Acute Unilateral Nephrectomy Elicits a Specific Increase in Plasma of Peptides Derived From the N-Terminal Region of Proopiomelanocortin

Changbin Qiu, Jean-Pierre Valentin, Xiao-Wen Chen, Eckehart Wiedemann, and Michael H. Humphreys

ABSTRACT

Acute unilateral nephrectomy (AUN) causes natriuresis from the contralateral, remaining kidney through neurohumoral reflex pathways (1, 2). We have shown that this natriuresis is accompanied by an increase in the plasma concentration of peptides derived from the N-terminal region of the ACTH precursor peptide proopiomelanocortin (POMC). To determine the specificity of these humoral changes, the concentrations in plasma of ACTH and two peptides arising from the N-terminal fragment (NTF) of POMC, NTF32-49 and γ-melanocyte-stimulating hormone (γ-MSH), and of another natriuretic peptide, atrial natriuretic peptide (ANP), were measured by RIA with highly specific antisera to these epitopes.

Group I experiments followed the course of sodium excretion (URaV) for 120 min after AUN or sham nephrectomy. URaV more than doubled within 60 min of AUN, and this natriuresis was maintained for the remainder of the experiment, whereas URaV in sham rats did not change. There was no difference in plasma immunoreactive (Ir) ACTH or Ir-ANP concentrations between sham and AUN rats 120 min after the procedure, but plasma Ir-NTF concentration was double in AUN rats compared with sham (P < 0.03).

In Group II experiments, animals were killed 30, 60, 90, or 120 min after AUN and the urinary response related to peptide concentrations in plasma. URaV rose rapidly after AUN, reaching a maximum value within 45 min that again was double the control value and remained stable for the duration of the experiment, up to 120 min after AUN. There was no significant change in Ir-ACTH or Ir-ANP at any point after AUN compared with values in sham AUN rats. However, plasma concentrations of both Ir-NTF and Ir-γ-MSH were elevated 30 min after AUN and reached values at 120 min that were again double the values in sham rats (P < 0.05 for both). These results indicate that AUN exerts a selective and specific effect to increase the plasma concentration of peptides derived from the N-terminal region of POMC without increasing ACTH concentration, in all likelihood reflective of increased pituitary secretion of these N-terminal peptides. The data support a role for them, most likely γ-MSH, in mediating the natriuretic response. These results on the other hand do not suggest a role for ANP in this response.

Key Words: Natriuresis, hormone, adrenocorticotropic hormone, γ-melanocyte-stimulating hormone, atrial natriuretic peptide

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sions of synthetic γ-MSH were natriuretic when given iv or directly into a renal artery (3).

Recently, this formulation has been questioned by experiments that strongly suggest a role of atrial natriuretic peptide (ANP) as mediator of the natriuresis after AUN. Plasma immunoreactive (ir) ANP concentration increased after AUN, accompanied by an increase in urinary cGMP excretion as well as natriuresis; natriuresis after AUN did not occur in animals that had undergone right atrial appendectomy, a maneuver that blocked the postnephrectomy increase in ir-ANP concentration (6). These results obscured the possible role of POMC-derived peptides in the response to AUN. One means to reconcile these divergent findings is to consider the possibility that the changes in plasma concentrations of peptides arising from cleavage of POMC reflect a generalized stress response. Because AUN could represent a stressful stimulus to the animal, a number of POMC peptides could be cosecreted along with ACTH as part of this stress response, yet have little bearing on the natriuresis. This possibility anticipates that ACTH concentration in plasma would increase in parallel with N-terminal POMC peptides after AUN.

A separate question concerns the temporal relationship between the increase in peptide concentrations in plasma and the natriuresis that occurs after AUN. A hormonal mediator of the natriuresis should have its concentration in plasma increase before or coordinately with the onset of the natriuresis. In all of our prior studies, peptide concentration was measured 90 to 120 min after AUN, at a time when the natriuresis was fully developed (2–5). Therefore, we evaluated the relationship among changes in plasma concentrations of ACTH and peptides derived from the N-terminal region of POMC after AUN and determined the temporal relationship between these changes and the postnephrectomy natriuresis. We also measured the plasma concentration of ir-ANP at various intervals after AUN to assess its possible role as a peptide mediator of the natriuresis. The results of our study suggest that the secretion of POMC-derived peptides is differentially regulated after AUN, with a selective increase in N-terminal peptides that is temporally related to the onset of the natriuresis but no change in ACTH release. We did not observe an increase in ir-ANP concentration at any point after AUN. Our data are therefore consistent with a role for these N-terminal peptides, most likely γ-MSH, in mediating the postnephrectomy natriuresis.

