Are Hypertensive Effects of Aldosterone, Angiotensin, Vasopressin, and Norepinephrine Chronically Additive?

ALLEN W. COWLEY JR., MEREDITH M. SKELTON, AND DAVID C. MERRILL

SUMMARY The effects of chronic combined administration of angiotensin II, norepinephrine, aldosterone, and arginine vasopressin were compared with the response to each of these hormones administered alone. The studies were performed in dogs to determine the extent to which moderately inappropriate elevations of these hormones could enhance each other's ability to produce chronic hypertension and influence Na and water homeostasis. Blood pressure sensitivity to Na intake was also evaluated by infusing the hormones for 11 days at normal levels of Na intake followed by 11 days at high Na intake with ad libitum drinking. Combined hormone administration did not enhance each hormone's singular hypertensive actions. With aldosterone infusion alone and normal Na intake, mean arterial pressure rose nearly 15 mm Hg and an additional 3 mm Hg during high Na intake. Combined hormone infusion also resulted in a nearly 15 mm Hg rise during normal Na intake and an additional 3 mm Hg rise in mean arterial pressure during high Na intake. Marked Na retention and hypernatremia were observed with aldosterone infusion, while hyponatremia characterized arginine vasopressin infusion. The combined hormone infusion resulted in a tendency toward hypernatremia, although daily Na balance was not significantly changed. Daily water turnover was substantially increased and urine osmolality fell to hyposmotic levels, despite elevated arginine vasopressin levels. Even with high Na intake, dogs receiving either angiotensin II, arginine vasopressin, or norepinephrine at the same concentrations showed 4 to 10 mm Hg increases in mean arterial pressure. Thus, humoral summation or synergism of these hormones probably does not play a major role in the development of chronic hypertension. (Hypertension 8: 332–343, 1986)

KEY WORDS • aldosterone • vasopressin • hypertension • angiotensin • norepinephrine • prostaglandins • sodium balance • water balance • dogs • blood pressure

I NAPPROPRIATELY high levels of plasma renin activity, aldosterone (ALDO), catecholamines, and vasopressin have been observed in various subgroups of subjects with essential and other common forms of hypertension.1–7 The importance of these neuroendocrine changes remains largely uncertain, however, since reported plasma hormone levels generally have been less than those required to produce hypertension experimentally.

To examine the ability of these various neurohormonal systems to modify each other's chronic actions, the present study addressed two issues: 1) whether modest elevations in several of these hormones could summate or synergize each other's hypertensive actions and 2) whether simultaneous, mild, inappropriate elevations of the activity of these systems could alter each other's actions on renal function and thereby influence fluid and electrolyte balance. Thus, we compared the combined and singular effects of angiotensin II (ANG II), norepinephrine (NE), ALDO, and arginine vasopressin (AVP) administration in dogs. Doses were chosen that resulted in only a small, chronic rise of arterial pressure when each agent was administered alone. In addition, blood pressure sensitivity to Na intake was evaluated by infusing the hormones for 2 weeks during normal Na intake and for 2 weeks during high Na intake. Daily fluid and electrolyte balances
were determined, mean arterial pressure was monitored continuously, and plasma hormone levels were measured.

We hypothesized that the multiple actions of these hormones would enhance hypertension through additive or synergistic vascular effects and by disabling the potent physiological counterregulatory systems that normally offset pressure elevations and that simultaneous elevations of antidiuretic (AVP) and antinatriuretic substances (angiotensin, ALDO, NE) would result in a greater rise of pressure in response to increased Na intake (salt sensitivity) by a greater net retention of sodium and water. Although we have not systematically examined this hypothesis of humoral summation or synergism in long-term studies, we have studied the chronic interaction of two hormones. In those studies, marked hypertension (150 mm Hg) was produced in dogs by 2-week intravenous infusions of ANG II. A second hormone, either AVP or ALDO, was then infused concurrently for an additional 2 weeks; neither hormone induced a further rise of mean arterial pressure. Possible multiple interactions of other systems have not been evaluated, however, and no studies have evaluated small elevations of hormone levels commonly observed in human essential hypertension.

Materials and Methods

Studies were performed on 21 well-conditioned mongrel dogs weighing an average of 21 ± 1 kg. At least 10 days before the study began, indwelling catheters were placed surgically into the aorta distal to the renal artery through the femoral artery and into the inferior vena cava through the femoral vein, then tunneled subcutaneously to the subscapular region, where they emerged through the skin. Chronic indwelling urinary bladder catheters were implanted as described previously. At least 1 week was allowed for recovery from this operation. Dogs were treated postoperatively with 1.2 million units of penicillin G benzathine (Bicillin; Parke-Davis, Detroit, MI, USA) intramuscularly and subsequently were given ampicillin (H. Schien, Port Washington, NJ, USA), 250 to 500 mg/day, throughout the study.

The dogs were fitted with lightweight canvas harnesses to protect catheters, infusion lines, and arterial pressure transducers. A strong flexible tubing was brought out through the top of the pen and counterweighted with a pulley to maintain it in a vertical position. This tubing was attached to the harness to protect infusion lines and electrical cables passing to and from the dogs. A 12-hour light, 12-hour dark cycle and a constant temperature were maintained automatically. Dogs readily adapted to this environment; arterial pressure and heart rate were stabilized at resting levels within 24 to 48 hours. Dogs were removed from harness each week and taken to an outdoor pen for exercise. Bladders were drained immediately before the dogs were removed from the metabolic cages on these occasions.

Experimental Protocols

One week after operation, a 7-day prehormone infusion control period was begun during which the dogs were fed a daily diet of two cans of food containing 5 mEq of Na and 56 mEq of K (Prescription Diet, Canned H-D, Hills, Topeka, KS, USA). Daily Na intake was supplemented by a continuous intravenous infusion of hypertonic saline (250 mEq/L), 200 ml/day, which delivered an additional 45 to 55 mEq of Na per day for a total Na intake of 50 to 60 mEq/day. During this period, and throughout all of the experimental studies, deionized tap water was available ad libitum to the dogs. Infusion volumes and electrolyte concentrations of the infusate were measured daily and used to calculate the Na intake to determine daily electrolyte balance. Daily fluid and electrolyte balances were determined, and arterial blood pressure was recorded continuously (see Blood Pressure Measurement).

