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Salt appetite: its neuroendocrine basis

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Abstract. Based on the early work of Richter (1936), showing that the adrenalectomized rat kept itself alive by drinking hypertonic NaCl solutions, Epstein and Stellar (1955) demonstrated that that salt appetite was innate, not dependent on learning. A scries of later papers by Epstein and his students made clear that in addition to the adrenal steroid, aldosterone, salt appetite depended upon the action of angiotensin II in the brain. Blocking either hormone in the brain reduced depletion-induced salt appetite in half; blocking both eliminated it. Two salt depletions enhanced depletion-induced salt appetite by nearly a factor of two even when the rats never had a chance to drink salt in the first depletion. With multiple depletions, need-free salt intake also increased when the rats were sodium replete, producing an chronic, elevated salt appetite. Strikingly, female rats drink almost twice as much as males and become more enhanced by prior depletions. The neural circuitry involved in the synergy of angiotensin and aldosterone is becoming clearer with lesions of the amygdala that reduce aldosterone's effects and lesions of the anterior wall of the third ventricle that reduce angiotensin's effects. The significance of salt appetite in nature, in body fluid homeostasis, and as a model system of the brain mechanisms of ingestive behavior is discussed.

Key words: salt appetite, angiotensin-aldosterone syngery, rats

It is a great personal honor for me to have my paper at the First International Congress of the Polish Neurnscience Society designated as the First Jerzy Konorski Memorial Lecture. Professor Konorski was recognized throughout the world as one of the great neuroscientists of this century, and he played an especially important role in the development of behavioral neuroscience. We first met in 1962 in Boston, USA, and I first visited the Nencki Institute in Warsaw in 1966. Shortly after that we became good colleagues and began a scientific exchange program between the Nencki Institute and the Institute of Neurological Sciences at the University of Pennsylvania that still exists to this day.

It is in this spirit of international cooperation in science that it is fitting to say that I originally planned to dedicate my lecture to the late Dr. Alan Epstein who was tragically killed in an automobile accident in Mexico in January 1992. It made sense to do this, for much of the research I want to report here was done in Alan's laboratory, and at the same time, the problem of salt appetite was of great interest to Professor Konorski.

At the time of his death, Alan was at the pinnacle of his career, and the study of salt appetite represented the crowning achievement in his search for neural mechanisms involved in the regulation of ingestive behavior - food intake, water intake, and salt in particular - and its implications for our understanding of the brain mechanisms of appetite and motivated behavior.

As many of you know, Alan was my student at the Johns Hopkins University and joined my laboratory 41 year ago in 1951 as an undergraduate. Our first research together was the study of salt appetite in the adrenalectomized rat, based on the striking finding by Curt Richter at Hopkins that the adrenalectomized rat made up for its obligatory loss of salt at the kidney by ingesting large quantities of concentrated saline (Fig. 1), and thus, kept itself alive (Richter 1936).

Now the appetite for sodium is a major phenomenon of nature. Animals, especially cattle, that live on a low-salt diet periodically become sodium-depleted, and begin the long trek toward outcroppings of minerals rich in sodium where they lick avidly and make up their sodium deficit. Even elephants have been seen to enter salt mines to satisfy their needs. And of course, the very existance of salt mines is testimony to the great craving humans have for salt. It was once literally worth its weight in gold. Even more dramatically, human avidity for salt was illustrated by the case of a three-year old boy described by Wilkins and Richter (1940). He licked the salt off of crackers and bacon rather than eat them. When he discovered the salt shaker, he literally ate salt by the teaspoonful. His first word was "salt". Because his general appetite was not good, he was admitted to the hospital for observation, and unfortunately, was put on a low salt diet and died within a week. Autopsy showed that tumors had destroyed his adrenal glands, and like