METHODS

We studied male Sprague-Dawley rats, weighing between 280 and 340 g, that were allowed free access to standard rat chow and tap water until the morning of the experiment, at which time they were brought to the research laboratory and anesthetized with Inactin® (Andrew Lockwood Associates, Sturtevant, WI) 120 mg/kg ip. They were placed on a heated table to maintain a rectal temperature of 37 ± 0.5°C. Surgical preparation involved placement of a tracheostomy tube and fine polyethylene catheters in the femoral artery for blood sampling and continuous recording of arterial blood pressure by means of a Statham P23ild transducer (Gould Instruments, Oxnard, CA) attached to a polygraph (Model 7; Grass Instruments, Quincy, MA). Through a small, supra-pubic incision, a catheter was placed in the urinary bladder for collection of urine. The right kidney was exposed by a dorsal approach. After completion of surgery, the dorsal wound was closed with clips.

During the surgical preparation, rats received an iv infusion of 5% BSA in saline in an amount equal to 0.5 to 1.0% body wt. At the completion of surgery, this infusion was changed to normal saline at a rate of 40 μL/min, which was maintained for the duration of the experiment. In some experiments, this infusion contained meglumine iothalamate (Conray 60; Mallinkrodt, St. Louis, MO) to determine the renal clearance of iothalamate as a measure of GFR. After a 60-min period of recovery, three control urine collections of 15- to 20-min duration were obtained in preweighed vials, after which the dorsal wound was reopened and the right kidney was either removed (AUN) or gently manipulated and left in place (sham AUN). The wound was reclosed, and urine collections were resumed. Experiments were conducted according to two different protocols. The initial set of experiments (Group I) was carried out in 20 animals (10 AUN and 10 sham AUN) in which we examined the effect of AUN on electrolyte excretion, iothalamate clearance, and plasma concentrations of ANP, ACTH, and immunoreactive material measured with an antiserum directed against the amino acid sequence 32 to 49 of the N-terminal fragment of the POMC molecule (ir-NTF) (7). After control urine collections, we carried out AUN or sham AUN; 30 min later, urine collection was resumed and six additional periods were obtained. Arterial blood samples (100 μL) were taken at the midpoints of alternate periods for measurement of hematocrit and iothalamate concentration; animals were killed by decapitation 120 min after AUN, and trunk blood (5 mL) was collected as described below. This sample was used for the subsequent measurement of plasma peptide concentrations by RIA. The other set of experiments (Group II) evaluated the time course of changes in electrolyte excretion and plasma POMC-derived peptide concentrations after AUN. Ten min after AUN, urine collection was resumed. The experiment continued until 30, 60, 90, or 120 min after AUN in different groups (Groups IIb through IIe, respectively, N = 8, 9, 8, and 10); at this time, 5 mL of trunk blood was obtained after the rats were decapitated in a chilled Vacutainer® tube containing EDTA and 500 KIU of appro
tinin (Sigma Chemical Co., St. Louis, MO) for the subsequent determination of the concentrations of ANP, ACTH, and peptides derived from the N-terminal region of POMC, as measured by two RIA recognizing ir-N-terminal fragment (ir-NTF) and ir-γ-MSH. Group IIa consisted of 12 rats undergoing sham nephrectomy that were killed at 30 (three rats), 60 (three rats), 90 (three rats), and 120 (three rats) min after the sham procedure. All samples for peptide hormone assay were immediately centrifuged at 4°C for 10 min at 4,000 rpm, the plasma was decanted, and aliquots were quickly stored at -70°C until the assay was performed.

We measured by RIA the concentrations of the POMC-derived peptides and ANP in extracted plasma. For ACTH, ir-γ-MSH, and ir-NTF assays, the plasma samples were thawed in the cold and extracted through SepPak C18 cartridges (Waters Co., Milford, MA) as previously described (2,3,5). The cartridge eluates were lyophilized and stored at -70°C until assayed, at which time they were reconstituted in assay buffer and centrifuged, and the supernatants were subjected to RIA with specific antisera. For ACTH, we used a commercial antiserum and [125I]-ACTH purchased from Peninsula Laboratories (San Carlos, CA). Ir-γ-MSH was measured with a highly specific rabbit antiserum raised against synthetic human γ2-MSH and [125I]-γ-MSH obtained by iodination of synthetic γ2-MSH (Peninsula Laboratories) as previously described (2,3). The peptide sequence immediately adjacent to the N-terminal end of γ-MSH on the POMC molecule is termed NTF32-49; it is recognized by a rabbit antiserum raised against this epitope. Results of assays with this antiserum and [125I]NTF32-49 yielded the concentration of ir-NTF. ANP concentration was determined with a commercial kit purchased from Peninsula Laboratories; the assay was carried out according to the manufacturer's instructions. The characteristics of the ir-γ-MSH and ir-NTF assays have been described previously (2,3,5).

