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

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Running title: alpha-MSH treatment in rats with BUO

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Abstract

The purpose of this study was to evaluate the effects of the anti-inflammatory hormone α-melanocyte stimulating hormone (α-MSH) treatment on renal function and expression of AQP5s and Na,K-ATPase in the kidney in response to 24 hours 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. 48 h after BUO-R demonstrated that α-MSH treatment almost completely prevented the decrease in GFR (non-treated: 271 ± 50 µl/min/100gbw; α-MSH: 706 ± 85 µl/min/100gbw; sham: 841 ± 105 µl/min/100gbw, \( P<0.05 \)) and ERPF (non-treated: 1139 ± 217 µl/min/100gbw; α-MSH: 2598 ± 129 µl/min/100gbw; sham: 2633 ± 457 µl/min/100gbw, \( P<0.05 \)) 48 hours after release of BUO. α-MSH treatment also partly prevented the downregulation of AQP1 and Na,K-ATPase expression in rats after release of BUO for 48 hours. 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.

**Key words:** urinary tract obstruction, water channels, sodium pump, and urinary concentrating defect.
Introduction

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 pathophysiological 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 pro-inflammatory mediators. This represents the pro-inflammatory state of ureteral obstruction and also contributes to the late development of interstitial fibrosis (37).

Aquaporins (AQP) are a family of membrane proteins and play key roles for the 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) (10; 11). AQP2 levels were persistently decreased up to two weeks 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 has 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 expression may also be involved in the disorders of renal water and salt regulation during post-obstructive diuresis (26; 27).

Alpha-melanocyte stimulating hormone (α-MSH) is a potent anti-inflammatory peptide and has been shown to prevent ischemia and 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 the decreased urinary concentrating ability (21; 22). Several mechanisms of the anti-inflammatory action induced by α-MSH treatment have been identified: 1) the inhibition of pyrogenic and proinflammatory effects of cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor (TNF) and interferon gamma (IFN gamma); 2) the inhibition of inflammation induced by other mediators of inflammation and by peripheral irritants; and 3) the 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 which were previously demonstrated in rats with post-obstructive 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 euolemic rats with BUO followed by release using a servo-controlled system. 2) To assess the effect of α-MSH treatment on AQPs and Na,K-ATPase expression in BUO and BUO followed by release.
Methods

Experimental Protocols

Protocols 1-3

Studies were performed on male Münich Wistar rats initially weighing 230 g (Møllegard 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 rats were kept in individual metabolic cages, with a 12-hour artificial light/dark cycle, a temperature of 21 ± 2 °C and a humidity of 55 ± 2%. Rats were allowed to acclimatize to the cages for 3 days prior to surgery.

The rats were anesthetized with halothane (Halocarbon Laboratories, NJ, USA) and during surgery, the rats were placed on a heated table to maintain rectal temperature at 37-38 °C. Through a midline abdominal incision both ureters were exposed and a 5 mm long piece of bisected polyethylene tubing (PE-50) was placed around the midportion of each ureter. The ureter was then occluded by tightening the tubing with a 5-0 silk ligature. After surgery rats were allowed to regain consciousness. Twenty-four hours later, rats were anesthetized again and the obstructed ureters were decompressed by removal of the ligature and the PE tubing. Using this technique the ureters could be completely occluded for 24 hours without evidence of subsequent functional impairment of ureteral function.

Rats were allocated to the protocols indicated below. Age and time matched sham-operated controls were prepared and observed in parallel with each BUO group in the following protocols.

Protocol 1: 1) Rats with BUO for 24 hours (n=28). The BUO animals were divided into two groups: non-treated group (n=14) and α-MSH treated group (n=14). Alpha-MSH (Phoenix Pharmaceutical Inc, Mountain View, CA, 50 µg, i.v.) was given at the onset of BUO and 12 hours later. The two kidneys were removed and separately prepared for semiquantitative immunoblotting or immunocytochemistry. 2) Sham-operated rats (n=14).

Protocol 2: 1) This protocol is identical to protocol 1 except that the ureter obstruction was released after 24 hours and rats followed for 5 hours after release. Alpha-MSH was given 3 times (at
onset of obstruction, and at 12 and 24 hours after release of obstruction). BUO non-treated (n=9), BUO-
alpha-MSH (n=9) and SHAM (n=9).

**Protocol 3:** 1) Rats with BUO for 24 hours and followed by release for 48 hours. The rats were
divided into two groups: non-treated group (n=5) and α-MSH treated group (n=6). Alpha-MSH (100
µg/24h) was given with micro-osmotic pump via jugular vein at the onset of BUO. The two kidneys
were removed and separately prepared for semiquantitative immunoblotting. 2) Sham-operated rats
(n=4). Sham-operated rats and non-treated rats were treated with vehicle (0.9% saline) with micro-
osmotic pump.

**Catheterization** The jugular vein was exposed at least 1 cm. The tip of the catheter was inserted
into the vein and pushed forwards towards the heart (about 2.5 cm). The catheter was then tied into the
blood vessel with the ligature. With a small incision made in the dorsal nape of the neck the catheter was
passed subcutaneously from the site of the entry of the catheter of the jugular vein to the dorsal incision
down the side of the neck to emerge anterior. And a micro-osmotic pump was connected with the end of
catheter.