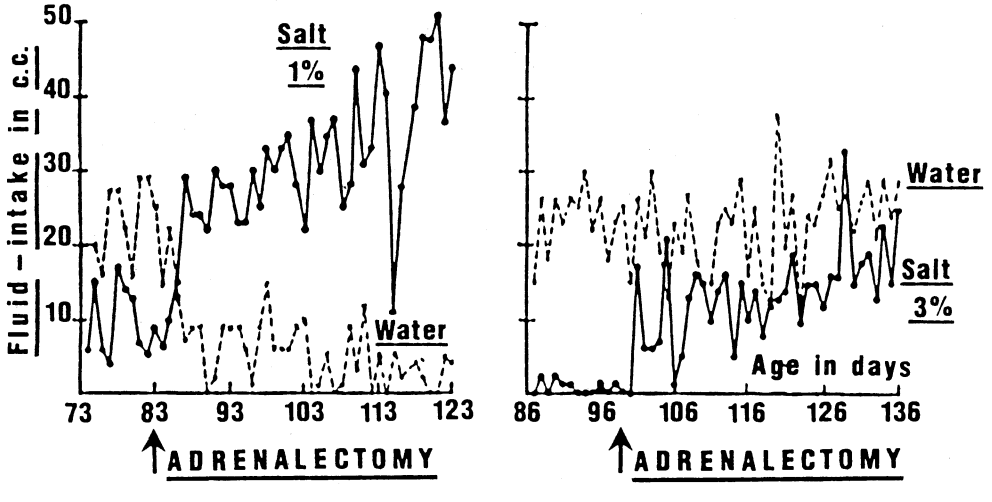

Fig. 1. Following adrenalectomy, rats show a prompt increase in their 24 hour intake of 1% and 3% NaCl solutions, thereby making up for NaCl lost at the kidney and keeping themselves alive.

Richter's adrenalectomized rats, he had made up for salt loss at the kidney by ingesting large quantities of salt, and thus kept himself alive.

What Richter learned from the boy and especially his studies of the rat, was that without the adrenal hormone, aldosterone, the kidney could not reabsorb sodium; replacement of aldosterone by injection or implant in the adrenalectomized animal prevented the salt loss and eliminated the excessive salt drinking. Curiously, however, if Richter gave large doses of aldosterone, both adrenalectomized and normal rats began drinking salt in large quantities. This is an important point I want to come back to later.

What Alan accomplished in his first experiment in my laboratory at Hopkins was to show that the salt appetite of the adrenalectomized rat was innate - that is, the rats began drinking excessive NaCl solutions immediately in a one-hour drinking test, once the sodium deficiency was allowed to build up. Secondly, he showed that the adrenalectomized rat had increased NaCl intake over all concentrations of the preference-aversion range. We published those findings together in 1955 in a paper we were both very proud of (Epstein and Stellar 1955). His most important discovery, however, was that he was inspired to a life-long urge to find out how the brain and the endocrines worked together to yield ingestive behavior and the avidity and motivation that go along with it.

He spent many productive years studying food and water intake in the rat with lateral hypothalamic lesions and in rats whose brains were injected with various hormones and chemicals that were candidates, in his mind, for the signals that could trigger ingestive behavior. Of all of these, angiotensin proved to be the most potent, for Alan and his coworkers discovered that femtomole quantities injected in the subfornical organ (SFO) of the brain could elicit robust water intake in the water-satiated rat (Simpson et al. 1978). It was in the course of follow-up experiments (Bryant et al. 1978) that Alan made the striking observation that continuous intracerebroventricular injection of angiotensin also elicited robust, long-lasting ingestion of 3% solutions of NaCl.

Now angiotensin is a molecule of great interest, for it is made in the periphery, catalyzed by renin released from the kidney by threats to body fluid homeostasis (Fig. 2). Its active form is the octapeptide that has multiple effects, on the brain through the circumventricular organs (SFO and the area postrema or AP) and it acts in the periphery to promote sodium and water retention at the kidney as well as to

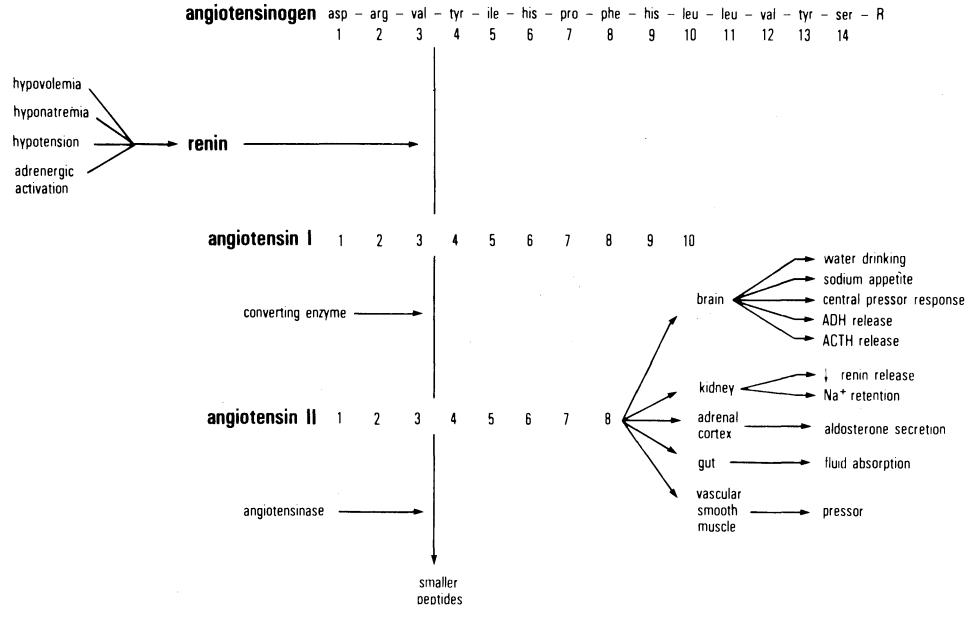

Fig. 2. Diagram showing how threats to body fluid homeostasis stimulate kidney renin that cleaves angiotensinogen to angiotensin I which is converted to angiotensin II through the action of the converting enzyme, leading to multiple physiological effects in the brain, kidney, adrenal cortex, gut and blood vessels, directed toward maintenance of body fluid balance.

