A γ-Melanocyte Stimulating Hormone-like Peptide Causes Reflex Natriuresis After Acute Unilateral Nephrectomy

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SUMMARY Previous studies have shown that acute unilateral nephrectomy stimulates sodium (UNaV) and potassium (UKV) excretion by the remaining kidney through reflex pathways requiring an intact pituitary gland, and the natriuresis is accompanied by an increase in the plasma concentration of a peptide or peptides derived from the adrenocorticotropic hormone-β-endorphin precursor molecule pro-opiomelanocortin. We tested the hypothesis that γ-melanocyte stimulating hormone (γ-MSH) was such a peptide involved in the postnephrectomy natriuresis. In six rats undergoing sham nephrectomy, no change in UNaV or UKV occurred and plasma immunoreactive γ-MSH-like material was 40 ± 18 (SD) fmol/ml 2 hours after the sham procedure. In 10 rats undergoing acute unilateral nephrectomy, UNaV and UKV from the remaining kidney increased significantly, and immunoreactive γ-MSH was 81 ± 36 fmol/ml (p<0.02). In individual studies, the increase in UNaV after nephrectomy correlated with the postnephrectomy concentration of immunoreactive γ-MSH (r= 0.75, p< 0.001).

In 17 rats injected with serum or globulin from control rabbits, unilateral nephrectomy led to the expected increases in UNaV and UKV. In 23 rats injected with serum or globulin from rabbits immunized against γ-MSH, no postnephrectomy natriuresis occurred and the kaliuresis was blunted. In hydropenic, mineralocorticoid-treated rats, intravenous infusion of synthetic γ-MSH led to natriuresis and kaliuresis with no change in inulin clearance; pretreatment with rabbit anti-γ-MSH antiserum blocked this effect of peptide infusion. In a final group of rats, synthetic γ-MSH infused directly into one renal artery at a rate of 300 fmol/min caused a prompt increase in UNaV from the infused kidney but not the contralateral kidney. These studies thus indicate that plasma immunoreactive γ-MSH activity is increased after acute unilateral nephrectomy in a manner related to the magnitude of the postnephrectomy natriuresis, that the natriuresis is not observed in rats exposed to antibodies to γ-MSH, and that γ-MSH itself possesses natriuretic properties through a direct renal action. γ-MSH or a related peptide therefore appears to be a critical element in the response to unilateral nephrectomy, functioning as a natriuretic hormone to stimulate the postnephrectomy natriuresis.

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Key Words • natriuretic hormone • sodium excretion • pro-opiomelanocortin • reflex natriuresis • potassium excretion

A CUTE unilateral nephrectomy (AUN) results in an increase in electrolyte excretion from the contralateral, remaining kidney through reflex pathways involving the central nervous system. A normally functioning pituitary gland is required for the reflex natriuresis to occur, and the natriuresis is associated with an increase in the plasma concentration of a peptide or peptides derived from the N-terminal region of the adrenocorticotropic hormone (ACTH)-β-endorphin precursor molecule pro-opiomelanocortin (POMC). A peptide sequence that possesses amino acid homology with α-melanocyte stimulating hormone (MSH) and β-MSH, termed γ-MSH, resides in the N-terminal region of POMC. Since MSH peptides, including γ-MSH, have been shown to possess natriuretic properties, we sought to test if an increase in...
plasma γ-MSH or a related peptide could participate in the natriuretic response to AUN. To do this, we measured immunoreactive γ-MSH-like material after AUN and found it to be increased in a manner related to the magnitude of the postnephrectomy natriuresis. We also prevented the postnephrectomy natriuresis by injecting antibodies to γ-MSH before AUN, suggesting that the increase in immunoreactive γ-MSH material was important in the natriuretic response to AUN. Finally, we found that γ-MSH infused directly into a renal artery produced a unilateral natriuresis, indicating that this peptide possesses direct natriuretic properties.