Infusions of the various hormones were begun at the end of the control period. All hormones were delivered by slow, continuous infusion using a Harvard syringe pump (Model 935; Millis, MA, USA) connected to the intravenous line through which the daily Na supplement, in a volume of 200 ml/day, was delivered by roller pump (Model MHRE, New Brunswick Scientific, Edison, NJ, USA). After 11 days of hormone infusion, daily Na intake was increased to 170 to 180 mEq/day by increasing the intravenous rate of delivery to 600 ml/day for another 11 days; levels of hormone administered were unchanged.

The four hormones infused in this study were NE HCl (Aldrich Chemicals, Milwaukee, WI, USA), 0.26 µg/kg/min; ANG II (Ciba Pharmaceutical, Summit, NJ, USA), 0.5 ng/kg/min; AVP (Sigma Chemical, St. Louis, MO, USA), 0.26 ng/kg/min; and ALDO (Ciba), 8.6 ng/kg/min. All syringes and lines were wrapped with aluminum foil to eliminate photooxidation of NE during delivery. Fresh solutions of all hormones were made daily.

The effects of the following infusions were studied: ALDO infusion in five dogs during normal and high Na intake; AVP infusion during normal Na intake in six dogs, four of which were also studied during high Na intake; combined infusion of ALDO, ANG II, and AVP in five dogs during normal Na intake; and combined infusion of ALDO, ANG II, AVP, and NE in five dogs during normal and high Na intake.

Collection of Blood Samples and Urinary Balance Determination

Blood samples (5 ml) for measurement of plasma Na, K, osmolality, and hematocrit were collected each morning before feeding (0900–1000) during the final 3 days of the control period, throughout the hormone infusion period, and for 3 days after hormone infusion ended. Additional blood (12 ml) was collected during each of the control and postcontrol days and every second or third day during the prolonged hormone infusion period for determination of plasma levels of
ALDO, AVP, NE, epinephrine, and plasma renin activity.

To measure volume, Na and K concentration, and osmolality, 24-hour urine samples were collected daily throughout the study. Dogs were kept in stainless steel metabolic cages designed to our specifications (Suburban Surgical Corporation, Wheeling, IL, USA), which enable a highly efficient recovery (90-95%). Urine was collected in a 4-L Erlenmeyer flask under the stainless steel collection tray. The bladder was drained into this flask at the same time each day. The cage and collection tray were then rinsed with 1 L of distilled water that was also collected and analyzed for Na and K (residue). This residue generally averaged 1.0 to 2.0 mEq/day of Na or K during normal Na intake and 4 to 6 mEq/day during high Na intake. The value of the residue was added to the calculated Na excretion to obtain total Na excretion.

On those days that urine prostaglandin E	extsubscript{2} level was determined, the 4-L collection flasks were placed in Styrofoam coolers containing a dry ice mixture. This precaution ensured a rapid freezing of the urine during the 24-hour collection period. These urine samples were thawed quickly, and aliquots were transferred to tubes containing 100 μg of meclofenamate.

The Na and K balances were calculated as the total amount of electrolyte given each day minus the amount excreted each day in the urine. The daily balances during the hormone infusion period were expressed as a change from the average of the 3 preinfusion control days, which was defined as the zero balance. Cumulative Na balance was calculated by adding the normalized net balance figures across either the normal or high Na period.

**Blood Pressure Measurement**

Mean arterial blood pressure was measured continuously from the last 3 days of the control period until 3 days after the hormone infusion ended, using transducers built in our laboratory with commercially available miniature piezoelectric solid state transducers (Micro Switch PC130, Honeywell, Freeport, IL, USA) interfaced with electronics of our own design. The output signals from the amplifiers were distributed through digital converters for data handling by a dedicated PDP 11/03 computer that was periodically interrogated by a remotely located PDP 11/44 computer (Digital Equipment Corporation, Maynard, MA, USA) interfaced with electronics of our own design. The data were stored on a computer disk for editing and reduction by batch processing, which provided hourly averages, statistics, and graphics, as described previously. Analog output signals of arterial pressure were also recorded on a Grass recorder (Model 7; Grass Instruments, Quincy, MA, USA) using a multiplex system of our own design, which allows three signals from a single pen to be displayed on a single paper channel.

**Chemical and Radioimmunoassay Analysis**

Plasma and urine Na and K concentrations were determined by standard flame photometry (IL 443, Instrumentation Laboratory, Boston, MA, USA). Urine osmolality was measured by freezing point depression (Osметte A, Precision Systems, Boston, MA, USA). Plasma osmolality was measured by vapor pressure osmometry (Model 5000C; Wescor, Logan, UT, USA). Samples were run routinely in triplicate to ensure results within plus or minus 0.4%. Using verified standards, frequent calibration checks were made during each measurement period to establish and subsequently confirm the slope and linearity of the calibration.

Plasma AVP concentration was determined using antiserum and radioimmunoassay procedures developed in this laboratory and described previously. Plasma renin activity was analyzed by the method of Sealey et al. using angiotensin I antisera provided by Dr. Jean Sealey. Plasma ALDO was extracted with dichloromethane and radioimmunoassayed using a highly specific antiserum and I	extsuperscript{125}I-labeled ALDO (Diagnostix Products Biochemistry, London, Ontario, Canada). Dextran-coated charcoal was used to separate the free and bound antigen. Urine prostaglandin E	extsubscript{2} was analyzed by radioimmunoassay using the techniques of Dray et al. as modified by Roman et al. Plasma ANG II concentration was determined by Dr. Ian Reid at the University of California (San Francisco, CA, USA), using a sensitive radioimmunoassay procedure. Plasma epinephrine and norepinephrine were determined by radioenzymatic assay (Upjohn, Kalamazoo, MI, USA).

**Statistical Analysis**

Values are expressed as means ± standard error (SE). A two-way analysis of variance followed by a Dunnett's t test for significance was performed for within-group comparisons. An unpaired Student's t test was used for between-group steady state comparisons. A p level less than 0.05 was considered statistically significant.

