Natriuretic and Hypertensive Activities Reside in a Fragment of ACTH

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SUMMARY The hypertensive and natriuretic effects of chronic administration of adrenocorticotropic hormone (ACTH) cannot be duplicated by the administration of glucocorticoids and/or mineralocorticoids. We investigated the effects of a fragment of this hormone (ACTH^4-10) and an analog of the fragment (D-Phe^7) ACTH^4-10 and found them to have pressor and cardioaccelerator actions in rats as determined by bolus intravenous (i.v.) injections of 30 to 1000 nmol/kg. The pressor and cardioaccelerator effects of (D-Phe^7) ACTH^4-10 were attenuated by α-receptor (phentolamine) and β-receptor (metoprolol) antagonists. The cardiovascular actions of ACTH^4-10 were produced in adrenalectomized or ganglionic-blocked (with mecamylamine) rats. At a lower dose (7 nmol/kg i.v.), ACTH^4-10 was natriuretic and had a pattern of activity similar to that of a larger ACTH fragment, alpha-melanocyte-stimulating hormone. Extraadrenal effects of the intact ACTH molecule or the in vivo production of an ACTH^4-10-like fragment from ACTH may contribute to the hypertensive and natriuretic actions associated with this hormone. (Hypertension 6: 468-474, 1984)

KEY WORDS • ACTH^4-10 natriuresis • sympathetic nervous system • pressor and cardioaccelerator actions

RECENT evidence from our laboratories suggests that it is possible for a single substance to regulate renal sodium excretion (a natriuretic hormone [NH]) and have cardiovascular effects. Investigators searching for a NH have isolated alpha-melanocyte-stimulating hormone (αMSH) and a fragment of adrenocorticotropic hormone (ACTH^11-13) from pituitaries based on their natriuretic activity and on a fragment of adrenocorticotropic hormone (ACTH^4-10) from pituitaries based on their natriuretic activity and on a fragment of adrenocorticotropic hormone (ACTH^4-10) from pituitaries based on their natriuretic activity. A smaller fragment of this hormone (ACTH^1-24) is natriuretic. Other investigators have produced hypertension by chronic administration of ACTH, although the underlying hypothesis in their work is that the hormone was working through an adrenocortical mechanism. Since natriuretic and pressor actions have been attributed to an NH, we hypothesized that all of these properties might be found in a small fragment of ACTH or βLPH. We therefore investigated the biological activity of the amino acid sequence common to all of these peptides, Met-Glu-His-Phe-Arg-Trp-Gly; ACTH-αMSH/βLPH. This peptide has previously attracted much attention due to its behavior-modifying properties and its ability to improve neuromuscular performance. We now report the natriuretic and cardiovascular effects of ACTH^4-10 and a related peptide, (D-Phe^7) ACTH^4-10.

Methods and Materials

ACTH^4-10 or (D-Phe^7) ACTH^4-10 (Bachem, Inc., Torrance, California) was dissolved in 75 mM saline at a concentration of 10^{-3} M, and the pH adjusted to 7 with NaH_2PO_4. Peptide solutions were made fresh daily, since in our hands degradation and loss of biological activity occurred in 1 to 3 days in frozen solutions even when they were acidic. The purity of the peptides was ascertained by reverse-phase high performance liquid chromatography (HPLC) with the use of a 10% to 40% propanol gradient in 0.5 M pyridine-formate, pH 3. Fresh peptide solutions yielded a single fluorescamine-positive peak upon chromatography, while solutions over 48 hours old had multiple fluorescamine-positive peaks. Control injections of 'nonsense-
peptides containing similar amino acids (e.g., Glu-Arg-Trp-His and Arg-Glu-Trp-His) at doses of 1 to 1000 nmol/kg were used to establish the specificity of responses to ACTH4-10.

