SUMMARY Extensive evidence reported here and elsewhere indicates a hormonal role for atrial natriuretic factor. In the light of this evidence, it appears that atrial natriuretic hormone is a more appropriate term for these peptides than atrial natriuretic factor. Plasma levels of immunoreactive atrial natriuretic hormone were measured daily in seven pigs before and 1 week after subcutaneous implantation of deoxycorticosterone acetate (DOCA). Nine other animals underwent daily measurements of mean arterial pressure and central venous pressure during similar treatments. Plasma immunoreactive atrial natriuretic hormone rose progressively during the first 3 days after implantation, from a basal level of 60 ± 9 pmol/L to a peak level of 159 ± 21 pmol/L (p < 0.05), and they remained significantly elevated throughout the rest of the 7-day observation period. In two animals that were restudied 6 weeks after DOCA implantation, plasma immunoreactive atrial natriuretic hormone had returned to preimplantation levels. The rise in plasma hormone levels after DOCA implantation closely paralleled the previously reported time course of mineralocorticoid escape. Whether atrial natriuretic hormone plays an important part in the escape phenomenon remains to be determined. (Hypertension 8 [Suppl II]: 11-16—11-20, 1986)

KEY WORDS • atrial natriuretic factor • mineralocorticoid hypertension • mineralocorticoid escape • experimental hypertension

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HEN mineralocorticoids are administered to animals and humans, there is a transient period of decreased urinary sodium excretion and positive sodium balance. Despite continued mineralocorticoid excess, urinary sodium excretion returns to initial levels, and a new steady state is achieved. This rise in urinary sodium excretion has been termed escape, and the mechanisms underlying the escape phenomenon have been the subject of much study.1

In previous studies we have characterized the changes in sodium and potassium metabolism that occur after the administration of deoxycorticosterone acetate (DOCA) to pigs.2-4 In this species, maximal sodium retention occurs within the first 24 hours, and escape is complete within 72 hours.

In the present study, we measured plasma levels of immunoreactive atrial natriuretic hormone (ANH) daily in pigs before and after subcutaneous implantation of DOCA. A consistent rise in immunoreactive ANH levels, paralleling the onset of escape, suggests that increased cardiac secretion of ANH may be an important mediator of escape in DOCA-treated pigs.

Methods

Sixteen male Yorkshire pigs weighing 39.3 ± 3.2 kg (mean ± SE) were studied. Seven pigs were studied for determination of plasma levels of immunoreactive ANH, sodium, and potassium and nine other pigs for sequential hemodynamic measurements. Instrumentation of animals for long-term study of hemodynamic changes was performed as previously described.5 Pigs were fed Purina Pig Chow (Ralston Purina, St. Louis, MO, USA) and given tap water ad libitum throughout the study. No excess salt was added to the diet. Strips of silicone rubber (Dow Corning, Midland, MI, USA) impregnated with DOCA (Sigma Chemical, St. Louis, MO, USA) were implanted subcutaneously with thiamyllal anesthesia in all 16 animals.2 Thiamylal was administered intravenously as a 4% solution to main-
tain a light surgical level of anesthesia. Daily direct recordings were made of mean arterial pressure and central venous pressure in the instrumented pigs.

Plasma samples were obtained daily through an indwelling arterial catheter. Samples for measurement of immunoreactive ANH were drawn into tubes of ethylenediaminetetraacetic acid on ice, centrifuged immediately at 4°C, separated, and stored at -70°C. Samples were extracted within 1 week of sampling, using C18 octadecylsilane cartridges (Sep-Pak, Waters Associates, Milford, MA, USA).

Plasma immunoreactive ANH was measured by radioimmunoassay. The method used was similar to that described for measurement of immunoreactive ANF in human plasma, with the exception that 5 ml of plasma was used for extraction. The assay uses atriopeptin III as a standard and antiserum directed against α-human atrial natriuretic polypeptide (1-28; both from Peninsula Laboratories, Belmont, CA, USA). Iodinated atriopeptin III was prepared with a chloramine-T procedure and purified by high-performance liquid chromatographic separation.