**Results**

Results of NE and ANG II given alone under similar conditions of Na and water intake have been reported previously and will not be repeated here.

**Aldosterone Infusion**

Average daily mean arterial pressure and plasma hormone levels associated with ALDO infusion are summarized in Figure 1. Mean arterial pressure increased gradually from 95 ± 9 to 110 mm Hg during the final 5 days of normal Na intake. Mean arterial pressure tended to rise another 2 to 3 mm Hg during high Na intake, but this small increase was not significantly different from that observed during the normal Na intake.

Plasma ALDO levels averaged 4.3 ± 1.4 ng/dl during the final 3 control days and increased to approximately 35 ng/dl during the hormone infusion period. Plasma renin activity averaged 1.6 ± 0.3 ng angiotensin I/ml/hr during the control period and decreased to 0.1 to 0.3 ng angiotensin I/ml/hr throughout the ALDO
infusion. Control levels of plasma AVP averaged 2.8 ± 0.4 pg/ml and were unchanged during the 11-day ALDO infusion at a normal Na intake. Small but statistically significant increases reaching 5.3 pg/ml were observed during high Na intake and returned to control values after cessation of ALDO infusion. Plasma NE and epinephrine levels were statistically unchanged throughout the control and experimental periods.

Urinary 24-hour excretion of prostaglandin E2 was determined in six dogs during the final day of the control and ALDO infusion periods at both normal and high Na intake. Control excretion rates averaged 428 ± 76 ng/day and increased with ALDO infusion to 1252 ± 373 ng/day during normal Na intake and to 1813 ± 629 ng/day during high Na intake (p < 0.05).

Figure 2 summarizes the observed changes in daily plasma Na, plasma K, Na excretion and balance, daily water intake and excretion, and urine osmolality during ALDO infusion. During the first day of ALDO infusion, plasma Na concentration rose 2.9 mEq/L above a control value of 141 ± 0.3 mEq/L and continued to increase to and be maintained at levels ranging from 6 to 9 mEq/L above control value during both the normal and high Na periods. Plasma K levels decreased progressively during ALDO infusion from control levels of 4.9 ± 0.1 to 3.7 ± 0.2 mEq/L by the end of the normal Na period and to 3.4 ± 0.3 mEq/L during high Na intake. Plasma osmolality tended to increase slightly throughout the Na infusion periods, but this change was not significant.

Figure 1. Mean arterial pressure (MAP) and plasma hormone levels during control period; 11-day aldosterone (ALDO) infusion with normal Na intake. 11-day ALDO infusion with high Na intake; and 3 days following ALDO infusion at normal Na intake. Shown are mean ± SE plasma concentrations of ALDO (P_{ALDO}), renin activity (PRA), arginine vasopressin (P_{AVP}), norepinephrine (P_{NE}), and daily urinary excretion of prostaglandin E2 (U_{PGE2}). Al = angiotensin I. Infused substances indicated by crosshatched bar graphs. Asterisks indicate significant difference (p < 0.05) compared with control values.

Figure 2. Plasma and urine electrolyte changes induced by continuous aldosterone (ALDO) infusion as described in Figure 1. Shown are the mean ± SE plasma Na (P_{Na}) and potassium (P_{K}) concentrations, daily Na excretion, normalized daily Na balance (NORM Na), daily total water intake (H2O IN) from food and drink, daily ad libitum drinking (DRINK), daily urine flow, and urine osmolality (U_{OSM}). Asterisks indicate significant difference compared with control values (p < 0.05).
By the end of the third day after hormone infusion ended, all electrolyte values had returned to levels that were not statistically different from the control level. Hematocrit remained within plus or minus 1% of the average control value throughout the normal and high Na infusion periods.

Excretion of Na decreased significantly the first day of ALDO infusion but returned to control levels by Day 3. During the period of high Na infusion, Na excretion increased significantly as expected. A significant retention of Na occurred during the first 2 days of ALDO infusion, totaling 48 mEq, and was followed by escape and achievement of Na balance. The cumulative Na balance during this period was 9 ± 35 mEq by Day 11. A further significant retention of Na occurred during the first 2 days of the high Na intake period, totaling 76 mEq, and again was followed by achievement of Na balance throughout most of the remaining ALDO infusion period. By Day 11 of high Na intake, cumulative Na balance was 169 ± 67 mEq. The ALDO infusion had no significant effect on K excretion.

Total water intake and urine flow increased progressively throughout ALDO infusion during normal Na intake, and this trend reached statistical significance by Day 11. Ad libitum drinking, which averaged 274 ± 93 ml/day during the 3 control preinfusion days, increased to 800 ± 270 ml/day by Day 7 and 1300 ± 530 ml/day (p < 0.05) by Day 11 of ALDO infusion during normal Na intake. Elevation of Na intake was associated with a significant rise of total water intake, to a maximum of 3781 ± 1017 ml/day on Day 7 of high Na intake, and a corresponding increase in ad libitum drinking, to a maximum of 3143 ± 1016 ml/day. Parallel changes were observed in daily urine excretion, which averaged 534 ± 101 ml/day during the final 3 days of the control period and increased progressively to 1551 ± 474 ml/day by Day 11 of normal Na intake. By Day 11 of high Na intake with ALDO infusion, urine excretion averaged 2630 ± 780 ml/day. Urine osmolality decreased gradually from a control value of 740 ± 107 to 481 ± 112 mosm/kg by Day 9 of normal Na with ALDO infusion. It then remained decreased significantly during the remaining period of normal Na intake and throughout high Na intake despite the fact that AVP levels were unchanged or slightly increased.

Arginine Vasopressin Infusion

Average daily mean arterial pressure and plasma hormone levels resulting from the continuous infusion of AVP alone at normal and elevated levels of Na intake are summarized in Figure 3. Only four of the six dogs were studied during both normal and high levels of Na intake. Although mean arterial pressure tended to rise during AVP infusion with normal and high Na intake, these increases were not significant.

