α-MSH prevents impairment in renal function and dysregulation of AQPs and Na-K-ATPase in rats with bilateral ureteral obstruction

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Submitted 2 August 2004; accepted in final form 22 September 2005

Li, Chunling, Yimin Shi, Weidong Wang, Chrysanthi Sardeli, Tae-Hwan Kwon, Klaus Thomsen, Thomas Jonassen, Jens Christian Djurhuus, Mark A. Knepper, Søren Nielsen, and Jørgen Frøkiær. α-MSH prevents impairment in renal function and dysregulation of AQPs and Na-K-ATPase in rats with bilateral ureteral obstruction. Am J Physiol Renal Physiol 290: F384–F396, 2006. First published September 27, 2005; doi:10.1152/ajprenal.00282.2004.—The purpose of this study was to evaluate the effects of the anti-inflammatory hormone α-melanocyte-stimulating hormone (α-MSH) on renal function and expression of aquaporins (AQPs) and Na-K-ATPase in the kidney in response to 24 h of bilateral ureteral obstruction (BUO) or release of BUO (BUO-R). In rats with 24-h BUO, immunoblotting revealed that downregulation of AQP2 and AQP3 was attenuated (AQP2: 38 ± 5 vs. 13 ± 4%; AQP3: 44 ± 3 vs. 19 ± 4% of sham levels; P < 0.05), whereas downregulation of Na-K-ATPase was prevented by α-MSH treatment (Na-K-ATPase: 94 ± 7 vs. 35 ± 5% of sham levels; P < 0.05). Immunocytochemistry confirmed the changes in AQP1 and Na-K-ATPase expression. Renal tubular cell apoptosis was confirmed in BUO kidneys, and α-MSH treatment virtually completely abolished apoptosis. Furthermore, we measured glomerular filtration rate (GFR) and effective renal plasma flow (ERPF), respectively. Forty-eight hours after BUO-R demonstrated that α-MSH treatment almost completely prevented the decrease in GFR (nontreated: 271 ± 50; α-MSH: 706 ± 85; sham: 841 ± 105 μl·min⁻¹·100 g body wt⁻¹, P < 0.05) and ERPF (nontreated: 1,139 ± 217; α-MSH: 2,598 ± 129; sham: 2,633 ± 457 μl·min⁻¹·100 g body wt⁻¹, P < 0.05). α-MSH treatment also partly prevented the downregulation of AQP1 and Na-K-ATPase expression in rats after BUO-R for 48 h. In conclusion, α-MSH treatment significantly prevents impairment in renal function and also prevents downregulation of AQP2, AQP3, and Na-K-ATPase during BUO or AQP1 and Na-K-ATPase after BUO-R, demonstrating a marked renoprotective effect of α-MSH treatment in conditions with urinary tract obstruction.

urinary tract obstruction; water channels; sodium pump; urinary concentrating defect

URINARY TRACT OBSTRUCTION is associated with long-term impairment in the ability of the kidney to regulate urinary excretion of water and sodium (2, 10, 11, 17, 25, 32). Hemodynamic changes are prominent, such as a reduction of renal blood flow (RBF) and glomerular filtration rate (GFR) (7, 31, 44). Moreover, abnormalities in tubular function are common in obstructive nephropathy, including a reduction in the urinary concentrating capacity, altered reabsorption of solutes and water, and an impaired excretion of hydrogen and potassium. In addition, urinary tract obstruction is also associated with a marked interstitial inflammatory response that has been suggested to play a key role in the pathophysiologial changes in renal function of the obstructed kidney. Consistent with this, there is infiltration of leukocytes, predominantly macrophages into the cortex and medulla of the kidney following ureteral obstruction (13) which produces proinflammatory mediators. This represents the proinflammatory state of ureteral obstruction and also contributes to the late development of interstitial fibrosis (37).

Aquaporins (AQPs) are a family of membrane proteins and play key roles in water reabsorption in the kidney. Aquaporin-2 (AQP2), which is the vasopressin-regulated water channel expressed at the apical plasma membrane and intracellular vesicles of the principal cells in the kidney collecting duct (33, 38), has been demonstrated to be significantly downregulated in the kidneys of rats with 24-h bilateral and unilateral ureteral obstruction (BUO and UUO, respectively) (10, 11). AQP2 levels were persistently decreased up to 2 wk after release of a 24-h period of BUO, indicating that reduced AQP2 protein expression may be important in the pathophysiology of post-obstructive diuresis (POD) and in the development of impaired urinary concentration (10, 25). Moreover, BUO, UUO, and release of BUO have been shown to be associated with downregulation of both collecting duct water channel AQP3 expression and proximal nephron water channel AQP1 expression (25). The decreased expression of both AQP3 and AQP1 also could contribute to the POD and the impaired urinary concentrating capacity. Na-K-ATPase, which maintains the driving force for active sodium reabsorption across the renal tubular epithelium, is heavily expressed in the basolateral plasma membrane of the renal tubule cells (16). The expression of Na-K-ATPase was significantly downregulated in the obstructed kidney, indicating that reduced Na-K-ATPase expres-
sion may also be involved in the disorders of renal water and salt regulation during postobstructive diuresis (26, 27).

