]. After hybridization the sections were exposed to a Kodak BIOMAX MR X-ray film. X-Ray films were scanned. The images were processed using NIH Image software.

For Western blotting assay of aquaporin-2 content the inner renal medulla and papillae were excised, homogenized, and prepared for electrophoresis in 12% polyacrylamide gel. After electrophoresis the protein was transferred onto a nitrocellulose membrane. The membrane was incubated with primary antibodies against aquaporin-2 (Dr. M. Knepper, US National Institute of Health) and secondary antibodies labeled with horseradish peroxidase (Pierce Chemical Co.). The signal was visualized using a chemiluminescence set with LumiGLO luminol (Kirkegaard and Perry Lab. MD) and Kodak light-sensitive film (165-1579). The image was processed by means of NIH Image software.

The renal medulla and papilla were excised, fixed with 4% paraformaldehyde in phosphate buffer (4°C), and embedded into epoxide resin [5]. Semithin sections (1 μ) were mounted on glasses. Epoxide resin was dissolved. The sections were incubated with primary antibodies against aquaporin-2 (LL127AP, 1:1000) and treated with secondary antibodies (anti-rabbit IgG, 1:100, Sigma). The reaction product was visualized by the peroxidase-antiperoxidase method using diaminobenzidine as the chromogen [5]. Stained sections were examined under an Olympus BX50 microscope connected to an Olympus PC10 digital camera.

The medulla and papilla were excised immediately after removal of the kidneys, frozen on dry ice, and stored at -70°C. RNA was isolated by the method of Chomczynski and Sacchi with modifications (TRIzol Reagent set, Gibro-BRL) and treated with DNase. RNA concentration was measured spectrophotometrically at 260 nm. The reverse transcription reaction was carried out using Cloned AMV First-Strand Synthesis Kit (Invitrogen) and 1 μg total RNA with polyT^{20} primers (20 μl mixture). Then 1 μl reaction mixture was used for PCR with MagAmp MIX kit (Dialat). We used the following pairs of primers: aquaporin-2 (AQP2s: 5' AGT-GCT-GGC-TGA-GTT-CTT-GG 3'→, AQP2as: 5' GCT-GTG-GCG-TTG-TTG-TGG-AG 3'←, 344 bp) and glycerol-3-phosphate dehydrogenase (GAPDHs: 5' GGA-CAT-TGT-TGC-CAT-CAA-CGA-C 3'→, GAPDHas: 5' ATG-AGC-CCT-TCC-ACG-ATG-CCA-AAG 3'←, 441 bp) [9]. PCR was conducted according to the following protocol: melting at 94°C for 2 min, annealing at 55°C for 45 sec (20 cycles), elongation at 72°C for 90 sec, and melting at 95°C for 20 sec; after 20 cycles final elongation at 72°C over 5 min was carried out. Products were analyzed by electrophoresis in 1.5% agarose gel, stained with ethidium bromide, and visualized in UV

TABLE 1. Plasma Na^+ Concentration and Urine Osmolarity after Injection of LPS (*M*±*m*)

Parameter	Control	3 h	6 h	12 h
Plasma Na^+, meq/liter	144±3	138±3	145.0±2.8	138.0±3.1
Urine osmolarity, mosmol/liter	2210±238	774±81*	1292±152*	1036±152*

Note. Here and in Table 2: *p<0.001 compared to the control.

TABLE 2. ^{3}H-Vasopressin Binding to Membranes in the Inner Renal Medulla and Aquaporin-2 Content in Rats Receiving LPS (*M*±*m*)

Parameter	Control	3 h	6 h	12 h
^{3}H-Vasopressin binding, pmol/liter	39.0±0.4	35.0±1.2	30.0±1.5	31.0±0.9
Aquaporin-2, arb. units				
glycosylated form	100.7±8.3	40.3±8.3*	49.0±2.3*	55.7±2.8*
non-glycosylated form	66.7±6.7	38.3±4.6	37.3±3.8	29.0±5.7

light. The molecular weight of PCR products was determined using 100 bp DNA ladder marker (Promega). For quantitative evaluation DNA content in different samples was standardized by the concentration of glycerol-3-phosphate dehydrogenase mRNA, which serves as a housekeeping gene, whose expression remains unchanged during inflammation. Each RNA sample was assayed in triplicate. The image was recorded on a DNA Analyzer video system (Liteks) and analyzed by means of Gel-Pro Analyzer 3.1 software (Media Cybernetics).