Antiserum, iodinated atriopeptin III, and unknown extract or standard were incubated overnight at 4°C, and bound and free hormone were separated with dextran charcoal.

Recovery of synthetic atriopeptin III added to pig plasma samples was 47.7 ± 4.4% (SE). Assay of serial dilutions of unknown pig plasma samples resulted in logit-log plots that were parallel to the standard curve (Figure 1). The slope of the standard curve was 2.64 × 10⁻¹² M⁻¹, and the mean slope for the unknown samples was 2.56 ± 0.13 × 10⁻¹² M⁻¹.

Two animals were maintained for 6 weeks after DOCA implantation. At the end of the 6-week period, plasma samples for measurement of immunoreactive ANH were obtained on 4 consecutive days.

Plasma sodium and potassium were measured with a National Instrument Laboratories flame photometer (IL443, Lexington, MA, USA).

Data are expressed as means ± SE. Immunoreactive ANH and hemodynamic measurements were evaluated by repeated measures analysis of variance. Daily measurements were compared with mean basal levels by a one-tailed Dunnett's test with an α level of 0.05. The mean basal sodium and potassium levels were compared with mean postimplantation levels by a paired t test. Daily levels were compared with mean basal levels by a paired t test with Bonferroni protection. The CLINFO system of the University of Michigan Clinical Research Center was used for data storage and analysis.

Results

Plasma immunoreactive ANH levels are shown in Figure 2. The mean level during the preimplantation period was 60 ± 9 pmol/L. After implantation there was a progressive rise in immunoreactive ANH during the first 3 days, reaching a peak of 159 ± 21 pmol/L. Levels remained elevated throughout the 7-day study period. Immunoreactive ANH levels rose in all 7 animals after implantation, and in each animal the peak level was at least double the basal level. The overall response was significant, as compared with basal levels (p < 0.05, analysis of variance), and the daily values from Day 3 to Day 7 were all significantly higher than basal levels.

Immunoreactive ANH levels returned to preimplan-
tation values after 6 weeks in the two pigs maintained for an extended period (Figure 3). This occurred despite the fact that hypertension persisted throughout the entire 6-week period in both animals.

The time course of changes in plasma sodium and potassium levels is shown in Figure 4. Sodium levels rose slightly during the first 2 days after implantation and remained elevated during the 7-day period. Although the increase in plasma sodium levels was small, the mean level during the postimplantation period was significantly higher than the mean preimplantation level (142.7 ± 0.9 vs 139.7 ± 1.4 mEq/L, p < 0.005 by paired t test). Plasma potassium levels fell throughout the 1-week postimplantation period. The decrease was significant, as compared with basal levels, by Day 4.

Mean arterial pressure and central venous pressure are shown in Figure 5. The rise in arterial pressure was significant by Day 2 after implantation, and it reached a plateau by Day 4. No significant changes were seen in central venous pressure throughout the study.

Discussion

Atrial natriuretic factor is the term that has been used most commonly to describe the natriuretic vasorelaxant peptides of cardiac origin. During the past year, extensive evidence has been reported that supports a hormonal role for these peptides. Circulating plasma levels have been measured in rats, humans, and pigs, and the circulating form of ANF in rat plasma has been described. Evidence of atrial secretion of ANF comes from both in vitro and in vivo studies. Several groups have reported increased ANF secretion in response to physiological stimuli and renal, vascular, and adrenal receptors have been described. Finally, Tikkanen et al. have shown that infusion of ANF in normal human subjects produces natriuretic effects, with plasma levels of ANF in the same range as those seen in patients with cardiac disease.

In view of this evidence, we agree with others that a hormonal role for these atrial peptides has been established. We believe that atrial natriuretic hormone is a more appropriate term for the peptides than atrial natriuretic factor.

