SYNERGY BETWEEN ANGIOTENSIN AND ALDOSTERONE IN EVOKING SODIUM APPETITE IN BABOONS

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Short Title: Angiotensin/Aldosterone Synergy in Sodium Appetite

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ABSTRACT

The synergy between angiotensin II (ANG II) and aldosterone (ALDO) in the induction of salt appetite, extensively studied in rats, has been tested in baboons. ANG II was infused intracerebroventicularly (ICV) at 0.5 or 1.0 µg/h; ALDO was infused subcutaneously at 20 µg/h. Separate infusions over 7 days had no significant effect on the daily intake of 300 mM NaCl. Concurrent infusions, however, increased daily NaCl intake ~10-fold and daily water intake ~ 2.5 fold. In addition, the combined infusions caused (i) a reduction in daily food intake, (ii) changes in blood composition indicative of increased vasopressin release, and (iii) changes of urinary excretion rates of cortisol and aldosterone indicative of increased ACTH release. Arterial blood pressure, measured in two baboons, rose during concurrent ANG II and ALDO treatment. These results indicate a potent synergy between central ANG II and peripheral ALDO in stimulating salt appetite in baboons. At the same time, other ANG II specific brain mechanisms concerned with water intake, food intake, vasopressin release, ACTH release, and blood pressure regulation appear to have been activated by the same type of synergy. These central enhancement processes have never been previously demonstrated in primates.
INTRODUCTION

Baboons develop a sodium appetite when depleted of sodium by diuretic administration (6). Recent experiments have shown that central infusion of angiotensin II (ANG II) stimulates the intakes of NaCl solution and water in baboons (2, 30). The physiological significance of these effects was increased by the findings that central infusion of AT1 receptor antagonists reduced the water intake of water restricted baboons given access to water, and the NaCl intake of sodium depleted baboons given access to NaCl solution (2, 30). Other experiments, assessing the role of brain ANG in baboons, disclosed that ANG III was as potent as ANG II in stimulating water and NaCl ingestion (1). These results indicated that brain ANG, acting alone, may be sufficient to stimulate water intake in dehydrated baboons and NaCl intake in Na deficient baboons.

An issue arising is whether this central activity of ANG II in physiological situations may involve interactions with other stimuli. Such an interaction could be the synergy of ANG II with mineralocorticoids that has been demonstrated for NaCl appetite in rats (7, 10, 23, 25, 45) and pigeons (19) but could not be demonstrated in sheep or rabbits (38). This synergy involves concurrent administration of ANG II centrally and deoxycorticosterone or aldosterone (ALDO) systemically at doses that, by themselves, do not stimulate NaCl intake. Those findings and their physiological significance have been reviewed (8, 9, 11, 24, 28). The synergy between the two major hormones of sodium deficiency might occur by (i) interaction between brain sites that are separately sensitive to ANG II (e.g., lamina terminalis) or ALDO (e.g., amygdala) as proposed by Schulkin (28) or, more directly than that, by (ii) interactions at the same brain site, e.g., by aldosterone upregulating ANG II receptors, increasing ANG II binding to receptors and enhancing responses to receptor activation (5, 11, 17, 32, 33, 40), or (iii) the inhibition of a central oxytocin mechanism (34, 37).
To test for synergy between ANG II and ALDO in evoking NaCl appetite in baboons, the animals were chronically infused intracerebroventricularly (ICV) with ANG II or subcutaneously (SC) with ALDO at doses that separately had no effect on daily NaCl intake. Then the infusions were given concurrently. The rate of ALDO infusion was calculated to equal the measured secretion rate of ALDO in severe sodium deficiency in animals of comparable size—sheep (3). Effects of these infusions on arterial blood pressure were measured in two baboons.
METHODS

Animals and Maintenance

Five adult male baboons weighing 29-32 kg were studied in individual metabolism cages fitted with stainless steel urine collection pans. All animals were habituated to the cages for 6-8 weeks before starting experimental observations. The daily food ration consisted of 500 g of pelleted food (Purina Monkey Chow 25-5045-6, Purina Mills, was the base for this diet) formulated by the SFBR experimental diet facility to contain 20 mmol Na/kg and ~190 mmol K/kg. The sodium content of ingested food was included in all sodium balance calculations. On this diet, the baboons maintained body weight and good health. Approximately 76% of the ingested sodium appeared in the daily urine loss.