**Protocol 4**

This protocol was identical to protocol 3. However, rats were used to measure renal hemodynamics
and tubular function by a servo-controlled system.

Studies were performed on female Wistar rats weighing 200 to 250 g (Møllegard Breeding Center,
Eiby, Denmark). Seven to 10 days before the baseline clearances study, rats were anesthetized with
Hypnorm® (Fentanyl citrate 0.315 mg/ml + Fluanisone 10 mg/ml, 400 µl/kg) and Dormicum®
(Midazolam 5 mg/ml, 800 µl/kg) and were placed on a heated table to maintain rectal temperature at 37-
38 °C. Using aseptic surgical techniques, sterile Tygon catheters (0.762 mm, Flexible plastic tubing,
TYGON®, Weyerhaeuser, Cleveland, OH, USA) were advanced into the abdominal aorta and the
inferior caval vein via the femoral vessels, and a stainless steel cannula was sewn into the bladder and
exteriorized through the ventral abdominal wall. The catheterizations were fixed, with smaller
modifications, as described by Petersen et al (36). After the operation the rats were returned to the animal
units and housed individually. After a recovery period of 4-5 days, the rats were acclimatized to restriction by three daily training sessions in the restraining cages. The duration of each daily session was increased stepwise from 1 to 3 hours a daily.

Clearance studies

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

Clearance study in protocol 4

The measurements of renal function were carried out in the conscious rats immobilized in restraining cages according the method described by Thomsen, et al (47). Each experiment comprised a 15 min bolus period for $[^{3}H]$-inulin and $[^{14}C]$-TEA, a 105 min equilibration period, then six 30 minutes urine collection periods. Blood specimens of 300 µl were collected from the arterial catheter each hour during the 180 minutes 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 units/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 (bolus 0.6ml, sustained 10 µl/min) containing $[^{14}C]$-TEA (New England Nuclear, Boston, USA) (bolus 0.84 μCi, sustained 0.014 μCi/min), $[^{3}H]$-inulin (Amersham International, Aylesbury, UK) (bolus 3.6 μCi, sustained 0.06 μCi/min) and LiCl (bolus 7.2 μmol, 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 in order 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-control 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\textsuperscript+] per second (Nova-biochemical, Waltham, MA, USA). Data on urine production (weight on scale) and [Na\textsuperscript+] were sampled continuously on an IBM-compatible computer, which in turn controlled the infusion rates of two infusion pumps. Urinary output of Na\textsuperscript+ and fluid were integrated over 5 min, thus allowing a 5-min delay in changes of Na\textsuperscript+ 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:

\[ C_x = U_x \cdot V / P_x; \]

Glomerular filtration rate was determined as GFR = C\textsubscript{Inulin}, and effective renal plasma flow (ERPF) = C\textsubscript{TEA} (35).

Fractional excretion of sodium (FE\textsubscript{Na}) was then calculated from the formula:

\[ FE_{Na} = \frac{Na_U}{Na_P} \times \frac{Inulin_P}{Inulin_U} \times 100 \]

Proximal water reabsorption: GFR - C\textsubscript{Li}.

Proximal sodium reabsorption: (GFR-C\textsubscript{Li}) \times P\textsubscript{Na}.

Distal water reabsorption: C\textsubscript{Li} - V.

Distal sodium reabsorption: (C\textsubscript{Li} - C\textsubscript{Na}) \times P\textsubscript{Na}.

Fractional distal water excretion: V/C\textsubscript{Li}.

Fractional distal sodium excretion: C\textsubscript{Na}/C\textsubscript{Li}.

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

Different treated groups were compared using ANOVA techniques followed by Dunnett’s test for individual comparisons between group means or comparisons between groups were made by unpaired t-test. A \( P \) values less than 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 (CHIP 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 alpha-1 subunit of Na,K-ATPase has previously been characterized (16). 5) AIF: A goat polyclonal antibody recognizing apoptosis inducing factor (AIF) which is commercial available from Santa Cruz Biotechnology, 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 ml (TK), 8 ml (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 phenylmethyl sulfonylfluoride), with 5 strokes of a motor-driven Potter-Elvehjem homogenizer, at 1250 rpm. This homogenate was centrifuged in a Universal 30RF centrifuge (Hettich, Tuttingen, Germany) at 4,000×g for 15 minutes at 4°C and the supernatant was pipetted off and solubilized at 65°C for 15 minutes in Laemmlli sample buffer containing 2% SDS, and then stored at -20°C.

**Electrophoresis and immunoblotting**

Protein samples were run 12% polyacrylamide minigels (Bio-Rad Mini Protean 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
electroelution 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, DAKO, Glostrup Denmark, diluted as 1:3000) using an enhanced chemiluminescence system (ECL, Amersham International, UK).