Materials and Methods

We studied male Sprague-Dawley rats weighing between 240 and 380 g (age, 8–13 weeks; Bantin-Kingman, Fremont, CA, USA). The rats were housed in constant temperature rooms and allowed access to laboratory chow and water until the day of the experiment, when they were brought to the laboratory and anesthetized first with ether and then with an intraperitoneal injection of thiobutabarbital (Inactin), 120 mg/kg. They were prepared for acute experimentation as previously described by our laboratory. Briefly, they breathed spontaneously through a surgically placed tracheostomy and had catheters placed in a femoral vein for infusion of solutions and a femoral artery for blood sampling and monitoring of arterial pressure by means of a Statham P23db pressure transducer (Gould, Oxnard, CA, USA) attached to a Grass Model 7D polygraph (Quincy, MA, USA). Through a midline abdominal incision, the kidneys were exposed and a fine polyethylene catheter inserted into one ureter. A ligature was loosely placed around the pedicle of the opposite kidney. The ureteral catheter was exteriorized, and the abdominal wound was closed. A solution of 3% bovine serum albumin in normal saline was infused during the surgical preparation in a volume of 1.5% body weight to replace surgically induced fluid losses. Following the completion of the operation, this albumin solution was discontinued and replaced with a normal saline solution that was infused at 40 μl/min for the duration of the experiment.

After a 30- to 45-minute period of equilibration, control urine collections were begun. On their completion, the abdominal wound was again opened and surgical nephrectomy was performed by ligating the renal pedicle, using the previously placed ligature, and removing the kidney. In experiments with sham nephrectomy, the ligature was simply removed and the kidney gently manipulated. The abdominal wound was reclosed, and after a 20-minute interval, urine collections were resumed for another 100 minutes. Two general protocols were performed using this format. In the first, the relationship of the postnephrectomy natriuresis to immunoreactive γ-MSH activity was evaluated. In this group of experiments, 10 animals underwent AUN as described, while six underwent a sham procedure. Then, 120 minutes after AUN or sham nephrectomy, 4 ml of blood was quickly withdrawn into chilled Vacutainer tubes containing EDTA and aprotonin and centrifuged at 4°C, and the plasma was decanted into storage tubes and frozen at −70°C until subsequently extracted and analyzed for immunoreactive γ-MSH.

The second type of experiment tested the importance of changes in circulating immunoreactive γ-MSH to the postnephrectomy natriuresis by performing AUN in rats given whole serum or globulin-enriched fractions of serum from rabbits immunized against γ-MSH. Synthetic γ-MSH (γ-MSH, Peninsula Laboratories, Belmont, CA, USA) was conjugated to bovine thyroglobulin by the carbodiimide method. New Zealand white rabbits were immunized by multiple site intradermal injections of 1.5 ml of an emulsion of conjugate (equivalent to 500 μg of ligand) and complete Freund’s adjuvant. Booster injections of conjugate and incomplete Freund’s adjuvant were given at monthly intervals. After 4 months, antiserum became available that formed the basis for the subsequent radioimmunoassay (RIA) developed to measure immunoreactive γ-MSH activity. Antiserum used in the RIA was also used in the experiments described next. Rats were surgically prepared for AUN exactly as described previously, except that, at the conclusion of surgical preparation and before the collection of control urine samples, they were injected with 300 μl of serum from control rabbits or rabbits immunized against γ-MSH. Group 1A consisted of seven rats given serum from a control rabbit, while Group 2A consisted of 13 rats given serum from a rabbit immunized against γ-MSH. To ensure that an adequate amount of antibody had been administered, additional rats were injected with a globulin-enriched fraction of rabbit serum. This fraction was obtained by (NH₄)₂SO₄ precipitation of rabbit serum. The globulin-enriched fraction was dialyzed against normal saline overnight. Then, 300 μl of the resulting solution obtained from control rabbit serum (Group 1B; n = 10) or from immunized rabbit serum (Group 2B; n = 10) was injected intravenously at the conclusion of surgical preparation. Experiments were then performed as just described. These experiments were conducted in a blinded manner, as the experimenter was not aware of the source of the injected serum or globulin until the conclusion of the study.