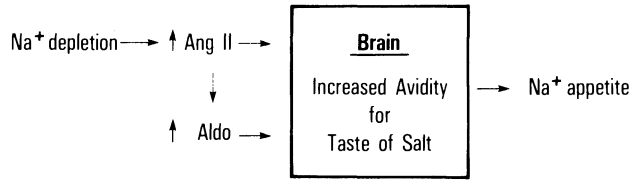

Fig. 3. Epstein's original hypothesis of the synergy of angiotensin and aldosterone in the brain to promote salt appetite.

promote vasoconstriction, thus reducing the vascular fluid compartment and defending blood pressure. Even more pertinent is the fact that the whole renin-angiotensin cycle takes place in the brain and that angiotensin acts as neurotransmitter and neuromodulator. Of especial interest to Alan was the fact that peripheral angiotensin promoted release of aldosterone from the adrenal cortex. This brought to mind the early Richter finding that aldosterone was essential for sodium retention at the kidney and that large doses of aldosterone promoted salt intake.

It was one of those rare insights that led Alan to realize that angiotensin and aldosterone might act together in the brain to promote salt appetite. As he hypothesized, subthreshold doses of aldosterone in the periphery and subthreshold doses of angiotensin in the ventricles of the brain produced a robust salt intake that neither one alone caused. This finding led Alan to the synergy hypothesis that sodium depletion causes an increase in angiotensin which increases aldosterone, the two hormones together producing a salt appetite in the brain (Fig. 3).

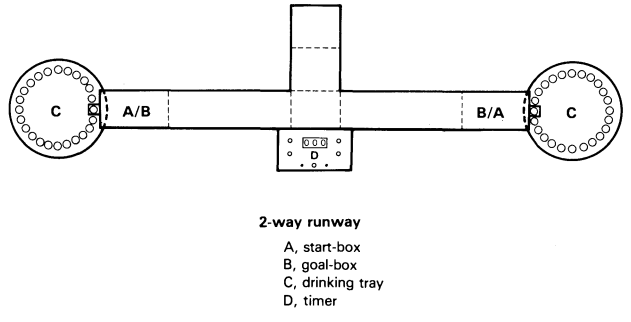

Fig. 4. Meter long runway to measure running speed of rats to small taste rewards (e.g. NaCl), yielding a measure of appetitive motivation or avidity (from Stellar and Epstein 1991).

To test this idea further, and to evaluate the avidity of salt appetite more directly, we turned to a runway test which measures the appetitive motivation of rats by recording how fast they run to tiny rewards of 3% NaCl of 0.1 ml or less (Fig. 4). From previous work, (Schulkin et al. 1985), we knew that normal, salt-replete rats do not find any salt solutions rewarding when they are mildly thirsty, even though they drink more of such solutions than of water (Fig. 5). But if they are primed with large doses of DOCA, an aldosterone mimic, they run avidly to strong salt solutions. In our experiment (Zhang et al. 1984), we found that we could get salt-replete rats to run to 3% salt rewards if they were primed with subthreshold peripheral doses of DOCA and triggered with a subthreshold injection of angiotensin into the ventricles (Fig. 6). So pro-