Water and 300 mM NaCl (when it was presented for experiments) were available ad libitum from containers connected to valves attached to the cage sides and activated by the baboon’s tongue (Lixit, Lixit Corp.). Twenty-four hour intakes of food, water and 300 mmol NaCl as well as 24-h urine volume were measured daily at 1200-1300 h. Infusion experiments were started when daily intakes of food and fluids were stable for at least 7 days.

All procedures and protocols were approved by the Southwest Foundation Institutional Animal Care and Use Committee.

Surgical Preparation

Animals were prepared for intracerebroventricular (ICV) infusion by placing a 22 g stainless steel cannula in the left lateral brain ventricle as described previously (2). ANG II or 0.9% NaCl was infused into cerebrospinal fluid from osmotic pumps (Alzet, Alza Corp., Palo Alto, CA) placed subcutaneously in the midscapular region of the baboon’s back. The surgical procedures (under 1-2% isoflurane anesthesia) used for replacing osmotic pumps for ICV
infusions have been described (2). Similar procedures were used for implanting and explanting ALDO-containing osmotic pumps at adjacent subcutaneous sites.

Infusions and Doses

ANG II (human, 1-8 octapeptide, Bachem, Torrance, CA) was dissolved in sterile 0.9% NaCl and ALDO (d-Aldosterone, Sigma, St. Louis, MO) was dissolved in 10% ethanol: 90% sterile 0.9% NaCl. Solutions were passed through sterile 0.22 µm filters using aseptic techniques into osmotic pumps.

The ICV dose of ANG II was established by knowledge from previous experiments in baboons (2, 30) in which 5 µg/h stimulated salt intake in every baboon, and by testing each baboon to prove that the selected dose of ANG II alone did not stimulate NaCl intake. The starting dose was 1.0 µg/h but a lower dose was tested when that dose was found to be stimulatory within the first three days of infusion. The ANG II solution was delivered at 1.0 µg/h (3 experiments) or 0.5 µg/h (2 experiments). The ALDO solution was delivered at 20 µg/h. This rate of infusion in 30 kg baboons is similar to the measured rates of aldosterone secretion in 30-40 kg sheep after prolonged sodium depletion (3).

Experimental Protocols

Each experiment began with a 7-day baseline period with ICV infusion of 0.9% NaCl. The intakes of food, water, and NaCl were measured and urine was measured and collected daily. Body weight was measured at the end of this period and a venous blood sample was taken. The daily measurements continued throughout the whole experiment but body weight and the blood sample were taken only when brief surgery was performed in order to change an osmotic pump at the end of each step of the experiment. (Fig. 1, days 8, 15, 22, 29, 36, and 43).
Two designs were used. (Fig. 1). When the responsiveness to ICV ANG II infusion had not been established for the baboon (3 experiments) the order of infusions was (a) ANG II, (b) ANG II plus ALDO, (c) ALDO. When the required dose of ANG II was known (2 experiments) the order was (a) ALDO, (b) ALDO plus ANG II, (c) ANG II. There was always a 7-day baseline period before infusion (a) and between (b) and (c), but there was no separate baseline immediately before (b). Thus all ANG II alone or ALDO alone infusions always had a preceding 7-day baseline period for comparisons, and the baseline for ANG II plus ALDO treatment was the period before infusion (a). ANG II plus ALDO treatment always had a 7-day recovery period. The vehicle 0.9% NaCl was infused ICV during all baseline and recovery periods. The order of infusions had no effects on the measured ingestive responses.

