Prevention of reflex natriuresis after acute unilateral nephrectomy by melanocortin receptor antagonists

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Prevention of reflex natriuresis after acute unilateral nephrectomy by melanocortin receptor antagonists. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R931–R938, 1998.—γ-Melanocyte-stimulating hormone (γ-MSH), atrial natriuretic peptide (ANP), and oxytocin have been identified as candidate hormonal mediators of the reflex natriuresis that follows acute unilateral nephrectomy (AUN). Pharmacological characterization of the third melanocortin receptor (MC3-R) indicates that it uniquely responds to physiological concentrations of γ-MSH. We tested the roles of γ-MSH, ANP, and oxytocin in the postnephrectomy natriuresis by carrying out AUN during continuous intrarenal infusion of specific antagonists for their cognate receptors. In anesthetized Sprague-Dawley rats, urinary sodium excretion (UNaV) increased from 0.34 ± 0.04 to 1.12 ± 0.11 µeq/min 90 min after AUN (P < 0.001). No change in UNaV occurred in rats undergoing a sham AUN procedure. Plasma immunoreactive γ-MSH concentration was 53 ± 8 fmol/ml after sham AUN but 112 ± 17 fmol/ml after AUN (P < 0.01); SHU-9119 and SHU-9005 are substituted derivatives of α-MSH with potent antagonist activity at the MC3-R in vitro. Infusion of these compounds at 5 pmol/min completely blocked the natriuretic response to AUN despite a similar elevation in plasma γ-MSH (111 ± 12 vs. 49 ± 8 fmol/ml in sham rats, P < 0.01). Intrarenal infusion of the ANP receptor antagonist A-71915 (5 pmol/min) or the oxytocin receptor antagonist (d(CH2)51, Tyr(Me)2, Orn8) vasotocin (10 pmol/min) effectively inhibited the natriuresis induced by intravenous infusion of ANP or oxytocin (each at 1 pmol/min), respectively, but did not block the natriuresis after AUN. Plasma immunoreactivity of these peptides was not increased after AUN. These results indicate that reflex natriuresis after AUN is accompanied by an increase in plasma γ-MSH but not ANP or oxytocin concentration and is prevented by intrarenal infusion of receptor antagonists with selectivity for MC3-R. The data indicate that γ-MSH or a closely related peptide mediates postnephrectomy natriuresis and provide further support for the possibility that γ-MSH may play a wider role in sodium homeostasis.

ACUTE UNILATERAL NEPHRECTOMY (AUN) elicits a prompt increase in sodium excretion (UNaV) from the contralateral kidney through a neurohormonal reflex (2, 12, 13, 17–21). Some elements in this natriuresis have been described (13), although the effector mechanism has not been definitively established. Three peptide hormones have been identified as candidate mediators of the postnephrectomy natriuresis: γ-melanocyte-stimulating hormone (γ-MSH) (13, 17, 24), atrial natriuretic peptide (ANP) (30, 31), and oxytocin (10). Evidence from our laboratory supporting a role for γ-MSH includes an increase in the plasma concentration of γ-MSH after AUN (13, 17, 24) and prevention of the postnephrectomy natriuresis by anti-γ-MSH antibodies (17). Furthermore, maneuvers that interrupt function of the pituitary gland, the major site of secretion of the peptide into the circulation, also prevent the natriuresis (19, 20). γ-MSH is differentially processed from its precursor, proopiomelanocortin (POMC), which also gives rise to the melanocorticotrophic α-MSH as well as adrenocorticotropic hormone (ACTH). Although γ-MSH shares a core sequence motif with α- and β-MSH and ACTH, it has very low affinity for the melanocortin-2 receptor (MC2-R) mediating adrenal steroidogenesis (22).