**Histological examination of kidneys**

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

**Immunocytochemistry**

The kidneys from BUO rats 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 micrometer on a rotary microtome (Leica, Germany). The sections were deparafinated and rehydrated. For immunoperoxidase labeling, endogenous peroxidase were blocked by 0.5% H₂O₂ in absolute methanol for 10 min at room temperature. To reveal antigens, sections were put in 1 mmol/l TRIS solution (pH 9.0) supplemented with 0.5 mM EGTA (3.6-di-oxa-octa-methylen-di-nitrilotetra-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 rinsing 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 (DAKO A/S, Glostrup, Denmark, P448, 1:200) diluted in PBS supplemented with 0.1% BSA and 0.3% Triton-X-100. The sections
were washed for 3 x 10 min and followed by incubation with diaminobenzidine for 10 minutes. The microscopy was carried out using a Leica DMRE light microscope (Leica, Heidelberg, Germany).

**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 unpaired t-test. A $P$ value less than 0.05 was considered to be statistically significant.
Results

**α-MSH treatment partially prevented urinary concentrating defect associated with BUO and release of BUO**

BUO-R was associated with an impaired renal tubular water reabsorption, polyuria and decreased urinary osmolality (Table 1). Consistent with this, in rats 48 hours after release of 24 hours-BUO (BUO-48hR) solute-free water reabsorption (T\textsuperscript{c}H\textsubscript{2}O) was markedly reduced compared with sham-operated controls (64 ± 11 vs. 186 ± 8 µl/min/kg, \(P<0.05\), Table 1), indicating an impaired renal tubular water reabsorption. Moreover, rats with BUO-R exhibited decreased GFR after release of BUO. Consistent with this, creatinine clearance was significantly decreased in rats with BUO-48hR compared with sham-operated controls (1.9 ± 1.0 vs. 9.6 ± 3.3 µl/min/kg, \(P<0.05\), Table 1). Moreover, plasma osmolality and plasma creatinine concentration were significantly increased in rats with BUO for 24 hours (BUO) and in rats 5 hours (BUO-5hR) or 48 hours (BUO-48hR) after release of 24 hours-BUO, respectively (Table 1), as demonstrated in previous studies (25; 26).

To investigate the effects of alpha-MSH on the kidney functions, rats with BUO and BUO-5hR or BUO-48 hours were treated with \(\alpha\)-MSH. \(\alpha\)-MSH treatment did not change the increased plasma osmolality in rats with BUO or BUO-5hR. In contrast, in \(\alpha\)-MSH-treated rats with BUO-48hR the increased plasma osmolality was attenuated (341 ± 8 mOsm/KgH2O in non-treated rats vs. 313 ± 3 mOsm/KgH2O in \(\alpha\)-MSH treated rats, \(P<0.05\), Table 1). Similarly, \(\alpha\)-MSH treatment was associated with decreased plasma creatinine levels in BUO-48R (214 ± 47 µmol/l in non-treated rats vs. 79 ± 12 µmol/l in \(\alpha\)-MSH treated rats, \(P<0.05\), Table 1). Moreover, \(\alpha\)-MSH treatment prevented the decrease in T\textsuperscript{c}H\textsubscript{2}O in BUO-48R (64 ± 11 µl/min/kg in the non-treated rats vs. 89 ± 5 µl/min/kg in \(\alpha\)-MSH treated rats, \(P<0.05\), Table 1).
Alpha-MSH treatment prevented the impairment of renal hemodynamics and tubular functions in rats with BUO-48hR

In order to further examine the protective effects of α-MSH treatment during the ureteral obstruction and the release of obstruction, renal hemodynamics and tubular functions were investigated in conscious, euvoletic rats with BUO-48hR by a servo-controlled system. GFR was measured by the inulin clearance and effective renal plasma flow (ERPF) was measured by the TEA clearance in rats with BUO-48hR. Forty-eight hours after release of BUO, GFR (271 ± 50 µl/min/100gbw in non-treated vs. 841 ± 105 µl/min/100gbw in sham-operated, P<0.05, Fig 1A) and ERPF (1,139 ± 217 µl/min/100gbw in non-treated vs. 2,633 ± 457 µl/min/100gbw in sham-operated, P<0.05, Fig 1B) were significantly decreased in non-treated BUO compared with sham-operated controls, respectively. α-MSH treatment prevented the reductions in GFR and ERPF in rats with BUO-48hR (Figs 1A and B).

Furthermore, renal tubular functions improved significantly after α-MSH treatment in rats with BUO-48hR. Non-treated rats with BUO-48hR 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 rats with BUO-48hR 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 of the fractional distal water and sodium excretion seen in non-treated rats with BUO-48hR was also attenuated by α-MSH treatment of rats with BUO-48hR (Table 2).

Alpha-MSH treatment prevents apoptosis

In sections from kidneys from rats subjected to 24 hours of BUO but without α-MSH treatment there were only marginal neutrophil cell infiltration (Fig 2A). In contrast, the untreated BUO kidneys demonstrated a relative high density of cells with densely stained nuclei likely to represent apoptotic cell (dense chromatin staining, arrows in Fig 2A). Renal tubular cell apoptosis was confirmed in sections from BUO kidneys stained for the presence of apoptotic nuclei with an antibody recognizing AIF,
consistent with previous evidence. Apoptosis occurred primarily in the renal medulla. Importantly, \( \alpha \)-MSH treatment virtually completely abolished the presence of apoptotic cells (Fig 2C and D) and did not differ from sham operated control kidneys (Fig 2E and F).