The rat natriuresis bioassay preparation has been previously described. Male Sprague Dawley rats weighing 140–160 g were anesthetized with pentobarbital, 50 mg/kg intraperitoneally. The femoral vein was cannulated with PE 50 tubing, the bladder catheterized suprapubically with PE 160, and the trachea cannulated with PE 240 tubing. The animals were placed on a heated pad, and their rectal temperatures were maintained at 37° to 39° C. A 75 mM saline/pentobarbital solution was infused intravenously at 100 μl/min throughout the entire assay procedure. To maintain a steady state of anesthesia, a protocol was used that employed increasing rates of pentobarbital infusion. The initial rate at the completion of surgery was 0.12 mg·g BW−1·min−1. The dose was changed to 0.35 mg·g BW−1·min−1 at 100 minutes after induction of anesthesia and again to 0.40 mg·g BW−1·min−1 at 380 minutes after induction of anesthesia. We have found this preparation to give a remarkably stable baseline sodium excretion approximately 2 hours after induction of anesthesia, which remains constant for up to 4 to 5 hours. ACTH4-10 or nonsense-peptides were administered as a bolus injection (i.v.) in 0.7 ml of 75 mM saline. Urine was collected in graduated containers at 10- or 20-minute intervals, its volume recorded, and Na and K concentrations determined by flame photometry. ACTH4-10 or the nonsense-peptides were dissolved in 0.7 ml 75 mM saline and given as a bolus injection over a 5-minute period in place of the anesthesia solution, or dissolved in the anesthetic solution and given as an infusion for 150 minutes. Changes in UNaV from baseline were tested at 10- or 20-minute intervals for statistical significance by the multiple comparison test of Dunnett, or the cumulative sodium excretion for 130 minutes was analyzed by Duncan’s new multiple range test. Above a minimally active dose, the bolus injections or infusions produced similar effects on sodium excretion. Thus, the data from these active doses were pooled.

Cardiovascular effects of ACTH4-10, (D-Phe4) ACTH4-10, or nonsense-peptides were assessed in anesthetized or unanesthetized rat preparations. The former bioassay preparation was similar to that described for natriuresis. In addition, arterial blood pressure was monitored via a femoral catheter connected to a pressure transducer and a Grass Polygraph recorder. A ganglionic blocker (mecamylamine, 10 mg/kg) was administered to achieve a steady baseline blood pressure, as well as to rule out direct central sympathetic nervous system effects of the peptides. ACTH4-10 or the nonsense-peptides were dissolved in 50 μl of 75 mM saline and administered as a bolus injection.

For the second cardiovascular assay preparation, 7-week-old male Sprague-Dawley rats were immobilized by the administration of ketamine (150 mg/kg, i.p.). Arterial catheters (PE 60) were implanted into the ascending aorta via the left carotid artery, and venous catheters (PE 50) were implanted into the right jugular vein. Both the arterial and venous catheter tips were made of silicone rubber tubing (Silastic, Dow-Corning, Midland, Michigan; 0.012 in. i.d. × 0.025 in. o.d.) and drawn over the outside wall of the PE tubing. The catheters were then passed under the skin and exteriorized to the back of the neck via a small incision. They were cut to approximately 4 cm in length and sealed with stainless steel stilettes. The animals were allowed to recover for at least 72 hours after surgery.

Individual rats were placed in a lucite cage (30 × 30 × 30 cm). The exteriorized arterial catheter was fitted to a swivel cannula that was attached to a low-volume displacement transducer (Electromedics, MS 20, Englewood, Colorado) secured above the cage to measure arterial blood pressure. Zero pressure was referenced to midchest level. Heart rate (HR) was electronically derived from the arterial pulse signal. Recordings were made on a Brush 8-channel recorder (Model 280, Cleveland, Ohio). Rats were acclimated in the cage for at least 30 minutes, after which control recordings were made for another 15 minutes. ACTH4-10 and (D-Phe4) ACTH4-10 were administered as an i.v. bolus dose in normal saline (100 μl·100 g BW−1·h−1).

Phentolamine (1.0 mg/kg, i.v.) was used to block α-receptors, while metoprolol (1.0 mg/kg, i.v.) was used to block β-receptors. Norepinephrine (1.0 μg/kg) and isoproterenol (0.5 μg/kg) were used to test the efficacy of the α- and β-blockade, respectively. Each agonist was administered 30 minutes before and 15 minutes after the respective receptor antagonist to ensure antagonism before the administration of (D-Phe4) ACTH.