A family of five melanocortin receptors (MC-Rs) has been identified, the members of which mediate the actions of POMC peptides (16, 22, 28). Pigmentation results from melanocortins acting through the MC1-R, and adrenal steroidogenesis occurs as a result of the interaction of ACTH with the MC2-R. Hypothalamic MC4-R mediates central inhibition of food intake (6, 15). The functions of MC3-R and MC5-R have not been clearly determined. Of these five receptors, γ-MSH has highest affinity for MC3-R (1, 28). The identification of these receptors has led to the synthesis of melanocortin analogs with selective agonist and antagonist activity (9). Receptor antagonists have traditionally provided an important pharmacological tool for helping to reveal the functions and physiological importance of receptor-agonist interactions. SHU-9119 and SHU-9005 are analogs of α-MSH substituted at the key Phe7 residue with bulky D-amino acid derivatives; they possess potent and selective antagonist activity at the MC3-R and the MC4-R in vitro while retaining full agonist activity at the MC1-R and the MC5-R (9). Because we had identified γ-MSH as the likely effector of the postnephrectomy natriuresis, the purpose of this study was to examine the effect of intrarenal MC-R antagonism with SHU-9119 or SHU-9005 on the natriuresis after AUN. In addition, we evaluated the effect of ANP and oxytocin receptor antagonists on this response. The doses of antagonists administered were each sufficient to block the effect of natriuretic infusions of the respective peptides.
METHODS

We studied male Sprague-Dawley rats weighing between 200 and 300 g, which were housed three to a cage in the Animal Care Facility at constant temperature and humidity and with 12:12-h light-dark cycles. The experimental protocol was reviewed and approved by the Committee on Animal Research of the University of California San Francisco. Rats were given free access to standard laboratory chow and tap water until the morning of the experiment, when they were anesthetized intraperitoneally (100 mg/kg) with Inactin (Charles Lockwood, Sturtevant, WI). They were placed on a heated table to maintain rectal temperature at 37 ± 0.5°C and underwent placement of a tracheostomy tube and fine polyethylene catheters in the jugular vein for infusion of solutions and in the carotid artery for recording of arterial pressure by means of a Statham P23ID transducer attached to a polygraph recorder (model 7D, Grass Instruments, Quincy, MA). The bladder was catheterized via a small suprapubic incision using a flanged catheter sewn to the dome. To maintain a euvolemic state, each animal received an intravenous infusion of 5% bovine serum albumin in normal saline during the surgery at an amount equal to 0.7% body wt. At the completion of surgery, this infusion was changed to normal saline containing 1 mg/ml each of bacitracin and bovine serum albumin at 30 µl/min (vehicle). The left kidney was exposed via a paraspinous incision, the left renal artery was identified, and a curved 30-gauge needle was inserted with the tip directed toward the kidney. A syringe mounted on a pump was connected to this needle with PE-10 tubing. Vehicle with or without antagonist was infused continuously at 10 µl/min. The left ureter was cannulated with PE-50 tubing for left kidney urine collection. Experiments were carried out under two different protocols.

AUN. For AUN or sham AUN, the right kidney was exposed via a paraspinous incision and a ligature was loosely placed around the renal pedicle. After 30–45 min of equilibration after completion of surgical preparation, urine was collected from each kidney at 15-min intervals for three control periods. Then AUN or sham AUN was carried out in five different groups of rats. In AUN experiments, the right paraspinous incision was opened again and the ligature around the renal pedicle was tied; the kidney remained in place. In experiments with sham nephrectomy, the kidney was gently manipulated, the ligature was removed, and the wound was closed. Urine collections were continued for a total of 90 min after AUN or sham AUN. In group 1, vehicle was infused into the left renal artery throughout the experiment; group 1A rats (n = 6) underwent sham AUN, whereas group 1B rats (n = 9) had the AUN procedure carried out. Group 2 rats received a continuous infusion (5 pmol/min) of Ac-Nle[2]-d-Asp5,d-Nal(2)2,Lys[10]-γ-MSH (4–10)-NH2 (SHU-9119) (9) into the left renal artery; group 2A rats (n = 6) were sham treated, whereas group 2B rats (n = 8) underwent AUN. Group 3 was similar except that the left renal artery infusion (5 pmol/min) contained [Nle2,d-Phe8]-γ-MSH(1–13)-NH2 (SHU-9005); eight sham (group 3A) and eight AUN rats (group 3B) were studied. In group 4, the natriuretic peptide antagonist [Arg6,Cha8,b-Tic10,Arg17,Cys31]ANP (6–18)-NH2 (A-71915) (33) was infused into the left renal artery at 5 pmol/min throughout the experiment; all animals (n = 6) received AUN. In group 5, the oxytocin receptor antagonist [d(CH2)6]Tyr(Me)2,Orn9] vasotocin (DOVT) (3) was infused (10 pmol/min) into the left renal artery continuously; all animals in this group (n = 6) underwent AUN. When the experiment was concluded in groups 1-3, a large blood sample was taken into chilled Vacutainer tubes (Becton-Dickenson, Rutherford, NJ) containing EDTA and 500 KIU aprotinin and centrifuged immediately at 4°C. The plasma was stored at −70°C for later determination of γ-MSH, ANP, and oxytocin concentrations.