In two baboons, arterial blood pressure and heart rate were monitored throughout the experiment by telemetry using a PhysiolTel® Multiplus™ implant (TA11PA-D70), receiver (RLA 2000), and calibrated pressure output adapter (R11CPA) Data Sciences International) coupled with a blood pressure processor (Coulbourn Instruments) and a Gould recorder. For installation of the transmitter and catheter, the animal was immobilized with ketamine (10 mg/kg, IM) and Valium (5 mg, IV), intubated, and anesthetized with isoflurane (1.5% V/V, IT). A midline incision from 2 cm below the umbilicus to the pubis was made to reveal the peritoneum. The colon was retracted to the right side to expose the left internal and external iliac arteries. The internal iliac artery was isolated with nonbinding ligatures to control bleeding, a small incision made in the artery, and the catheter connected to the telemetry transmitter introduced retrograde into the artery. The proximal ligature was tensioned to prevent bleeding while the distal ligature was tightened to occlude the artery and then tied around the catheter to anchor it. The transmitter was attached to the abdominal wall at 4 points and just above the pubis. Fascia was closed with
an interrupted horizontal mattress; skin was closed with a simple continuous suture. The animal was monitored to recovery. Postoperative analgesia was Buprenorphine (0.15 mg/kg, IM, IBD) for 3 days.

Analytical Procedures

Urinary sodium concentration was measured by flame photometry (Corning Model 450) and used to calculate daily Na balance (total intake in food and NaCl solution – loss in urine). Twenty-four hour excretion rates of aldosterone and cortisol were measured by radioimmunoassay (Diagnostic Products Corp., Los Angeles, CA). Urine samples were collected into ice-chilled containers holding 500 ml of 4.1% boric acid. The urine was extracted with dichloromethane for cortisol analysis, and with ethyl acetate for aldosterone analysis. Hematocrit and plasma concentrations of creatinine, urea, total proteins, glucose, sodium, and potassium were measured by standard clinical methods.

Statistical Analysis

Data are presented as means ± SE. The effects of treatments on ingestive behaviors and on Na balance were analyzed by a two-factor (experiment and day) repeated measures analysis of variance. Significant differences between days were determined by post hoc analysis (Tukey’s LSD). The effects of treatments on hematocrit and plasma composition and on urinary corticosteroid excretion rates were analyzed by paired t-tests.
RESULTS

In an individual baboon (Fig. 2) only the concurrent ICV infusion of a low dose, 1 µg/h, of ANG II and 20 µg/h of ALDO subcutaneously substantially increased daily NaCl intake (top panel). It rose from a baseline of 13.0 ± 3.3 mmol/day to ~100 mmol/day on days 2-6 of treatment. In other periods, baseline/recovery, ALDO alone, and ANG II alone, NaCl intake fluctuated from 3-40 mmol/day. Calculated daily Na balance (total Na intake – urinary Na output) was 13.1 ± 3.4 mmol during the baseline period (accounted for by the unmeasured Na loss in feces). The balance was unaltered during ALDO infusion alone, increased to high positive values on some days of ANG II/ALDO treatment, fell to negative values on some recovery days, and tended to stabilize at 10-30 mmol during the final recovery period. Daily Na balance paralleled the daily intake of 300 mM NaCl except for the wide variations during ANG II/ALDO treatment and the recovery from it. The increase in NaCl intake was not preceded by a period of negative Na balance.

Daily water intake also increased during ANG II/ALDO treatment (Fig. 2). This baboon consistently reduced water intake on the days of surgery to change osmotic pumps. Food intake was usually reduced on the same days (bottom panel – Fig. 2). Food intake was 400-500 g on almost every other day.