The effect of γ-MSH infusions was tested in a third group of studies. Animals were prepared as described previously, except that no albumin-saline solution was given during the operation and bladder rather than ureteral urine was collected from a polyethylene catheter surgically placed in the bladder through a small suprapubic incision. The saline maintenance solution contained [¹⁴C]methoxymelin (New England Nuclear, Boston, MA, USA) for measurement of inulin clearance, and each rat received an intramuscular injection of deoxycorticosterone acetate in oil (Organon, West Orange, NJ, USA), 0.5 mg, during surgical preparation. Rats also received 250 μl of either the control rabbit serum or the anti-γ-MSH antiserum at the conclusion of the operation. Normal saline vehicle containing bacitracin, 1 mg/ml, and bovine serum albu-
min, 1 mg/ml, was infused through the venous catheter at 10 \( \mu l/min \). After four 15-minute urine collections had been obtained, 100 \( \mu l \) of vehicle alone or vehicle containing 250 pmol synthetic \( \gamma \)-MSH was injected intravenously. In experiments receiving the peptide, the infusion of vehicle was changed to vehicle containing \( \gamma \)-MSH to deliver 34 pmol/min. We collected urine for four additional periods 30 to 90 minutes after the peptide or vehicle injection. Arterial pressure was monitored throughout; 50 \( \mu l \) of plasma was obtained in glass capillary tubes in alternate periods; and urine and plasma were counted for \( ^{14}C \) activity in a liquid scintillation counter. Four groups of rats were studied in this manner: Group 3A \( (n=5) \) received control rabbit serum and vehicle infusion, Group 3B \( (n=17) \) received control rabbit serum and \( \gamma \)-MSH infusion, Group 3C \( (n=8) \) received anti-\( \gamma \)-MSH antiserum and vehicle infusion, while Group 3D \( (n=8) \) received the antiserum with \( \gamma \)-MSH infusion.

We further tested the natriuretic effect of \( \gamma \)-MSH in a fourth group of 12 anesthetized rats. Through a midline abdominal incision, the left renal pedicle was identified and a 30-gauge curved needle attached to PE-10 tubing inserted into the left renal artery. In these experiments, urine was collected from each kidney through ureteral catheters. To achieve a high basal rate of urinary sodium excretion (\( U_{\text{Na}} \)), the rats received an intravenous infusion of 3\% albumin in saline, amounting to 2\% body weight, during surgical preparation to be comparable to the assay system reported by Lymangrover et al.\(^9\) Normal saline vehicle was infused through the renal artery catheter at a rate of 25 \( \mu l/min \). Urine was collected for three 15-minute periods, following which the renal arterial infusion was changed to the saline vehicle with \( \gamma \)-MSH in a concentration calculated to achieve an infusion rate of 300 fmol/min. Three additional 15-minute urine collections followed, at the conclusion of which the arterial infusion was changed back to the saline vehicle. This sequence was then repeated. In four of these experiments the same procedure was followed, except that denervation of the infused kidney was performed as previously described by us\(^{10}\) by stripping the renal vessel of all visible nerves and coating the pedicle with 2 \( N \) phenol. Blood pressure was monitored by a catheter in the femoral artery throughout the experiment. At the conclusion of the study, the renal arterial line was disconnected from the infusion pump; prompt reflux of arterial blood indicated successful placement of the needle in the renal artery.

**RIA of \( \gamma \)-MSH-Like Material**

Plasma samples were thawed in ice and extracted according to the method of Bennett et al.\(^{11}\) by passing 1.5-ml aliquots twice through a Sep-Pak C\(_{18}\) cartridge (Waters Associates, Milford, MA, USA) that had been primed by wetting with 3 ml of solvent (acetonitrile/water/trifluoroacetic acid [TFA], 80:19.9:0.1 vol/vol) and washed with 10 ml of 0.1\% TFA. After loading, the cartridge was again washed with 10 ml of 0.1\% TFA. Plasma extracts were lyophilized and stored frozen until reconstituted for the assay. The samples were then assayed for immunoreactive \( \gamma \)-MSH-like activity using the RIA established in our laboratory and described elsewhere (E. Wiedemann et al., unpublished data, 1987). Briefly, an antisera raised in a male New Zealand white rabbit against a conjugate of synthetic \( \gamma \)-MSH (Peninsula) with bovine thyroglobulin (Sigma Chemical, St. Louis, MO, USA) was used in a final dilution of 1:100,000. This antisera displays no apparent cross-reactivity with \( \alpha \)-MSH or \( \beta \)-MSH. For the tracer, \( \gamma \)-MSH was labeled with iodine-125 to a specific activity of approximately 150 \( \mu Ci/\mu g \) by a modified lactoperoxidase method. Tracer was added to the assay tubes after a 48-hour preincubation. We precipitated antibody-bound tracer after another 48-hour incubation using goat anti-rabbit gamma globulin suspended in 7\% polyethylene glycol containing 0.7\% nonimmune rabbit serum. After centrifugation, the supernatant was removed by suction and the pellet was counted for \( ^{125}I \) activity in a gamma counter. The sensitivity of the assay was 4 fmol/tube, and half-maximal tracer displacement occurred at 100 fmol/tube. Intra-assay and interassay coefficients of variation at this concentration were 6 and 10\%, respectively. Rat pituitary extracts produced tracer displacement in parallel with the synthetic \( \gamma \)-MSH standard.

