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Review article

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NEUROENDOCRINE FACTORS IN SALT APPETITE *

We dedicate this paper to Curt P. Richter, father of the study of salt appetite, who died recently at the age of 94. Richter first demonstrated that the adrenalectomized rat's voracious appetite for salt kept it alive (1936) and showed the same in humans (1940). Our first paper in 1955 demonstrated that salt appetite was an innate response to salt depletion. Since then, we have pursued the notion that the neuroendocrine consequences of sodium depletion create a brain state that raises salt appetite. In Epstein's laboratory, it was shown that angiotensin and aldosterone, the hormones of salt retention in the periphery, act synergistically in the brain to produce salt appetite in the rat. Block either hormone and the appetite is reduced by half; block both and the appetite is eliminated despite severe bodily need. With repeated depletions or treatments of the brain with angiotensin and aldosterone, salt ingestion increases, reaching an asymptote by the third depletion. Need-free intake of NaCl also increaes, especially in female rats which ingest more NaCl than male rats. In Stellar's laboratory, running speed to salt solutions in a runway is used as a measure of salt appetite. When the appetite is raised with large doses of DOCA, a mimic of aldosterone, rats run rapidly for a taste of strong salt solutions as high as 24% (almost 4 molar). Using ingestion as a measure, the role of the atrial natriuretic peptide (ANP), an antagonist of angiotensin's physiological effect, was investigated as a modulator of salt appetite. When angiotensin is involved is producing salt appetite, following sodium depletion by a diuretic combined with a low-salt diet, ANP reduced salt intake by 40%. When salt appetite was raised by DOCA, however, ANP either had no effect or reduced salt ingestion by only 10%. The subfornical organ, the lateral preoptic area, and the central and medial nuclei of the amygdala are being investigated as major components of the limbic circuit underlying salt appetite produced by the actions of angiotensin, aldosterone and ANP in the brain.

In 1954, one of us envisaged a motivational system in the brain, centered in the hypothalamus, but involving other structures as well (1) *(Fig. 1)*. Now, 36 years later, we know that the system involves the much broader circuits of the limbic system *(Fig. 2)*. Extensive experimental evidence makes it clear that the limbic system, including the hypothalamus, is involved in physiological regulations, motivated behavior, reward and reinforcement, and hedonic

* This paper is dedicated to the late C. P. Richter of Johns Hopkins who was the pioneer in laboratory studies of salt appetite and who got us started on this work.

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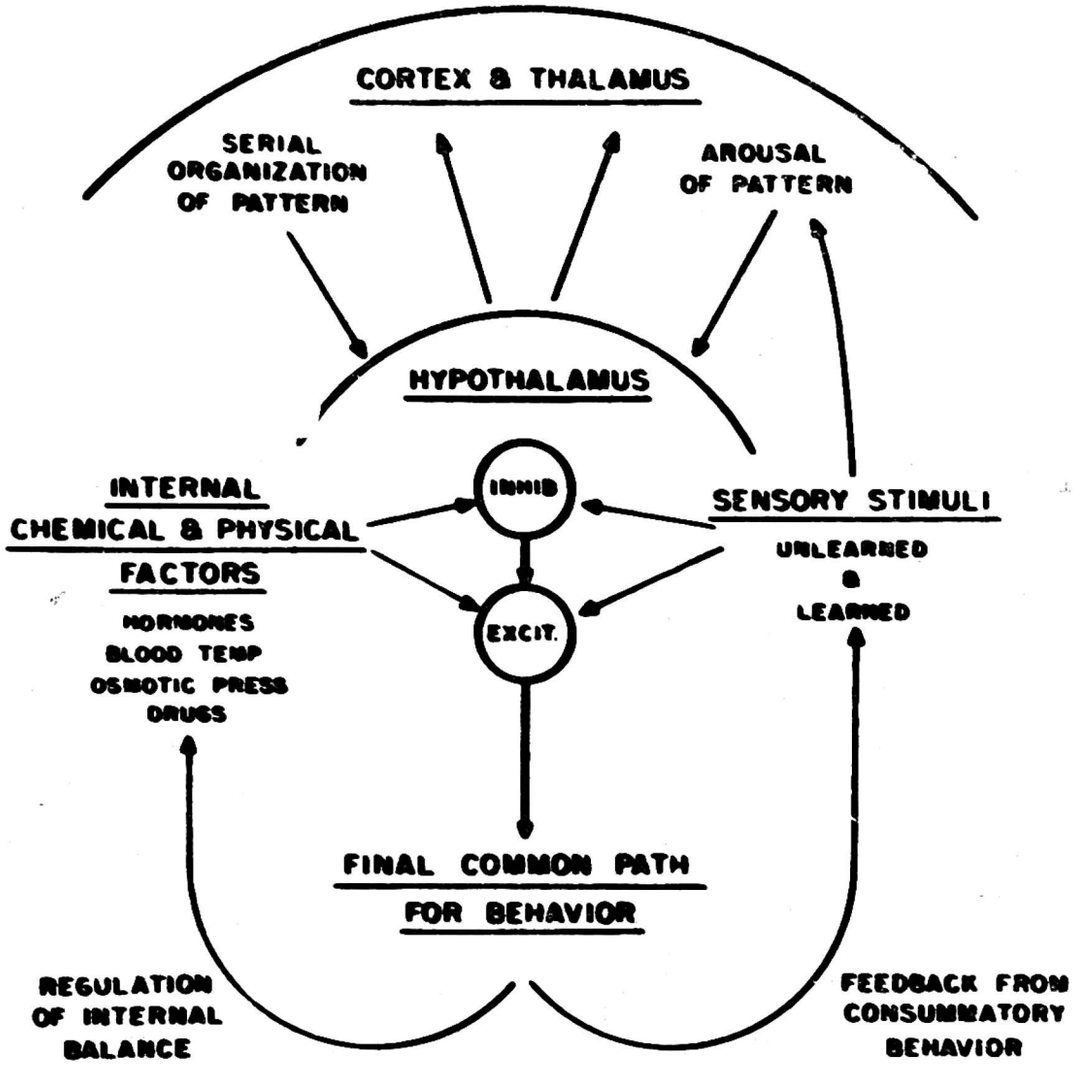