Because of the large volume of plasma required for these measurements, only one blood sample could be obtained from each rat, precluding paired comparison with each rat. In each group of experiments, all measurements of a given peptide were carried out in the same assay to minimize interassay variation. Loss of samples during processing and assay resulted in fewer peptide measurements than animals studied in most of the groups; however, no group had less than seven measurements of any given peptide.

In all experiments, urine flow rate was determined gravimetrically and urine sodium and potassium concentrations were measured by flame photometry. Lanthamate concentration in plasma and urine was measured by fluorescence excitation (8); these values and urine flow rate were used to calculate urinary clearance as a marker of GFR. Control values for all renal variables were expressed for a single kidney by halving the total kidney function. We felt this to be a valid approach because previous investigations from our laboratory in which function of each kidney was measured have demonstrated no difference between the two kidneys (2,9,10). Data are expressed as mean ± 1 SE. We used a paired t test to assess the significance of differences within a group; because measurements of some peptides were not normally distributed, we used the Kruskal Wallis statistic for comparisons among the groups. A P value less than 0.05 was taken as the criterion for statistical significance.
nephrectomy as half of total GFR, occurred after either AUN or sham AUN. One hundred twenty minutes after AUN, the concentration of ir-NTF was double the value seen in the sham-operated animals, a statistically significant elevation \( (P < 0.05) \) that duplicated results we reported previously (4,5). However, no significant changes in plasma ir-ACTH or ir-ANP concentration occurred between sham-operated animals and rats undergoing AUN. These results consequently suggested that the plasma concentrations of POMC-derived peptides may be differentially regulated after AUN but offered no evidence of any change in plasma ANP concentration as a result of AUN.

To explore these issues further and to establish the temporal profile of changes in plasma peptide concentrations, we carried out the second set of studies in which the experiments were ended 30, 60, 90, and 120 min after AUN. The results are summarized in Figure 2, which shows the pattern of the increase in \( U_{NaV} \) after AUN in these four groups. All of these groups had a control rate of \( U_{NaV} \) that was similar, and in each group, \( U_{NaV} \) increased after AUN to a comparable extent and followed a similar time course. Group IIa, studied for 120 min after AUN, resembled Group Ib in its response (Figure 1). These results thus indicated that these experiments were suitable to evaluate the hormonal response to AUN.

The results of measurements of the three POMC-derived peptide concentrations, as well as plasma ir-ANP concentration, in these experiments are shown in Figure 3. Because results in Group IIa animals were similar at every time point after sham AUN, they have been pooled for analysis. The value of ACTH in animals undergoing sham nephrectomy (Group IIa) averaged 87.4 \( \pm \) 7.8 fmol/mL and was statistically unchanged at any time point after AUN (Groups IIb through IIe), thus extending the observation shown earlier in Group I experiments on the lack of any increase in ir-ACTH levels 120 min after AUN. On the other hand, a different pattern emerged with respect to the plasma concentrations of two other peptides, derived from the N-terminal region of POMC. ir-NTF concentration rose immediately after AUN; the values 30, 60, and 120 min after AUN were all significantly elevated over the sham value. The results at 90 min, although elevated, did not reach statistical significance \( (P = 0.085) \). The concentration 120 min post-AUN was nearly twice as much, as was also seen in Group I. As with ir-NTF, ir-\( \gamma \)-MSH concentration increased immediately after AUN, and values 30, 90, and 120 min after AUN were all significantly higher than those of sham controls. There was no significant change in ir-ANP at any time point after AUN compared with that in sham-operated animals.