Bilateral adrenalectomy was performed in five rats that had previously been implanted with chronic indwelling arterial and venous catheters. The rats were anesthetized with ether, and the adrenal glands were removed through a dorsal (retroperitoneal) incision. Animals were allowed to recover for 24 hours during which time they were given saline 150 mM solution and food ad libitum.

Results

Vehicle control experiments showed only a small, transient, insignificant increase in UNaV during the first 30 minutes after injection (Figure 1 C). Bolus injections of 7–35 nmol/kg ACTH4-10 in 12 rats caused a progressive increase in UNaV from baseline of 4.1 ± 0.34 μEq/min to a peak of 5.4 ± 0.46 μEq/min at 50 to 70 minutes after the injection (Figure 1 A). The increment for a single 10- or 20-minute collection period was not significantly different from the control. However, cumulative UNaV for the 130 minutes of the assay after ACTH4-10 was significantly (p < 0.05) elevated to 129 ± 40 μEq/130 minutes compared to 0.25 ± 38 μEq/130 minutes in rats given vehicle control. Injections of nonsense peptides caused no changes in UNaV above that seen with vehicle control.
A constant infusion of 175 to 350 pmol/kg/min of ACTH$_{4-10}$ in 13 rats raised $U_{NaV}$ from a baseline of 4.5 ± 0.31 μEq/min to a level of about 6.1 μEq/min within 30 to 50 minutes after the beginning of the infusion. This rate of $U_{NaV}$ was maintained fairly constant during the infusion period (Figure 1 B). Cumulative $U_{NaV}$ during the constant infusion was 154 ± 39 μEq/130 minutes ($p < 0.05$ compared to vehicle control). The difference between cumulative $U_{NaV}$ in the bolus injection experiments and the constant infusion experiments was not statistically significant ($p > 0.1$).

No significant natriuretic response was observed with an ACTH$_{4-10}$ infusion of 70 pmol/kg/min.

Initially, the pressor effects of ACTH$_{4-10}$ were evaluated in the anesthetized rat preparation. Each rat was allowed to stabilize for at least 40 minutes after mecamylamine, and the mean blood pressure did not differ by more than ±5% for 20 minutes prior to an injection. Figure 2 depicts the blood pressure increase seen after injection of 700 nmol/kg and 1.7 μmol/kg of ACTH$_{4-10}$. The average increase in mean arterial blood pressure (MAP) for a 700 nmol/kg injection was 15 ± 5 mm Hg, while for 1.7 μmol/kg it was 32 ± 8 mm Hg ($n = 5$ for both doses). The smallest dose required to give a significant pressor response was 50 nmol/kg. Injection of nonsense peptides ($n = 5$) produced no change in blood pressure.

It is well known that anesthetized bioassay preparations may not always give results that are identical to those observed in conscious, unrestrained animals. We therefore repeated and extended our studies with ACTH$_{4-10}$ as well as with (D-Phe$^7$) ACTH$_{4-10}$ in the conscious rat. The use of the latter peptide was based on the possibility that a sterically hindered analog of ACTH$_{4-10}$ might show enhanced activity.

Control MAP in 11 unanesthetized freely moving rats averaged 111 ± 3.5 mm Hg, while mean HR
Figure 3. Dose-dependent effects of bolus injections of ACTH\textsuperscript{4-10} and (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} on mean arterial pressure (MAP) in conscious rats. Values are the mean response at that dose ± standard error.

Figure 4. Dose-dependent effects of bolus injections of ACTH\textsuperscript{4-10} and (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} on heart rate (HR) in conscious rats. Values are the mean response at that dose ± standard error.