α-Melanocyte stimulating hormone (α-MSH) is a potent anti-inflammatory peptide and has been shown to prevent ischemia-reperfusion-induced renal injury (5). α-MSH treatment of rats with acute renal failure (ARF), which was induced by bilateral renal ischemia and reperfusion, markedly prevented the reduction in the expression of AQP1, AQP2, and AQP3 as well as partially preventing decreased urinary concentrating ability (21, 22). Several mechanisms of the anti-inflammatory action induced by α-MSH treatment have been identified: 1) inhibition of pyrogenic and proinflammatory effects of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF), and interferon-γ (IFN-γ); 2) inhibition of inflammation induced by other mediators of inflammation and by peripheral irritants; and 3) inhibition of infiltration of macrophages and neutrophils (4, 28). The effects of α-MSH are likely to be mediated by melanocortin receptors found expressed in macrophages, neutrophils, and possibly in the renal tubules. We hypothesize that interstitial inflammation and tubular cytokine production could also significantly contribute to the renal injury and dysregulation of renal AQPs and major renal transporter proteins that were previously demonstrated in rats with postobstructive polyuria (24–27).

In this study, we therefore tested whether α-MSH has any protective effects on renal injury in rats with BUO and BUO followed by release. The following objectives were addressed: 1) to assess the effect of α-MSH on renal tubular functions and hemodynamics in euvolemic rats with BUO followed by release using a servo-controlled system; and 2) to assess the effect of α-MSH treatment on AQPs and Na-K-ATPase expression in rats subjected to BUO and BUO followed by release.

METHODS

Experimental Protocols

Studies were performed in male Munich-Wistar rats initially weighing 230 g (Møllegaard Breeding Centre, Eiby, Denmark). The rats were maintained on a standard rodent diet (Altromin, Lage, Germany) with free access to water. During the entire experiment, the rats were kept in individual metabolic cages, with a 12:12-h artificial restriction by three daily training sessions in the restraining cages. The duration of each daily session was increased stepwise from 1 to 3 h daily.

Clearance Studies

Body weight, water intake, food intake, and urine output were observed while the rats were maintained in the metabolic cages. Urine was collected during 24-h periods throughout the study. In protocols 1–3, clearance studies were measured as previously described (23, 24).

For protocol 4, the measurements of renal function were carried out in conscious rats immobilized in restraining cages according to the method described by Thomsen and Olesen (47). Each experiment comprised a 15-min bolus period for [3H]inulin and [14C]TEA, a 105-min equilibration period, then six 30-min urine collection periods. Blood specimens (300 µl) were collected from the arterial catheter each hour during the 180-min urine collection periods. Blood specimens were replaced immediately with same volume of heparinized donor blood. Through the pressure transducer, a continuous intra-arterial infusion of 25 mM glucose solution containing heparin...
(100 U/ml) at a rate of 5 μl/min was given to keep the arterial catheter open. Through the vein catheter, the animals received throughout the experiment an intravenous infusion of 25 mM glucose solution (0.6-ml bolus, sustained 10 μl/min) containing [14C]TEA (0.84-μCi bolus, sustained 0.014 μCi/min, New England Nuclear, Boston, MA), [3H]inulin (3.6-μCi bolus, sustained 0.06 μCi/min, Amersham International, Aylesbury, UK), and LiCl (7.2-μmol bolus, sustained 120 nmol/min). In addition, 25 mM glucose was given throughout the experiment at a rate adjusted so that the total infusion rate of the above-mentioned solutions, including that given in the artery pump, was maintained at a rate of 45 μl/min to keep an adequate minimum urine flow rate necessary for elimination of bladder-emptying errors. Throughout the experiment, water and Na⁺ balance was maintained by a computer-driven servo-controlled system, as a previous study described (43). In brief, from the bladder catheter, urine passed a Na⁺-sensitive electrode that performed one measurement of urinary [Na⁺] per second (Nova Biochemical, Waltham, MA). Data on urine production (weight on scale) and [Na⁺] were sampled continuously on an IBM-compatible computer, which, in turn, controlled the infusion rates of two infusion pumps. Urinary output of Na⁺ and fluid were integrated over 5 min, thus allowing a 5-min delay in changes in Na⁺ and glucose infusion rates.

Calculations of Renal Function and Statistics in Protocol 4

Renal clearances (C) were calculated by the standard formula, where U is the urine concentration, V is the urine flow rate, P is the plasma concentration:

\[ C = \frac{U \cdot V}{P} \]

GFR was determined as GFR = C_inulin, and effective renal plasma flow (ERPF) = \( \frac{C_{\text{TEA}}}{P} \) (35).

Fractional excretion of sodium (FENa) was then calculated from the formula

\[ \text{FENa} = \frac{\text{Na}_U}{\text{Na}_P} \times \frac{\text{Inulin}_P}{\text{Inulin}_U} \times 100 \]

Other calculations are as follows:

- Proximal water reabsorption: \( GFR - C_{Li} \times P_{Na} \)
- Distal water reabsorption: \( C_{Li} - V \)
- Distal sodium reabsorption: \( C_{Li} - C_{Na} \times P_{Na} \)
- Fractional distal water excretion: \( V/C_{Li} \)
- Fractional distal sodium excretion: \( C_{Na}/C_{Li} \)

The urine water and sodium excretions expressed as fraction of amount delivered from the distal nephron are given by \( V/C_{Li} \) and \( C_{Na}/C_{Li} \), respectively (46).