Fig. 1. 1954 diagram of the multifactor system underlying drive and motivation with its center in the hypothalamus.

experience in humans (2, 3). It is also clear, as C. P. Richter pointed out in 1942 (4), that behavioral mechanisms play a crucial role in physiological regulations and homeostasis, utilizing the same neuroendocrine mechanisms in the brain. Examples can be seen in hunger, the regulation of food intake, and energy balance and in thirst and salt appetite, the regulation of water and salt intake, and body fluid homeostasis. The same limbic mechanisms also appear to be at work in temperature regulation and thermoregulatory behavior; sexual, maternal, and parental behavior; aggression; sleep and wakefulness; emotion, etc.

Especially fruitful research along these lines has been the investigation of salt appetite, begun by us in 1955 (5), on the basis of prior work by Richter, dating back to 1936 (6). In this paper, we want to report our recent findings that show that angiotensin and aldosterone, hormones of salt conservation at the kidney, are hormones of salt appetite, working in synergy in the brain. These are mechanisms that are part of the neuroendocrine machinery of body fluid homeostasis and blood pressure regulation as well as salt appetite and motivated behavior.

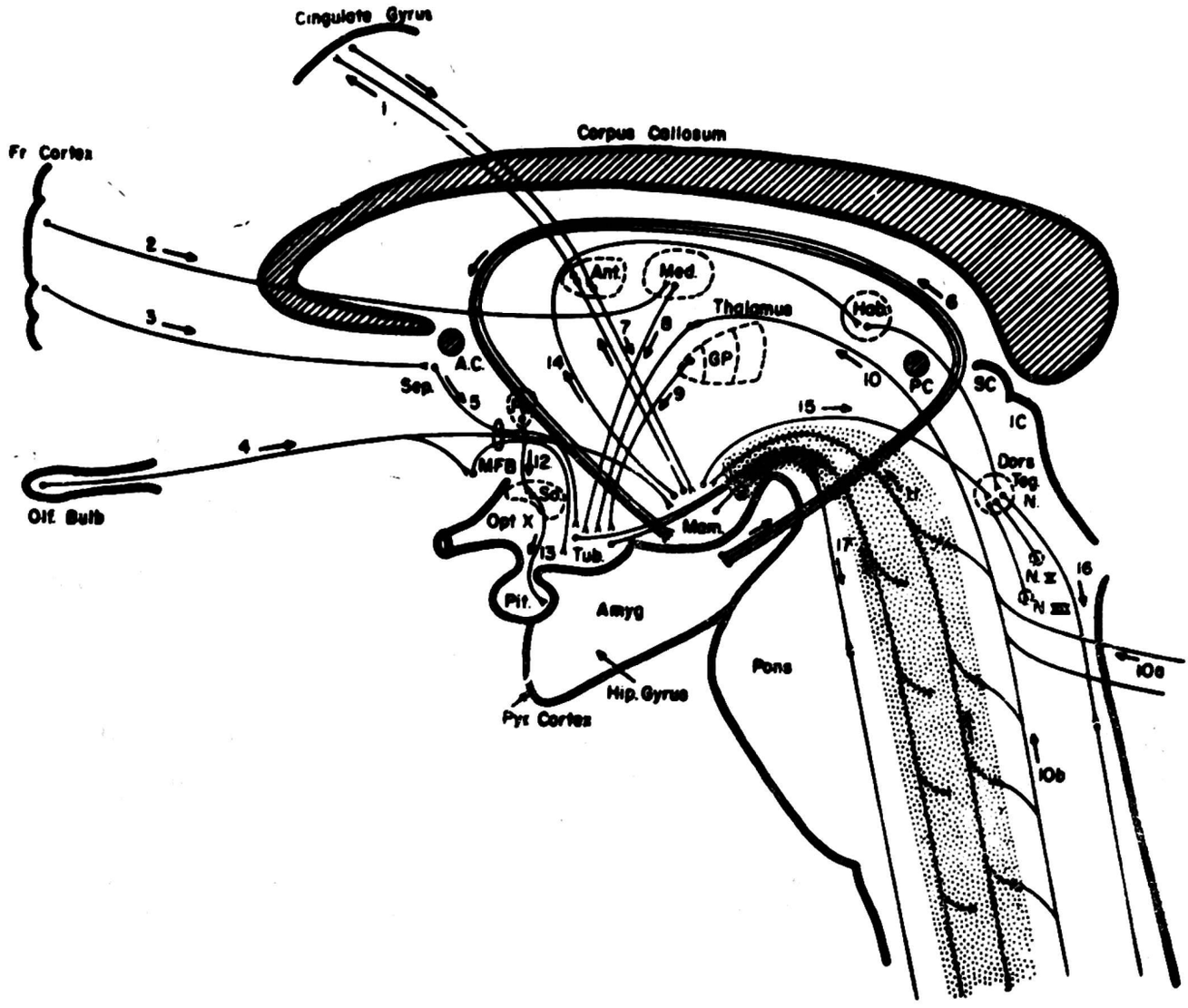

Fig. 2. Diagram of brain circuits, showing the hypothalamus as part of the broader limbic system.