The changes in 300 mM NaCl intake in the 5 experiments (Fig. 3) show that only the concurrent infusion of ANG II and ALDO significantly increased the intake of this solution above its baseline (P<0.001). The variability in response was largely due to persistent differences between baboons. The baboons had individually consistent daily NaCl intakes (mmol/day) during the baseline period (note the small SEs); three were low: 13.0 ± 3.3, 20.9 ± 2.9, and 9.3 ± 1.4, and two were high: 68.6 ± 7.6 and 85.9 ± 8.6. During ANG II plus ALDO infusion, NaCl intakes
increased (P<0.001) in all 5 baboons to peaks that were approximately 10 times the baseline value. This peak occurred on day 5 or 6 of infusion in 4 experiments. Intakes declined in all experiments on day 7 of infusion and had declined to baseline values on day 2 of recovery. The infusions of ANG II alone or ALDO alone had no significant effects on NaCl intake. The effect of ANG II plus ALDO infusion was highly significant compared with either ANG II alone or ALDO alone, or with the sum of these effects (P<0.001).

The ingestive behavior in the 5 experiments is grouped in Fig. 4 (mean ± SE of the mean values for each baboon during the baseline or infusion period). The increase in daily NaCl intake during ANG II plus ALDO infusions was associated with an almost 2.5-fold increase in daily water intake (P<0.001) and a significant fall in daily food intake (P<0.001). Although the NaCl intakes were greatly increased during ANG II plus ALDO infusions, the calculated daily Na balance (total Na intake – Na loss in urine) tended to remain ~ plus 20 mmol, near to baseline values. There was no evidence that the increases in daily NaCl or water intakes were preceded by a natriuresis or diuresis. The ANG II and the ALDO infusions alone did not alter the daily Na balance, or water and food intakes.

**Effects on Plasma Composition and Hematocrit**

The plasma samples were taken immediately after sedation for surgery to change osmotic pumps, starting and ending infusions, and therefore indicate composition changes that occurred during prior infusion periods. Plasma [Na], [K], total protein, and hematocrit were unaltered by ANG II alone infusion. ALDO alone infusion increased [Na] from 144.0 ± 0.6 to 145.8 ± 0.2 mmol/l (P<0.05) and reduced [K] from 3.8 ± 0.1 to 3.1 ± 0.3 mmol/l (P<0.05); total proteins fell slightly from 6.7 ± 0.1 to 6.5 ± 0.1 g%, (P<0.05); and hematocrit fell from 42.0 ± 0.8 to 40.0 ± 1.0% (P<0.05). All of those small changes were reversed during the recovery period.
Large and different changes occurred during ANG II plus ALDO infusion. Plasma [Na] fell from 145.0 ± 1.1 to 137.8 ± 2.0 mmol/l (P<0.05) and [K] fell from 3.7 ± 0.1 to 2.3 ± 0.4 mmol/l (P<0.05). Plasma total protein fell in 2 experiments and rose in 3 experiments, and similar changes occurred in hematocrit so that the mean effects on these two parameters were almost zero. The individual changes in all of these 5 parameters returned to baseline during the recovery period.

Effects on Body Weight

Body weight was unchanged during ANG II infusion, rose slightly in the 5 baboons, from 30.6 ± 0.3 to 31.1 ± 0.3 kg (P<0.05) during ALDO infusion, and fell in 4 of 5 baboons during ANG II plus ALDO infusion, overall 30.7 ± 0.4 down to 29.5 ± 0.2 kg (P<0.05).

Effects on Corticosteroid Excretion

The infusion of ANG II alone had no effect on daily excretion rates of aldosterone or cortisol. The infusion of ALDO alone had no effect on cortisol excretion but mean daily aldosterone excretion rate increased from baseline 36 ± 10 to 89 ± 18 nmol/24 h (P<0.05). The infusion of ANG II plus ALDO increased the rate to 140 ± 30 nmol/24 h (P<0.05). Daily cortisol excretion rate rose to very high levels during ANG II plus ALDO infusion, particularly during the first 3-4 days. Mean daily excretion rates of cortisol increased 1.5-fold in one baboon and 11- to 17-fold in the others. The overall mean excretion rate increased from baseline 380 ± 104 to 3,743 ± 1,351 nmol/24 h (P<0.05), much of this variation being due to the baboon with just 1.5-fold increase in excretion rate.