Intravenous peptide infusion. Separate experiments were carried out to determine the effectiveness of receptor antagonists in blocking natriuresis during intravenous peptide infusion. In these experiments, vehicle was infused intravenously at 30 µl/min for three 15- or 20-min control periods. The infusion was changed to vehicle containing [Nle2,d-Phe8]-γ-MSH (NDP-γ-MSH), a stable analog of γ-MSH (29), at 2 pmol/min or ANP or oxytocin at 1 pmol/min for an additional three periods and was changed back to vehicle alone for a final three periods. Throughout the experiment, the MC-R antagonist SHU-9119 (5 pmol/min), the natriuretic peptide receptor-A antagonist A-71915 (5 pmol/min), or the oxytocin receptor antagonist DOVT (10 pmol/min) was infused into the left renal artery at 10 µl/min. Six animals were studied in each group. To determine the effectiveness of MC-R blockade by SHU-9005, a different protocol was followed. An initial control period of three 20-min collections in which vehicle was infused into both the left renal artery (10 µl/min) and a peripheral vein (30 µl/min) was followed by three periods during which SHU-9005 dissolved in vehicle was infused at 1 pmol/min (n = 6) or 5 pmol/min (n = 11) into the left renal artery. This was then followed by a third series of three periods during which intravenous vehicle was changed to vehicle containing NDP-γ-MSH to achieve an infusion rate of 2 pmol/min, and the intrarenal infusion of SHU-9005 continued without interruption. In a separate group of experiments, the effect of SHU-9119 infusion into the left renal artery (5 pmol/min) on the natriuresis resulting from intravenous infusion of ANP (100 pmol/min) was studied (n = 6).

In all experiments, urine flow rate was determined gravimetrically, and urine sodium and potassium concentrations were measured by flame photometry with cesium as internal standard (model 943, Instrumentation Laboratories, Lexington, MA), ANP, oxytocin, A-71915, and DOVT were purchased from Peninsula Laboratories (Belmont, CA). Bovine serum albumin and bacitracin were purchased from Sigma (St. Louis, MO). SHU-9119, SHU-9005, and NDP-γ-MSH were synthesized in the laboratory of V. J. Hruby, University of Arizona (9).

The plasma concentrations of immunoreactive (IR) γ-MSH (groups 1 and 2) and ANP and oxytocin (group 3) were determined by radioimmunoassay using commercially available kits (Peninsula Laboratories). Plasma samples were extracted using Sep-Pak C18 cartridges (Waters, Milford, MA), as previously described (13, 17, 20, 21). After extraction, samples were lyophilized in a SpeedVac concentrator (Savant Instruments, Farmingdale, NY) and stored at −70°C until assayed. For the assays, samples were reconstituted in buffer; the assays were carried out according to the manufacturer’s instructions. The antisemur used in the assay for γ-MSH was raised against γ-MSH and has 0.5% cross-reactivity to γ1-MSH, 0.3% cross-reactivity to γ2-MSH, and zero cross-reactivity to α-MSH, β-MSH, ACTH, or β-endorphin, according to the manufacturer’s characterization. Sensitivity is 1 fmol/tube. Intra- and interassay coefficients of variation are 5 and 15%, respectively.