The work reported here is part of a larger endeavor that includes a number of investigators, collaborating in an NIMH program project grant *(Fig. 3)*, aimed at levels of analysis that go all the way from the behavior to the underlying neural circuits in the limbic system used by angiotensin and aldosterone, to the cellular and molecular mechanisms involved in the synergy that produces the salt hunger.

The key molecule is angiotensin *(Fig. 4)*, called into action by renin in the periphery and in the brain as well. Angiotensin stimulates the release of aldosterone from the adrenal glands, and together with vasopressin, these hormones promote salt and water retention at the kidney. In the brain, angiotensin, in synergy with aldosterone, promotes salt and water intake. These behavioral and physiological effects, taken together with angiotensin's vasopressor effects protect the integrity of vascular volume and blood pressure.

To study the neuroendocrine basis of salt appetite specifically, rats are treated with the diuretic natriuretic drug, furosemide (two 5 mg. doses subcutaneously), are given a low-salt diet, are kept in cages cleaned of ambient sodium and are allowed to restore their water loss by free access to water.

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An NIMH Program Project for Research on

The Neurohormonal Mechanisms of Ingestive Behavior

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Richard R. Miselis
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Pennsylvania State University
(Hershey)
James S. Schwaber
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Fig. 3. Members of a program project grant, dedicated to a reductionist analysis of salt appetite that extends from behavior through the neurendocrine circuits involved to the cell and molecular levels.

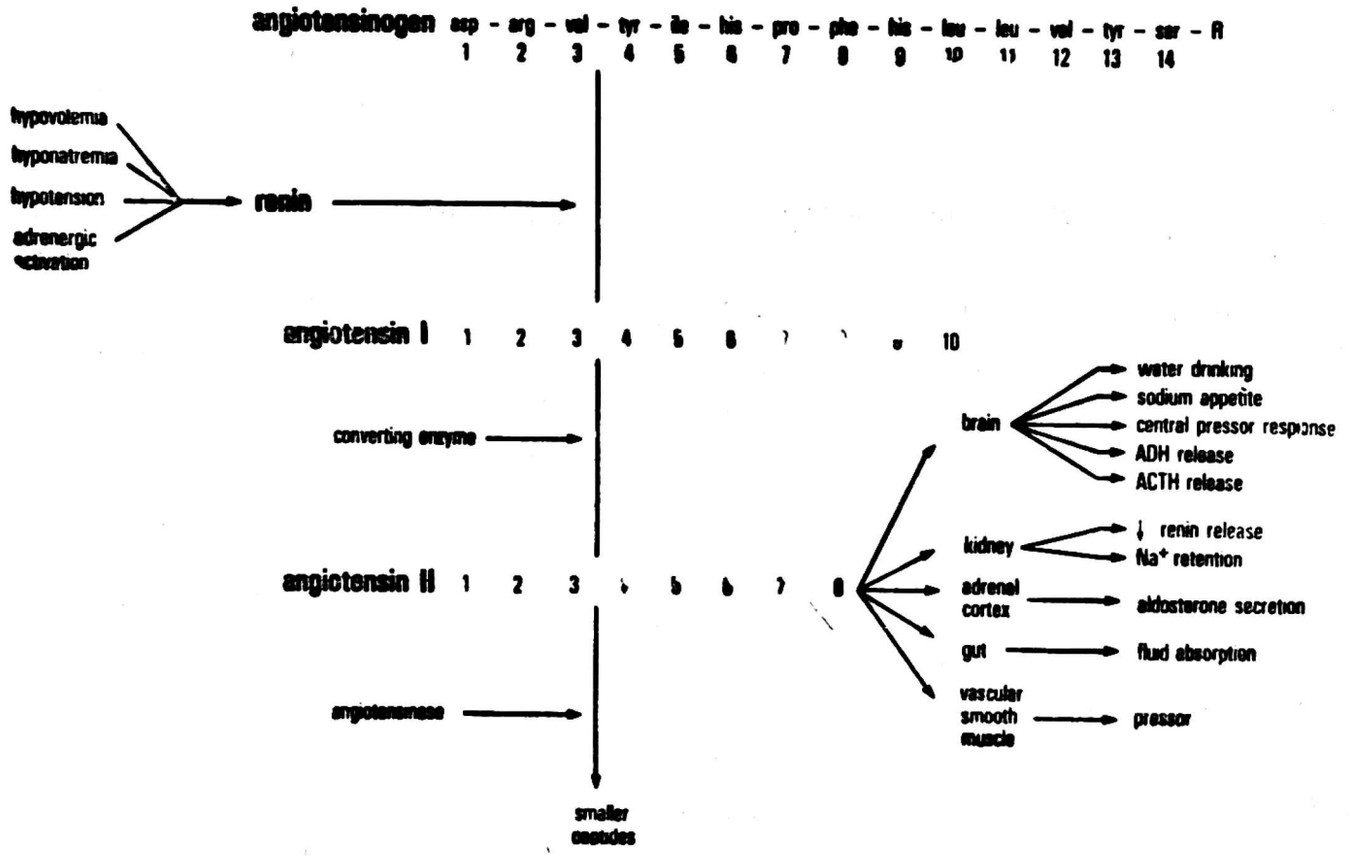

Fig. 4. Multiple functions of the angiotensin molecule.