**Analytical Methods and Statistical Evaluation**

Urine collections lasted 10 to 15 minutes, and two to five such collections were obtained in the control period and averaged to provide a single control value for each rat. Similarly, after AUN or sham nephrectomy, urine collections were averaged for the 20 to 120 minutes after the procedure. Urine volume was determined gravimetrically, and urine sodium and potassium concentration were determined by flame photometry. Data are presented as the mean ± 1 SD of the average of control and postprocedure values. The paired or unpaired Student’s \( t \) test was used to determine statistical significance when two means were compared; when more than two means were tested, repeated-measures analysis of variance was used, with the Scheffe’s \( t \) test applied to identify the different group when analysis of variance indicated a significant difference to exist. A \( p \) value less than 0.05 was taken to indicate statistical significance.

**Results**

AUN led to a brisk increase in urine flow, \( U_{\text{Na}} \), and urinary potassium excretion (\( U_{\text{K}} \)), in accorance with multiple earlier observations from this and other laboratories (Figure 1).\(^1\)\(^{-5}\)\(^\text{, }\)\(^\text{10}-\text{14}\) \( U_{\text{Na}} \) increased from 1027 ± 447 to 1828 ± 742 nEq/min \(( p<0.005 \) ), while \( U_{\text{K}} \) rose from 1610 ± 412 to 2560 ± 851 nEq/min \(( p<0.005 \) ). Sham nephrectomy had no effect on urinary electrolyte excretion. The plasma concentration of immunoreactive \( \gamma \)-MSH was measured 120 minutes after AUN or sham nephrectomy, and the results are presented in Figure 2. In the sham nephrectomy group, immunoreactive \( \gamma \)-MSH concentration averaged 40 ± 18 fmol/ml, whereas in the group undergoing...
SHAM AUN

Figure 1. Changes in urinary sodium (UNaV) and potassium (UKV) excretion in response to sham nephrectomy or acute unilateral nephrectomy (AUN). No change in cation excretion occurred after the sham procedure, whereas both UNaV and UKV rose significantly after AUN. Lines connect average values from individual experiments during control and for 30 to 120 minutes after the procedure (Experiment); larger symbols represent means ± 1 SE. Units for both UNaV and UKV are nEq/l min.

AUN, the value at the same point in time was doubled at 81 ± 36 fmol/ml; this difference was significant (p < 0.02). A relationship existed between the post-nephrectomy immunoreactive γ-MSH concentration and the increase in UNaV caused by AUN, as shown in Figure 3; the more elevated plasma immunoreactive γ-MSH occurred in those rats with the biggest increase in UNaV after AUN.

To determine if the observed increase in immunoreactive γ-MSH concentration after AUN was functionally important in the natriuretic effect of this maneuver, we performed additional studies in rats treated with either serum or globulin-enriched fractions from control rabbits or rabbits immunized against γ-MSH. The results of pooled data are shown in Table 1 and Figure 4. Analysis of variance indicated that all four groups of studies were comparable during the control period, that is, no differences in urine flow, UNaV, or UKV could be detected as a result of the treatment with serum or globulin fractions. Similarly, no differences could be detected between groups receiving serum or globulin fractions; consequently, the data from Groups 1A and 1B are presented as Group 1 in Table 1, and Groups 2A and 2B as Group 2. AUN caused the expected increase in electrolyte excretion in rats pretreated with control rabbit globulin or serum. In contrast, pretreatment with globulin or serum from the rabbit immunized against γ-MSH resulted in no significant increase in UNaV, while the kaliuresis following AUN, although still present, was significantly reduced compared with Group 1 experiments (see Figure 4). Thus, pretreatment with serum or globulin fractions from a rabbit immunized against γ-MSH abolished the post-nephrectomy natriuresis and reduced the postnephrectomy kaliuresis by approximately 50%.