**Alpha-MSH attenuated downregulation of AQP2, -3 and -1 in rats with BUO, BUO-5hR or BUO-48hR**

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, AQP3 and AQP1 expression compared with sham-operated controls (Fig 3-6 and Fig 8, Table 3). In contrast, \( \alpha \)-MSH treatment significantly attenuated the inner medullary reduction in AQP2 expression in BUO rats (38 ± 5% of sham levels, \( P < 0.05 \)) compared with non-treated BUO rats (13 ± 4% of sham levels, Fig 3A and B). Moreover, \( \alpha \)-MSH treatment also attenuated downregulation of inner medullary AQP3 expression (44 ± 3% of sham levels, \( P < 0.05 \)) compared with non-treated BUO rats (19 ± 4% of sham levels, Fig 3C and D). Whole kidney expression of AQP1 was decreased in both non-treated BUO and \( \alpha \)-MSH-treated BUO compared with sham-operated rats (Fig 4A and B, Table 3).

In rats with BUO-5hR, \( \alpha \)-MSH treatment attenuated downregulation of inner medullary AQP3 expression (30 ± 2% of sham levels, \( P < 0.05 \)) compared with non-treated BUO rats (15 ± 5% of sham levels, Fig 5C and D), whereas inner medullary AQP2 expression was unchanged in \( \alpha \)-MSH treated rats (Fig 5A and B, n.s.). This is consistent with the maintained polyuria. In contrast, \( \alpha \)-MSH treatment prevented downregulation of whole kidney AQP1 expression (81 ± 21% of sham levels, n.s., Fig 6A and B) and attenuated the downregulation of AQP1 in cortex and outer medulla (62 ± 9% of sham levels, \( P < 0.05 \), Fig 6C and D), compared with non-treated rats.

Immunocytochemistry confirmed the downregulation of AQP1 abundance in the proximal tubules in untreated kidneys in response to 24h BUO and BUO 5hR (Fig 7A and D). The labeling was markedly attenuated in BUO-5hR rats in response to \( \alpha \)-MSH treatment (Fig 7E), demonstrating virtually the same labelling density as sham-operated controls (Fig 7F).
In rats with BUO-48hR, 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 non-treated rats with BUO-48hR, respectively. In contrast, the expression of inner medullary AQP2 and AQP3 was unchanged in rats with BUO-48hR in response to α-MSH treatment (Fig 8B and C, Table 3).

**Alpha-MSH prevented downregulation of Na,K-ATPase in response to BUO, BUO-5hR and BUO-48hR**

Release of BUO was associated with an increased fractional excretion of sodium to urine (\( \text{FE}_{\text{Na}} \)) 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 rats with BUO (35 ± 5% of sham levels in the whole kidney expression, \( P<0.05 \), Fig 4C 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 9A-D), and BUO-48hR (39 ± 3% 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 rats with BUO, BUO-5hR and BUO-48hR. Semiquantitative immunoblotting using whole kidney proteins prepared from the rats with BUO (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 non-treated rats (35 ± 5% of sham levels, Fig 4C and D, Table 3). Furthermore, in rats with BUO-5hR (protocol 2) the whole kidney expression of Na,K-ATPase was normalised completely in response to α-MSH-treatment (100 ± 14% of sham levels, \( P<0.05 \)) compared with non-treated rats (50 ± 11% of sham levels, Fig 9A 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 non-treated rats ($34 \pm 5\%$ of sham levels, Fig 9C and D, Table 3). Consistent with this, in rats with BUO-48hR (protocol 3), $\alpha$-MSH treatment prevented downregulation of Na,K-ATPase. $\alpha$-MSH-treatment of rats partially prevented downregulation of whole kidney expression of Na,K-ATPase expression ($68 \pm 6\%$ of sham levels, $P<0.05$) compared with non-treated rats ($39 \pm 3\%$ of sham levels, Table 3). Similarly, in the C+OM $\alpha$-MSH treatment was associated with normalized expression of Na,K-ATPase ($113 \pm 4\%$ of sham levels, $P<0.05$) compared with non-treated rats ($63 \pm 6\%$ of sham levels, Fig 8D, Table 3). These results suggested a potential preventive role of $\alpha$-MSH treatment on 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 10A and D). This was markedly attenuated in response to $\alpha$-MSH treatment (Fig 10B and E), demonstrating virtually the same labelling density as sham-operated controls (Fig 10C and F).
Discussion

The present results demonstrated that complete ureteral obstruction for 24-hours caused significant reductions in both GFR and ERPF, which remained decreased for the next 48 hours after release of obstruction. Rats with BUO and short-term release of BUO showed an impaired urinary concentrating ability, which was associated with downregulation of AQP1, AQP2, AQP3 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 AQPs and Na,K-ATPase, in comparison with non-treated rats with BUO or especially in the setting of release of BUO. Thus, the present data strongly indicate that α-MSH treatment may be of significant value to protect against BUO-induced impaired renal function with downregulation of AQPs and Na,K-ATPase.

α-MSH treatment prevented the 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 24h-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 of GFR and ERPF. Moreover, α-MSH treatment in rats with release of BUO prevented significantly the decline of proximal and distal water and sodium reabsorption. In particular, the 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 on the proximal nephron compared with the distal nephron including the collecting duct in rats with release of BUO.
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 the 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 post-obstructed 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 glomerular filtration rate following the ureteral obstruction (18). Consistent with this, it has been demonstrated that inhibition of the inflammatory cell infiltration in the obstructed kidney by irradiation blunted the TXA2 generation and improved GFR, RBF and partially corrected the 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 of 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 in 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 (NO) (5).