As you can see from *Fig. 5*, a robust intake of 3% sodium chloride solution is produced in a two-hour intake test. Even more remarkable, this salt appetite is greatly enhanced if the treatment is repeated a week later as a second

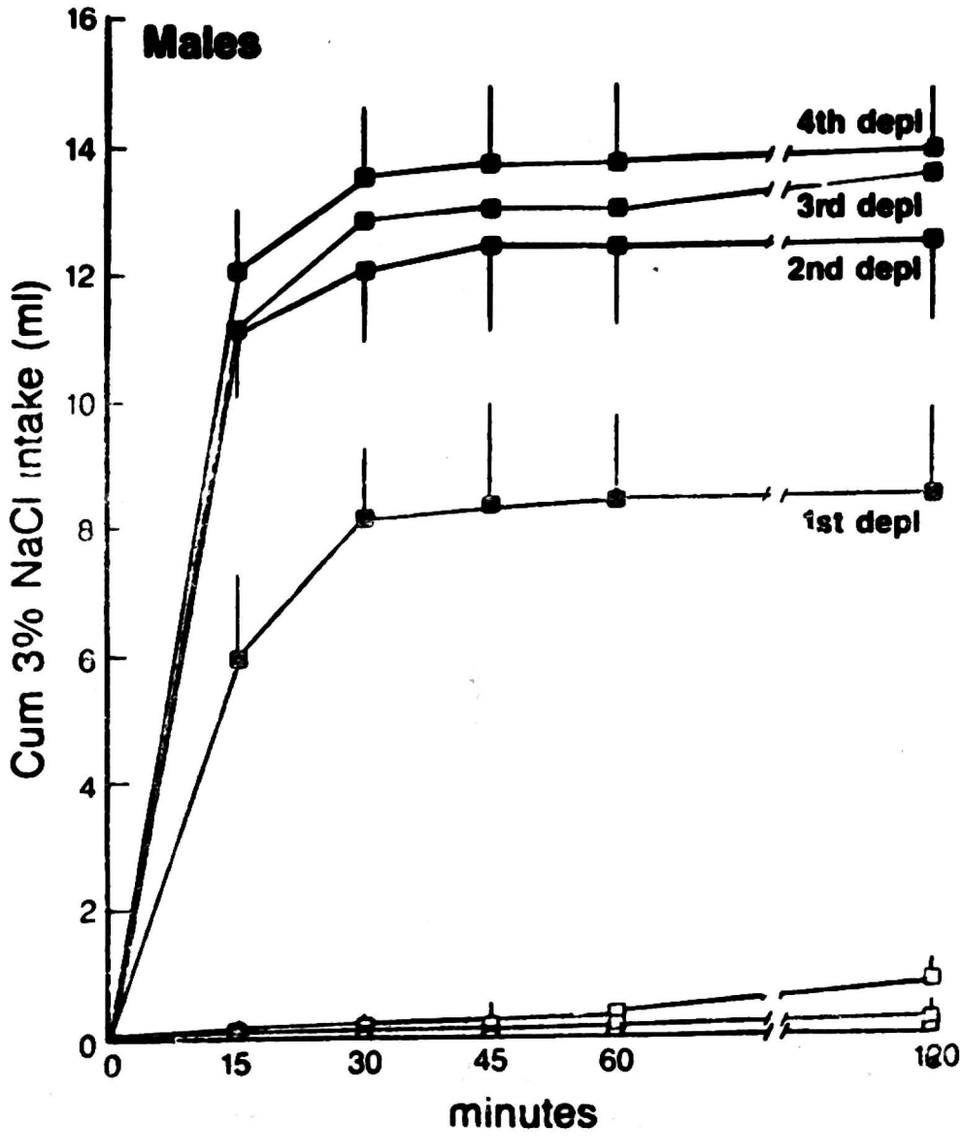

Fig. 5. High level of intake of 3% NaCl following a salt depletion treatment with furosemide. Note that when the treatment is repeated a week later, the intake is greatly increased.

depletion, and the intake is doubled as Sakai, Fine Frankmann & Epstein (7) showed. The same enhancement can be produced even if the rats never have the experience of drinking salt solutions, but restore their sodium balance by eating their normal diet, rich in salt. More remarkable, the enhanced level of salt intake can be produced by treating rats with aldosterone, and then administering a pulse injection of angiotensin into the brain ventricles one week prior to the first sodium depletion. So the experience of salt depletion or the synergistic action of angiotensin and aldosterone produce salt appetite and change the brain to enhance it.

Further demonstration of this neuroendocrine synergy can be made by blocking aldosterone receptors with RU 28318 or the production of angiotensin II by captopril (8) *(Fig. 6)*. Using one blocker alone reduces enhanced salt intake by one-half. Using both blockers eliminates salt intake entirely. Even more dramatic is the effect the angiotensin II blocker in eliminating the