In a third protocol, synthetic γ-MSH was infused intravenously (see Table 1). These rats received no fluid volume replacement but were injected with deoxycorticosterone acetate during surgical preparation; baseline rates of UNaV and UKV were consequently very low but were not different among the various groups. In Group 3A, which received control rabbit serum, vehicle infusion had no effect on UNaV or UKV in the 30- to 90-minute infusion period compared with the control. In Group 3B, γ-MSH infusion to rats pretreated with control rabbit serum led to significant increases in both UNaV and UKV (p < 0.001 for both) without an increase in glomerular filtration rate or arterial pressure (see Table 1). Group 3C rats received rabbit anti-γ-MSH antiserum; vehicle infusion again caused no significant change in UNaV or UKV. Group 3D rats were also pretreated with anti-γ-MSH anti-
serum and, during the experimental period, received the same γ-MSH infusion as rats in Group 3B. However, in marked contrast to the results in Group 3B, the peptide failed to produce natriuresis or kaliuresis in these rats. The increase in $U_{Na}V$ observed in Group 3B was significantly greater ($p < 0.001$) than the changes seen in the other groups, while the increase in $U_{Na}V$ caused by infusion of the peptide, although highly significant within the group, was not statistically greater than the changes seen in the other groups. These studies therefore indicate that γ-MSH infused intravenously leads to natriuresis and kaliuresis without an increase in glomerular filtration rate or arterial pressure. Furthermore, this effect of the peptide was not observed in rats pretreated with anti-γ-MSH antiserum.

In eight volume-expanded rats with intact renal nerves, γ-MSH was infused directly into the renal artery; the results of these experiments are shown in Figure 5. Baseline rates of $U_{Na}V$ and $U_{K}V$ from both the infused and the contralateral kidney were comparable in these volume-expanded rats, suggesting that the infusion per se did not materially affect solute excretion from that kidney. Changing from the saline vehicle to vehicle containing γ-MSH resulted in a prompt, large increase in $U_{Na}V$ that was progressive during the three 15-minute periods, reaching a peak rate nearly double that seen during the control period by the third period of the infusion. This effect was confined to the infused kidney, as $U_{Na}V$ did not change from the contralateral kidney during the experiment. On resumption of vehicle infusion, $U_{Na}V$ from the infused kidney decreased to the baseline level and increased promptly with reinitiation of γ-MSH infusion, again without effect on $U_{K}V$ from the contralateral kidney. Qualitatively similar but much less marked effects on $U_{K}V$ were also observed (see Figure 5). In four additional experiments, a similar protocol was followed, except that the kidney receiving the arterial infusion of γ-MSH was acutely denervated during surgical preparation. Intra-arterial infusion of γ-MSH increased $U_{Na}V$ from 2587 ± 506 to 4265 ± 1399 nEq/min ($p < 0.05$).

### Table 1. Effect of Anti-γ-MSH Antibodies on Electrolyte Excretion After Acute Unilateral Nephrectomy or During Intravenous Infusion of Synthetic γ-MSH

<table>
<thead>
<tr>
<th>Variable</th>
<th>C (μl/min)</th>
<th>E (μl/min)</th>
<th>C (nEq/min)</th>
<th>E (nEq/min)</th>
<th>C (nEq/min)</th>
<th>E (nEq/min)</th>
<th>GFR* (ml/min)</th>
<th>MAP (mm Hg)</th>
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<tr>
<td>Acute unilateral nephrectomy</td>
<td></td>
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<tr>
<td>Group 1 (n = 17)</td>
<td>8.7 ± 4.8</td>
<td>12.4 ± 5.8</td>
<td>1100 ± 624</td>
<td>2076 ± 1785</td>
<td>1220 ± 362</td>
<td>2183 ± 683</td>
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<td>Group 2 (n = 23)</td>
<td>7.8 ± 3.4</td>
<td>9.8 ± 4.6</td>
<td>1317 ± 829</td>
<td>1564 ± 1002</td>
<td>1463 ± 734</td>
<td>1983 ± 695</td>
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<td>—</td>
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<tr>
<td>Intravenous infusion of synthetic γ-MSH</td>
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<tr>
<td>Group 3A (n = 5)</td>
<td>4.1 ± 0.8</td>
<td>4.8 ± 1.0</td>
<td>349 ± 146</td>
<td>256 ± 93</td>
<td>512 ± 249</td>
<td>575 ± 309</td>
<td>4.1</td>
<td>4.6</td>
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<tr>
<td>Group 3B (n = 17)</td>
<td>5.6 ± 3.7</td>
<td>14.4 ± 10.3</td>
<td>240 ± 121</td>
<td>552 ± 337</td>
<td>367 ± 126</td>
<td>368 ± 174</td>
<td>5.7 ± 0.8</td>
<td>5.1 ± 1.2</td>
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<tr>
<td>Group 3C (n = 8)</td>
<td>4.3 ± 1.2</td>
<td>8.9 ± 6.6</td>
<td>300 ± 199</td>
<td>800 ± 387</td>
<td>384 ± 330</td>
<td>564 ± 353</td>
<td>5.1 ± 1.4</td>
<td>4.9 ± 1.1</td>
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<tr>
<td>Group 3D (n = 8)</td>
<td>4.6 ± 1.3</td>
<td>6.1 ± 1.5</td>
<td>279 ± 141</td>
<td>323 ± 193</td>
<td>572 ± 417</td>
<td>599 ± 312</td>
<td>5.7 ± 1.8</td>
<td>5.2 ± 1.1</td>
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</table>