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### Table 1. Effect of AUN or sham nephrectomy on iothalamate clearance and the concentration of plasma peptides

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham AUN (N = 10)</th>
<th>AUN (N = 10)</th>
<th>Co (ml/min)</th>
<th>MAP (mm Hg)</th>
<th>Hct</th>
<th>ir-ACTH (fmol/mL)</th>
<th>ir-NTF (fmol/mL)</th>
<th>ir-ANP (fmol/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.13 ( \pm ) 0.09</td>
<td>1.03 ( \pm ) 0.05</td>
<td>115 ( \pm ) 5</td>
<td>124 ( \pm ) 5</td>
<td>45.8 ( \pm ) 0.3</td>
<td>45.2 ( \pm ) 0.2</td>
<td>21.0 ( \pm ) 3.9</td>
<td>21.1 ( \pm ) 4.5</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>-0.04b</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Group IIa</td>
<td>1.16 ( \pm ) 0.11</td>
<td>1.31 ( \pm ) 0.09</td>
<td>122 ( \pm ) 4</td>
<td>126 ( \pm ) 4</td>
<td>45.8 ( \pm ) 0.3</td>
<td>45.6 ( \pm ) 0.3</td>
<td>114.2 ( \pm ) 8</td>
<td>84.7 ( \pm ) 8.1</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

\( ^a \) Data are means \( \pm \) SE of average values of iothalamate clearance (\( C_o \)), mean arterial pressure (MAP), and hematocrit (Hct) during control (Con) and after sham AUN or AUN (Exp). Plasma peptide concentrations were measured 120 min after sham AUN or AUN. NS, not significant. Exp versus Con.

\( ^b \) Exp greater than Con by paired t test.

\( ^c \) AUN value greater than sham, \( P < 0.05 \).
These results confirm and extend the result shown earlier in the Group I experiments on the lack of any increase in ir-ANP level 120 min after AUN and suggest a temporally appropriate relationship between plasma concentrations of N-terminal peptides and the postnephrectomy natriuresis.

The likelihood of a specific effect of AUN on plasma concentrations of N-terminal peptides was strengthened when data from all Group II experiments in which measurements of both peptides were available were compared (Figure 4). A correlation between the plasma concentrations of ir-γ-MSH and ir-NTF occurred, although no such correlation could be demonstrated between ir-NTF and ir-ACTH or between ir-γ-MSH and ir-ACTH (data not shown).

DISCUSSION

The results of these experiments provide evidence that AUN exerts a specific and selective effect on the plasma concentration of the peptide hormones assayed. In view of the evidence linking the natriuretic response after AUN to increases in circulating levels of peptides originating in the N-terminal region of POMC (2–5), it became important to determine whether these changes were specific or merely reflected a nonspecific discharge of POMC peptides in response to the stress of the nephrectomy procedure. The results show clearly that AUN exerts trivial effects on plasma ir-ACTH concentration for up to 120 min after the maneuver, compared with that in sham-operated animals. We conclude from these data that any stress to these anesthetized animals caused by the nephrectomy is not reflected by an increase in ir-ACTH concentration over the time course of our experiments. On the other hand, our data suggest a different pattern after AUN for the plasma concentration of immunoreactive material derived from the N-terminal region of POMC. The concentrations of both γ-MSH–like and NTF_{32-49}-like peptides.
immunoreactivity were significantly elevated over values from sham control animals at the earliest time point 30 min after AUN, and in general remained elevated at the subsequent measurements, including 120 min after AUN, the interval we had chosen in our initial studies (Figure 1 and references 2–5). This could not be born out statistically at 90 min for ir-NTF and 60 min for ir-γ-MSH, although mean values in the groups at these times were equivalently elevated (Figure 3). In part this is because the changes in peptide concentrations after AUN are relatively small, increasing only by a factor of two or less (our results; 2–5). Moreover, the study design precluded any comparison of the postnephrectomy value to the control value in the same rat. Such a design could not be used because of the large volume of plasma required to perform the various peptide RIA. In earlier studies measuring only the changes in ir-NTF concentration, the smaller volume of plasma permitted a paired design that uniformly demonstrated an increase in the plasma concentration of this peptide after AUN over the control value in the same animal (4,5). Thus, it seems possible that the mean values of ir-NTF and ir-γ-MSH at 90 and 60 min, respectively, could also reflect increases similar to those seen at the other time points. In any case, the data overall indicate that the plasma concentrations of these N-terminal peptides are elevated shortly after AUN in a manner consistent with their postulated involvement in the natriuretic response.