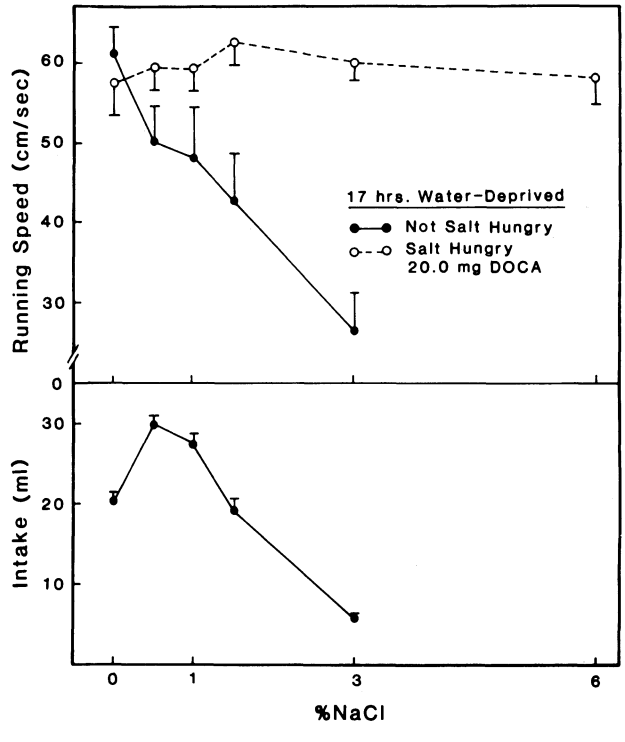

Fig. 5. Normal, moderately thirsty rats which show increasing intake of hypertonic NaCl solutions (lower, rising and falling curve), run slower and slower to every increasing concentration of NaCl reward (solid, descending curve in upper figure). When salt appetite is induced with DOCA, they run very rapidly to all concentration of NaCl, even as high as 6% (dashed curve) (from Stellar and Epstein 1991).

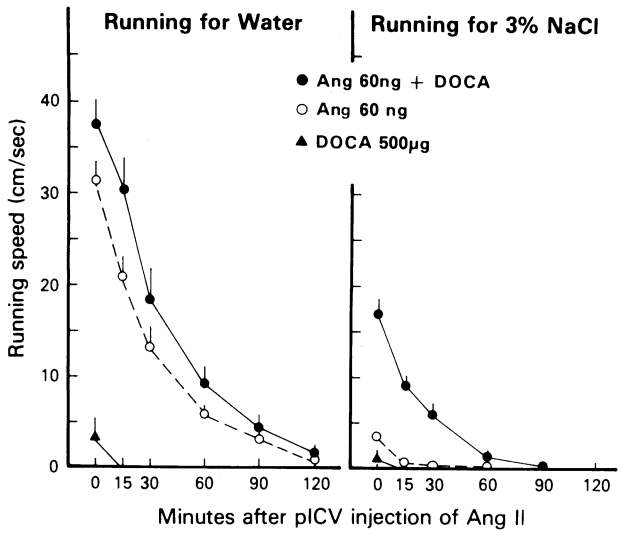

Fig. 6. Subthreshold doses of peripheral DOCA and intracerebroventricular angiotensin act together to generate significant running speed to 3% NaCl rewards (right hand curves) (from Stellar and Epstein 1991).

viding the right hormonal conditions in the brain increases the avidity for salt and the reward-value of salt solutions.

By this time, Alan had organized a group of investigators under an NIMH Program Project Grant without walls to pursue the question of the neurohormonal basis of salt appetite further, investigating it at different levels of analysis from the behavioral to the neural systems and to the cell and molecular levels. Let me report here on some aspects of the progress we have made.

In Harvey Grill's laboratory at Penn, it was shown earlier that the decerebrate rat does not respond to sodium depletion by either ingesting 3% NaCl intake of solutions dripped into the mouth through an intraoral cannula or by showing positive facial responses to salt the way the intact rat does (Grill et. al. 1986). As you know, Grill and Norgren had previously shown that decerebrate rats respond to sucrose solutions dripped into the mouth when food -deprived but not when satiated (Grill and Norgren 1978). So the decerebrate is competent to respond with ingestive behavior in these tests, and this means that the mechanisms essential for the expression of salt intake are in the forebrain, not in the brainstem.

Yet we know from Ralph Norgren's work at Hershey that much information about the basis of salt appetite is processed in the brainstem. First of all, the detection of salt is conveyed by very specific, "salt best" fibers of the chorda tympani of the VIIth cranial nerve. This information is processed in the rostral nucleus of the solitary tract (NTS) and elaborated in the parabrachial nucleus (PBN). Lesions in the PBN, however, do not interfere with the detection of salt, but severely reduce the rat's responsiveness to it (Flynn et. al. 1991) and greatly attenuate salt's rewarding value in the runway (research in process).

In Stylianos Nicolaidis' laboratory in Paris, unit recording in the septal area following iontophoresis of angiotensin or aldosterone or both together shows that there are some units that respond only to both angiotensin and aldosterone given together and not to either one alone. As Nicolaidis points out, these may be "synergy neurons", and it will be extremely interesting to study them further (Stellar et al. 1992).