Infusion of AVP increased plasma AVP concentration from a control value of 2.8 ± 0.3 pg/ml to approximately 13 pg/ml during normal Na intake. These concentrations were uninfluenced by the increased level of Na intake and returned to control levels following AVP infusion. Infusion of AVP had no significant effect on plasma ALDO concentration. Plasma renin activity decreased significantly during AVP infusion (control, 1.6 ± 0.5; normal Na intake, 0.3-0.5; high Na intake, <0.3 ng angiotensin I/ml/hr) and returned toward control values after the AVP infusion ended. Plasma NE level decreased from a control value of 312 ± 49 pg/ml to 168 ± 40 pg/ml on Day 4 of normal Na intake and tended to stay at levels that were nearly 50% of control throughout the high Na intake period. Plasma epinephrine concentration averaged 95 ± 17 pg/ml during the control period and duplicated the pattern of plasma NE concentration. By the third day
after the infusion, all plasma hormone concentrations had nearly returned to control levels.

Urinary prostaglandin \( E_2 \) excretion was determined in four dogs with 24-hour urine samples collected during the final day of the control and post control periods and the final day of AVP infusion during normal and high Na intake. Significant increases occurred during AVP infusion (control, \( 2013 \pm 340 \); normal Na intake, \( 5109 \pm 270 \); high Na intake, \( 4126 \pm 500 \) ng/day).

Figure 4 summarizes the daily plasma Na, plasma K, Na excretion and balance, daily water intake and excretion, and urine osmolality during AVP infusion. Plasma Na concentration decreased significantly with AVP infusion from control levels of \( 144 \pm 0.7 \) mEq/L to levels averaging \( 125 \) mEq/L by the end of the normal Na intake period and remained at these levels throughout the high Na intake period. Plasma K level tended to decrease 0.1 to 0.2 mEq/L during both periods of AVP infusion, but this decrease was significant on only a few of the days (see Figure 4). Plasma osmolality decreased to \( 274 \pm 4 \) mosm/kg by Day 4 of AVP infusion with normal Na intake from a control value of \( 292 \pm 1 \) mosm/kg and continued to fall during high Na intake (average, \( 254-258 \) mosm/kg during the last 5 days). Hematocrit decreased significantly to \( 28 \pm 2\% \) by Day 7 of normal Na intake from control levels of \( 35 \pm 1 \) % and remained at similar levels throughout the period of high Na intake. These values returned to control levels 1 day after the AVP and high Na infusion ended.

Excretion of Na remained unchanged throughout AVP infusion at normal Na intake and increased as expected with high Na intake. Neither net Na balance nor K excretion changed significantly during AVP infusion. Total water intake decreased significantly to \( 852 \pm 32 \) ml/day from a control level of \( 955 \pm 48 \) ml/day by Day 2 of AVP infusion with normal Na intake because of a nearly complete cessation of drinking, which extended throughout both the normal and high Na intake periods. This decreased drinking did not greatly influence total water intake, since dogs continued daily to receive \( 600 \) ml of fluid in the form of canned food and another \( 200 \) and \( 600 \) ml by intravenous infusion during normal and high Na intake, respectively. With high Na intake, total water intake tended to rise above control levels as a result of the higher intravenous infusion rate and ad libitum drinking remained suppressed. Daily urine excretion decreased significantly by the second day of AVP infusion but thereafter was not significantly different from control. Daily urine excretion tended to increase approximately \( 300 \) ml/day during high Na intake. Urine osmolality increased significantly from a control value of \( 631 \pm 27 \) mosm/kg to levels of \( 1377 \) mosm/kg with AVP infusion but after Day 7 was not significantly different from control even during high Na intake.

**Combined Hormone Infusion**

Figure 5 summarizes the average daily mean arterial pressure and plasma hormone levels achieved during simultaneous infusion of ALDO, ANG II, AVP, and NE at both levels of Na intake. Mean arterial pressure increased by nearly the same amount as was observed with infusion of ALDO alone. From an average 3-day control value of \( 106 \pm 2 \) mm Hg, arterial pressure increased 10 to 11 mm Hg during normal Na intake and 14 to 15 mm Hg above control during high Na intake. This rise in pressure was significantly greater but only about 2 mm Hg higher than that achieved with ALDO infusion alone.

Plasma ALDO levels increased significantly during combined hormone infusion to 15 to 16 ng/dl from control levels of \( 3.2 \pm 0.5 \) ng/dl and were not influenced measurably by Na intake. These plasma ALDO levels were statistically less than those achieved when only ALDO was infused (see Figure 1). Plasma renin
activity decreased significantly (control, 2.6 ± 0.2; combined infusion, <0.3 ng angiotensin I/ml/hr). Plasma ANG II level did not change significantly during normal Na intake. Analysis of samples for ANG II during high Na intake was not performed. Plasma AVP levels increased 5 to 7 pg/ml from control levels of 3.2 ± 0.4 pg/ml during both normal and high Na intake periods. As seen with ALDO infusion, plasma AVP concentration was significantly less than that during infusion of AVP alone (see Figure 2). Plasma NE increased from control levels of 323 ± 46 pg/ml to concentrations reaching 2100 pg/ml throughout the hormone infusion periods. Plasma epinephrine level decreased significantly (control, 189 ± 35; combined infusion, 100 pg/ml) during the initial days of normal and high Na intake; however, all values returned to control levels 3 days after infusion with the exception of plasma renin activity, which remained significantly below control levels. Urinary prostaglandin E_2 excretion rates averaged 2121 ± 368 ng/day during the high Na hormone infusion period and were significantly greater than those observed on the third postinfusion day (996 ± 116 ng/day).

Figure 6 summarizes the daily plasma Na, plasma K, Na excretion and balance, daily water intake and excretion, and urine osmolality during the combined hormone infusion. Although plasma Na concentration increased during normal Na intake, it was significantly elevated only on Day 6. During high Na intake, plasma Na gradually returned toward control levels. Plasma K level fell somewhat less than that observed during ALDO infusion alone, decreasing nearly 0.5 and 1.0 mEq/L during normal and high Na intake, respectively. Plasma osmolality increased gradually, from a control value of 290 ± 1 mosm/kg to 298 mosm/kg, during the last 5 days of normal Na infusion and then tended to fall toward control values during high Na intake in parallel with changes in plasma Na levels. Hematocrit was decreased 3 to 4% during both normal and high Na infusion but did not reach statistical significance.