Experimental results are expressed as means ± SE. Student’s t-test for paired or unpaired data was used to assess differences between groups, and one-way and repeated-measures analysis of variance with the Bonferroni post hoc test was used for multiple differences within and among groups. P < 0.05 was taken to indicate a significant difference.
RESULTS

AUN. The effect of AUN or sham AUN on $U_{NaV}$ is presented in Fig. 1A. AUN led to a large increase in $U_{NaV}$ from the vehicle-infused left kidney of group 1B rats that was evident within 30 min of the procedure and persisted for the 90-min duration of these studies, consistent with multiple previous reports. $U_{NaV}$ increased from $0.34 \pm 0.04$ to $1.12 \pm 0.11 \mu$eq/min 90 min after AUN ($P < 0.001$). Parallel increases in urine flow and potassium excretion ($U_K$) also occurred (Table 1). No change in $U_{NaV}$ from either kidney occurred in group 1A rats undergoing the sham AUN procedure. The effect of SHU-9119 on $U_{NaV}$ after AUN or sham AUN in group 2 experiments is presented in Fig. 1B. SHU-9119 infusion into the left renal artery at 5 pmol/min had minimal effect on basal $U_{NaV}$ or $U_{NaV}$ after sham AUN. However, it completely blocked the natriuretic response to AUN, with $U_{NaV}$ actually decreasing from $0.42 \pm 0.08$ to $0.30 \pm 0.06 \mu$eq/min after AUN ($P < 0.05$). There was no change in $U_{NaV}$ in SHU-9119-infused rats undergoing the sham nephrectomy (group 2A). Similar results were observed with SHU-9005 infused into the left renal artery at 5 pmol/min (group 3); no increase in urine flow or $U_{NaV}$ occurred after AUN or sham AUN (Fig. 1 and Table 1). However, a significant kaliuresis still occurred after AUN in groups 2B and 3B (Table 1).

The effect of AUN or sham AUN on the plasma concentration of γ-MSH 90 min later in groups 1 and 2 is shown in Fig. 2. The plasma γ-MSH concentration in rats undergoing AUN in both groups was more than double the value in sham-operated rats, as noted previously (13, 17, 24). The values in sham and AUN rats were not affected by SHU-9119 infusion. These results indicate that intrarenal infusion of the MC-R antagonist SHU-9119 completely prevented the postnephrectomy natriuresis despite an equivalent increase in the plasma concentration of γ-MSH, the putative mediator of the natriuresis.

To determine if ANP and oxytocin make some contribution to the natriuretic response induced by AUN, we used two selective receptor antagonists. A-71915 is a small, substituted peptide analog of ANP that is a potent inhibitor of ANP-dependent guanosine 3',5'-cyclic monophosphate (cGMP) stimulation (33). DOVT is a substituted analog of vasotocin with potent inhibition of oxytocin in an in vitro rat uterus bioassay (3). The influence of these agents on the natriuretic response to AUN is presented in Fig. 3. Infusion of A-71915 at 5 pmol/min into the left renal artery had no appreciable effect on basal $U_{NaV}$ and did not materially affect the response to AUN as $U_{NaV}$ rose (Fig. 3A), much as in vehicle-infused rats (Fig. 1A). Similarly, DOVT infused at 10 pmol/min into the left renal artery also did not markedly alter the response to AUN (Fig. 3B and Table 1). Plasma concentrations of ANP and oxytocin were measured in group 3A and 3B experiments after sham AUN or AUN; the results are presented in Fig. 2. Plasma ANP concentration was $4.7 \pm 1.1$ fmol/ml after sham nephrectomy and was statis-
Intravenous peptide infusion.