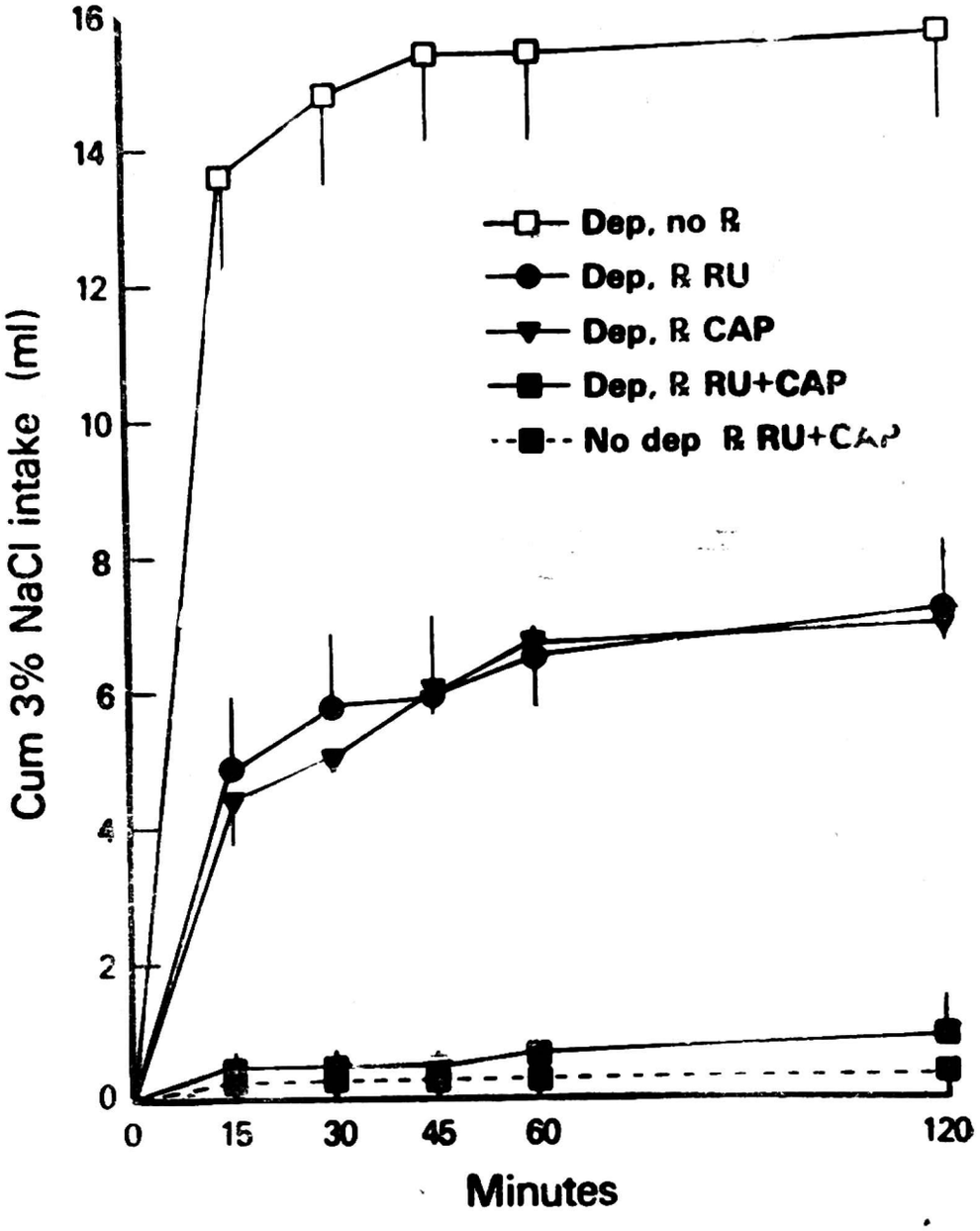

Fig. 6. Blocking angiotensin II synthesis with captopril and aldosterone receptors in the brain with intracerebroventricular infusion of RU-28318 virtually eliminates salt appetite induced by furosemide treatment. Blocking only one of the hormones reduces salt intake, but doesn't eliminate it.

salt appetite of the adrenalectomized rat that has no aldosterone and will die unless it ingests large amounts of salt (9).

Two other findings are of interest. One is that the salt appetite of the female rat is normally about twice that of the male rat (10), suggesting an important role for this neuroendocrine mechanism in maintaining salt levels during pregnancy and lactation when the demands on the female for salt are high. The second is that salt depletion produces enhanced need-free salt intake, so that rats come to ingest much more 3% sodium chloride when they are salt replete (10). In other words, there is excessive salt intake after an experience of depletion, even when the rat doesn't need salt. Both of these effects have possible health implications. The sexual dimorphism and the enhanced need-free intake are shown in *Fig. 7*.