No significant changes were seen in daily Na excretion during hormone infusion at normal levels of Na intake. Excretion increased to the expected range during periods of high Na intake. There was no trend or significant changes in daily Na balance during the two periods of salt intake. The cumulative amount of Na retained during combined hormone infusion did not differ significantly from that observed with infusions of ALDO or AVP alone. Excretion of K averaged 49 ± 2 mEq/day during the control period, 44 ± 6 mEq/day on Day 11 of normal Na intake, and 48 ± 2 mEq/day during high Na intake. Total water intake, drinking, and urine flow increased gradually during combined hormone infusion with normal Na intake, reaching significance after the seventh day of hormone infusion. Drinking increased from control levels of 450 ± 117 ml/day to 1740 ± 376 ml/day by the end of the normal Na intake period. During high Na intake, total water intake increased to a maximum of 4700 ± 1080 ml/day on Day 5, at which time drinking had increased to 3500 ± 990 ml/day. Parallel increases were observed in daily urine excretion rates. Urine osmolality averaged 363 mosm/kg during the last 3 days of normal Na infusion (control, 525 ± 72 mosm/kg), fell further during high Na infusion (reaching hypoosmotic levels of 275 mosm/kg during the final 3 days of infusion; p < 0.05), and returned toward control values 2 days after infusion.
Combined Hormone Infusion Without Norepinephrine

A separate group of five dogs was evaluated before other experiments were performed. In this group, three hormones (ALDO, ANG II, and AVP) were infused simultaneously for 11 days during normal Na intake (55 mEq/day). Mean arterial pressure averaged 101 ± 2 mm Hg during the 3 control days, 115 ± 2 mm Hg by the end of the first week of hormone infusion, and 120 ± 3 mm Hg by the end of the second week of infusion. Although higher, these values were not significantly different from those achieved when NE was included in the combined hormone infusion. Plasma ALDO level increased from 4.4 ± 2.0 ng/dl to levels ranging from 13 to 21 ng/dl during hormone infusion; this increase was similar to the changes seen with the four-hormone infusion (see Figure 5) but less than those seen with ALDO infusion alone. Plasma AVP concentration increased from 4.4 ± 0.6 pg/ml to 18 pg/ml, which was similar to levels obtained with infusion of AVP alone but significantly greater than those observed with the four-hormone infusion (see Figure 5). Plasma NE levels averaged 285 ± 50 pg/ml and tended to decrease, although not significantly, to 163 ± 12 pg/ml during combined hormone infusions. Plasma renin activity was suppressed to less than 0.3 ng angiotensin I/ml/hr. (Plasma ANG II concentration was not determined in these dogs.)

Daily Na excretion was not altered significantly throughout the three-hormone infusion period. Plasma Na level increased gradually but not significantly during the final 5 days of hormone infusion. Plasma K concentration decreased significantly from 4.2 ± 0.1 to 3.2 mEq/L during the final week of hormone infusion. Hematocrit averaged 33.4 ± 0.9% during control and gradually declined to 26 ± 1.5% during the final 5 days of infusion.

Discussion

The present results indicate that mild, inappropriate overactivity of four major neuroendocrine systems known to be involved in arterial pressure regulation under chronic conditions does not result in a summation or a synergism of the hypertensive actions of these hormones. The combinations of hormones studied represented near-threshold hypertensive amounts when administered alone to simulate conditions likely to be observed in persons with moderate hypertension. The doses administered resulted in elevated plasma levels of AVP, ALDO, and NE, but not ANG II because of the offsetting fall of renin activity. However, since plasma ANG II levels normally would be suppressed by administration of ALDO and AVP, the "normal" ANG II levels seen in these studies should be considered inappropriately elevated.

Each of the four hormones has now been infused chronically alone with ad libitum water intake and at similar levels of Na intake in either the present (ALDO and AVP) or previous studies (ANG II and NE). Figure 7 summarizes the average arterial pressure responses to each of these hormones infused alone and in combination. Interestingly, combined hormone administration did not elevate arterial pressure more than did ALDO infusion alone. Elevation of plasma NE levels also did not alter arterial pressure when added to the combined infusion of the other vasoconstrictor and salt-retaining hormones.

Since hormonal levels maintained constant by in-
All results and statistics were reported in earlier studies (p < 0.05).

Figure 7. Influence of chronic infusions of norepinephrine (NE), angiotensin II (All), arginine vasopressin (AVP), aldosterone (ALDO), and the combination of these substances (COMBO) on the change in mean arterial pressure (ΔMAP) at normal and elevated levels of Na intake. Control and postinfusion control data at normal Na intake are included. The NE and All results and statistics were reported in earlier studies (p < 0.05).

Values for ALDO, AVP, and COMBO are expressed as means ± SE. Significant changes from baseline values are indicated by asterisks (p < 0.05).

Elevations of ALDO in dogs appear to have less effect than does angiotensin on the salt-sensitive nature of the hypertension (see Figure 1). Small increases of pressure in high ALDO states with large increases of Na intake have also been observed by Lohmeier et al. who reported that arterial pressure increased by only 6 mm Hg when Na intake was increased from 75 to 190 mEq/day during a 2-week intravenous administration of excess ALDO.

It is possible that the observed stimulation of renal prostaglandin E2 secretion offset the expected salt-sensitive nature of the hypertension. We have observed previously that chronic intrarenal infusion of prostaglandin E2 tends to produce a natriuresis despite chronic elevations of plasma renin activity and ALDO. Alternatively, the elevated levels of AVP may also have had natriuretic consequences, since elevations of plasma AVP with volume expansion lead to increased Na excretion.

The observed changes in arterial pressure would not have been predicted based on observations of the acute pressor actions of these hormones. Ishikawa et al. have recently reported that, in conscious rats, subpressor doses of NE, ANG II, or vasopressin potentiate the blood pressure response to a pressor dose of any of the other vasoactive hormones. Furthermore, combined subpressor doses of two of these three hormones resulted in a significant rise in blood pressure, even though each hormone by itself did not alter the pressure. Previous observations of mild synergism between AVP and catecholamines have also been observed in anesthetized dogs, rats, and cats. Subpressor doses of ANG II have also been reported to potentiate the pressor effect of NE in normal animals.