To document that the postnephrectomy natriuresis is not mediated by changes in plasma peptide levels, we measured in rats receiving intrarenal infusion of SHU-9005, SHU-9119, and DOVT the plasma immunoreactivity for oxytocin, atrial natriuretic peptide (ANP), and γ-melanocyte-stimulating hormone (γ-MSH). Plasma γ-MSH immunoreactivity was 14.2 ± 2.3 fmol/ml after sham AUN and 13.5 ± 4.5 fmol/ml after AUN (P = not significant). Thus no significant change in the plasma concentration of these two peptides occurred after AUN, nor did intrarenal infusion of antagonists of their respective receptors blunt the postnephrectomy natriuresis.

An acute, transient rise in mean arterial pressure (MAP) after AUN is thought to be the stimulus initiating the reflex natriuresis (2). One minute after AUN, MAP had increased equivalently in vehicle and SHU-9119-infused rats (14 ± 2 mmHg, P = not significant), indicating that a blunted stimulus was not responsible for the lack of natriuresis in SHU-9119-infused animals (Fig. 4). In group 4 experiments, blood pressure had increased 14 ± 1 mmHg, and in group 5, pressure had increased 13 ± 1 mmHg, values identical to those seen in the other AUN groups.

Intravenous peptide infusion. To document that the intrarenal infusions of SHU-9119, SHU-9005, A-71915, and DOVT were adequate to block the effects of natriuretic levels of melanocortins, ANP, and oxytocin, respectively, we infused the peptides intravenously during continuous infusion of the corresponding receptor antagonist into the left renal artery at 5 pmol/min (SHU-9119, SHU-9005, and A-71915) or 10 pmol/min (DOVT). The results are shown in Fig. 5. Intravenous infusion of

Values are means ± SE of 3 measurements before (control) and 45–90 min after acute unilateral nephrectomy or sham nephrectomy (exp). 

V, urine flow rate; U[Na], sodium excretion; U[K], potassium excretion; MAP, mean arterial pressure. *Different from corresponding control value, P < 0.05 or greater by paired t-test; †greater than groups 1A, 2A, 2B, and 3B and ‡greater than groups 1A, 2A, and 3A, P < 0.05 by 1-way ANOVA.

![Figure 2](http://ajpregu.physiology.org/)

**Fig. 2.** Plasma immunoreactive γ-melanocyte-stimulating hormone (γ-MSH), atrial natriuretic peptide (ANP), or oxytocin concentrations after sham AUN (open bars) or AUN (filled bars). γ-MSH was measured in rats with intrarenal infusion of vehicle (left bars under γ-MSH) or SHU-9119 (right bars under γ-MSH); ANP and oxytocin were measured in rats receiving intrarenal infusion of SHU-9005. *P < 0.01 vs. sham AUN; n = 6 for each group. NS, not significant.

![Figure 3](http://ajpregu.physiology.org/)

**Fig. 3.** Change in U[Na]V after AUN (arrow) in rats receiving a continuous infusion of natriuretic peptide receptor antagonist A-71915 (5 pmol/min; A) or oxytocin receptor antagonist DOVT (10 pmol/min; B) in left renal artery. Neither compound blunted postnephrectomy natriuresis. Asterisks indicate values after AUN that are significantly greater than control (*P < 0.05, repeated-measures ANOVA); n = 6 for each group.
NDP-γ-MSH (2 pmol/min) led to a prompt, significant increase in UNaV from the right, control kidney, whereas UNaV from the left kidney infused with SHU-9119 did not change (Fig. 5A). Similar results were seen with intravenous infusion of ANP (Fig. 5C) or oxytocin (Fig. 5D) for 45 min, as UNaV from the right kidney increased progressively, reaching a magnitude comparable to that seen after AUN by the third period of infusion. However, natriuresis from the left kidney in response to ANP was completely blocked in the presence of the A-71915 infusion, indicating that the rate of infusion of the antagonist was sufficient to prevent the intrarenal action of a natriuretic level of ANP. Natriuresis due to intravenous oxytocin infusion was likewise markedly attenuated from the left kidney receiving the intrarenal infusion of DOVT, although a small increase in UNaV occurred. UNaV tapered back toward baseline after cessation of intravenous hormone infusion. The effect of intrarenal SHU-9005 infusion on natriuresis induced by intravenous NDP-γ-MSH is shown in Fig. 5B. The change from vehicle to SHU-9005 (5 pmol/min) led to a small but significant increase in UNaV, suggesting that this agent possesses partial agonist activity. Subsequent intravenous infusion of NDP-γ-MSH led to a brisk natriuresis from the right kidney but no change in UNaV from the left kidney. Intrarenal infusion of SHU-9005 at 1 pmol/min did not increase UNaV and only attenuated the natriuresis from intravenous NDP-γ-MSH (data not shown). SHU-9119 and SHU-9005 also prevented the natriuresis from intravenous infusion of γ-MSH (not shown). These studies thus indicate that the respective receptor antagonists are effective at the doses infused in blocking or blunting natriuretic doses of infused melanocortins, ANP, or oxytocin.