Differently treated groups were compared using ANOVA techniques followed by Dunnett’s test for individual comparisons between groups means, or comparisons between groups were made by an unpaired t-test. A P value <0.05 was considered to be statistically significant.

Primary Antibodies

For semiquantitative immunoblotting and immunocytochemistry, previously characterized affinity-purified polyclonal or monoclonal antibodies were used: 1) AQP1 (CHP serum or LL266AP): immune serum or an affinity-purified antibody to AQP1 has previously been characterized (45); 2) AQP2 (LL127 serum or LL127AP): immune serum or affinity-purified antibody to AQP2 has previously been described (8, 29); 3) AQP3 (LL178AP): an affinity-purified polyclonal antibody to AQP3 has previously been characterized (9); 4) Na-K-ATPase: a monoclonal antibody against the α₁-subunit of Na-K-ATPase has previously been characterized (16); and 5) AIF, a goat polyclonal antibody recognizing apoptosis-inducing factor (AIF) which is commercially available from Santa Cruz Biotechnology, Santa Cruz, CA.

Membrane Fractionation for Immunoblotting

For removal of kidneys, rats were anesthetized with halothane. One total kidney (TK) was kept and another kidney was split into cortex plus outer medulla (C+OM) and inner medulla (IM). All of them were frozen in liquid nitrogen. Tissue was minced finely and homogenized in 9 (TK), 8 (C+OM), or 1 ml (IM) of dissecting buffer (0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, pH 7.2, and containing the following protease inhibitors: 8.5 μM leupeptin, 1 mM phenylmethylsulfonyl fluoride), with five strokes of a motor-driven Potter-Elvehjem homogenizer at 1,250 rpm. This homogenate was centrifuged in a Universal 30RF centrifuge (Hettich, Tuttinglen, Germany) at 4,000 g for 15 min at 4°C, and the supernatant was pipetted off and solubilized at 65°C for 15 min in Laemmli sample buffer containing 2% SDS and then stored at −20°C.

Electrophoresis and Immunoblotting

Protein samples were run on 12% polyacrylamide minigels (Bio-Rad Mini Protein II) for AQP1, -2, -3, and Na-K-ATPase. For each gel, identical gel was run in parallel and subjected to Coomassie staining to assure identical loading (45). Then, gels were subjected to immunoblotting. After transfer by electrophoresis to nitrocellulose membranes, blots were blocked with 5% milk in PBS-T (80 mM Na₂HPO₄, 20 mM NaH₂PO₄, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 h and incubated overnight at 4°C with affinity-purified primary antibodies (see above). The labeling was visualized with horseradish peroxidase (HRP)-conjugated secondary antibodies (P448 or P447 diluted at 1:3,000, DAKO, Glostrup Denmark) using an enhanced chemiluminescence system (ECL; Amersham International).

Histological Examination of Kidneys

Paraffin sections (2 μm) of perfusion-fixed kidneys were stained with hematoxylin and eosin for light microscopic analysis. Specifically, the presence of neutrophil infiltration and dense nuclei (as a measure of apoptotic cells) were examined in the sections.

Immunocytochemistry

The kidneys from BUO and sham-operated rats were fixed by retrograde perfusion via the abdominal aorta with 3% paraformaldehyde in 0.1 M cacodylate buffer, pH 7.4. For immunoperoxidase microscopy, kidney blocks containing all kidney zones were dehydrated and embedded in paraffin. The paraffin-embedded tissues were cut at 2 μm on a rotary microtome (Leica, Heidelberg, Germany). The sections were deparaffinized and rehydrated. For immunoperoxidase labeling, endogenous peroxidase was blocked by 0.5% H₂O₂ in absolute methanol for 10 min at room temperature. To reveal antigens, sections were put in 1 M PBS-T solution (pH 9.0) supplemented with 0.5 mM EGTa (3.6-di-oxa-octa-methylen-di-nitrilo-tetra-acetic acid) and heated using a microwave oven for 10 min. Nonspecific binding of immunoglobulin was prevented by incubating the sections in 50 mM NH₄Cl in 30 min followed by blocking in PBS supplemented with 1% BSA, 0.05% saponin, and 0.2% gelatin. Sections were incubated overnight at 4°C with primary antibodies diluted in PBS supplemented with 0.1% BSA and 0.3% Triton X-100. After being rinsed with PBS supplemented with 0.1% BSA, 0.05% saponin, and 0.2% gelatin for 3 x 10 min, the sections were washed, then incubated with horseradish peroxidase-conjugated immunoglobulin (P448, 1:200, DAKO) diluted in PBS supplemented with 0.1% BSA.
and 0.3% Triton X-100. The sections were washed for 3 × 10 min, followed by incubation with dianibenzidine for 10 min. The microscopy was carried out using a Leica DMRE light microscope (Leica).

**Statistical Analyses**

For densitometry of immunoblots, samples from kidneys were run on each gel with corresponding sham kidneys. Renal AQP1, -2, -3, and Na-K-ATPase expression in the samples from the experimental animals was calculated as a fraction of the mean sham control value for that gel. Parallel Coomassie-stained gels were used for correction of minor differences in loading. All values are presented as means ± SE. Comparisons between groups were made by an unpaired t-test. A P value <0.05 was considered to be statistically significant.