In contrast, angiotensin and isoproterenol in mildly pressor amounts recently were reported to decrease the pressor responses to AVP in conscious rats.

The second unpredictable result of these studies was the lack of mutual enhancement of endocrine activity on Na retention. The combined infusion of ALDO, ANG II, NE, and AVP resulted in no measurable change in daily Na balance and an insignificant rise in plasma Na level. This effect occurred even though plasma ALDO was elevated to levels observed with chronic Na depletion and plasma ANG II remained at normal levels.

Elevations of plasma AVP and mild volume expansion could explain the lack of Na retention despite elevations of the Na-retaining hormones. Nearly 500 ml of fluid retention occurred in dogs infused chronically with the same amount of AVP used in the current

Blood pressure responses to increased Na intake during combined hormone infusion were less than we expected. Based on previous studies, in which large amounts of these substances were infused individually, humoral summation or synergism was expected to enhance the blood pressure response to Na intake in these animals. Specifically, the inability of the renin-angiotensin system to respond to changes in Na intake has been shown to enhance the salt sensitivity of arterial pressure. Angiotensin, in particular, when infused at high, excessive levels or held at constant, low levels with captopril, greatly enhanced chronic arterial pressure responsiveness to Na intake. In the present studies, this effect was not apparent when ANG II was given in combination with the other hormones. Elevations of ALDO in dogs appear to have less effect than does angiotensin on the salt-sensitive nature of the hypertension (see Figure 1). Small increases of pressure in high ALDO states with large increases of Na intake have also been observed by Lohmeier et al. who reported that arterial pressure increased by only 6 mm Hg when Na intake was increased from 75 to 190 mEq/day during a 2-week intravenous administration of excess ALDO.

It is possible that the observed stimulation of renal prostaglandin E2 secretion offset the expected salt-sensitive nature of the hypertension. We have observed previously that chronic intrarenal infusion of prostaglandin E2 tends to produce a natriuresis despite chronic elevations of plasma renin activity and ALDO. Alternatively, the elevated levels of AVP may also have had natriuretic consequences, since elevations of plasma AVP with volume expansion lead to increased Na excretion.

The observed changes in arterial pressure would not have been predicted based on observations of the acute pressor actions of these hormones. Ishikawa et al. have recently reported that, in conscious rats, subpressor doses of NE, ANG II, or vasopressin potentiate the blood pressure response to a pressor dose of any of the other vasoactive hormones. Furthermore, combined subpressor doses of two of these three hormones resulted in a significant rise in blood pressure, even though each hormone by itself did not alter the pressure. Previous observations of mild synergism between AVP and catecholamines have also been observed in anesthetized dogs, rats, and cats. Subpressor doses of ANG II have also been reported to potentiate the pressor effect of NE in normal animals. In contrast, angiotensin and isoproterenol in mildly pressor amounts recently were reported to decrease the pressor responses to AVP in conscious rats.

The second unpredictable result of these studies was the lack of mutual enhancement of endocrine activity on Na retention. The combined infusion of ALDO, ANG II, NE, and AVP resulted in no measurable change in daily Na balance and an insignificant rise in plasma Na level. This effect occurred even though plasma ALDO was elevated to levels observed with chronic Na depletion and plasma ANG II remained at normal levels.

Elevations of plasma AVP and mild volume expansion could explain the lack of Na retention despite elevations of the Na-retaining hormones. Nearly 500 ml of fluid retention occurred in dogs infused chronically with the same amount of AVP used in the current

Blood pressure responses to increased Na intake during combined hormone infusion were less than we expected. Based on previous studies, in which large amounts of these substances were infused individually, humoral summation or synergism was expected to enhance the blood pressure response to Na intake in these animals. Specifically, the inability of the renin-angiotensin system to respond to changes in Na intake has been shown to enhance the salt sensitivity of arterial pressure. Angiotensin, in particular, when infused at high, excessive levels or held at constant, low levels with captopril, greatly enhanced chronic arterial pressure responsiveness to Na intake. In the present studies, this effect was not apparent when ANG II was given in combination with the other hormones. Elevations of ALDO in dogs appear to have less effect than does angiotensin on the salt-sensitive nature of the hypertension (see Figure 1). Small increases of pressure in high ALDO states with large increases of Na intake have also been observed by Lohmeier et al. who reported that arterial pressure increased by only 6 mm Hg when Na intake was increased from 75 to 190 mEq/day during a 2-week intravenous administration of excess ALDO.

It is possible that the observed stimulation of renal prostaglandin E2 secretion offset the expected salt-sensitive nature of the hypertension. We have observed previously that chronic intrarenal infusion of prostaglandin E2 tends to produce a natriuresis despite chronic elevations of plasma renin activity and ALDO. Alternatively, the elevated levels of AVP may also have had natriuretic consequences, since elevations of plasma AVP with volume expansion lead to increased Na excretion.

The observed changes in arterial pressure would not have been predicted based on observations of the acute pressor actions of these hormones. Ishikawa et al. have recently reported that, in conscious rats, subpressor doses of NE, ANG II, or vasopressin potentiate the blood pressure response to a pressor dose of any of the other vasoactive hormones. Furthermore, combined subpressor doses of two of these three hormones resulted in a significant rise in blood pressure, even though each hormone by itself did not alter the pressure. Previous observations of mild synergism between AVP and catecholamines have also been observed in anesthetized dogs, rats, and cats. Subpressor doses of ANG II have also been reported to potentiate the pressor effect of NE in normal animals. In contrast, angiotensin and isoproterenol in mildly pressor amounts recently were reported to decrease the pressor responses to AVP in conscious rats.

The second unpredictable result of these studies was the lack of mutual enhancement of endocrine activity on Na retention. The combined infusion of ALDO, ANG II, NE, and AVP resulted in no measurable change in daily Na balance and an insignificant rise in plasma Na level. This effect occurred even though plasma ALDO was elevated to levels observed with chronic Na depletion and plasma ANG II remained at normal levels.