In a final set of experiments we tested whether intrarenal infusion of SHU-9119 altered the natriuretic response. A: infusion of SHU-9119 (5 pmol/min) into left renal artery prevented natriuresis seen from right kidney caused by intravenous infusion of NDP-γ-MSH (2 pmol/min). B: SHU-9005 (5 pmol/min) infused into left renal artery after 60 min (left arrow) caused a mild but significant increase in UNaV but completely blocked natriuresis from intravenous NDP-γ-MSH at 120 min (right arrow). C: infusion of A-71915 (5 pmol/min) into left renal artery blocked natriuresis induced from right kidney by intravenous ANP (1 pmol/min). D: infusion of DOVT (10 pmol/min into left renal artery) blunted natriuresis resulting from intravenous infusion of oxytocin (1 pmol/min) compared with right side. Six rats were studied in each group; horizontal bar in A, C, and D indicates duration of agonist infusion. Small asterisks indicate values significantly greater than control for that kidney, P < 0.05 by repeated-measures ANOVA; large asterisks indicate values from right kidney that are significantly greater than left kidney, P < 0.05.

Fig. 4. Mean arterial pressure after AUN in group 1 (○, vehicle infusion in left renal artery) and group 2 (○, SHU-9119 infusion in left renal artery). Within 1 min of AUN, pressure rose 13–14 mmHg in each group, after which it returned to control levels and remained there for rest of experiment. Values are means ± SE of measurements in 6 animals in each group.
response to ANP infused intravenously. The rate of ANP infusion (100 pmol/min) caused a robust natriuresis from both kidneys that was not influenced by intrarenal SHU-9119 infusion, because the natriuretic response was not different between left and right kidneys. Before ANP infusion, $U_{NaV}$ was $0.71 \pm 0.15$ and $0.70 \pm 0.09 \mu$equiv/min from SHU-9119-infused left and control right kidneys, respectively, and rose to $3.38 \pm 0.87$ and $3.21 \pm 0.87 \mu$equiv/min during ANP infusion ($P = \text{not significant, left vs. right kidneys}$). These results indicate that there is no appreciable interaction of SHU-9119 with biologically active receptors for ANP in the kidney.

**DISCUSSION**

The present studies were designed to provide data supporting or refuting a role for one of three candidate hormone mediators of the reflex natriuresis after AUN by using selective receptor antagonists infused directly into the kidney. Data suggesting a role for ANP as mediator of the postnephrectomy natriuresis are strong. The natriuresis was accompanied by an increase in urinary cGMP excretion, a reflection of ANP action in the kidney (34), and by a more than doubling of plasma ANP concentration (31). Both these changes were prevented and the natriuresis was blocked in rats with right atrial appendectomy, a major source of circulating ANP. Additional studies demonstrated that the postnephrectomy natriuresis could be prevented by administration of a monoclonal antibody to ANP (30). However, an earlier study from our laboratory could not confirm an elevation in plasma ANP concentration at any time from 30 min to 2 h after AUN (24), and the present data likewise did not demonstrate any increase. These latter observations argue against its role as the effector of the postnephrectomy natriuresis.