**RESULTS**

**α-MSH Treatment Partially Prevented Urinary Concentrating Defect Associated with BUO and BUO Release**

The release of BUO was associated with impaired renal tubular water reabsorption, polyuria, and decreased urinary osmolality (Table 1). Consistent with this, in rats 48 h after release of 24 h-BUO (BUO-48hR) solute-free water reabsorption (T\*H\textsubscript{2}O) was markedly reduced compared with sham-operated controls (64 ± 11 vs. 186 ± 8 \textmu ml\textsuperscript{-1} kg\textsuperscript{-1}, P < 0.05, Table 1), indicating an impaired renal tubular water reabsorption. Moreover, rats with BUO release exhibited decreased GFR afterward. Consistent with this, creatinine clearance was significantly decreased in BUO-48hR rats compared with sham-operated controls (1.9 ± 1.0 vs. 9.6 ± 3.3 \textmu ml\textsuperscript{-1} kg\textsuperscript{-1}, P < 0.05, Table 1). Moreover, plasma osmolality and plasma creatinine concentration were significantly decreased in BUO-48hR rats (214 ± 47 \textmu mmol/l in nontreated rats vs. 79 ± 12 \textmu mmol/l in α-MSH treated rats, P < 0.05, Table 1). Similarly, α-MSH treatment was associated with decreased plasma creatinine levels in BUO-48R rats (214 ± 47 \textmu mmol/l in nontreated rats vs. 79 ± 12 \textmu mmol/l in α-MSH treated rats, P < 0.05, Table 1). Moreover, α-MSH treatment prevented the decrease in T\*H\textsubscript{2}O in BUO-48R rats (64 ± 11 \textmu ml\textsuperscript{-1} kg\textsuperscript{-1} in nontreated rats vs. 89 ± 5 \textmu ml\textsuperscript{-1} kg\textsuperscript{-1} in α-MSH treated rats, P < 0.05, Table 1).

**α-MSH Treatment Prevented Impairment of Renal Hemodynamics and Tubular Functions in BUO-48hR Rats**

To further examine the protective effects of α-MSH treatment during ureteral obstruction and the release of obstruction, renal hemodynamics and tubular functions were investigated in conscious, euvoletic BUO-48hR rats with a servo-controlled system. GFR was measured by inulin clearance, and ERPF was measured by the TEA clearance in BUO-48hR rats. Forty-eight hours after release of BUO, GFR (271 ± 50 \textmu ml\textsuperscript{-1} 100 g body wt\textsuperscript{-1} in nontreated vs. 841 ± 105 \textmu ml\textsuperscript{-1} 100 g body wt\textsuperscript{-1} in sham-operated, P < 0.05, Fig. 1A) and ERPF (1,139 ± 217 \textmu ml\textsuperscript{-1} 100 g body wt\textsuperscript{-1} in nontreated vs. 2,633 ± 457 \textmu ml\textsuperscript{-1} 100 g body wt\textsuperscript{-1} in sham-operated, P < 0.05, Fig. 1B) were significantly decreased in nontreated BUO rats compared with sham-operated controls, respectively. α-MSH treatment prevented the reductions in GFR and ERPF in BUO-48hR rats (Fig. 1, A and B).

Furthermore, renal tubular functions improved significantly after α-MSH treatment in BUO-48hR rats. Nontreated BUO-48hR rats were associated with significantly decreased renal tubular reabsorption of sodium and water in both the proximal and the distal segments of the kidney tubules (Table 2), suggesting a dysregulation of water and sodium handling in the whole-nephron segments. In contrast, α-MSH treatment in BUO-48hR rats partially prevented the reduction in water and sodium reabsorption in both the proximal and the distal segments of the kidney tubules (Table 2). The increase in fractional distal water and sodium excretion seen in nontreated rats with BUO release of BUO.

**Table 1. Changes in renal functional data in rats subjected to BUO for 24 h or followed by release of BUO for 5 and 48 h treated with or without α-MSH and sham-operated controls (protocols 1–3)**

<table>
<thead>
<tr>
<th></th>
<th>α-MSH</th>
<th>Sham</th>
<th>BUO-5hR</th>
<th>BUO-48hR</th>
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</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
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<td>P\textsubscript{osmol}</td>
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<td>132±2</td>
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<td>P\textsubscript{cre}</td>
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<td>6.1±0.2*</td>
<td>4.9±0.1*</td>
<td>5.9±0.5*</td>
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<tr>
<td>P\textsubscript{cre}</td>
<td>377±10*</td>
<td>366±11*</td>
<td>203±17*</td>
<td>214±24*</td>
</tr>
<tr>
<td>U\textsubscript{cre}</td>
<td>10±3</td>
<td>14±2</td>
<td>21±4*</td>
<td>10±4*</td>
</tr>
<tr>
<td>U\textsubscript{cre}</td>
<td>10±3</td>
<td>14±2</td>
<td>21±4*</td>
<td>10±4*</td>
</tr>
<tr>
<td>T*H\textsubscript{2}O</td>
<td>10±3</td>
<td>14±2</td>
<td>21±4*</td>
<td>10±4*</td>
</tr>
<tr>
<td>Cl\textsubscript{cre}</td>
<td>10±3</td>
<td>14±2</td>
<td>21±4*</td>
<td>10±4*</td>
</tr>
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</table>

Values are means ± SE. BUO, bilateral ureteral obstruction; n, no. of rats; α-MSH, α-melanocyte-stimulating hormone; P\textsubscript{osmol}, plasma osmolality; P\textsubscript{cre}, plasma sodium; P\textsubscript{cre}, plasma potassium; U\textsubscript{cre}, plasma creatinine; U\textsubscript{cre}, urine volume; U\textsubscript{osmol}, urine osmolality; T\*H\textsubscript{2}O, solute-free water reabsorption; Cl\textsubscript{cre}, clearance of creatinine. BUO-5hR and BUO-48hR, rats subjected to BUO and then release 5 or 48 h later, respectively. *P < 0.05 compared with sham-operated controls.