Understanding of the processing and secretion of POMC-derived peptides has focused chiefly on ACTH, regarded as the product of the most biologic significance from this precursor. A prevailing viewpoint has been that, in the anterior lobe, the chief products of POMC processing are ACTH and β-lipotropin, whereas in the neurointermediate lobe, the precursor is cleaved into the lower-molecular-weight peptides α-MSH, corticotropin-like intermediate peptide, and β-endorphin (7,11). Although the highly conserved nature of the amino acid sequences in N-terminal POMC has been recognized, relatively little attention has been paid to their regulation and secretion. In part, this stems from the lack of a clearly defined role for such peptide(s), although some evidence suggests that they may be involved in the regulation of aldosterone secretion (12,13). The results of our experiments clearly indicate that, insofar as changes in plasma concentration of these POMC-derived peptides are due to changes in their secretion from the pituitary, N-terminal peptides are differentially regulated compared with ACTH. This conclusion was hinted at by an earlier study in which we demonstrated an increase in plasma ir-NTF concentration after AUN without change in ir-β-endorphin, a peptide sequence at the C-terminus of POMC (5). Although a decrease in the metabolic clearance of these peptides resulting from the unilateral nephrectomy could have produced a similar increase in their plasma concentrations, there seems to be little reason to entertain such a possibility: other peptides of similar size, namely ACTH, β-endorphin (5), and ANP, were not affected by AUN, and AUN by itself does not result in an increase in the plasma concentration of ir-NTF or ir-γ-MSH unless also accompanied by natriuresis (2,4,5).

Recent data indicate that AUN may elicit an increase in plasma ir-ANP concentration, which in turn mediates the natriuresis (6). Although the data in support of this formulation appeared strong, we were unable, with a different antiserum, to measure any change in ir-ANP concentration for up to 2 h after AUN. The stimulus for any increase in ANP secretion after AUN is unclear: right atrial pressure, regarded as the major stimulus to ANP secretion (14), actually fell after AUN (6), leaving unanswered the mechanism by which the signal for ANP release reached the heart. In addition, renal denervation, known to prevent the postnephrectomy natriuresis (2,10), has not been linked to any impairment in ANP secretion or action on the kidneys. Also, AUN leads to natriuresis, in part, through a decrease in reabsorption in the proximal tubule (15–17), a nephron segment in which involvement of ANP in the regulation of reabsorption is still unclear (14). However, it is still possible that these two peptides may be linked after AUN despite our inability to measure any increase in ir-ANP concentration in plasma. A hypothalamic/pitui-
tary factor has been postulated to be necessary for increased ANP secretion in response to volume expansion (18, 19), and specific binding of γ-MSH peptides has been demonstrated in cardiac tissue (20). We have recently been able to show that iv infusion of synthetic γ-MSH causes a threefold increase in plasma ir-ANP concentration in association with natriuresis and an increase in urinary cGMP excretion (21). Recent studies have also indicated that amino-terminal peptides of proANP also possess natriuretic activity (22) and inhibit sodium transport–dependent oxygen consumption in inner medullary collecting duct cells (23). This latter effect of ANP31–67 was not accompanied by an increase in cGMP accumulation but was inhibited by ibuprofen (23), suggesting that it may have a different cellular mechanism of action than ANP99–126. The role of such peptide(s) in the postnephrectomy natriuresis is unknown, but they provide a number of pathways by which the γ-MSH system may interact with proANP peptides to participate in the regulation of UNaV.

Our data provide further evidence in support of a role of one or more N-terminal peptides from POMC in the postnephrectomy natriuresis. Previous studies from our laboratory have shown that all circumstances in which AUN leads to natriuresis from the contralateral kidney are accompanied by an increase in the plasma concentration of either ir-NTF or ir-γ-MSH, whereas maneuvers that block the natriuretic response also prevent the increase in concentration of these peptides (2–5). This study has shown that the peptides measured by the two assays are coordinately increased in plasma after AUN. We have presented evidence that the γ-MSH sequence itself likely causes the natriuresis: antiserum to γ-MSH blocks the natriuresis when injected into the animal before AUN (3), and infusions of the peptide are natriuretic when administered iv (3, 21, 24) or directly into a renal artery (3, 21). However, the nephron locus at which γ-MSH inhibits reabsorption is as yet undetermined, and neither the precise structure nor the size of the N-terminal natriuretic peptide in circulation is known. By gel exclusion chromatography under dissociating conditions and by HPLC, considerable size heterogeneity of ir-NTF has been demonstrated in extracts of both rat plasma and rat putitary (25). This is also true for ir-γ-MSH (E. Wiedemann, unpublished observations). It is possible that the circulating hormone is a peptide containing both the NTF32–49 epitope and the NTF51–62 (i.e., γ-MSH) epitope, although the whole NTF, i.e., POMC1–76, in the rat is not natriuretic (E. Wiedemann, unpublished observations). Alternatively, more than one natriuretic N-terminal peptide may exist in plasma. Further work will be necessary to identify the precise nature of the POMC peptide most closely associated with the postnephrectomy natriuresis.

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