Elevations of plasma AVP and mild volume expansion could explain the lack of Na retention despite elevations of the Na-retaining hormones. Nearly 500 ml of fluid retention occurred in dogs infused chronically with the same amount of AVP used in the current

Blood pressure responses to increased Na intake during combined hormone infusion were less than we expected. Based on previous studies, in which large amounts of these substances were infused individually, humoral summation or synergism was expected to enhance the blood pressure response to Na intake in these animals. Specifically, the inability of the renin-angiotensin system to respond to changes in Na intake has been shown to enhance the salt sensitivity of arterial pressure. Angiotensin, in particular, when infused at high, excessive levels or held at constant, low levels with captopril, greatly enhanced chronic arterial pressure responsiveness to Na intake. In the present studies, this effect was not apparent when ANG II was given in combination with the other hormones. Elevations of ALDO in dogs appear to have less effect than does angiotensin on the salt-sensitive nature of the hypertension (see Figure 1). Small increases of pressure in high ALDO states with large increases of Na intake have also been observed by Lohmeier et al. who reported that arterial pressure increased by only 6 mm Hg when Na intake was increased from 75 to 190 mEq/day during a 2-week intravenous administration of excess ALDO.

It is possible that the observed stimulation of renal prostaglandin E2 secretion offset the expected salt-sensitive nature of the hypertension. We have observed previously that chronic intrarenal infusion of prostaglandin E2 tends to produce a natriuresis despite chronic elevations of plasma renin activity and ALDO. Alternatively, the elevated levels of AVP may also have had natriuretic consequences, since elevations of plasma AVP with volume expansion lead to increased Na excretion.

The observed changes in arterial pressure would not have been predicted based on observations of the acute pressor actions of these hormones. Ishikawa et al. have recently reported that, in conscious rats, subpressor doses of NE, ANG II, or vasopressin potentiate the blood pressure response to a pressor dose of any of the other vasoactive hormones. Furthermore, combined subpressor doses of two of these three hormones resulted in a significant rise in blood pressure, even though each hormone by itself did not alter the pressure. Previous observations of mild synergism between AVP and catecholamines have also been observed in anesthetized dogs, rats, and cats. Subpressor doses of ANG II have also been reported to potentiate the pressor effect of NE in normal animals. In contrast, angiotensin and isoproterenol in mildly pressor amounts recently were reported to decrease the pressor responses to AVP in conscious rats.

The second unpredictable result of these studies was the lack of mutual enhancement of endocrine activity on Na retention. The combined infusion of ALDO, ANG II, NE, and AVP resulted in no measurable change in daily Na balance and an insignificant rise in plasma Na level. This effect occurred even though plasma ALDO was elevated to levels observed with chronic Na depletion and plasma ANG II remained at normal levels.

Elevations of plasma AVP and mild volume expansion could explain the lack of Na retention despite elevations of the Na-retaining hormones. Nearly 500 ml of fluid retention occurred in dogs infused chronically with the same amount of AVP used in the current
study and permitted to drink ad libitum. This finding conforms with the 3 to 4% fall in hematocrit seen in the present studies with combined hormone infusion. We previously reported that the hyponatremic effects of AVP do not occur when fluid expansion is prevented by use of chronic servocontrolled volume techniques. In the present study, the combined hormone infusion was clearly associated with increased prostaglandin E₂ excretion, which could have led to natriuretic actions.

Differences in plasma hormone levels achieved during combined infusions may account for some of the apparent lack of mutual hormone enhancement observed in present study (see Figure 6). Both plasma AVP and ALDO levels were lower in the group of dogs infused with combined hormones, despite the same rates of infusion. The reasons for this finding are unclear but could be related to the elevation of plasma NE, since the combined hormone infusion exclusive of NE yielded plasma AVP and ALDO levels similar to those obtained with infusion of AVP or ALDO alone. A reduction in AVP release has been reported with infusions of both NE and clonidine. Enhanced clearance of AVP and ALDO could also account for such differences. Based on previous studies, however, it is unlikely that significant differences in chronic arterial pressure levels would be expected from the differences in plasma ALDO or AVP observed in these studies. When increased by infusion to levels of 15 or 30 ng/dl, plasma ALDO produced no significant differences in levels of hypertension. Similarly, an increase in plasma AVP level from 8 to 12 pg/ml has not been shown to have a measurable influence on arterial pressure, even in dogs with total autonomic blockade.

Finally, the absence of sodium retention could be explained by the simultaneous increase in the activity of the combined hormones. This increase could influence glomerular filtration and tubular reabsorption in opposing manners, since small elevations of ANG II and AVP have been shown to elevate glomerular filtration rates and decrease tubular Na reabsorption by ANG II and ALDO.

Enhancement of water turnover was unexpectedly observed during the combined hormone infusion including NE despite substantial elevations of plasma AVP level (see Figure 6). This finding could be explained by the 5 mEq/L increase of plasma Na level and the osmotic stimulation of thirst, although these increases were statistically significant only on a few days. Interestingly, this exaggerated increase in water turnover was not observed in the group of dogs in which NE was excluded from the combined hormone infusion. Since β-adrenergic agonists have been reported to stimulate water intake while α-adrenergic agonists appear to have opposite effects, it would have been difficult to predict the outcome of these factors in the present studies. These data suggest either an upward resetting of the threshold or a blunting of the sensitivity of the osmocontrol system with infusion of ALDO or the combined hormones.

Subjects with essential and other common forms of hypertension often exhibit inappropriately elevated plasma or urinary concentrations of vasoconstrictor agents and antinatriuretic or antidiuretic substances. The most extensively characterized of these agents have been the components of the renin-angiotensin-aldosterone system, the autonomic nervous system, and vasopressin. Under various conditions of Na and water intake, experimentally induced overactivity of these systems can lead to a chronic state of hypertension. It is also known clinically that pharmacological inhibition of one or more of these systems often results in lowering or normalization of pressure in subjects with hypertension. It is therefore generally assumed that the overactivity of one or more of these systems could be responsible for the development or maintenance of various clinical forms of hypertension, including essential hypertension.