Functional data from the present experiments also argue strongly against the involvement of ANP in the natriuresis that results from AUN. Intrarenal infusion of A-71915 at a dose sufficient to block entirely the natriuresis caused by intravenous infusion of ANP had no discernible effect on the postnephrectomy natriuresis. $U_{NaV}$ rose promptly after AUN in these studies in a manner qualitatively and quantitatively similar to the results of AUN in rats infused with vehicle alone. This observation makes it difficult to invoke a role for ANP in the reflex natriuresis after AUN.

Similar data have been presented supporting an important role for oxytocin in the response to AUN. These include an increase in plasma oxytocin concentration after AUN and prevention of the natriuresis by intravenous infusion of an oxytocin receptor antagonist (10). We obtained data that make an important role for oxytocin as the effector of postnephrectomy natriuresis unlikely. Plasma oxytocin concentration did not increase significantly after AUN. Furthermore, intrarenal infusion of the oxytocin receptor antagonist DOVT was successful in blunting natriuresis after intravenous infusion of a natriuretic dose of oxytocin yet had no effect to alter the increase in $U_{NaV}$ observed after AUN. Thus, as was the case with ANP, the absence of an increase in plasma oxytocin concentration as well as the preservation of the postnephrectomy natriuresis in the presence of an oxytocin receptor antagonist argue against a major role of circulating oxytocin in the postnephrectomy natriuresis.

Such was not the case, however, in the studies of AUN during intrarenal infusion of the MC-R antagonists SHU-9119 and SHU-9005. SHU-9119 was shown to be a potent, selective antagonist at the human MC3-R and MC4-R ($pA_2 = 8.3$ and 9.3, respectively) in an in vitro assay system measuring melanocortin-stimulated cAMP accumulation, while retaining agonist activity at MC1-R and MC5-R (9). Infusion of this compound into the left renal artery at a rate of 5 pmol/min did not appreciably affect basal $U_{NaV}$ but completely prevented the postnephrectomy natriuresis despite a doubling of plasma IR $\gamma$-MSH concentration. It also blocked natriuresis during intravenous infusion of NDP-$\alpha$-MSH. Although not examined in the present experiments, SHU-9119 was shown to possess partial agonist activity at human MC4-R and mouse MC1-R and MC5-R (R. A. Kesterson, V. J. Hruby, and R. D. Cone, unpublished observations). The data shown in Fig. 5 suggest that SHU-9005 may also possess partial agonist activity at the renal MC-Rs mediating natriuresis, because $U_{NaV}$ rose slightly during its infusion. However, it was still capable of blocking the postnephrectomy natriuresis.

$\gamma$-MSH has relatively low affinity for MC-Rs except for the MC3-R, where it was shown to have an $EC_{50}$ of $3.8 \times 10^{-9}$ M, approximately equal to that of $\alpha$-MSH at this receptor (1, 28). Thus our studies not only indicate that a melanocortin peptide closely related to the $\gamma$-MSH primary sequence is the likely mediator of natriuresis after AUN but also demonstrate that signaling likely occurs through MC3-R or MC4-R pathways. In view of the high affinity of $\gamma$-MSH for the MC3-R, we propose that this receptor that mediates the postnephrectomy natriuresis. Because antagonism of these compounds was demonstrated against human rather than rat MC3-R and MC4-R in vitro (9), it is possible that differences in primary structure of MC3-R between these species would alter this conclusion, although a high degree of homology exists among mammalian MC-Rs so far studied (16, 22, 28).

These observations help to explain several puzzling aspects of the response to AUN. The MC3-R appears to be expressed primarily in nervous tissue (28), and presumably the receptors within the kidney reside on renal nerve terminals. This would account for the observation that renal denervation prevents natriuresis after AUN (13, 27) and also blocks the natriuretic effect of intrarenal infusion of $\gamma$-MSH (4, 14). It also would rationalize the failure to identify specific binding of $\gamma$-MSH in rat kidney by emulsion autoradiography or in membrane fractions from renal cortex (Ref. 23 and J.-P. Valentin, C. Qiu, E. Wiedemann, and M. H. Humphreys, unpublished observations), because these...
techniques lack the sensitivity necessary for detection of minor binding sites on nerve endings.