Values are means ± 1 SD of multiple measurements during control (C) and after nephrectomy (Groups 1 and 2) or peptide or vehicle infusion (Group 3). $V = \text{urine flow}; E = \text{experiment}; U_{Na}V = \text{urinary sodium excretion}; U_{K}V = \text{urinary potassium excretion}; GFR = \text{glomerular filtration rate}; MAP = \text{mean arterial pressure}.$

Group 1 = rats pretreated with control rabbit serum or globulin; Group 2 = rats pretreated with rabbit anti-γ-MSH antiserum or globulin; Group 3A = control rabbit serum, vehicle infusion; Group 3B = control rabbit serum, γ-MSH infusion; Group 3C = anti-γ-MSH antiserum, vehicle infusion; Group 3D = anti-γ-MSH antiserum, γ-MSH infusion.

*GFR was measured in two rats in Group 3A, in five rats in Group 3B, in six rats in Group 3C, and in five rats in Group 3D.

$\ddagger p < 0.05$, $\ddagger p < 0.01$, $\ddagger p < 0.02$, $\ddagger p < 0.001,$ compared with control values (by Student's $t$ test for paired data).
The change in urinary sodium (\( U_{NaV} \)) and potassium (\( U_{KV} \)) excretion after acute unilateral nephrectomy (AUN) in 17 rats injected with control rabbit serum or globulin (●) compared with 23 rats injected with serum or globulin from rabbits immunized against γ-MSH (▲). Repeated-measures analysis of variance indicated that the groups were indistinguishable with respect to baseline rates of cation excretion. AUN caused significant increases in both \( U_{NaV} \) and \( U_{KV} \) in rats treated with control rabbit serum or globulin. In rats exposed to antibodies to γ-MSH, the postnephrectomy natriuresis did not occur and the kaliuresis, although still significant within the group, was blunted compared with the response in the control rats.

Discussion

Previous studies on the postnephrectomy natriuresis have indicated that it takes place without discernible changes in the volume or composition of the blood, major alterations in renal hemodynamics, or changes in mineralocorticoid or vasopressin activity, suggesting that other, presumably neurohumoral mechanisms must be involved in the natriuresis. In this regard, denervation of either the kidney to be removed or the kidney remaining in place significantly altered the response to AUN by abolishing the natriuresis and reducing the kaliuresis. These studies suggested that both afferent and efferent renal nerves could be involved or that something about the process of renal denervation altered the state of the kidney so that it could no longer respond to other natriuretic stimuli activated by AUN. The potential for a humoral mediator of the postnephrectomy natriuresis received support from observations that an intact pituitary gland was required for increased \( U_{NaV} \) to occur after AUN and by the demonstration that a relationship existed between the increased concentration of a peptide or peptides derived from the N-terminal fragment (NTF) of POMC and the postnephrectomy natriuresis. Maneuvers that prevented the rise in plasma concentration of NTF, such as hypophysectomy, dexamethasone pretreatment, ventriculocisternal perfusion with the opiate receptor antagonist naloxone, or production of a metabolic lesion in the arcuate nucleus of the hypothalamus and the anterior lobe of the pituitary, also prevented the postnephrectomy natriuresis from taking place.