Seven days after stopping the ANG II plus ALDO infusion, mean cortisol excretion rate was 405 ± 84 nmol/24 h (P<0.05) during the next 7 days and mean aldosterone excretion rate fell to 11 ± 3 (P<0.05) nmol/24 h.
Effects on Arterial Blood Pressure and Heart Rate

Arterial blood pressures (BP) was measured during the final two experiments only (Fig. 5, daily means of ~ 140 values/day). Mean arterial blood pressure increased 10-15 mm Hg through the ANG II/ALDO infusion period. The ALDO alone infusion appeared to increase mean BP by 5-10 mm Hg, and the ANG II alone infusion (1.0 µg/h) appeared to be without effect.
DISCUSSION

The major finding from these experiments was that the combination of an ICV infusion of ANG II and a peripheral infusion of ALDO, both of which alone had no effects on ingestion, caused large increases in NaCl intake and water intake. These two intakes followed similar time courses during the 7-day infusion period but the daily water intake was not in a fixed proportion to daily NaCl intake in all 5 baboons. The increases in intakes were not secondary to natriuresis or diuresis.

The combined infusions also reduced daily food intake in 4 of 5 baboons, associated with a small reduction in body weight, and caused significant reductions in plasma [Na] and [K]. The combined infusions caused a significantly greater increase in daily aldosterone excretion rate than aldosterone infusion alone. This response may have been related to an almost 10-fold increase in daily cortisol excretion rate, suggesting a large increase in ACTH release. Recent studies have shown that exogenous ACTH, in contrast to findings in other species, did not stimulate sodium appetite in baboons (31). A rise in systemic arterial pressure was the other characteristic response to the combined infusions. All of these changes were reversed during the 7-day recovery period confirming that they were attributable to the treatment combination.

The interaction between central infusion of ANG II and peripheral administration of ALDO to evoke salt intake confirms the extensive findings of Epstein and his colleagues (7, 10, 19, 23, 25, 45) particularly in rats and pigeons. The overall conclusion from many studies was summarized as follows: “salt appetite is aroused by a synergy of angiotensin II and aldosterone, uniting the behavioral and renal contributions to sodium homeostasis in the same hormonal network” (7, 8) with “cerebral rather than blood-borne angiotensin as the agent that participates in this synergy” (9, 44). At the moment, the effect of blood-borne ANG II on NaCl appetite in
baboons is not known but prolonged ICV infusion of ANG II at 5 µg/h (5-10 times the dose used in the present experiments) certainly causes a large and prolonged increase in NaCl intake (2, 30). Even more importantly, the central infusion of an AT1 receptor antagonist ZD7155 inhibits the high sodium intake caused by sodium deficiency (2, 30), indicating that the inhibition of central ANG II alone is sufficient to block the behavior.

But, before discussing possible mechanisms underlying the ingestive responses, inspection of the other features of the treatments and responses may be helpful. First, these experiments involved prolonged infusions of the agonists rather than bolus injections or combinations of infusions and bolus injections. Second, these baboons started out with a wide range of baseline intakes of NaCl, some baboons routinely drinking 20-40 ml/day of NaCl solution, and others drinking 200-300 ml/day. This differential was also observed during ANG/ALDO stimulation with intakes rising 10-fold over baseline on the average.

Plasma [Na] increased during ALDO infusion but, because this was not associated with an increase in NaCl intake, it was probably due to Na retention. There was a small increase of Na balance ~ 10 mmol/day, on only the first two days of ALDO infusion. Daily water intake was not altered by this small increase in plasma [Na]. Plasma [Na] fell in all experiments during ANG/ALDO infusions and substantially in 4 of them, 6-11 mmol/l, despite the very large increases in NaCl intake. The response indicates water retention in excess of Na retention despite the high aldosterone excretion rates (higher in these experiments than in the ALDO alone experiments). Possibly the kidney was in “escape” from aldosterone towards the end of the combined infusions, but sodium balance calculations did not indicate Na deficiency at any time during the infusion. It is more likely that the excess of water to Na in the plasma was due to the
cerebral ANG II/ALDO combination evoking ANG II stimulation of vasopressin release (26, 42) and water retention.