In Alan's laboratory meantime, some of the fundamental behavioral and neuroendocrine factors related to salt appetite have been uncovered. The main method of producing sodium depletion and inducing salt appetite for these studies is to treat the rat with a natriuretic and diuretic, furosemide, put them on a salt-free diet, clean the cages of ambient sodium, and let them have all the water they want. When tested in a 2-hour intake test with 3% NaCl solutions, they drink much more than when not treated (minimal intake). When treated and tested a week later(Fig. 7) they drink almost twice as much (Sakai et al. 1987). This is not the result of learning to drink salt, for they show the enhanced intake even when they do not drink salt in the first test, but are allowed to make up their depletion by eating their normal, salt-rich diet. Eliminating the first depletion, but simply treating the rats with peripheral aldosterone and intracerebroventricular angiotensin produces enhanced drinking on the next depletion. The strong suggestion is that the synergistic action of aldosterone and angiotensin in the brain, not only produces salt appetite, but it changes the brain so

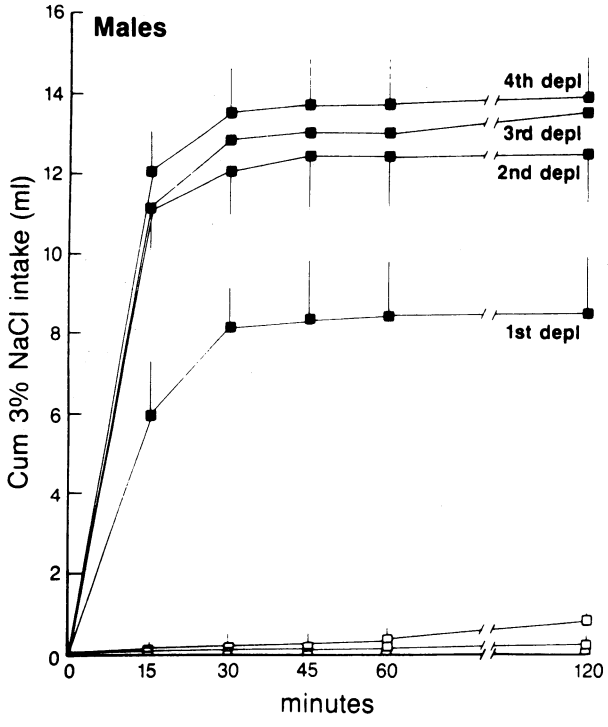

Fig. 7. Enhancement of NaCl intake in a two-hour test after a second and later salt-depletions as a consequences of a prior depletion (from Stellar and Epstein 1991).

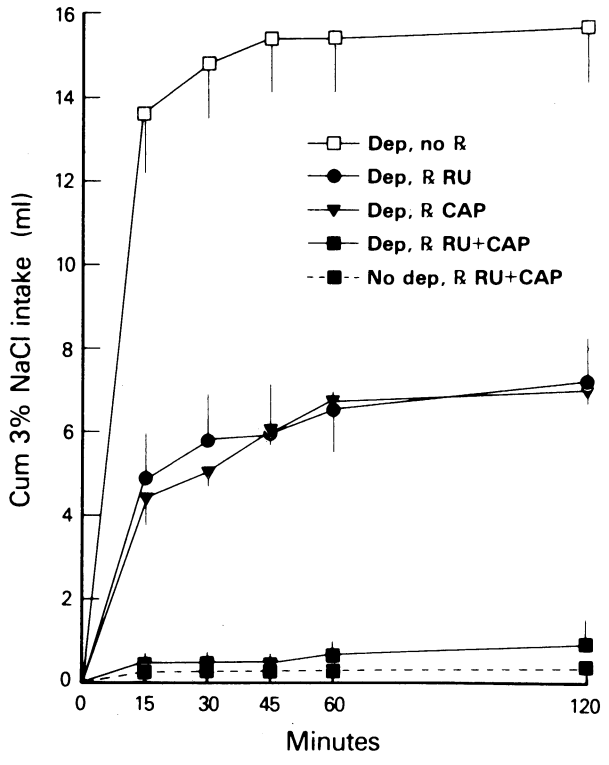

Fig. 8. Blocking either angiotensin or aldosterone in the brain reduces NaCl intake in a two-hour test by about one-half: blocking both eliminated NaCl intake (from Stellar and Epstein 1991).

that it is more reactive to subsequent effects of these hormones.

Further proof that these hormones are working together in the brain to produce salt appetite can be seen in a study in which aldosterone receptors are blocked in the brain by intracerebroventricular infusion of the aldosterone receptor blocker, RU28318, and blocking angiotensin II synthesis by administering captopril in the brain by the same route (Sakai et al. 1986). Either blocker alone reduces salt intake by about half (Fig. 8). However, when both blockers are given, salt appetite is eliminated even though the animals have been sodium depleted with furosemide. In the case of the adrenaleclomized rat which has no aldosterone, blocking angiotensin in the brain will eliminate salt appetite, even though the rat will die without ingesting salt (Sakai and Epstein 1990).

When the rat is salt replete, it drinks very little 3% NaCl, even in a 24-hour period. But following each successive depletion, the rats begin to ingest more and more 3% NaCl until after four depletions, they are drinking very significant amounts of 3% NaCl per 24 h that they do not need (Fig. 9) (Sakai et al. 1989). They have what we call a chronic, need-free sodium appetite. Evidently the change in the brain can be expressed as increased response to salt, even in the absence of depletion.