The present studies provide further strong support for a humoral mechanism in postnephrectomy natriuresis. The γ-MSH peptide sequence resides on the N-terminal portion of POMC immediately adjacent to the sequence NTF-(32-49) against which the antibody used in our previous studies was raised. MSH peptides, including α-MSH, β-MSH, and γ-MSH and ACTH(4-10), all possess amino acid homology, and all produce natriuresis when infused into experimental animals. We therefore sought to test whether immunoreactive γ-MSH-like material could be secreted after AUN and participate in the postnephrectomy natriuresis.
Our results clearly indicate that the plasma concentration of immunoreactive γ-MSH increases after AUN in a manner that relates to the magnitude of the postnephrectomy natriuresis. Levels of immunoreactive γ-MSH after sham nephrectomy, presumably reflecting the baseline steady state level, were similar in magnitude to those we have observed for NTF-(32–49). In addition, levels observed in rats undergoing AUN were elevated over those of the sham-operated group by an amount also quite similar to that observed for the increase in NTF-(32–49) that we have reported. These considerations must be tempered in view of the fact that technical considerations prevented a study design in which each animal served as its own control. The volume of blood required for determination of immunoreactive γ-MSH concentration is sufficiently large that we were concerned that hemodynamic alterations induced by the sampling would interfere with the response to AUN. Consequently, our design was unpaired to avoid this problem. Despite this limitation, it seems quite clear that increased immunoreactive γ-MSH occurs regularly after AUN. Moreover, this increase in immunoreactive γ-MSH appears critical to the excratory response of the remaining kidney, since injection of serum or globulin fractions from rabbits immunized against γ-MSH markedly altered the response. The rabbit serum and the globulin fraction derived from it contained antibodies to γ-MSH; their administration during surgical preparation did not produce any effect to alter basal rates of UnV and UxV. However, no increase in UnV occurred after AUN, and the increase in UxV was reduced in magnitude about 50% compared with that observed in rats receiving control rabbit serum or globulin. That this effect of the anti-γ-MSH antisera to block the postnephrectomy natriuresis is related to binding and inactivation of native γ-MSH peptide(s) is further supported by our finding that infused synthetic γ-MSH produced natriuresis in rats exposed to control rabbit serum, but was ineffective in rats treated with the antisera. We interpret these studies to indicate that γ-MSH, or a peptide of similar primary structure, is secreted after AUN and is a key participant in the natriuretic response of the remaining kidney. When this newly secreted peptide (or peptides) is bound by exogenously administered antibody, it cannot act to trigger the natriuretic response.

Our data indicate that γ-MSH itself is natriuretic when infused intravenously or directly into a renal artery. Lymangrover et al. administered bolus injections of γ-MSH into a peripheral vein and observed a modest increase in UnV over the subsequent 2 hours. Using continuous infusions of synthetic γ-MSH, we have also observed significant increases in UnV and UxV, although the amount infused in our studies was greater than the single doses used by Lymangrover et al. Our rats were hydropenic, with very low basal UnV and a modest, albeit highly significant, increase during the infusion, whereas the rats studied by Lymangrover et al. were volume-expanded, which may have rendered them more responsive to the peptide. In support of this idea, we have observed that volume expansion of anesthetized rats amplifies the natriuretic response to AUN. The present studies indicate that the natriuresis resulting from γ-MSH infusion occurs without changes in glomerular filtration rate or mean arterial pressure and in the presence of exogenously administered mineralocorticoid, suggesting a change in tubular reabsorption not mediated by these factors.