The intensity of this stimulus causing water retention is clear on inspection of the comparative increases in NaCl and water intakes. It is interesting that this high water intake continued despite the low plasma [Na] and presumably low plasma osmolality, so the thirst was driven by the infusion, not plasma hypertonicity. All baboons sustained elevated NaCl and water intakes throughout ANG II/ALDO infusion, and some proportionality between the two increments might have been predicted, e.g., mean daily intakes of NaCl and water that maintained plasma osmolality. Such was not the case however. Although ANG II/ALDO infusions caused baboons with high NaCl intake baselines to drink more NaCl than the baboons with low baseline intakes there was no proportionality, animal to animal, between the mean increases of NaCl and water consumption. Two baboons, one a high NaCl drinker and one a low NaCl drinker, almost balanced daily mean NaCl and mean water intakes to the concentration 145 mmol/l (mean plasma [Na]). However two baboons drank at least 2.0 l/day of water in excess of that proportion; and one baboon drank 1.5 l/day less than that value. A possible explanation of this variability might be that the two ingestive mechanisms driven by the ANG II/ALDO synergy could be acting independently, leaving the peripheral systems, e.g., circulation, kidneys, and gastrointestinal tract to manage any imbalance. A similar situation, of course, applies under baseline conditions where some baboons with relatively low daily NaCl intakes may have relatively high daily intakes of water, and vice versa.

Another interesting feature of these experiments was the evidence that the ANG II/ALDO synergy also appeared to cause a strong stimulation of ACTH release and to affect arterial blood pressure. The very large increase in cortisol excretion rate in most experiments is indicative of an
ACTH release that could have been sufficient to stimulate aldosterone secretion (3, 4, 14) and that stimulation was confirmed by the finding that aldosterone excretion rate during ANG II/ALDO infusion was significantly higher than during ALDO infusion alone, although the ALDO infusion rates were equal.

Conclusions about effects on blood pressure must be limited with data coming from the final two experiments only. A small pressor effect of ALDO alone might be attributable to fluid retention and expansion of vascular volume, as evidenced by the small but significant reductions in hematocrit and plasma total protein concentration. However those changes were not consistent during ANG II plus ALDO infusion so the larger rise in blood pressure with that combination may have been caused more directly by the central action of the subpressor dose of ANG II after enhancement by ALDO. Upregulation of brain ANG II receptors by mineralocorticoids is well established (5, 11, 17, 32, 33, 40) and central ANG (21, 36, 42) is involved in blood pressure regulation. Another possibility is that the pressor response was initiated by ACTH and high cortisol levels as illustrated in baboons (31), sheep (29), and humans (39).

Turning to the issue of possible mechanisms for the synergy between ANG II and ALDO in stimulating NaCl appetite, Epstein and coworkers (8, 9, 23, 25) concluded that cerebral rather than peripheral blood ANG II is the agent that participates in the synergy. Epstein (9) also concluded that supernormal levels of ANG II or ALDO produce sodium appetite in rats but high normal levels of ANG II and ALDO together are sufficient to induce sodium appetite under physiological conditions e.g., sodium deficiency. Epstein at that time did not discuss brain pathways for synergy or enhancement mechanisms and the primary interest was in sodium appetite not thirst. In rats, the threshold ICV dose of ANG II for stimulating thirst was below the threshold for stimulating sodium appetite (45) so that the pulse ICV injections of ANG II used in
synergy studies had a substantial effect on water intake when administered alone. Therefore, in the presence of exogenous mineralocorticoids, the injections of ANG II in rats caused remarkable increases in sodium appetite from a low baseline whereas the increases in thirst were not so remarkable, although significant (45). That situation did not apply in these baboon studies because the ICV doses of ANG II infused chronically for 7 days were subthreshold for both NaCl and water intakes and both intakes were increased remarkably by the combination of agents. At the moment, it is unknown whether there is any dose of ALDO SC alone that would stimulate NaCl intake in baboons whereas, in rats, it is long established that aldosterone or desoxycorticosterone alone may have measurable effects on NaCl intake (12, 22, 28, 41).