RESULTS

Urine osmolarity decreased to 65% of the basal level 3 h after intraperitoneal injection of LPS and remained low over 24 h (50% below normal, Table 1).

LPS suppressed binding of radioactive ligand to V_2-receptors on the membranes of collecting ducts (Table 2). These changes were accompanied by a significant decrease in the concentration of V_2-receptor mRNA (Fig. 1). The content of V_2-receptor mRNA decreased by more than 90% 3 and 6 h after LPS injection. This parameter progressively increased by the 24th hour, but did not return to the basal level.

Aquaporin-2 content significantly decreased 3 h after LPS injection and remained low by the 24th hour (Table 2). It should be noted that significant decrease was noted only for glycosylated aquaporin-2 with a molecular weight of 36-45 kDa.

The intensity of staining for aquaporin-2 decreased 6 h after LPS injection, which primarily concerned the apical membrane of the chief cells in collecting ducts, but general staining pattern (strongly stained apical membrane and weakly stained cytoplasm) was preserved [7] (Fig. 2).

Expression of aquaporin-2 mRNA decreased most significantly 3 and 6 h after LPS injection, but did not differ from the basal level by the 12th hour. Moreover, 24 h after treatment expression of aquaporin-2 mRNA

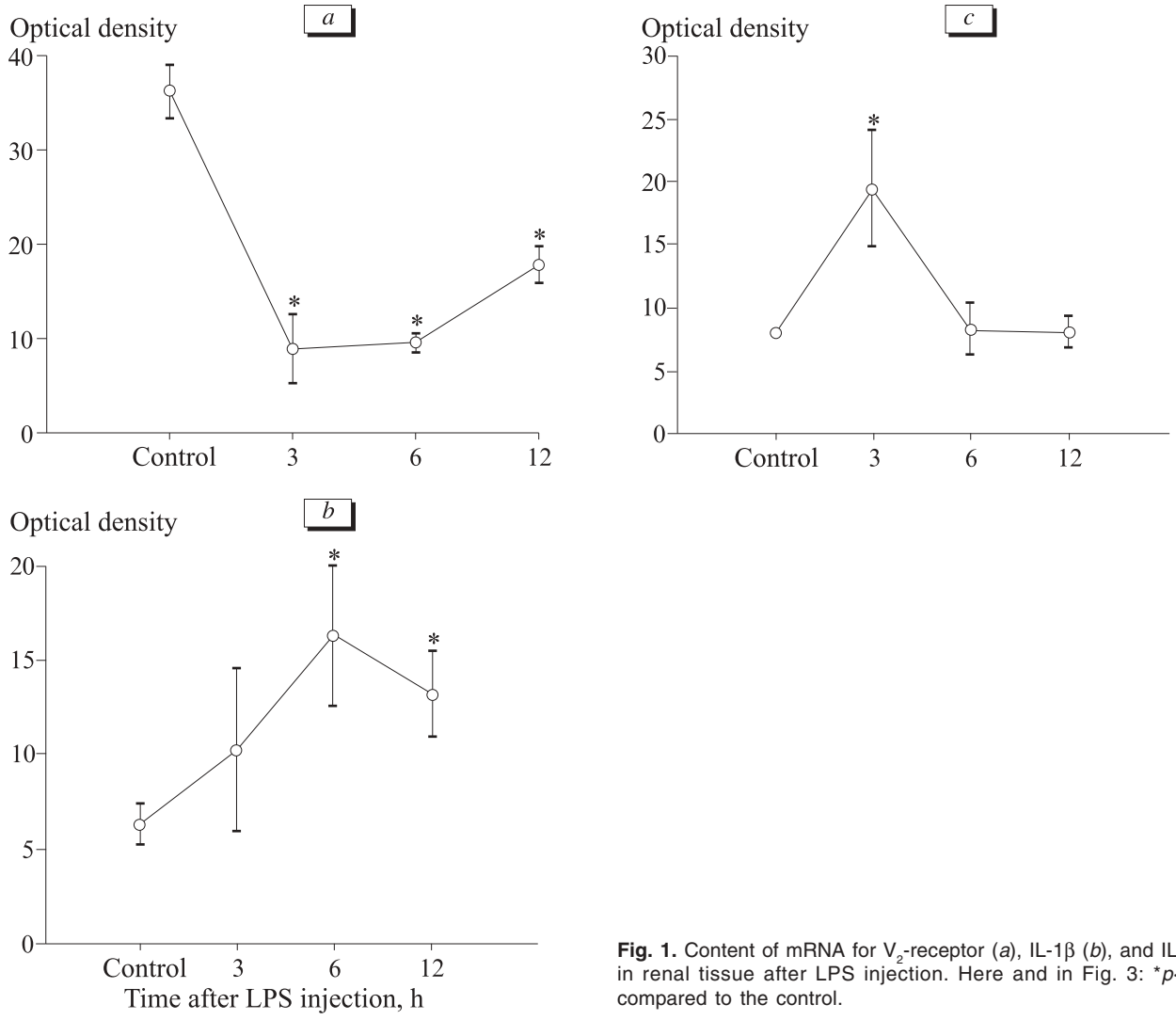

Fig. 1. Content of mRNA for V_2-receptor (*a*), IL-1β (*b*), and IL-6 (*c*) in renal tissue after LPS injection. Here and in Fig. 3: **p*<0.01 compared to the control.

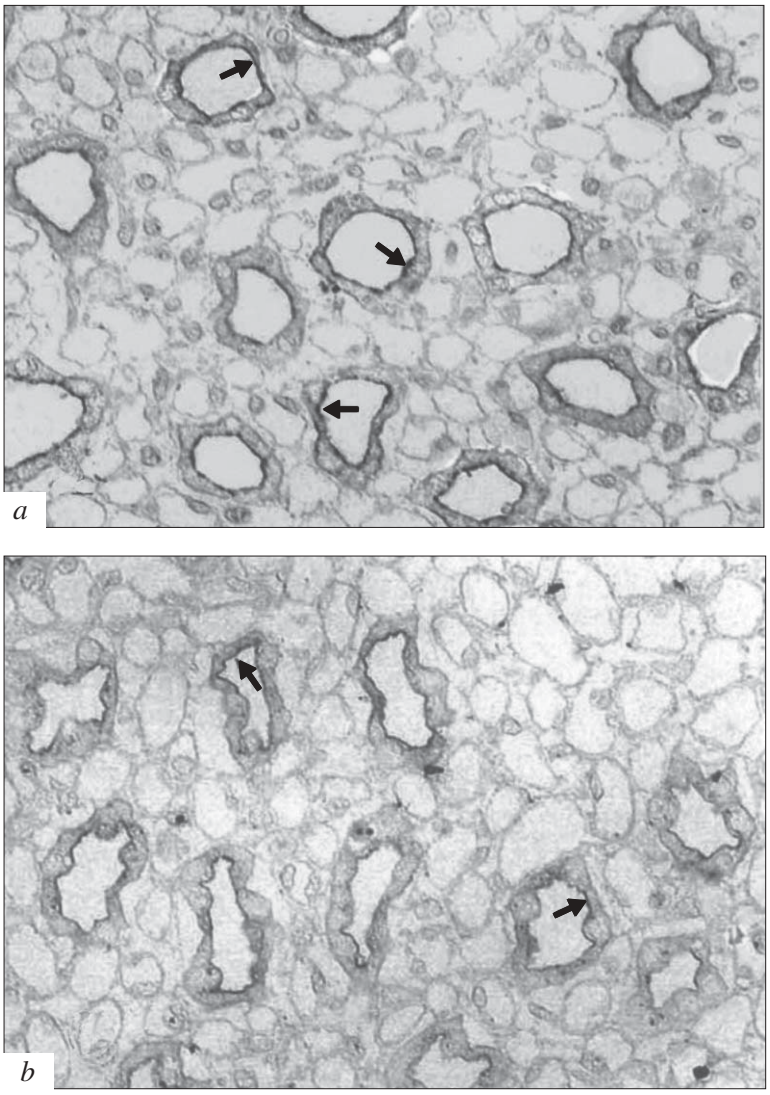

Fig. 2. Immunohistochemical staining of renal tissue for aquaporin-2. Control (*a*) and 6 h after LPS injection (*b*). Arrows: staining of apical membranes in collecting ducts, ×40.

exceeded the control by 20% (statistically insignificant, Fig. 3).