†P < 0.05 compared with nontreated rats with BUO or release of BUO.
apo-poptosis was confirmed in sections from BUO kidneys stained for the presence of apoptotic nuclei with an antibody recognizing AIF (Fig. 2B), consistent with previous evidence. Apoptosis occurred primarily in the renal medulla. Importantly, α-MSH treatment virtually completely abolished the presence of apoptotic cells (Figs. 2, C and D) and did not differ from sham-operated control kidneys (Figs. 2, E and F).

α-MSH Attenuated Downregulation of AQP2, -3, and -1 in BUO, BUO-5hR, or BUO-48hR Rats

Consistent with previous studies (25), immunoblotting revealed that BUO (protocol 1), BUO-5hR (protocol 2), and BUO-48hR (protocol 3) were associated with a significant downregulation of AQP2, -3, and -1 expression compared with sham-operated controls (see Figs. 3–6, 8 and Table 3). In contrast, α-MSH treatment significantly attenuated the inner medullary reduction in AQP2 expression in BUO rats (38 ± 5% of sham levels, *P < 0.05) compared with nontreated BUO rats (13 ± 4% of sham levels, Fig. 3, A and B). Moreover, α-MSH treatment also attenuated downregulation of inner medullary AQP3 expression (44 ± 3% of sham levels, *P < 0.05) compared with nontreated BUO rats (19 ± 4% of sham levels, Fig. 3, C and D). Whole kidney expression of AQP1 was decreased in both nontreated BUO and α-MSH-treated BUO compared with sham-operated rats (Fig. 4, A and B, Table 3).

In BUO-5hR rats, α-MSH treatment attenuated downregulation of inner medullary AQP3 expression (30 ± 2% of sham levels, *P < 0.05) compared with nontreated BUO rats (15 ± 5% of sham levels, Fig. 5, C and D), whereas inner medullary AQP2 expression was unchanged in α-MSH-treated rats (Fig. 5, A and B, not significant). This is consistent with the maintained polyuria. In contrast, α-MSH treatment prevented downregulation of whole kidney AQP1 expression (81 ± 21% of sham levels, not significant, Fig. 6, A and B) and attenuated the downregulation of AQP1 in C+OM (62 ± 9% of sham levels, *P < 0.05, Fig. 6, C and D) compared with nontreated rats.

Immunocytochemistry confirmed the downregulation of AQP1 abundance in the proximal tubules in untreated kidneys in response to 24-h BUO and BUO-5hR (Fig. 7, A and D). The labeling was markedly attenuated in BUO-5hR rats in response to α-MSH treatment (Fig. 7E), demonstrating virtually the same labeling density as sham-operated controls (Fig. 7F).

Table 2. Changes in reabsorption of water and sodium in proximal and distal tubules in BUO-48hR rats treated with or without α-MSH compared with sham-operated controls (protocol 4)

<table>
<thead>
<tr>
<th></th>
<th>Nontreated</th>
<th>α-MSH</th>
<th>Sham</th>
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<tr>
<td>U\text{\textsubscript{Na}} × U\text{\textsubscript{V\textsubscript{u}}}</td>
<td>0.34±0.05*</td>
<td>0.34±0.05*</td>
<td>0.53±0.05</td>
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<tr>
<td>F\text{\textsubscript{E\text{\textsubscript{Na}}}}</td>
<td>1.00±0.13*</td>
<td>0.37±0.07†</td>
<td>0.47±0.06</td>
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<tr>
<td>Proximal water reabsorption, μl/min−1·100 g body wt</td>
<td>215±39*</td>
<td>553±55†</td>
<td>552±116</td>
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<tr>
<td>Proximal sodium reabsorption, μmol/min−1·100 g body wt</td>
<td>28.6±5.4*</td>
<td>76.5±7.1†</td>
<td>76.5±15.7</td>
</tr>
<tr>
<td>Distal water reabsorption, μl/min−1·100 g body wt</td>
<td>38±18*</td>
<td>129±34†</td>
<td>263±41</td>
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<td>Distal sodium reabsorption, μmol/min−1·100 g body wt</td>
<td>7.3±2.7*</td>
<td>20.8±4.5†</td>
<td>39.7±5.7</td>
</tr>
<tr>
<td>Fractional distal sodium excretion ((C_{\text{Na}}/C_{\text{Li}}))</td>
<td>0.071±0.020*</td>
<td>0.020±0.005†</td>
<td>0.014±0.002</td>
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<td>Fractional distal water excretion ((V/C_{\text{Li}}))</td>
<td>0.51±0.19*</td>
<td>0.20±0.05†</td>
<td>0.10±0.02</td>
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</table>