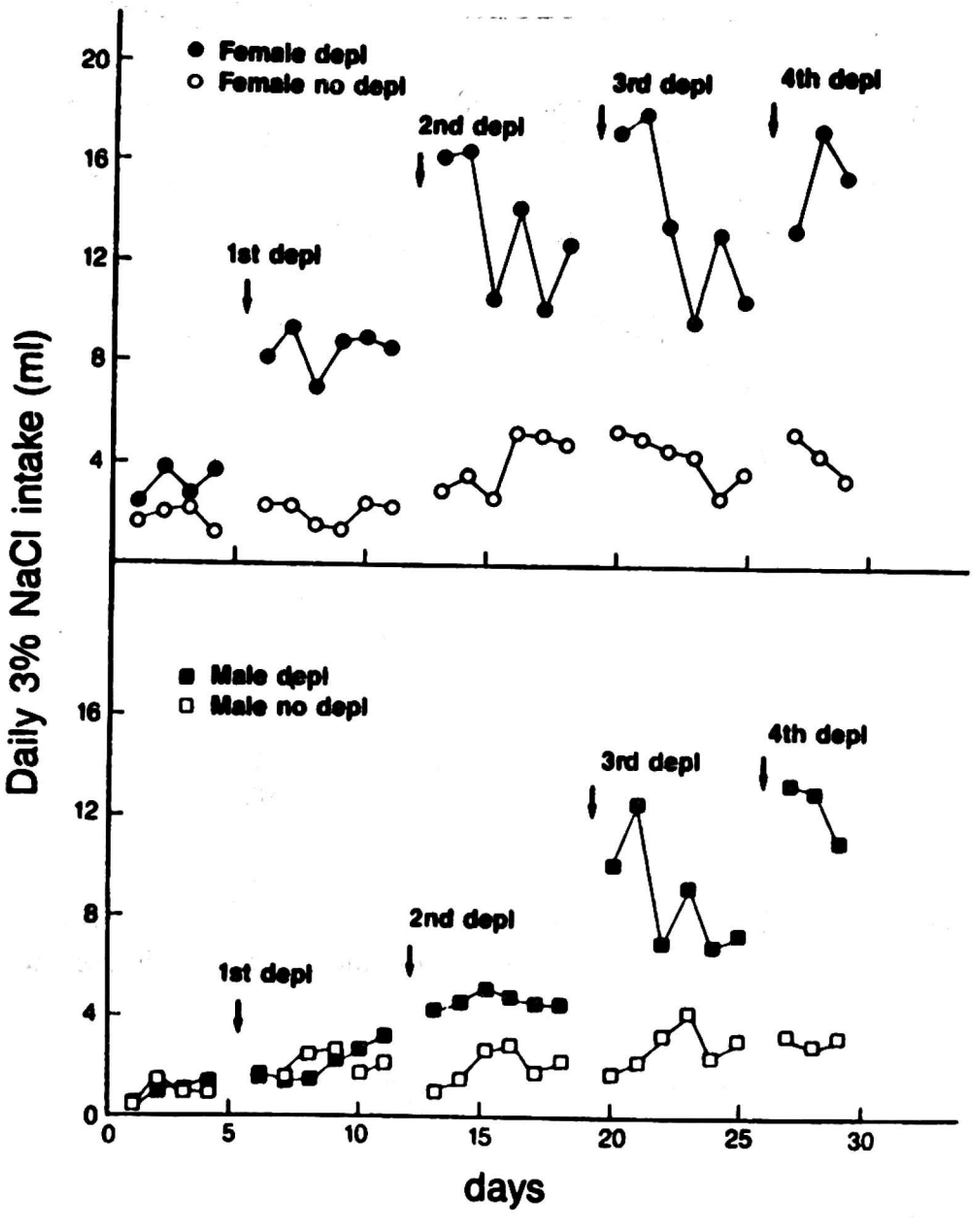

Fig. 7. The increase in need-free intake of 3% NaCl over a series of salt-depletion treatments with furosemide. Note that female rats drink more than twice as much sodium chloride as male rats and are more responsive to repeated depletion.

A third hormone, the atrial natriuretic peptide, ANP, plays a role in modulating salt intake and salt retention, primarily by counteracting the effects of angiotensin. ANP, made in the atrium of the heart and also synthesized in the brain, has a clear and consistent effect of reducing the salt intake produced by furosemide-induced salt depletion *(Fig. 8)* — about a 40% reduction of intake. When salt appetite is induced through subcubtaneous DOCA injections, the modulation of intake is minimal, *(Fig. 9)*. So ANP appears to be a modulator of salt intake that acts mainly through the angiotensin component of the angiotensin-aldosterone synergy.

Finally, we have begun experiments, under Jay Schulkin's leadership, exploring sites in the limbic system where the synergy of angiotensin and aldosterone might take place in the production of salt hunger. Guided by where concentrations of angiotensin and aldosterone receptors occur, Schulkin

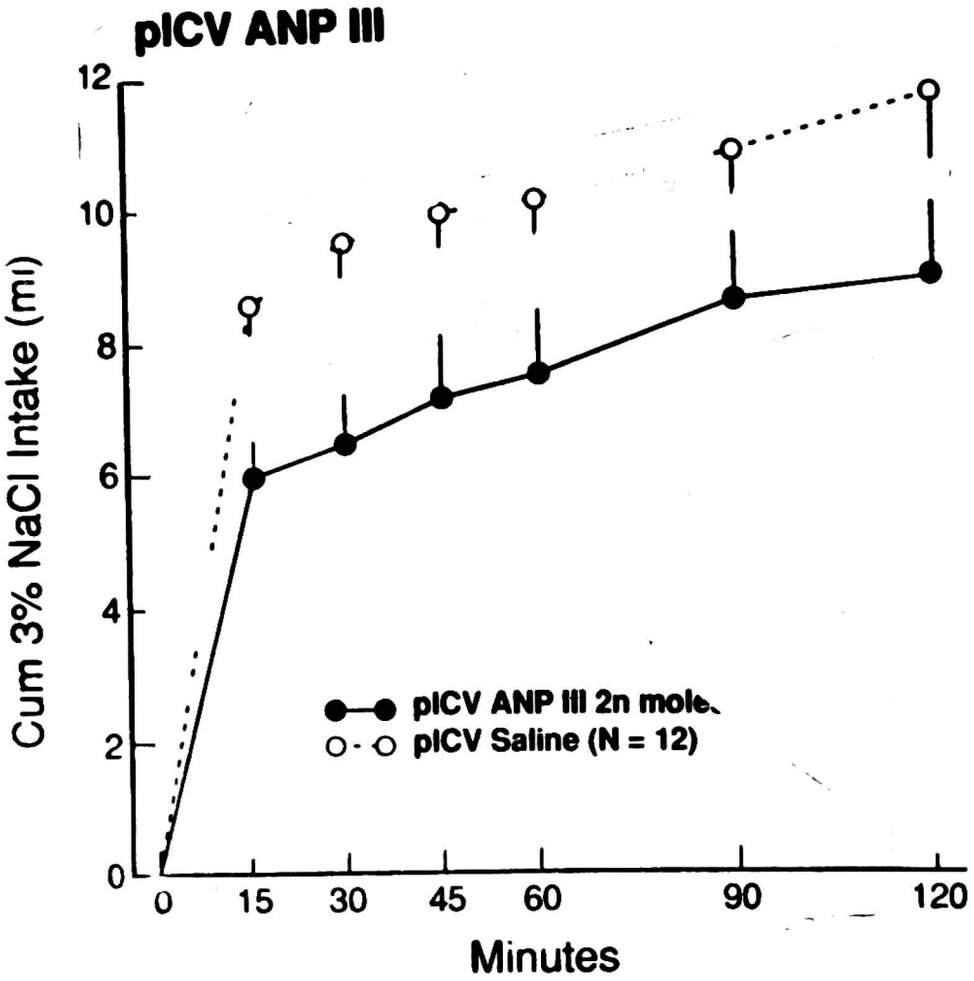

Fig. 8. The suppression of 3% NaCl intake by pulse intracerebroventricular (pICV) injection of 2 nmoles of atrial natriuretic peptide (ANP) when furosemide treatment is used to produce salt depletion.