In situ hybridization did not detect mRNA for IL-1β and IL-6 in renal tissue before LPS injection. The concentration of IL-1β mRNA significantly increased after LPS injection, reached maximum 6 h after treatment, and remained high by the 12th hour (Fig. 1, *b*). The concentration of IL-6 mRNA increased 3 h after LPS injection, decreased 6 h after treatment, and did not differ from the control by the 12th hour (Fig. 1, *c*). Both cytokines were mainly expressed in the inner medullary layer and renal papilla. Expression of cytokines in the renal cortex was low (data not shown).

ADH is the major factor regulating concentration capacity of the kidneys [3]. One can hypothesize that the decrease in urine osmolarity caused by inflammation is associated with reduced plasma ADH concentration. However, recent studies showed that plasma ADH concentration remains unchanged in this model of inflammation [1]. Another possible explanation is insensitivity of the collecting ducts to ADH. Decreased number of V_2-receptors and aquaporin-2 content confirmed this assumption.

Expression of aquaporin-2 primarily depends on the state of V_2-receptors. Changes in activity of V_2-receptors are most likely to precede the decrease in the content of aquaporin-2 and specific mRNA.

The sequence of changes in the content of mRNA and protein in the system V_2-receptor—aquaporin-2 is of particular interest. This system is hierarchically subordinate, *i.e.*, expression of the aquaporin-2 gene, mRNA synthesis, and protein traffic to the apical membrane are regulated by V_2-receptors and secondary messengers (cAMP). This hierarchical relationship explains the decrease in the number of active V_2-receptors on cell membrane and reduction of the content of aquaporin-2 and specific mRNA. This problem requires further investigations.

Inflammation produced by intraperitoneal injection of LPS is followed by a decrease in urine osmo-

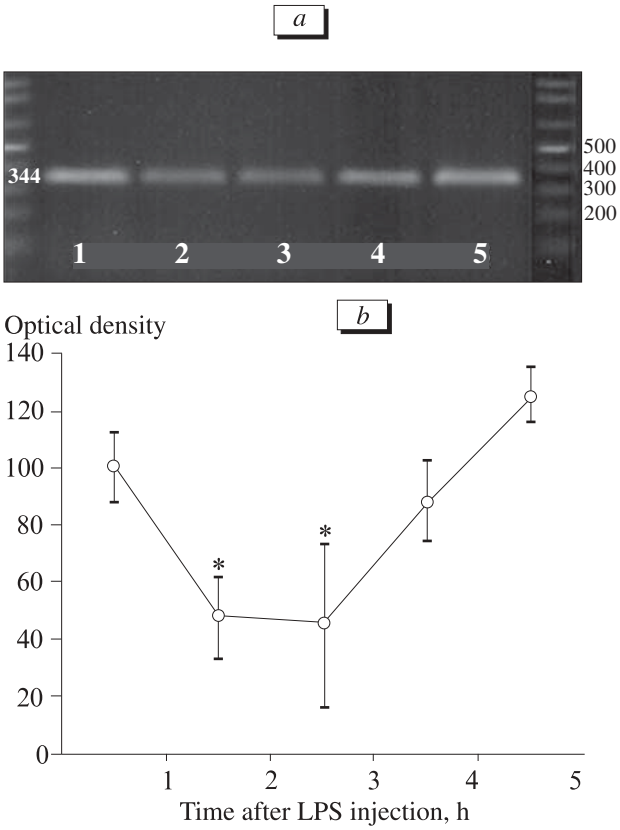

Fig. 3. Expression of aquaporin-2 mRNA after LPS injection. Electrophoresis of RT-PCR products in 1.5% agarose gel (*a*). Numerals: size of DNA fragments (bp). Marginal bands: 100 bp DNA marker. Primers for aquaporin-2. Aquaporin-2 mRNA after LPS injection (*b*). Control (*1*) and 3 (*2*), 6 (*3*), 12 (*4*), and 24 h (*5*) after LPS injection.

larity, which reflects impairment of the concentration capacity in the kidneys. We revealed a decrease in the expression of V_2-receptors and aquaporin-2 in renal collecting ducts. These data indicate that ADH loses control over the kidneys. It can be associated with the influence of cytokines expressed in the renal medulla. Our results show that the ADH—V_2-receptor—aquaporin-2 system plays an important role in the impairment of the concentration capacity in the kidneys and shift in the water-electrolyte metabolism during acute inflammation.

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