ANH causes marked natriuresis and diuresis in animals and humans. The effect occurs within minutes of injection and persists for up to 1 hour with continued infusion. ANH induces increases in the glomerular filtration rate and filtration fraction, most likely through relaxation of the afferent arterioles. Although several studies have failed to show direct effects of ANH on epithelial transport, natriuresis may be effected by tubular as well as glomerular actions. Burnett et al. reported that ANH increases fractional reabsorption of lithium and phosphate, and Hammond et al. reported that ANH administration in vivo results in subsequent inhibition of sodium-coupled transport in proximal tubules in vitro.

The factors that regulate ANH secretion have not been fully determined, but several studies suggest that volume expansion is a major regulator of secretion. Dietz reported that isolated heart-lung preparations released bioassayable ANH in response to increased central venous pressure. Lang et al. reported that saline infusion in rats increased plasma ANH levels, and the rise was proportional to the increase in central venous pressure. We have reported that ANH levels are higher in normal subjects on a high sodium diet than in those on a low sodium diet and that patients with volume expansion due to cardiac failure also have high ANH levels. The increase in plasma ANH levels...
in the patients with congestive heart failure was closely correlated with the increase in right atrial pressure. Patients with volume expansion due to renal failure also have increased ANH levels.19 Manning et al. have recently reported that infusion of several pressor agents in rats causes an increase in plasma levels of ANH.14 Nonpressor analogues of vasopressin were without effect, and the rise in ANH was directly related to the increase in arterial pressure. Central venous pressure was not measured.

Mineralocorticoid escape has been described in rats, rabbits, dogs, pigs, sheep, and humans.1-4 It occurs 2 to 10 days after the start of mineralocorticoid administration. Although the mechanism of escape is not established, it is clear that it is not due to circulating antagonists of mineralocorticoids, since there is no escape from the sodium-retaining effects on the gastrointestinal tract or salivary or sweat glands.30-32 Micro-puncture studies have shown that the handling of sodium in the distal nephron is not altered during escape, suggesting a proximal site of action. Direct measurements of proximal sodium handling have not, however, shown consistent increases during escape.1 Glomerular filtration rates are increased during mineralocorticoid escape.33 Hall et al. have reported that when renal arterial pressure was held constant in aldosterone-treated dogs, escape did not occur.33 Since ANH appears to be ineffective in animals with renal artery clips, these observations are consistent with a role for ANH in mineralocorticoid escape (S. A. Atlas et al., personal communication, 1985).

The observation that plasma levels of ANH rise during the development of DOCA hypertension supports a role for ANH in mediating escape. The time course of the rise in ANH closely parallels the previously reported changes in urinary sodium excretion; escape is complete within 72 hours,2-4 just as plasma ANH levels are reaching their peak.

In addition to directly promoting natriuresis, increased secretion of ANH may contribute to the suppression of renin and aldosterone levels that occurs during the first week of mineralocorticoid administration. ANH directly suppresses aldosterone secretion in vitro and in vivo34-36 and plasma renin activity in vivo.36

It is also possible that ANH secretion limits DOCA-induced hypertension and that more severe elevations in blood pressure would occur in the absence of ANH. Examination of this possibility will probably have to await the development of specific ANH antagonists.

The return of plasma ANH to basal levels after 6 weeks of DOCA treatment suggests that ANH is not required for the long-term maintenance of sodium balance in a state of mineralocorticoid excess. A larger number of animals must be studied to verify this observation.

The stimulus for increased ANH secretion in DOCA hypertension is not apparent from these studies. Increased atrial pressure in response to sodium and water retention would be an attractive explanation, but no change in central venous pressure occurred in our pigs. It is possible that the increase in arterial pressure is a direct stimulus for ANH release.14 In addition, since mineralocorticoids produce rapid changes in membrane sodium fluxes,37 an altered transmembrane sodium gradient in atrial cells or in the central nervous system may initiate ANH secretion.

In summary, ANH levels rise during the first 3 days after DOCA treatment in pigs, and the rise parallels the onset of mineralocorticoid escape. ANH levels remain significantly elevated throughout the first week of treatment. In two pigs studied over a 6-week period, ANH levels returned to pretreatment levels despite the persistence of hypertension.

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