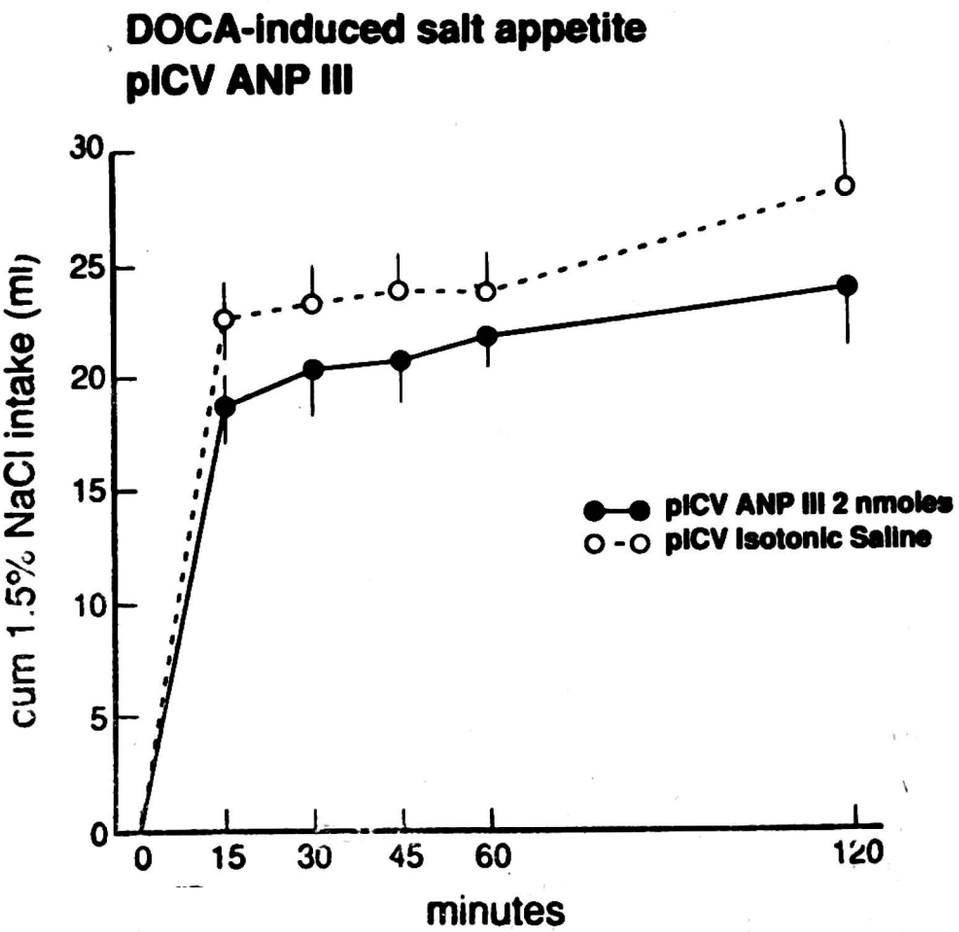

Fig. 9. The failure of pICV ANP to produce a significant reduction in salt intake produced by subcutaneous DOCA injections.