Another striking feature of the figure is the sexual dimorphism, seen not only in the higher (almost double) intake of the female rats, but also in the greater enhancement of their ingestion by depletions, including their need-free intake. This has been a consistent finding in the Epstein laboratory and suggests that the female rat may be more sensitive to sodium depletions because she has to meet extra demands for sodium in pregnancy and lactation. Further experiments show that the sexual dimorphism is a result of testosterone blunting the mechanism of salt appetite, for neonatal castration

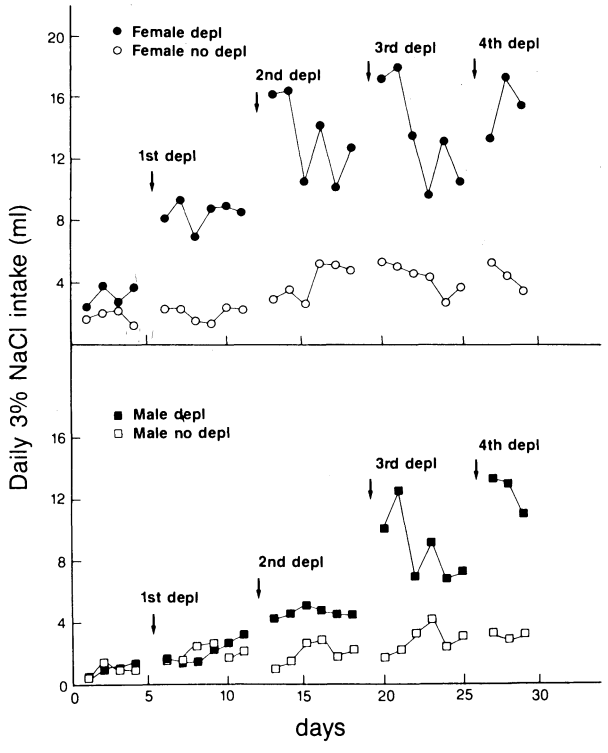

Fig. 9. Enhancement of 24-hour, need-free NaCl intake of salt-replete rats as a result of prior depletions. Note that the NaCl intake of female rats is almost double that of male rats (from Stellar and Epstein 1991).

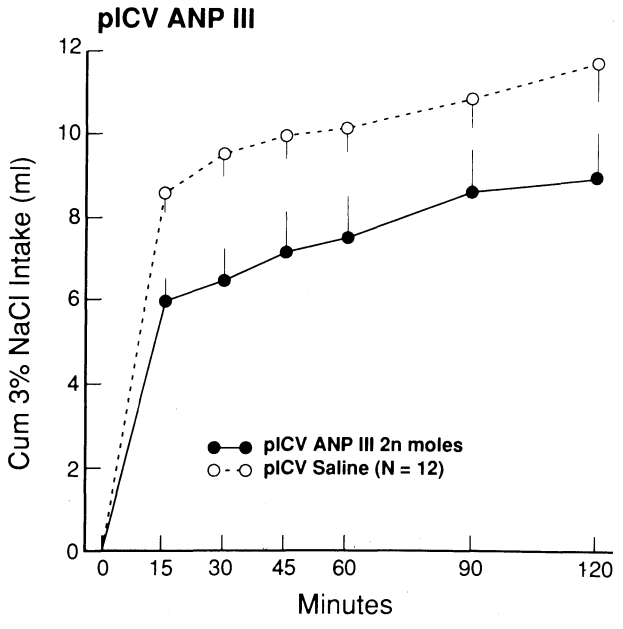

Fig. 10. Inraventricular injection of atrial natiuretic peptide (ANP) attenuates depletion-induced NaCl intake by about 40% (from Stellar and Epstein 1991).

make males ingest salt like females and administering testosterone neonatally reduces female salt intake to male levels (Chow et al. 1992).

Much of the successs of further behavioral experiments depends upon the investigations in Steven Fluharty's laboratory at Penn of angiotensin II receptors in the brain and in Bruce McEwen's laboratory at Rockefeller of aldosterone and corticosterone receptors in the brain. In both cases, quantitative autoradiographic studies and immunohistochemistry not only help identify areas of the brain rich in these receptor systems, but also identify the critical receptor subtypes, and even more intriguing, the interactions among these neuroendocrine systems. For example, in Fluharty's laboratory it has been shown that it is the Type 1 Angiotensin II receptor that is mainly involved in salt appetite and that our sodium depletion procedure produces enduring upregulation of these receptors and an amplification of signal transduction that might account for the enhancement of salt intake after one or more depletions (Stellar et al. 1992). In McEwen' s laboratory, it has been found that corticosterone also upregulates angiotensin receptors and increases angiotensin production (Stellar et al. 1992). So instead of just a synergy of angiotensin and aldosterone, we may have a trinity, involving corticosterone as well.