A mechanism that may account for the synergy that increased NaCl intake has been suggested by the studies of Verbalis, Stricker, and colleagues (34, 37). They described central inhibition of salt intake by oxytocin in rats, and how sodium depletion or administered mineralocorticoid potentiated ANG II-induced NaCl intake by inhibition of central oxytocin secretion.

Schulkin (28) proposed separate neural circuits for ANG II- and mineralocorticoid-induced Na appetite, medial anterior third ventricle for ANG II and medial amygdala for ALDO, and that there must be a region where the effects of the two hormones interact for synergy. He concluded from evidence of lesion experiments that the central nucleus of the amygdala was the most likely candidate and interactions there may involve changes in receptor function or in second messenger efficiency.

More recent studies and reviews have concluded that mineralocorticoids act on brain nuclei, particularly in the amygdala, to influence sodium intake through both genomic mechanisms (involving cytosolic receptors) and nongenomic mechanisms involving, for example,
GABA receptors or unique receptors of their own (11, 24). Other studies have been concerned with the role of mineralocorticoids (and glucocorticoids) in modulating the expression and function of ANG II receptors at specific brain sites (reviewed in 11, 16). For example, glucocorticoids augment the sodium appetite of rats treated with mineralocorticoids (18), the water intake in rats treated with ANG II (13), and the central hypertensive action of ANG II (27). These findings indicate that glucocorticoids may facilitate mineralocorticoid function and ANG II function in the brain. Evidence accumulates that mineralocorticoids and glucocorticoids induce ANG II receptor expression and binding in specific brain sites (11, 32, 33, 40) and other tissues (35). Brain sites of interest in those and other studies are the bed nucleus of the stria terminalis, subfornical organ, area postrema, and hypothalamic paraventricular nucleus.

It is possible that the responses to the ANG II/ALDO synergy were enhanced by the induced increase in cortisol production. Evidence, cited above, that glucocorticoids may potentiate the central effects of both ALDO and ANG II, suggests that the induced cortisol may act as a positive feed-back element in the synergy. This element might not be involved in the stimulation of salt appetite because exogenous ACTH did not stimulate salt appetite in baboons (31). Also, in one baboon in the present study (Fig. 2), the 10-fold increase in NaCl intake and the increase in water intake occurred although cortisol excretion rate rose only 1.5-fold in that animal; food intake was only suppressed for one day. A large increase in cortisol may not be necessary for the salt and water responses but the effects on food intake and blood pressure may be influenced in that way.

Throughout this manuscript it has been argued that the ANG/ALDO synergy involves the octapeptide, ANG II. Our recent study in baboons reported that ANG III was as potent as ANG II in stimulating NaCl and water intake (1). That line of investigation was opened because new
evidence (20, 21, 46) strengthened earlier evidence (15, 42, 43) that certain central actions of ANG II may actually be mediated by ANG III. These actions include water intake, pressor and vasopressin release—three of the actions that were pertinent to these experiments in baboons. It is therefore possible that the synergies under investigation here may actually involve the heptapeptide ANG III.

Finally, it appears likely that the sustained increased NaCl and water intakes caused only by the ANG II/ALDO infusion combination in these experiments in baboons were due to the synergy originally described by Epstein et al., (7, 8, 9, 10, 25) by a mechanism involving ALDO induction of ANG II receptor expression, binding and cellular activity. The synergy, presumably involving other brain sites, also reduced food intake, and probably increased ACTH and vasopressin release and arterial blood pressure. The synergy involving sodium appetite is well established for rats, and the thirst response has also been noted, but these are all novel findings in a primate. The findings also emphasize that the synergy is not highly specific for sodium appetite and thirst but may be incorporated into a broad range of ANG II-based brain functions.
PERSPECTIVES

The synergism of aldosterone and angiotensin II determined in a baboon as distinct from a rodent has significant implications for further studies in primates including humans. The measurements in these experiments involved primarily salt appetite, thirst and food intake but some information on release of pituitary hormones and blood pressure has been obtained.