It has been difficult to reconcile the fact, however, that in human hypertension, these hormones generally are not elevated to those ranges required to produce hypertension experimentally. Sodium-replete normal subjects and dogs exhibit plasma ANG II levels of 5 to 15 pg/ml and require fivefold to 10-fold acute plasma elevations to raise mean arterial pressure 25 to 30 mm Hg. Infusion rates that raise plasma levels 40 to 60 pg/ml (5.0 ng/kg/min) are required to experimentally produce a sustained 25 to 30 mm Hg elevation of pressure with a normal Na intake. In moderate essential hypertension, plasma ANG II, plasma renin activity, and ALDO seldom reach levels that exceed twice the normal range.

The role of autonomic dysfunction in essential hypertension has been difficult to assess by measurement of circulating catecholamine levels. Many investigators, however, have demonstrated increased catecholamine plasma levels in hypertensive subjects, as reviewed by Goldstein. As with the renin-angiotensin system, it is unusual for plasma NE levels to exceed twice the normal range (150–300 pg/ml) in uncomplicated essential hypertension, although subjects with pheochromocytoma can have plasma levels ranging from 500 to 6000 pg/ml. We have reported that chronic intravenous infusion of NE, in amounts that raised plasma levels to about 2000 pg/ml in the present study, resulted in only about a 10 mm Hg elevation of mean arterial pressure in dogs while Na intakes were increased from 5 to 200 mEq/day.
study, each level of Na intake was maintained for 3 days and these same amounts of NE then were infused directly into the renal artery for another 3 days until the next level of Na intake was initiated.18, 46 Even though arterial pressure rose significantly during intrarenal infusions (30-40 mm Hg), it always returned to within 10 mm Hg of the low Na intake control value obtained during the previous intravenous infusion of NE.

Although AVP has been found to be elevated in 25 to 30% of male subjects with moderate essential hypertension, this finding is rare in female subjects.7, 47 Nonetheless, plasma AVP levels generally do not exceed twice the normal range. The contribution of these AVP elevations to essential hypertension is not yet understood. It is clear, however, that hypertension is not commonly observed in subjects with primary excess secretion of AVP (syndrome of inappropriate secretion of antidiuretic hormone),48 nor can hypertension readily be sustained by chronic infusion of AVP in amounts used in the present study, even when excess fluid is administered.20 Hypertension can be sustained with AVP only in dogs in which renal excretory ability has been severely compromised by surgical reduction of reninal mass to one third of normal.49

The aforementioned studies are cited to emphasize that primary excess secretion or chronic administration of any of these substances can, in sufficiently large amounts, produce chronic hypertension. However, in most subjects with mild to moderate essential hypertension, large elevations of these neuroendocrine substances are rarely observed and it is difficult to account for the observed increases of pressure with the observed changes in hormone levels. Although enhanced sensitivity to these substances may, in part, account for some of these discrepancies, it has been difficult to assess the relative contribution of such changes. The hypothesis of humoral summation or synergism offered an alternative or additional explanation whereby mild elevations of these hormones could amplify their influence on arterial pressure, but evidence to support this hypothesis was not found in the present study. The results clearly demonstrate that it is not yet possible to predict the influence of changes in multiple, complex interacting neurohumoral systems on arterial pressure or fluid and electrolyte balance in chronic states.

Acknowledgments
The authors are grateful to Mr. Vaughn Carroll for his expert assistance with the experimental animal studies; Ms Deborah Schoenborn, Ms Sharon Taylor, and Ms Leslie Sutherland for their careful analytic and radioimmunoassay work, Ms Ruth Scherkenbach for her careful statistical analysis of the data; and Ms. Therese Gauthier for her excellent secretarial and editorial assistance. We also thank Dr. Ian Reid at the University of California at San Francisco for the analysis of Group 3 plasma ANG II samples.

References
1 Goldstein DS. Plasma noradrenelin in essential hypertension a study of the studies Hypertension 1981;3:48-52
4 DeQuattro V, Campese V, Muura Y, Meyer D. Increased plasma catecholamines in high-renin hypertension Am J Cardiol 1976,38:801-807
6 Catt KJ, Crane E, Zimmnt PZ, Best JR, Cain MD, Coghlan JP. Angiotensin II blood-levels in human hypertension Lancet 1971,1:459-463
7 Cowley AW Jr, Cushman WC, Quillen EW Jr, Skelton MM, Langford HG. Vasopressin elevation in essential hypertension and increased responsiveness to sodium intake Hypertension 1981,3(suppl 1):1-93-1-100
13 Sealey JE, Laragh JH. How to do a plasma rennin assay In Laragh JH, ed Topics in hypertension New York Yorke Medical Publishers, 1980.244-256
16 Ben LK, Maselli J, Keil LC, Reid IA. Role of the renin-angiotensin system in the control of vasopressin and ACTH secretion during the development of renal hypertension in dogs Hypertension 1984;6:35-41
17 Dunnett CW. New tables for multiple comparisons with a control. Biometrics 1964;20:482-491
22 Cowley AW Jr, McCaee RA. Acute and chronic dose-response relationships for angiotensin, aldosterone, and arterial pressure at varying levels of sodium intake Circ Res 1976;39:788-796
25 Ishikawa S, Goldberg JP, Schner DM, Assenbrey GA, Schner RW. Interrelationship between suppressor effects of vasopres-
sin and other vasoactive hormones in the rat Miner Electrolyte Metab 1984,10:184–189
26 Bartelstone HJ, Nasmyth PA Vasopressin potentiation of catecholamine actions in dog, cat, and rat aortic strip Am J Physiol 1965,208:754–762
28 Commarato MA, Lum BKB Cardiovascular interactions of amphetamine and epinephrine with norepinephrine and with vasopressin Eur J Pharmacol 1969,7:125–134
49 Manning RD, Guyton AC, Coleman TG, McCaa RE. Hypertension in dogs during antidiuretic hormone and hypotonic saline infusion Am J Physiol 1979,236:H314–H322
Are hypertensive effects of aldosterone, angiotensin, vasopressin, and norepinephrine chronically additive?

A W Cowley, Jr, M M Skelton and D C Merrill

Hypertension. 1986;8:332-343
doi: 10.1161/01.HYP.8.4.332

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/8/4/332

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/