And of course we know it is more complicated than that, for other peptides are involved in modulating salt as well as water intake. In my laboratory, we have shown, as have others, that the atrial natriuretic peptide, made in the atrium of the heart and in the brain as well, reduces depletion-induced salt appetite by 40% (Fig. 10) and has much less of an effect on DOCA-induced appetite (Fig. 11), suggesting its main mode of action is through attenuating the effects of the angiotensin limb of the synergy. In a similar way, Alan's collaboration with colleagues in Camerino, Italy, shows that the tachykinins also attenuate salt appetite to a similar degree (Massi and Epstein 1989).

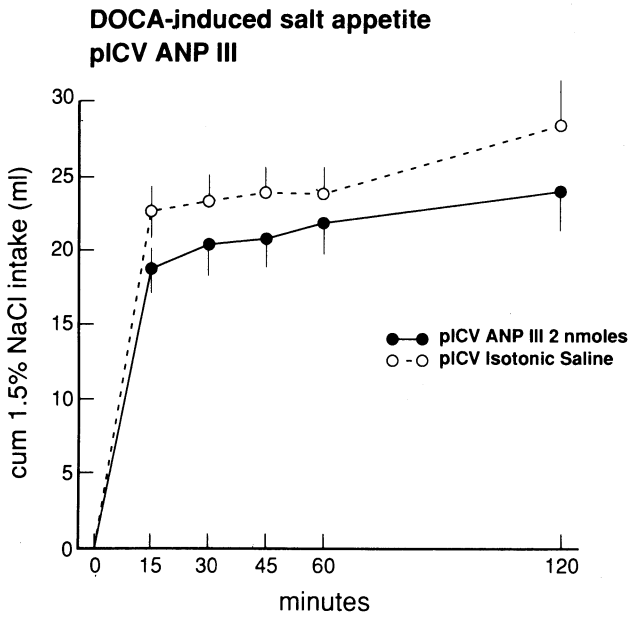

Fig. 11. Intracerebroventricular injection of ANP reduces DOCA-induced NaCl intake by only about 10% (from Stellar and Epstein 1991).

Taking advantage of autoradiographic studies, Jay Schulkin and Alan and their students have begun to make selective lesions in an effort to eliminate either the angiotensin or the aldosterone limb of the neural circuity. Initial studies with electrolytic lesions are encouraging and are being followed by more discrete ibotenic acid lesions. In summary, it has been found that lesions of the medial amygdala where aldosterone binds, eliminates the salt intake raised by both DOCA and aldosterone injections, but has no effect on depletion-induced salt appetite where angiotensin is believed to be the main factor (Schulkin et al. 1989). Similarly, lesions of the anterior wall of the third ventricle, the AV3V region rich in angiotensin receptors, block renin angiotensin-induced salt intake but not DOCA-induced; depletion-induced appetite is greatly attenuated (De Luca et al. 1991). Interesting enough need-free intake is not effected by the AV3V lesion. The next step now is to introduce specific agonists and antagonists of aldosterone and angiotensin into the medial amygdala and the AV3V region respectively.

On the basis of this work, Schulkin has drawn preliminary maps of the circuitry involved (Fig. 12), particularly the connections between the medial amygdala and the AV3V region as well as the NTS and the PBN circuits involved in processing the