We have previously demonstrated that α-MSH treatment prevented the reduction of AQPs expression, reduction in urinary concentration, and reduction in GFR in kidneys of rats with ischemia-reperfusion induced acute renal failure (22). Also this was consistent with the previous studies on mice and rats with ischemia-reperfusion-induced ARF (5). α-MSH treatment in rats and mice with ischemia-induced ARF significantly reduced the neutrophil infiltration and erythrocyte congestion in the medullary region, in association with a marked 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 to prevent the decline in renal function, in addition to the anti-inflammation actions. As demonstrated previously, the dramatic effect of alpha-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 AQPI, AQP2, AQP3 and Na,K-ATPase in rats with BUO and release of BUO.**

Recently, the aquaporins, which are 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 the renal sodium reabsorption as well as for establishment of the driving force for water reabsorption and urine concentration. Previously we demonstrated that reduced expression in AQPs and major renal sodium transporters was associated with an impaired urinary concentrating ability and renal sodium wasting in the experimental animal models with BUO, UUO, and after release of BUO 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 hours after the release of BUO demonstrating that downregulation persists immediately after release of 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). 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 hours or 48 hours. Importantly α-MSH treatment prevented downregulation of Na,K-ATPase during obstruction and after release of BUO for 5 hours or 48 hours, which was associated with a complete recovery of sodium and water reabsorption in proximal tubules 48 hours 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. And polyuria and decreased urine osmolality were not affected by the treatment in rats with the release of BUO for 48 hours despite that the downregulation of AQP1 and Na,K-ATPase was prevented 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 was associated with a reduction in GFR and in ERPF, as well as an impaired urinary concentrating ability in conjunction with downregulation of renal AQP1, AQP2, AQP3, 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, in comparison with non-treated rats with BUO and release of BUO. Thus, the protective mechanisms of α-MSH might be multifactorial and broad, including prevention of both hemodynamics and tubule functions. The present data suggested that α-MSH treatment might play a potential protective role against 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.
Acknowledgements

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). 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 Advanced Medical Technology Cluster for Diagnosis and Prediction at KNU from MOCIE (THK), and The intramural budget of the NHLBI, NIH.
Legends to figures

**Figure 1.** Effect of α-MSH treatment on changes in glomerular filtration rate (GFR, panel A) and effective renal plasma flow (ERPF, panel B) in rats followed by release of BUO for 48 h (protocol 4). Before operation, GFR and ERPF levels were not different between non-treated, α-MSH treatment and sham groups. Both GFR and ERPF significantly decreased in non-treated BUO-48hR rats compared with sham-operated controls. α-MSH treatment prevented the reduction in GFR and ERPF 48 hours after release of BUO. *P<0.05 compared with sham-operated controls. # P<0.05 compared with non-treated BUO-48hR rats.

**Figure 2.** Paraffin sections (2 µm) of perfusion fixed kidneys stained with hematoxylin and eosin for light microscopic analysis (A, C and E) and immunoperoxidase microscopy of AIF (B, D and F). In untreated BUO kidneys (A), there was very limited neutrophil infiltration in the sections (asterisk). In contrast, a relative high density of cells with densely stained nuclei was observed (arrows in A), likely to represent apoptotic cells with dense chromatin staining. Apoptotic cells were further demonstrated with AIF immunolabelling and were shown to be associated with IMCD cells and other cells in the renal inner medulla (B). Alpha-MSH treatment virtually completely abolished the presence of such apoptotic cells (C and D). The pattern observed after α-MSH treatment did not differ from that seen sham operated control kidneys (E and F). Magnification: A-C, ×250.

**Figure 3.** Semiquantitative immuno blotting of membrane fractions of kidney inner medulla (IM) from rats with BUO for 24 hours or sham-operated rats (protocol 1). A: The immunoblot was reacted with affinity-purified anti-aquaporin-2 (anti-AQP2) and revealed 29 kDa and 35-50 kDa AQP2 bands, representing non-glycosylated and glycosylated forms of AQP2. B: Densitometric analysis revealed that α-MSH treatment prevented the downregulation of AQP2 expression in non-treat BUO rats from 13 ± 4% to 38 ± 5% of sham levels (# P<0.01). C: Immunoblotting was reacted with affinity-purified anti-AQP3 and revealed 27-kDa and 33- to 40-kDa AQP3 bands. D: Densitometric analysis revealed that AQP3 levels
were significantly reduced in non-treated BUO rats, from 100 ± 7% to 19 ± 4%, compared with control rats (*P<0.01). In α-MSH treated rats the expression of AQP3 was increased significantly to 44 ± 3% of sham levels (#P<0.01). *P<0.01 compared with sham-operated controls. # P<0.01 compared with non-treated BUO rats.