Values are means ± SE; n, 5/group. U\text{\textsubscript{Na}} × U\text{\textsubscript{V\textsubscript{u}}}, urinary sodium excretion; F\text{\textsubscript{E\text{\textsubscript{Na}}}}, fractional excretion of sodium. *P < 0.05 compared with sham-operated rats. †P < 0.05 compared with nontreated BUO-48hR rats.
In BUO-48hR rats, α-MSH treatment also markedly and significantly prevented downregulation of whole kidney AQP1 expression (83 ± 2% of sham levels, \( P < 0.05 \), Table 3) as well as cortical and outer medullary AQP1 expression (73 ± 8% of sham levels, \( P < 0.05 \), Fig. 8A, Table 3) compared with nontreated BUO-48hR rats, respectively. In contrast, the expression of inner medullary AQP2 and AQP3 was unchanged in BUO-48hR rats in response to α-MSH treatment (Fig. 8, B and C, Table 3).

Table 3. Expression of AQPs and Na-K-ATPase in rats subjected to 24-h BUO and followed by release of BUO for 5 and 48 h treated with or without α-MSH and sham-operated controls (protocols 1–3)

<table>
<thead>
<tr>
<th></th>
<th>AQP2 (IM)</th>
<th>AQP3 (IM)</th>
<th>AQP1 (TK)</th>
<th>AQP1 (C + OM)</th>
<th>Na-K-ATPase (TK)</th>
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<tr>
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<td>19±4%*</td>
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<td>100±7%</td>
<td>100±13%</td>
<td>100±6%</td>
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<tr>
<td>Nontreated</td>
<td>5</td>
<td>32±11%*</td>
<td>15±5%*</td>
<td>7±2%*</td>
<td>50±11%*</td>
<td>34±5%*</td>
</tr>
<tr>
<td>α-MSH</td>
<td>5</td>
<td>27±3%*</td>
<td>30±2%*†</td>
<td>81±21%†</td>
<td>100±14%†</td>
<td>63±10%†</td>
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<tr>
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<td>100±4%</td>
<td>100±9%</td>
<td>100±22%</td>
<td>100±15%</td>
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<tr>
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<td>13±5%*</td>
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</tr>
<tr>
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<td>100±1%</td>
<td>100±1%</td>
<td>100±7%</td>
<td>100±3%</td>
<td>100±4%</td>
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</table>

Values are means ± SE. \( n \), No. of rats; AQP, aquaporin; IM, inner medulla; TK, total kidney; C + OM, cortex plus outer medulla. *\( P < 0.05 \) compared with sham-operated control rats. †\( P < 0.05 \) compared with nontreated rats.
α-MSH TREATMENT IN RATS WITH BUO

Release of BUO was associated with an increased fractional excretion of sodium to urine compared with sham-operated rats (Table 2). This indicated that BUO and BUO-R were associated with an impairment of the tubular reabsorption of filtered sodium.

Consistent with this, Na-K-ATPase expression was markedly decreased in BUO (35 ± 5% of sham levels in the whole kidney expression, $P < 0.05$, Fig. 4, C and D), BUO-5hR (50 ± 11% of sham levels in the whole kidney expression or 34 ± 5% of sham levels in the C+OM, $P < 0.05$, Fig. 9, A–D), and BUO-48hR rats (39 ± 4% of sham levels in the whole kidney expression or 63 ± 6% of sham levels in the C+OM, $P < 0.05$, Fig. 8D, Table 3) compared with sham-operated control rats, respectively.

Next, we examined the effects of α-MSH treatment on the expression of Na-K-ATPase in BUO, BUO-5hR, and BUO-48hR rats. Semiquantitative immunoblotting using whole kidney proteins prepared from BUO rats (protocol 1) and sham-operated rats revealed that α-MSH treatment significantly prevented downregulation of whole kidney expression of Na-K-ATPase (94 ± 7% of sham levels, $P < 0.05$) compared with nontreated rats (35 ± 5% of sham levels, $P < 0.05$, Fig. 9, A and B). Similarly, in the C+OM α-MSH treatment significantly prevented downregulation of Na-K-ATPase expression (63 ± 10% of sham levels, $P < 0.05$) compared with nontreated rats (34 ± 5% of sham levels, $P < 0.05$, Fig. 9, C and D, Table 3). Consistent with this, in BUO-48hR rats (protocol 3), α-MSH treatment prevented downregulation of Na-K-ATPase, α-MSH treatment of rats partially prevented downregulation of whole kidney expression of Na-K-ATPase expression (68 ± 6% of sham levels, $P < 0.05$) compared with nontreated rats (39 ± 3% of sham levels, Table 3). Similarly,
in the C+OM α-MSH treatment was associated with normalized expression of Na-K-ATPase (113 ± 4% of sham levels, \( P < 0.05 \)) compared with nontreated rats (63 ± 6% of sham levels, Fig. 8D, Table 3). These results suggested a potential preventive role of α-MSH treatment in the regulation of Na-K-ATPase in response to ureteral obstruction.

Immunocytochemistry confirmed the immunoblotting analysis demonstrating downregulation of Na-K-ATPase abundance in the proximal tubules in untreated kidneys in response to 24-h BUO and BUO-5hR (Fig. 10, A and D). This was markedly attenuated in response to α-MSH treatment (Fig. 10, B and E), demonstrating virtually the same labeling density as sham-operated controls (Fig. 10, C and F).