The rise in MAP, but not HR, was significantly reduced by pretreatment with the \(\alpha\)-antagonist phentolamine (Table 1). Similarly, the rise in HR, but not MAP, was significantly reduced by pretreatment with the \(\beta\)-antagonist metoprolol (Table 1). Typical blood pressure and HR responses to (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} in the presence of \(\alpha\)- or \(\beta\)-receptor blockade are shown in Figure 5.

Table 1. Mean Arterial Blood Pressure (MAP) and Heart Rate (HR) Response to (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} (300 nmol/kg, i.v.) Before and After \(\alpha\)-Adrenergic or \(\beta\)-Adrenergic Receptor Blockade

<table>
<thead>
<tr>
<th></th>
<th>Control ((n = 3))</th>
<th>(D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} + antagonist</th>
<th>(D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} alone</th>
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<tr>
<td></td>
<td>MAP (mm Hg)</td>
<td>165 ± 2.9</td>
<td>97 ± 1.7</td>
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<td></td>
<td>HR (bpm)</td>
<td>444 ± 24.0</td>
<td>396 ± 20.7</td>
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<tr>
<td>Phentolamine ((\alpha)-receptor antagonist)</td>
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<tr>
<td>Metoprolol ((\beta)-receptor antagonist)</td>
<td>MAP (mm Hg)</td>
<td>148 ± 1.6</td>
<td>107 ± 8.9</td>
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<tr>
<td></td>
<td>HR (bpm)</td>
<td>504 ± 13.8</td>
<td>368 ± 8.0</td>
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Values are means ± SEM. (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} was injected 15 minutes before and 5 minutes after receptor blockade. *Significantly smaller change than with (D-Phe\textsuperscript{7}) ACTH\textsuperscript{4-10} alone, by one-way analysis of variance and Duncan's multiple-range test: \(p < 0.01\).

After adrenalectomy, the same dose of the peptide increased MAP by 36 ± 1.3 mm Hg and increased HR by 54 ± 5.4 bpm. Control MAP before and after adrenalectomy was not significantly different. However, control HR after adrenalectomy increased by 89 ± 9.5 bpm.

Discussion

ACTH-induced blood pressure elevations have been reported in a number of species,\textsuperscript{7-10} including humans.\textsuperscript{10} In almost all of these reports the blood pressure effects have been suggested to result from adrenocortical stimulation, even though a consistent correlation between increased circulating levels of a specific adrenal steroid and blood pressure was not demonstrated. In addition, in at least one report the renin-angiotensin system was found to be normal or suppressed in states...
of high circulating ACTH. Recently, Lohmeier and Carroll extensively investigated the chronic effects of ACTH administration on blood pressure and suggested that the hypertensive effects might be due to increased secretion of an unknown pressor factor. Attempts to totally duplicate the hypertensive effects of ACTH infusions by glucocorticoid and/or mineralocorticoid infusions were not successful. No one has previously suggested that the hypertensive effects of ACTH might be directly due to an extraadrenal effect of this hormone. However, support for this hypothesis is found in clinical studies that show a high incidence (>75%) of hypertension associated with the natural course of Cushing's disease, while the incidence of hypertension in steroid-treated patients is between 5% and 16%. The delayed pattern of natriuresis observed with bolus injections of ACTH (peak effect at 40–80 minutes) is similar to the effect of αMSH in the rat. Orias and McCann reported that bolus injections of αMSH produced a natriuretic effect that plateaued at 40 to 80 minutes postinjection. These natriuretic patterns are similar to those seen with extracts prepared from plasma of saline-expanded dogs but are slightly different in that the peak of natriuresis occurred about 20 minutes later than that of plasma extracts.

It is difficult to compare the dose relationship between ACTH and ACTH or αMSH with regard to the renal and cardiovascular effects due to the different assays used, the approaches to data analysis, and the routes of administration. For example, the natriuretic effects of ACTH and αMSH in rats have usually been demonstrated with bolus injections, while the pressor actions are always shown by infusions. However, our work indicates that with bolus injections of ACTH, natriuresis occurs at 1/35th the dose needed to induce a significant pressor effect. This dose-dependent relationship is similar to the findings of Lohmeier and co-workers, who showed that subpressor infusions of ACTH in dogs induced a significant natriuresis.