**Figure 4.** Semiquantitative immunoblotting of membrane fractions of total kidney (TK) in rats with BUO for 24 hours and sham operated rats (protocol 1). *A:* The immunoblot from total kidney was reacted with affinity-purified anti-aquaporin-1 (anti-AQP1). *B:* Densitometric analysis revealed a marked reduction in AQP1 expression in non-treated and α-MSH treated rats compared with sham-operated controls (48 ± 7% and 65 ± 8% of sham levels, *P<0.01). *C:* Immunoblots were reacted with α isoform specific monoclonal antibody to Na,K-ATPase and revealed a single ~96-kDa band. *D:* Densitometric analysis revealed that α-MSH treatment prevented the downregulation of Na,K-ATPase from 35 ± 5% in response to 24 h BUO to 94 ± 7% of sham levels. *P<0.01 compared with sham-operated controls. #P<0.05 compared with non-treated rats.

**Figure 5.** Semiquantitative immunoblotting of membrane fractions of kidney inner medulla (IM) in rats with BUO followed by release for 5 hours (BUO-5hR) and sham-operated controls (protocol 2). *A:* The immunoblot was reacted with anti-AQP2. *B:* Densitometry revealed a marked reduction in AQP2 expression in non-treated and α-MSH treated rats compared with sham-operated controls (32 ± 10% and 27 ± 3% of sham levels, *P<0.01). *C:* The immunoblot was reacted with anti-AQP3. *D:* Densitometric analysis revealed that in α-MSH treated rats the expression of AQP3 was increased significantly from non-treated group 15 ± 5% to 30 ± 2% of sham levels. *P<0.01 compared with sham-operated controls. # P<0.05 compared with non-treated BUO rats.

**Figure 6.** Semiquantitative immunoblotting of membrane fractions in rats with BUO-5hR and sham-operated controls (protocol 2). The immunoblots from membrane fraction obtained from total kidney
(TK; panels A and B) or from cortex plus outer medulla (C+OM; panels C and D) were reacted with anti-
AQP1.  

**B:** Densitometric analysis revealed that AQP1 expression from TK was decreased markedly in
non-treated animals (*P<0.05). With α-MSH treatment the level of AQP1 expression was greatly
increased to 81 ± 21% of sham levels compared with non-treated BUO-5hR rats (# P<0.05). 

**D:** Densitometric analysis in C+OM revealed that α-MSH treatment prevented the downregulation of AQP1
expression levels in the non-treated rats from 35 ± 2% to 62 ± 9% of sham levels, #P<0.05. *P<0.05
compared with sham-operated controls. # P<0.05 compared with non-treated BUO rats.

**Figure 7.** Immunoperoxidase microscopy of AQP1 in the kidney cortex. AQP1 labeling was present at
the apical plasma membrane (arrows) and basolateral plasma membrane domains of the proximal tubules
in non-treated BUO rats (A and D), alpha-MSH-treated BUO rats (B and E), and sham-operated control rats (C and F). Immunoperoxidase microscopy demonstrated decreased AQP1 immunolabeling in the
proximal tubules in non-treated kidneys in response to 24h BUO and BUO-5hR (A and D). In contrast,
alpha-MSH treatment in rats subjected to 24h BUO and BUO-5hR was associated with similar AQP1
immunolabeling in the proximal tubule (B and E) to the labelling density observed in sham-operated

**Figure 8.** Semiquantitative immuno blotting of membrane fractions from kidneys of rats with BUO-48hR
or sham-operated controls (protocol 3). The immunoblots were reacted with anti-AQP1 in C+OM (A),
anti-AQP2 in IM (B), anti-AQP3 in IM (C) and anti-α1-Na,K-ATPase in C+OM (E). In the C+OM,
AQP1 expression level was decreased in non-treated rats with BUO-48hR, which was attenuated by α-
MSH treatment, similar to band density to the sham operated control levels (A). Both AQP2 and AQP3
expression levels in the inner medulla were decreased significantly in non-treated and α-MSH treated
rats compared with sham operated control rats (B and C). In C+OM, Na,K-ATPase expression was
decreased in non-treated animals with BUO-48hR. This was attenuated by α-MSH treatment and band
density was similar to that seen in the sham operated control rats (D).
Figure 9. Semiquantitative immunoblotting of membrane fractions in rats with BUO-5hR and sham-operated controls (protocol 2). A and C: Immunoblots were reacted with anti-α-Na,K-ATPase. B: Densitometric analysis of all samples from total kidney (TK) revealed α-MSH treatment prevented the downregulation of Na,K-ATPase in non-treated BUO-5hR rats from 50 ± 11% to 100 ± 14% of sham levels, #P<0.05 compared with non-treated rats. D: Densitometric analysis of all samples from C+OM revealed that α-MSH treatment prevented the downregulation of Na,K-ATPase in non-treated BUO-5hR rats from 34 ± 5% to 63 ± 10% of sham levels. *P<0.05 compared with sham-operated controls. # P<0.05 compared with non-treated BUO rats.

Figure 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 (asterisks) and distal convoluted tubule or connecting tubules in non-treated BUO rats (A and D), alpha-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 (asterisks) in non-treated kidneys in response to 24 h BUO and BUO-5hR (A and D). In contrast, alpha-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 labelling density observed in sham-operated controls (C and F). Magnification: A-F, ×630.
Table 1. Changes of renal functional data in rats subjected to BUO for 24 hours or followed by release of BUO for 5 and 48 hours treated with or without α-MSH and sham-operated controls (Protocols 1-3).