**DISCUSSION**

The present results demonstrated that complete ureteral obstruction for 24 h caused significant reductions in both GFR and ERPF, which remained decreased for the next 48 h after the release of the obstruction. Rats with BUO and short-term release of BUO showed an impaired urinary concentrating ability, which was associated with downregulation of AQP1, -2, and -3 water channels and Na-K-ATPase in kidney tubules. α-MSH treatment significantly prevented the deterioration of renal function in rats with release of BUO: 1) the hemodynamic changes (e.g., reduction in GFR and ERPF) were not observed in rats with release of BUO, which were treated with α-MSH; 2) moreover, α-MSH treatment, to a major degree, prevented the downregulation of AQP and Na-K-ATPase, compared with nontreated BUO rats or especially in the setting of BUO release. Thus the present data strongly indicate that α-MSH treatment may be of significant value in protecting against BUO-induced impaired renal function with downregulation of AQP and Na-K-ATPase.

**α-MSH Treatment Prevented Deterioration of Renal Hemodynamics and Renal Tubular Functions in Response to BUO**

It is well known that urinary tract obstruction is associated with a sustained intraluminal tubular pressure (7) and progressive reduction of RBF and GFR. Following release of 24-h
BUO, intratubular pressure returns from elevated levels to normal, but renal plasma flow decreases due to the afferent arteriolar vasoconstriction, resulting in a persistent decrease in GFR (19). Importantly, we demonstrated that α-MSH treatment in rats with release of BUO significantly prevented the decline in GFR and ERPF. Moreover, α-MSH treatment in rats with release of BUO significantly prevented the decline in proximal and distal water and sodium reabsorption. In particular, proximal water and sodium reabsorption was totally unchanged with α-MSH treatment in rats with release of BUO compared with sham-operated rats (Table 2), suggesting a preferential effect of α-MSH treatment, accelerating recovery in the proximal nephron compared with the distal nephron, including the collecting duct.

Acute ureteral obstruction is characterized by an infiltration of macrophages and lymphocytes into the kidney (41). A time-dependent infiltration of inflammatory cells, predominantly macrophages and suppressor T lymphocytes, has been demonstrated in both the cortex and medulla following ureteral obstruction (40). This indicates that BUO and release of BUO are associated with a severe inflammatory response. Thus it is possible that the infiltrating cells may play a potential role in decreasing the GFR and the RBF, possibly via stimulation of the production of vasoactive mediators, such as angiotensin II (19) and thromboxane A2 (TXA2) (13). TXA2 is able to contract mesangial cells in culture and therefore can potentially reduce the glomerular capillary area available for filtration. Moreover, inhibition of thromboxane synthesis increases GFR and RBF significantly in the postobstructed kidney (20). Thus TXA2 released by infiltrating cells may play an important role in the decrease of renal plasma flow and, possibly, the decline in single-nephron GFR following ureteral obstruction (18).

Consistent with this, it has been demonstrated that inhibition of inflammatory cell infiltration in the obstructed kidney by irradiation blunted TXA2 generation and improved GFR and RBF and partially corrected renal sodium and water handling (41). This suggests that infiltrating inflammatory cells may modulate the changes in renal function following ureteral obstruction. It is likely that the effect of α-MSH treatment in preventing the decline in tubular functions in the obstructed kidney could be due to its anti-inflammatory mechanisms, but this needs to be examined in detail in future studies.

It is well known that urinary tract obstruction induces a progressive loss of renal mass through apoptotic renal cell death. Immunocytochemistry demonstrated renal tubular cell apoptosis in BUO kidneys, and α-MSH treatment virtually abolished the presence of apoptotic cells, thus preventing apoptosis, consistent with the results from previous studies where α-MSH treatment decreased cyclosporine A-induced apoptosis in the proximal tubule (15, 23). α-MSH is known to be an inhibitory cytokine produced by macrophages that dampens local inflammatory responses. α-MSH inhibits the production and actions of proinflammatory cytokines and chemokines and the inhibition of neutrophil migration and infiltration (3). Moreover, α-MSH has been demonstrated to inhibit serum-activated lipopolysaccharide (SA-LPS)-induced NF-κB activation not only in macrophages but also in a T cell line and human neutrophils isolated from the peripheral blood (39). Furthermore, α-MSH has been demonstrated to inhibit the production of nitric oxide (5).

We have previously demonstrated that α-MSH treatment prevented the reduction in AQP expression, urinary concentration, and GFR in kidneys of rats with ischemia-reperfusion-induced acute renal failure (ARF) (22). Also, this was consis-
tent with previous studies in mice and rats with ischemia-reperfusion-induced ARF (5). α-MSH treatment in rats and mice with ischemia-induced ARF significantly reduced neutrophil infiltration and erythrocyte congestion in the medullary region, in association with markedly improved renal function (5, 22). Thus these results could support the view that infiltrating inflammatory cells in the obstructed kidneys may significantly contribute to the changes in renal function. Accordingly, α-MSH treatment could prevent the deterioration of renal function by inhibiting the inflammatory process, which is known to be associated with BUO and release of BUO.

It should also be emphasized that it is not known whether α-MSH treatment has effects mediated by other mechanisms, in addition to the anti-inflammation actions, that prevent the decline in renal function. As demonstrated previously, the dramatic effect of α-MSH in ischemia-reperfusion kidney failure decreases renal injury through inhibition of neutrophil-independent pathways, suggesting that it inhibits tubular injury by direct tubular effects (6). It should be underscored that further studies are needed to clarify the underlying mechanisms of the beneficial effects of α-MSH treatment after ureteral obstruction.