It is not clear at present how to reconcile the extensive data pointing to γ-MSH as the mediator of the postnephrectomy natriuresis (Refs. 13, 17, and 24 and present studies) with the reports arguing for a role of ANP (30, 31) or oxytocin (10). An increase in the plasma concentration of a peptide hormone cannot by itself be taken as evidence for a role of the hormone in causing the natriuresis, because the increased concentration could reflect a reduction in the metabolic clearance of the peptide due to the reduction in renal mass rather than an increase in hormone secretion stimulated by the unilateral nephrectomy. The kidneys are a recognized site of peptide hormone degradation (25). In the case of ANP, it has been pointed out that natriuretic effects are only seen when the plasma concentration was more than triples (reviewed in Ref. 7). Blockade of the postnephrectomy natriuresis by intravenous infusion of an oxytocin receptor antagonist (10) but not by intrarenal infusion (present study) raises the possibility of an intermediate step involving oxytocin receptors outside the kidney in the reflex pathway initiated by AUN. A recent study has presented evidence that oxytocin mediates the increase in plasma ANP concentration after blood volume expansion (8), suggesting a possible relationship between these two peptides. However, oxytocin itself possesses natriuretic properties separate from those related to any increase in plasma ANP it may cause (5, 10, 32). Data have also shown that an intrathecal injection of an oxytocin receptor antagonist blocks the postnephrectomy natriuresis, suggesting a site of action in the spinal cord for oxytocin in the reflex natriuresis after AUN (11). In any case, the absence of any significant change in the blood concentration of either ANP or oxytocin after AUN in the present experiments indicates that they play no role as circulating hormones in the postnephrectomy natriuresis.

The present experiments also indicate that the postnephrectomy kaliuresis can be partially dissociated from the natriuresis. Increased UNaV occurred after AUN in groups 2B and 3B despite complete blockade of the postnephrectomy natriuresis in these experiments with intrarenal infusion of MC-R antagonists. The magnitude of this kaliuresis, although less than in vehicle-infused rats (group 1B), was still greater than the minimal changes seen in sham-operated animals. Previous studies have also revealed a persistent kaliuretic effect of AUN in two other conditions in which the natriuresis has been blocked: treatment with anti-γ-MSH antibodies (17) or AUN after renal denervation (27). These observations have contributed to the speculation that an as-yet-unrecognized mechanism involving the central nervous system may participate in regulation of UNaV after AUN (26).

In summary, the present data further strengthen the contention that a γ-MSH-like peptide mediates the increase in UNaV after AUN. Because this reflex pathway must have significance beyond the unusual circumstance of unilateral nephrectomy, it may play a more general role in sodium metabolism. In this regard, we have recently shown that plasma γ-MSH concentration and pituitary POMC messenger RNA abundance are increased in rats ingesting a high-sodium diet (21), leading to the possibility that these changes are involved in the adjustments to an increase in sodium intake. Further work will be necessary to identify the true role of this peptide hormone system in the maintenance of sodium balance, both acutely and chronically.

Perspectives

Reflex control of the circulation and extracellular fluid volume involves a complex interplay of neural and humoral systems, many of which influence the regulation of renal UNaV. The model of AUN has been used to study the pathways involved in rapid (<1 h) increases in UNaV, because it initiates a reflex leading to natriuresis without discernible change in the volume or composition of the blood and extracellular fluid volumes. Among numerous natriuretic hormones, three have been argued to participate in this postnephrectomy natriuresis: γ-MSH, ANP, and oxytocin. The present studies were carried out to evaluate the roles of each of these peptides in the natriuresis. The results indicate that γ-MSH, but not ANP or oxytocin, is the mediator of the postnephrectomy natriuresis, thereby lending support to the contention that this peptide may play a wider role in sodium metabolism.

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