<table>
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<tr>
<th></th>
<th>n</th>
<th>P_Osm</th>
<th>P_Na</th>
<th>P_K</th>
<th>P_Cr.</th>
<th>U_Vol</th>
<th>U_Osm</th>
<th>TcH2O</th>
<th>Cl_Cr.</th>
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<tbody>
<tr>
<td></td>
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<td>(mOsm/kg H2O)</td>
<td>(mmol/l)</td>
<td>(µmol/l)</td>
<td>(µl/min/kg)</td>
<td>(mOsm/kg H2O)</td>
<td>(µl/min/kg)</td>
<td>(µl/min/kg)</td>
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<tr>
<td>BUO</td>
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<tr>
<td>Non-treated</td>
<td>10</td>
<td>332 ± 4*</td>
<td>135 ± 2*</td>
<td>6.3 ± 0.3*</td>
<td>377 ± 10*</td>
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<tr>
<td>α-MSH</td>
<td>10</td>
<td>345 ± 5*</td>
<td>139 ± 1#</td>
<td>6.1 ± 0.2*</td>
<td>366 ± 11*</td>
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<tr>
<td>SHAM</td>
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<td>302 ± 1</td>
<td>139 ± 1</td>
<td>3.9 ± 0.1</td>
<td>31 ± 1</td>
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<tr>
<td>BUO-5hR</td>
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</tr>
<tr>
<td>Non-treated</td>
<td>5</td>
<td>336 ± 2*</td>
<td>139 ± 0.2</td>
<td>4.9 ± 0.1*</td>
<td>203 ± 17*</td>
<td>247 ± 31*</td>
<td>539 ± 56*</td>
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<tr>
<td>α-MSH</td>
<td>5</td>
<td>344 ± 3*</td>
<td>141 ± 1</td>
<td>5.6 ± 0.2*#</td>
<td>234 ± 17*</td>
<td>206 ± 33*</td>
<td>733 ± 188*</td>
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<td>139 ± 1</td>
<td>4.2 ± 0.1</td>
<td>33 ± 2</td>
<td>29 ± 3</td>
<td>1969 ± 221</td>
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</tr>
<tr>
<td>Non-treated</td>
<td>5</td>
<td>341 ± 8*</td>
<td>134 ± 2</td>
<td>5.9 ± 0.5*</td>
<td>214 ± 47*</td>
<td>104 ± 10*</td>
<td>559 ± 41*</td>
<td>64 ± 11*</td>
<td>1.9 ± 1.0*</td>
</tr>
<tr>
<td>α-MSH</td>
<td>6</td>
<td>313 ± 3*#</td>
<td>140 ± 1</td>
<td>4.9 ± 0.2*</td>
<td>79 ± 12*#</td>
<td>103 ± 13*</td>
<td>615 ± 58*</td>
<td>89 ± 5*#</td>
<td>3.4 ± 0.3*</td>
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<tr>
<td>SHAM</td>
<td>4</td>
<td>302 ± 2</td>
<td>139 ± 1</td>
<td>4.0 ± 0.1</td>
<td>30 ± 1</td>
<td>30 ± 3</td>
<td>2262 ± 232</td>
<td>186 ± 8</td>
<td>9.6 ± 3.3</td>
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</table>

Values are means ± SE. BUO, bilateral ureteral obstruction; n, number of rats; P_Osm, plasma osmolality; P_Na, plasma sodium; P_K, plasma potassium; P_Cr, plasma creatinine; U_Vol, urine volume; U_Osm, urine osmolality; TcH2O, solute-free water reabsorption; Cl_Cr, clearance of creatinine. *P<0.05 compared to sham-operated controls. #P<0.05 compared to non-treated rats with BUO or release of BUO.
Table 2. Changes in reabsorption of water and sodium in proximal and distal tubules in rats with BUO followed by release for 48 h compared with sham-operated controls (Protocol 4).