α-MSH Treatment Partially Prevented Downregulation of AQP1, -2, and -3, and Na-K-ATPase in BUO and BUO-R Rats

Recently, the AQPs, a family of membrane proteins that function as water channels, have been demonstrated to play pivotal roles in the physiology and pathophysiology of renal regulation of body water balance (1, 34). Furthermore, sodium (co)transporters in the individual renal tubule segments have been identified, and the regulation of these transporters is fundamental for renal sodium reabsorption as well as for establishment of the driving force for water reabsorption and urine concentration. Previously, we demonstrated that reduced expression of AQPs and major renal sodium transporters was associated with impaired urinary concentrating ability and renal sodium wasting in the experimental animal models with BUO, UUO, and after release of BUO for up to 30 days (24–27). In this study, we further demonstrated that the expression levels of AQP2, -3, and -1 and Na-K-ATPase were significantly decreased in rats 5 h after the release of BUO, demonstrating that downregulation persists immediately after the release of the obstruction. Our clearance study confirmed...
the previous findings that showed a decreased fractional reabsorption of sodium and water in both the proximal and distal tubule and an impairment of water reabsorption in the collecting duct in BUO and BUO-R (12, 14, 30, 42, 48) rats. α-MSH treatment partly prevented the downregulation of AQP2 during 24-h BUO and the decreased expression of AQP3 during BUO and immediately after release of BUO. Also, it prevented downregulation of AQP1 after release of BUO for 5 or 48 h. Importantly, α-MSH treatment prevented downregulation of Na-K-ATPase during obstruction and after release of BUO for 5 or 48 h, which was associated with a complete recovery of sodium and water reabsorption in proximal tubules 48 h after release of BUO. In contrast, the reabsorption of sodium and water in distal tubules was only partly recovered compared with sham-operated controls after α-MSH treatment. Additionally, polyuria and decreased urine osmolality were not affected by the treatment in rats with the release of BUO for 48 h despite the prevention of the downregulation of AQP1 and Na-K-ATPase by α-MSH treatment. Consistent with this, α-MSH-treated rats (BUO-48hR) had markedly improved GFR, but they had still decreased osmotic water permeability in the collecting duct, which is also evidenced by the decreased expression of inner medullary expression of AQP2 and AQP3. Further investigations are needed to define the underlying mechanisms in the downregulation of AQPs and Na-K-ATPase in response to urinary tract obstruction and the protective effect of α-MSH treatment.

**Summary**

BUO and short-term release after BUO were associated with a reduction in GFR and in ERPF, as well as an impaired urinary concentrating ability in conjunction with downregulation of renal AQP1, -2, and -3 and Na-K-ATPase expression. α-MSH treatment dramatically prevented the deterioration of renal function in rats with release of BUO: 1) the hemodynamic changes (e.g., reduction in GFR and ERPF) were not observed in rats with release of BUO, which were treated with α-MSH; 2) moreover, α-MSH treatment, to some degree, prevented the downregulation of AQPs and Na-K-ATPase compared with nontreated rats with BUO and release of BUO. Thus the protective mechanisms of α-MSH might be multifactorial and broad, including prevention of both hemodynamic and tubule functions. The present data suggest that α-MSH treatment might play a potentially protective role regarding the impaired renal function and the downregulation of AQPs and Na-K-ATPase seen during BUO and after release of BUO. These findings may potentially provide relevant clinical value in obstructive nephropathy.

**ACKNOWLEDGMENTS**

The authors thank Gitte Kall, Jette Birk, Dorte Wulff, Inger Merete Paulsen, Mette Vistisen, Helle Høyer, Zhila Nikrozi, Lotte Valentin Holbech, and Line Nielsen for expert technical assistance. The Water and Salt Research Centre at the University of Aarhus is established and supported by the Danish National Research Foundation (Danmarks Grundforskningsfond).

**GRANTS**

Support for this study was provided by The Karen Elise Jensen Foundation, The Commission of the European Union (QLRT-2000–00987 and QLRT-2000–00778), The Human Frontier Science Program, The WIRED program (Nordic Council and the Nordic Centre of Excellence Program in Molecular Medicine), The Novo Nordisk Foundation, The Danish Medical Research Council, The University of Aarhus Research Foundation, The Skovgaard Foundation, The Danish Research Academy, The University of Aarhus, The

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**Fig. 10.** Immunoperoxidase microscopy of Na-K-ATPase in the kidney cortex. Na-K-ATPase labeling was associated with the basolateral plasma membrane of the proximal tubules (*) and distal convoluted tubule or connecting tubules in nontreated BUO rats (A and D), α-MSH-treated BUO rats (B and E), and sham-operated control rats (C and F). Immunoperoxidase microscopy demonstrated decreased Na-K-ATPase immunolabeling in the proximal tubules (*) in nontreated kidneys in response to 24-h BUO and BUO-5hR (A and D). In contrast, α-MSH treatment in rats subjected to 24-h BUO and BUO-5hR was associated with similar Na-K-ATPase immunolabeling in the proximal tubule (B and E) to the labeling density observed in sham-operated controls (C and F). Magnification: ×630 (A–F).
REFERENCES