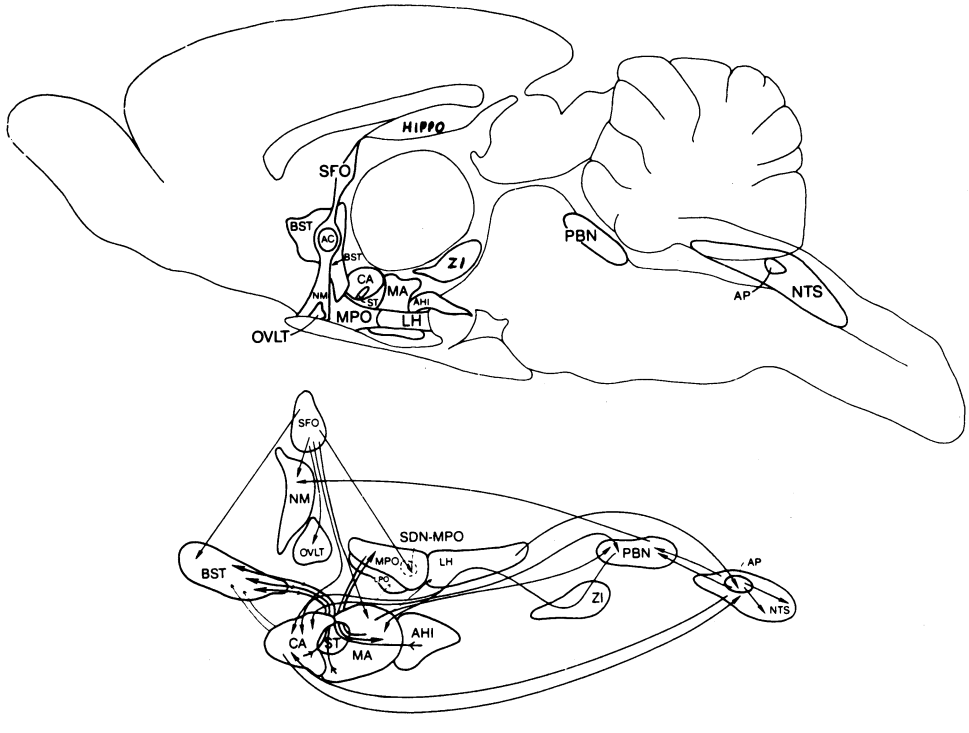

Fig. 12. Schulkin's conception of the connections involved in the circuits responsible for the synergistic effects of angiotensin (acting in the anterior wall of the third ventricle, SFO, NM, SST, OVLT) and aldosterone (acting in the medial amygdala, MA).

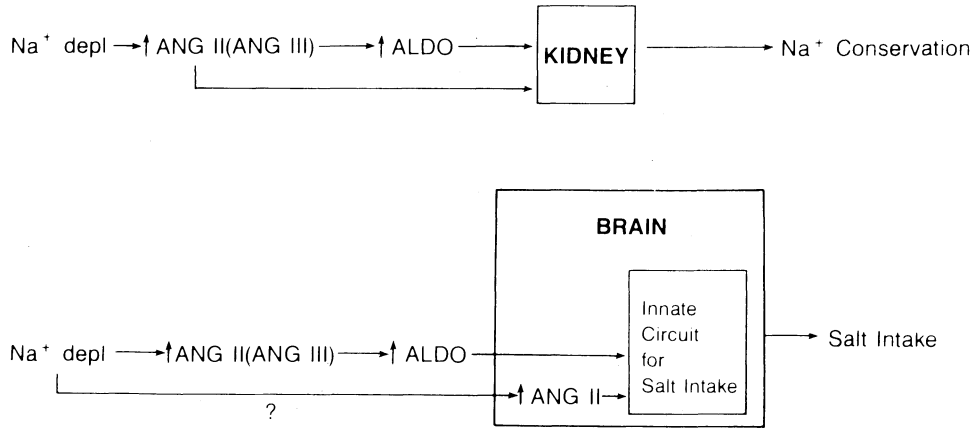

Fig. 13. The latest version of the Epstein synergy hypothesis, showing that angiotensin and aldosterone that act to conserve sodium at the kidney, act together in the brain in response to sodium depletion to arouse an innate circuite for salt intake.

taste afferents. He and his students now plan to use knife cuts to interrupt these connections and to learn whether the synergy is disrupted by any of them. The first results, transecting the stria terminalis, did not interfer with salt intake induced either by sodium depletion or DOCA adminstration (Black et al. 1992). Carrying out other transections will be important next steps.

This is how far we have gotten. Let me conclude with a brief summary.

1. The synergistic actions of angiotensin and aldosterone in the brain are the result of sodium depletion and lead to salt appetite and salt intake. It is interesting that these two hormones are also key to the conservation of salt at the kidney (Fig. 13).

2. Other peptides and steroids are involved in modulating salt appetite, especially ANP and the tachykinins and corticosterone. Stricker and his colleagues have also demonstrated the importance of oxytocin in controlling salt appedte (Stricker and Verbalis 1987)

3. We have entertained two possible mechanisms for the synergy. One is the "synergy neurons" that Nicolaidis has identified in the septal area and the other is the connections between the medial amygdala and the AV3V region in the anterior wall of the third ventricle. There are undoubtedly other mechanisms involved.

4. Prior episodes of sodium depletion alter the sensitivity of the brain to further depletions and result in increased salt intake. This may be related to upregulation of angiotensin receptors the brain.

5. The enhancement effect extends to need-free salt intake when the rats are sodium-replete and results in chronic elevated salt intake when there is no need for it.

6. Salt appetite is sexually dimorphic. Female rats ingest much more salt than males and are more enhanced by prior depletions.

7. Studies of changes in the receptors for angiotensin and aldosterone in the brain with sodium depletion are preparing the ground for a cell and molecular analysis of the mechanisms involved in salt appetite.

8. Finally, we consider the neuroendocrine basis for salt appetite a relatively simple model system of the brain mechanisms controlling ingestive behavior and yielding motivation, rewards, and hedonic experience.

ACKNOWLEDGEMENT

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