<table>
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<th>SHAM</th>
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<tbody>
<tr>
<td></td>
<td>n = 5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>( U_{Na} \times U_{Vol} ) (μmol/min/100gbw)</td>
<td>0.34 ± 0.05*</td>
<td>0.34 ± 0.05*</td>
<td>0.53 ± 0.05</td>
</tr>
<tr>
<td>( FE_{Na} ) (%)</td>
<td>1.00 ± 0.13*</td>
<td>0.37 ± 0.07#</td>
<td>0.47 ± 0.06</td>
</tr>
<tr>
<td>Proximal water reabsorption (μl/min/100gbw)</td>
<td>215 ± 39*</td>
<td>553 ± 55#</td>
<td>552 ± 116</td>
</tr>
<tr>
<td>Proximal sodium reabsorption (μmol/min/100gbw)</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/100gbw)</td>
<td>38 ± 18*</td>
<td>129 ± 34*#</td>
<td>263 ± 41</td>
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<tr>
<td>Distal sodium reabsorption (μmol/min/100gbw)</td>
<td>7.3 ± 2.7*</td>
<td>20.8 ± 4.5*#</td>
<td>39.7 ± 5.7</td>
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<tr>
<td>Fractional distal sodium excretion ( (C_{Na}/C_{Li}) )</td>
<td>0.071 ± 0.020*</td>
<td>0.020 ± 0.005#</td>
<td>0.014 ± 0.002</td>
</tr>
<tr>
<td>Fractional distal water excretion ( (V/C_{Li}) )</td>
<td>0.51 ± 0.19*</td>
<td>0.20 ± 0.05#</td>
<td>0.10 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( n = \) number of rats. \( U_{Na} \times U_{Vol} \), urinary sodium excretion; \( FE_{Na} \), fractional excretion of sodium. *P<0.05 compared with sham-operated rats. # P<0.05 compared with non-treated rats followed by release of BUO for 48 hours.
Table 3. Expression of AQPs and Na,K-ATPase in rats with 24 h of 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>
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<tr>
<th></th>
<th>n</th>
<th>AQP2 (IM)</th>
<th>AQP3 (IM)</th>
<th>AQP1 (TK)</th>
<th>AQP1 (C+OM)</th>
<th>Na,K-ATPase (TK)</th>
<th>Na,K-ATPase (C+OM)</th>
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<tr>
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<td>13 ± 4%*</td>
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<td>48 ± 7%*</td>
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<td>35 ± 5%*</td>
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<tr>
<td>α-MSH</td>
<td>10</td>
<td>38 ± 5%*#</td>
<td>44 ± 3%*#</td>
<td>65 ± 8%*</td>
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<td>94 ± 7%#</td>
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<td>100 ± 7%</td>
<td>100 ± 13%</td>
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<td>100 ± 6%</td>
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<tr>
<td><strong>BUO-5hR</strong></td>
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<tr>
<td>Non-treated</td>
<td>5</td>
<td>32 ± 11%*</td>
<td>15 ± 5%*</td>
<td>7 ± 2%*</td>
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<td>30 ± 2%*#</td>
<td>81 ± 21%#</td>
<td>62 ± 9%#</td>
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<td>5</td>
<td>100 ± 4%</td>
<td>100 ± 9%</td>
<td>100 ± 22%</td>
<td>100 ± 19%</td>
<td>100 ± 15%</td>
<td>100 ± 14%</td>
</tr>
<tr>
<td><strong>BUO-48hR</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>5</td>
<td>13 ± 5%*</td>
<td>10 ± 5%*</td>
<td>63 ± 8%*</td>
<td>52 ± 4%*</td>
<td>39 ± 3%*</td>
<td>63 ± 6%*</td>
</tr>
<tr>
<td>α-MSH</td>
<td>6</td>
<td>22 ± 10%*</td>
<td>27 ± 10%*</td>
<td>83 ± 2%*#</td>
<td>73 ± 8%#</td>
<td>68 ± 6%*#</td>
<td>113 ± 4%#</td>
</tr>
<tr>
<td>SHAM</td>
<td>4</td>
<td>100 ± 1%</td>
<td>100 ± 1%</td>
<td>100 ± 7%</td>
<td>100 ± 11%</td>
<td>100 ± 3%</td>
<td>100 ± 4%</td>
</tr>
</tbody>
</table>

Values are means ± SE. BUO, bilateral ureteral obstruction; n=number of rats; BUO-5hR, release of BUO for 5 hours; BUO-48hR, release of BUO for 48 hours; 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 non-treated rats.
References


Figure 1

A

Glomerular Filtration Rate (µl/min/100gbw)

Before BUO 48 h after release of BUO

non-treated (n=5)

MSH (n=5)

Sham (n=5)

B

Renal clearance of TEA (ERPF) (µl/min/100gbw)

Before BUO 48 h after release of BUO

* #
Figure 2

BUO-24h
Figure 3

(A) 24hBUO AQP2 (IM)

(B) AQP2 Expression (Fraction of Sham)

(C) 24hBUO AQP3 (IM)

(D) AQP3 Expression (Fraction of Sham)

n=10

* p < 0.05
# p < 0.01
Figure 4

**A** 24hBUO AQP1 (TK)

**B** AQP1 Expression (Fraction of Sham)

**C** 24hBUO Na,K-ATPase (TK)

**D** Na,K-ATPase Expression (Fraction of Sham)
Figure 5:

A. BUO-5hR AQP2 (IM)

B. AQP2 Expression (Fraction of Sham)

C. BUO-5hR AQP3 (IM)

D. AQP3 Expression (Fraction of Sham)
Figure 6

A. BUO-5hR AQP1 (TK)

B. AQP1 Expression (Fraction of Sham)

C. BUO-5hR AQP1 (C+OM)

D. AQP1 Expression (Fraction of Sham)
Figure 7
Figure 8

AQP1 (C+OM)

A

AQP2 (IM)

B

AQP3 (IM)

C

Na,K-ATPase (C+OM)

D

Figure 8
Figure 9

BUO-5hR Na,K-ATPase (TK)

A

BUO-5hR Na,K-ATPase (C+OM)

C

**Figure 9**

**A** BUO-5hR Na,K-ATPase (TK)

**B** Na,K-ATPase Expression (Fraction of Sham)

**C** BUO-5hR Na,K-ATPase (C+OM)

**D** Na,K-ATPase Expression (Fraction of Sham)

**Figure 9**
Figure 10