This approach, however, cannot identify whether the peptide stimulated UnV through a direct action on the kidneys or indirectly through other neurohumoral mechanisms. We therefore infused it directly into a renal artery to produce natriuresis. One can calculate in our studies the approximate level of γ-MSH obtained in renal artery plasma: If one assumes that renal plasma flow to the infused kidney was roughly 4 ml/min, then the infusion rate of 300 fmol/min should have increased plasma γ-MSH concentration by 75 fmol/ml. If resting plasma concentration was about 40 fmol/ml (see Figure 2), then the plasma concentration distal to the infusion needle should have been in the range of 115 fmol/ml. This concentration is within the range we observed in postnephrectomy plasma (see Figures 2 and 3) and thus is physiologically relevant. Moreover, the rapidity of onset, as well as the magnitude, of the natriuresis suggests that the peptide was acting directly on the kidney. The finding that UnV from the contra-lateral kidney was not markedly altered by the intrarterial γ-MSH infusion supports the contention that the peptide-induced natriuresis occurred through a direct action on the infused kidney. It also suggests that the systemic concentration of γ-MSH was not increased sufficiently to achieve a level capable of inducing natriuresis from the contralateral kidney; by inference, it would indicate a substantial degree of catabolism or inactivation of the infused peptide by the kidney receiving the infusion. These studies do not, however, rule out an additional, indirect action of γ-MSH to influence UnV: the protracted natriuresis observed by Lymangrover et al. following intravenous bolus injections contrasts markedly with the falloff in UnV on cessation of peptide infusion into the renal artery in our studies (see Figure 5), raising the possibility that the peptide may have a systemic as well as direct renal action. Hypertensive actions of MSH-related peptides have been suggested to result from central stimulation of sympathetic nervous outflow. This approach, however, cannot identify whether the physiological γ-MSH-like peptide responsible for the postnephrectomy natriuresis. There is no evidence as yet that γ-MSH circulates as such in the rat; rather, the predominant form may be NTF-(51–74), which is γ-MSH with an N-terminal lysine. As described earlier, we have previously demonstrated a positive correlation between the natriuresis following AUN and the plasma concentration of NTF-like immunoreactive material using an antibody that recognizes an epitope within the sequence NTF-(32–49). At least five peptides containing this sequence, and ranging in apparent size from 2 to 23 kDa, are detectable both in the pituitary and plasma of the rat, and similar heterogeneity exists.
with regard to γ-MSH-like peptides (E. Wiedemann et al., unpublished observations, 1987). It is possible that a larger peptide containing both NTF-(32–49) and γ-MSH could be the physiological peptide. Alternatively, the similar increases in plasma NTF-(32–49)-like and immunoreactive γ-MSH-like material after AUN could simply be due to cosecretion of peptides containing the respective sequences. At any rate, the active peptide must contain the γ-MSH sequence, since in the present study antisera raised against this sequence prevented the natriuretic response. Both α-MSH and β-MSH possess natriuretic activity\(^6\),\(^8\),\(^9\) and share sequence homology with γ-MSH. However, these other POMC-derived peptides play no major role in the natriuretic response to AUN, since our antisera showed virtually no cross-reactivity with α-MSH or β-MSH.

The results of the experiments with immunized rabbit serum or globulin are strikingly similar to those previously observed on the effects of renal denervation on postnephrectomy natriuresis.\(^3\) In this earlier study, denervation of either the kidney to be removed or the kidney remaining in situ blocked the natriuretic response to AUN but reduced only by about 50% the kaliuresis.\(^10\) The results of the present studies are virtually identical and raise the possibility that immunoreactive γ-MSH could participate in postnephrectomy natriuresis through a pathway involving the renal nerves. This possibility seems unlikely in view of our observation that γ-MSH infused into the renal artery of acutely denervated kidneys produces natriuresis much as in innervated kidneys. Alternatively, renal denervation could impair the secretion of POMC-derived peptides after AUN, as renal afferent nerves project to the central nervous system\(^24\),\(^25\) and have been shown to participate in renorenal reflexes\(^26\),\(^27\) and cardiovascular regulation.\(^28\) Moreover, renal afferent nerve stimulation activates neurons controlling vasopressin secretion in the supraoptic nucleus.\(^29\) It is thus conceivable that the deafferentation resulting from acute renal denervation in some way interferes with increased secretion of immunoreactive γ-MSH after AUN. In support of this possibility, we have observed in preliminary studies that plasma immunoreactive γ-MSH concentration is reduced in rats with acute unilateral renal denervation and does not increase after AUN. Thus, renal afferent nerves may exert a permissive effect on both tonic and stimulated γ-MSH secretion. Further studies are necessary to establish these findings.

The current results lead to the conclusion that γ-MSH, or a peptide containing the γ-MSH sequence, functions as a natriuretic hormone involved in reflex natriuresis stimulated by AUN. Although speculative, it seems reasonable to consider that AUN triggers release of γ-MSH, presumably from the anterior pituitary, into the circulation, where it reaches the remaining kidney and initiates natriuresis through a direct action to inhibit tubular reabsorption. Further studies examining this system should extend our understanding of the factors involved in the regulation of extracellular fluid volume.

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