The involvement of aldosterone in angiotensinergic brain pathways regulating three ingestive behaviors in a primate argues against the proposal that the synergy is specifically concerned with salt appetite. The emphatic effect on corticosteroids indicates that the synergy may also be implicated in the stress response, allied with the pressor response noted in two experiments.

The contributions of angiotensin II and, recently, aldosterone to cardiovascular disease have become apparent. Primary aldosteronism is known to be more common than thought hitherto, and aldosterone can cause cardiac hypertrophy and fibrosis. The use of spironolactone can prolong life in patients with cardiac failure. Angiotensin is a direct contributor in many forms of hypertension but also may be involved in end organ complications. Whether the synergy in physiological processes reported here extends to pathological processes is an area for further inquiry.
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LEGENDS TO FIGURES

Fig. 1: Design of experiments in baboons with successive 7-day periods for infusion of angiotensin II (ANG II) or aldosterone (ALDO) alone or combined. ANG II was infused into a lateral brain ventricle (ICV) and ALDO was infused subcutaneously (SC). Sterile saline (0.9% NaCl) was infused ICV during baseline and recovery periods. Days of surgery to change infusion pumps, collect venous blood samples, and weight the baboons, are shown by arrows (1-6).

Fig. 2: Individual baboon: effects of subcutaneous infusion of aldosterone (ALDO SC) alone, then ALDO SC plus intracerebroventricular infusion of angiotensin II (ANG II ICV), and then ANG II ICV alone, on daily intakes of 300 mM NaCl, water and food. Daily Na balance (Na intake in food and NaCl solution – Na loss in urine) is also shown. Sterile saline (0.9% NaCl) was infused ICV during baseline and recovery periods.

Fig. 3: Summary of 5 experiments: comparison of daily intakes of 300 mM NaCl during 3 treatments, intracerebroventricular infusion of angiotensin II (ANG ICV), subcutaneous infusion of aldosterone (ALDO SC), and both infusions concurrently. The baseline values for ANG II ICV alone (?) and ALDO SC alone (?) were the values for the 7 days immediately preceding those infusions. The baseline values for ANG II ICV+ALDO SC (?) were the values for the 7 days preceding any infusion. The broken line between the left and center panels indicates that the data were not continuous. Sterile 0.9% NaCl was infused ICV during all baseline and recovery periods. Values are means ± SE. ***P<0.001 for response to combined ANG + ALDO infusions compared with its baseline, and with the responses to infusions of ANG or ALDO alone.
Fig. 4: Summary of 5 experiments: effect on daily intakes of 300 mM NaCl, water, and food, and on daily Na balance (total Na intake in food and 300 mM NaCl – Na loss in urine) of 3 treatments. Left panel, intracerebroventricular infusion of angiotensin II (ANG II ICV); center panel, subcutaneous infusion of aldosterone (ALDO SC); right panel, concurrent infusions of ANG II ICV and ALDO SC. Sterile saline (0.9% NaCl) was infused ICV during baseline periods. Values are the mean ± SE of the mean values for each baboon during 7 baseline days (B, open bars) and during 7 days of infusion (I, solid bars). The baseline values for ANGII ICV alone and for ALDO SC alone were the mean values for the 7 days immediately preceding those infusions. The baseline values for ANG II ICV+ALDO SC were the values for the 7 days preceding any infusion. ***P<0.001 compared with baseline.

Fig. 5: Two individual baboons: effects on daily mean arterial blood pressure of intraventricular infusion of angiotensin II (ICV ANG II) alone, then ICV ANG II plus subcutaneous infusion of aldosterone (SC ALDO), and then SC ALDO alone. Sterile saline (0.9% NaCl) was infused ICV during baseline and recovery periods. Values are means of approximately 140 measurements each day.
Surgeries for Changing Osmotic Pumps

Baseline ANG II or ALDO

Recovery and Baseline

ALDO or ANG II

Recovery
Figure 3
Figure 4
Figure 5