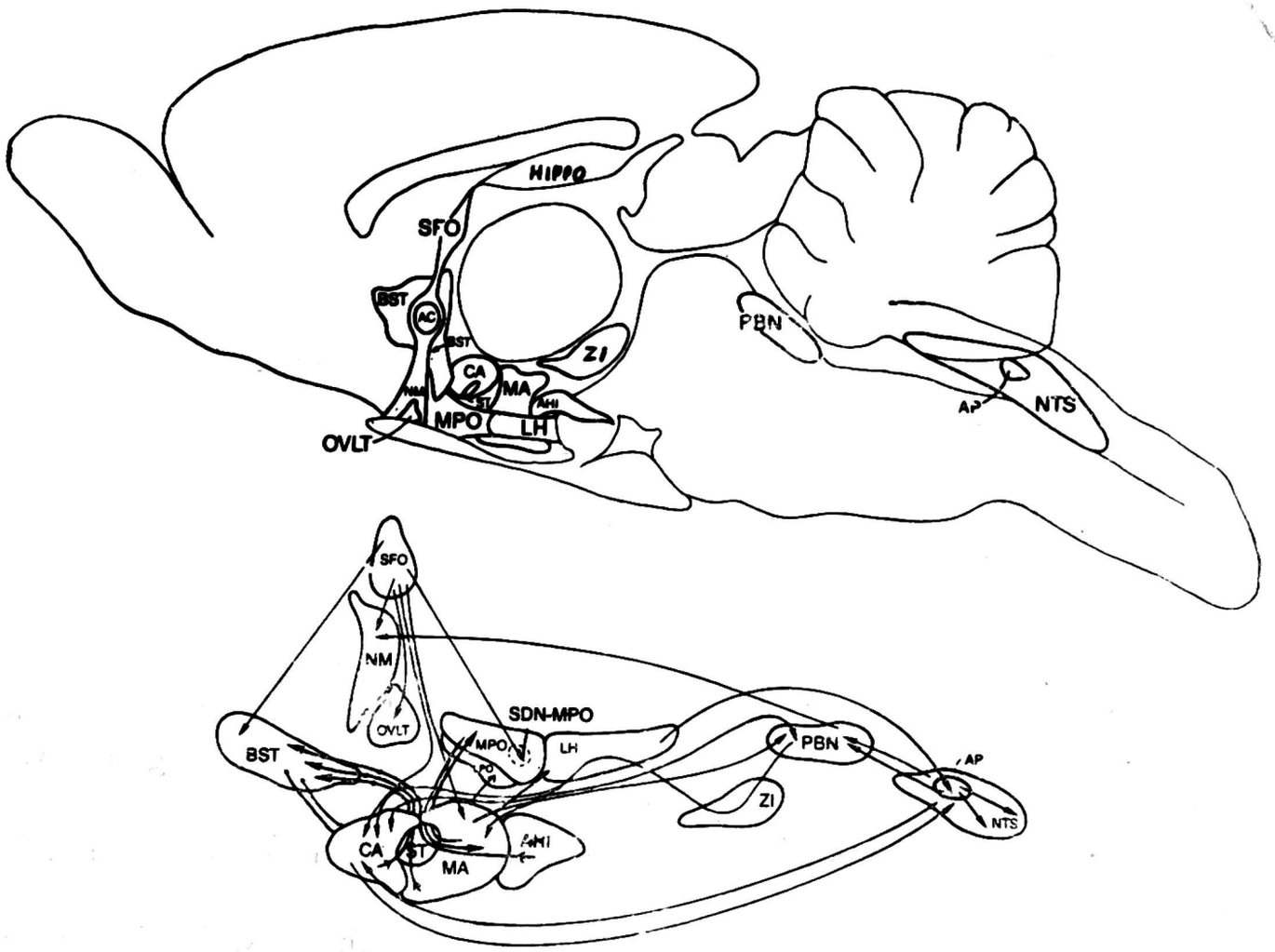

Fig. 10. Proposed map of the limbic structures involved in the neuroendocrine synergy that produces salt appetite. Relevant structures are: the medial amygala (MA) and the AV3V area consisting of the subfornical organ (SFO), nucleus medianus (NM), organum vasculosum of the lamina terminalis (OVLT), and the bed nucleus of the stria terminalis (BST), and the connections from the taste afferents reaching the nucleus tractus solitarius (NTS) and the parabrachial nucleus (PBN).

and Epstein and their students have been making lesions in the medial amygdala where aldosterone binds and in the anterior wall of the third ventricle, the AV3V region, where angiotensin binds. Lesions of the medial amygdala block salt appetite, raised by both DOCA and aldosterone injections, but not depletion-induced salt appetite (11, 12). Lesions of the AV3V area appear to block renin-angiotensin-induced salt appetite, but not DOCA-induced or depletion-induced salt appetite. These findings have led Schulkin and colleagues to develop preliminary maps of the limbic structures and possible connections involved in the synergistic action of angiotensin and aldosterone in producing salt appetite *(Fig. 10)*. These include connections between the amygdala and the AV3V area (SFO, N. medianus, OVLT, BST) and connections from the taste afferents reaching the NTS and PBN. Now we are in a position to do knife-cut experiments and to focus on the cellular and molecular mechanisms in these regions where the hormones act and the sexual dimorphism may reside.

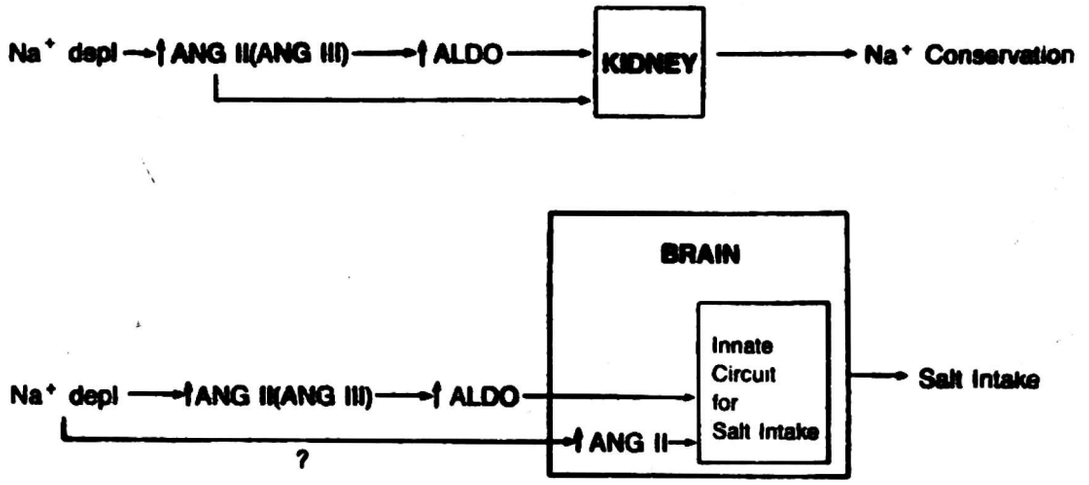

Fig. 11. Diagram showing how angiotensin and aldosterone, hormones of salt conservation at the kidney, work in synergy in the brain to produce salt appetite.

In summary, then, our work has revealed that angiotensin and aldosterone, the hormones that play a role in conserving salt at the kidney, also work in synergy in the brain to raise salt appetite *(Fig. 11)*. From the forgoing, we conclude:

1) There is a hard-wired, innate mechanism in the limbic system that is triggered by salt depletion.

2) Salt depletion activates both peripheral angiotensin II, which increases aldosterone output, and also brain angiotensin II.

3) It is the synergy of angiotensin II and aldosterone in the brain, modulated by ANP, that arouses salt appetite.

4) We speculate that the limbic circuit involved includes the medial amygdala for aldosterone effects, the AV3V region for angiotensin effects, and their connections for the neuroendocrine synergy. It is at these sites that we can get at the cell and molecular mechanisms involved.

5) Finally, the biological significance of this particular limbic mechanism lies in its role in body fluid homeostasis, yielding the motivated behavior we observe as salt appetite.

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