The bolus injections of ACTH (i.v.) or αMSH (i.p.) used to produce a significant natriuretic effect in rats vary from 16.5 nmol (ACTH) to 30 nmol (αMSH). These doses, respectively, produced cumulative sodium excretions of approximately 125 μEq/5 hr and 165 μEq/100 min. In comparison, a 7 to 35 nmol/kg bolus of ACTH produced a net sodium excretion of 129 μEq/130 min. While one might infer that ACTH is more potent than ACTH or αMSH, this difference in natriuretic activity was most likely due to the use of different bioassay approaches.

The ability of α- and β-receptor antagonists to reduce the pressor and cardioaccelerator effects of ACTH suggests that catecholamines play a role in its action. There is the possibility that some of the cardiovascular effects occur through a central-nervous-system-induced action. However, the use of ganglionic blocked rats for the pressor studies and the rapidity of onset of these effects strongly indicate that there is a major peripheral component to the action of ACTH-like peptides.

The bolus doses of ACTH used to produce in vivo effects are too large to be considered physiological. However, enzymatic degradation and/or renal excretion may be limiting its biological half-life. In support of this is our evidence that infusions of ACTH at
175–350 pmol/kg/min (1/20 the bolus natriuretic dose) induced a significant natriuresis. Lohmeier and Carroll have shown that subpressor ACTH infusions potentiate the pressor effect of norpinephrine. Other investigators have shown that ACTH may potentiate norpinephrine-induced contractions of atrial muscle preparations. To further link the hypertensive effects of ACTH to the ACTH-10 sequence, it would be interesting to demonstrate that infusions of the latter peptide produce a hypertensive state with at least some of the hallmarks of ACTH infusions, for example, norpinephrine potentiation.

It is possible that an extraadrenal effect of the entire ACTH molecule or of an ACTH 4-10-containing catabolite of ACTH may contribute to the renal and cardiovascular effects of ACTH. Hudson and McMartin have shown that the administration of ACTH 4-24 to rats produces circulating fragments as small as 3-14 and 3-15. While these peptides would have vastly reduced steroidogenic capability, it is conceivable that their renal and cardiovascular effects would be similar to ACTH 4-10.

Our data suggest that the ACTH 4-10 sequence may be responsible for the effects of ACTH and αMSH or βMSH on the kidney and thus may contribute to the hypertensive actions of ACTH. Recently, a new class of receptors that can bind substances containing the ACTH 4-10 sequence has been described. The presumed physiological ligand for these receptors is γMSH. This is a recently described fragment of proopiocortin, which contains an amino acid sequence analogous to ACTH 4-10 (Met-Gly-His-Phe-Arg-Trp-Asp). The γMSH receptor is not the classic MSH receptor since γMSH has only a fraction of the biological activity of αMSH in a melanophore assay. However, γMSH is two to three times more powerful than ACTH or αMSH for competition in this new class of receptors. showed that ACTH-induced potentiation of norpinephrine inotropism in canine atrial muscle was associated with specific binding of ACTH to the tissue. We have found that γMSH-containing peptides (such as γ3MSH, a didecapeptide) are 10 to 100 times more potent than ACTH 4-10 in cardiovascular and natriuresis studies (unpublished observations).

Placing aside consideration of the physiological significance of the ACTH 4-10 sequence, we believe our data clearly demonstrate a peptide that is natriuretic and has catecholamine-dependent cardiovascular effects. We have previously proposed in our work with a natriuretic factor and a volume-expansion-released pressor factor that NH-like compounds might well have cardiovascular effects at substantially higher doses than those needed for natriuresis. These properties can be demonstrated in this fragment.

In conclusion, the identification of a natriuretic and hypertensive fragment of ACTH provides a unique explanation for the renal and cardiovascular effects seen with chronic ACTH administration. The development of ACTH 4-10 analogs with enhanced peripheral actions may be the forerunners of a new class of drugs that regulate the renal and cardiovascular systems.

Acknowledgement
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