Resistance to Outflow of Cerebrospinal Fluid after Central Infusions of Angiotensin

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Abstract. **Infusions of artificial cerebrospinal fluid (CSF) into the cerebroventricles of conscious rats can raise CSF pressure (CSFp). This response can be modified by some neuropeptides. One of these, angiotensin, facilitates the rise in CSFp. We measured CSFp in conscious rats with a computerized system and evaluated resistance to CSF outflow during infusion of artificial CSF, with or without angiotensin, from the decay kinetics of superimposed bolus injections. Angiotensin (10 ng/min) raised CSFp ($P < 0.05$) compared with solvent, but the resistance to CSF outflow of the two groups was similar ($P > 0.05$). Because CSFp was increased by angiotensin without an increase in the outflow resistance, a change in some volume compartment is likely. Angiotensin may raise CSFp by increasing CSF synthesis; this possibility is supported, since the choroid plexuses contain an intrinsic isorenin-angiotensin system. Alternatively, angiotensin may dilate pial arteries, leading to an increased intracranial blood volume.**

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An expanding literature suggests that various neuropeptides in cerebrospinal fluid (CSF) affect CSF pressure (CSFp) in normal and pathophysiological conditions. These include angiotensin (1, 2), vasopressin (3, 4), somatostatin (5), and atrial natriuretic peptide (6, 7). Another experimental observation, reported by several laboratories (6, 8–10), is that cerebroventricular infusions of sterile, isosmotic, balanced salt solutions, at rates similar to endogenous CSF synthesis, gradually increase CSFp after a lag of several hours. A potential reason for such a solvent effect is that it alters the activity of an endogenous neuropeptide(s) that contributes to the normal regulation of CSFp.

One neuropeptide, angiotensin, raised CSFp during a 30-min cerebroventricular infusion, whereas solvent was without effect (1). When these experiments were prolonged for 5 hr, angiotensin again increased CSFp rapidly, but the artificial CSF solvent, between hr 2 and 5, also raised CSFp (8). In general, CSFp is thought to be independent of the rate of volume input into the ventricular system, unless there is an obstruction to flow. For example, Betz *et al.* (11) state: "A normal human can absorb CSF at a rate up to four to six times the normal rate of CSF formation with only a moderate increase in intracranial pressure." (See also Ref. 12).

In the experiments cited, the inflow rate was 2 μl/min, which is the approximate rate of CSF synthesis in rats (13). Based on this moderate input rate, the rapidity of the CSFp increase after angiotensin, and the long delay before solvent effects, we proposed (8) that the peptide elevated CSFp by affecting resistance to CSF outflow (Ro). On the other hand, the choroid plexuses appear to have a complete, intrinsic renin-angiotensin system (14). The normal function of choroidal tissue is to synthesize CSF.

There are, to our knowledge, no experimental data to judge whether an angiotensin-stimulated increase in CSFp is related to either the synthesis or resistance components of CSF dynamics. Therefore, the experiment to be described was designed to specifically evaluate Ro in conscious rats whose CSFp was elevated by a central infusion of angiotensin.

Materials and Methods

The subjects of the experiments were male Sprague-Dawley rats weighing between 300 and 400 g. They

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were cared for in accordance with the *Guide for Care and Use of Laboratory Animals* (DHEW/NIH publication no. 86-23, 1985). The rats underwent a surgical procedure (pentobarbital, 45 mg/kg ip, and methoxyflurane by inhalation, if needed) to implant a lateral cerebroventricular cannula (15). The cannula was centered in a hollow freezer vial cap and cemented, along with a small stainless steel anchor screw, to the skull. At least 48 hr were allowed for recovery.

On the experimental day, the rats were placed in individual metabolism cages, with food and water available. A fluid-filled needle/tubing system was inserted into the preimplanted guide cannula. The tubing was connected to a liquid swivel which in turn was connected to an ultralow Harvard pressure transducer and sealed silastic ports that could be connected to infusion pumps as required. The output from the transducers was amplified, digitized, and stored on the hard disk of a microcomputer at a rate of 1 sample/sec. A cathode ray tube screen permitted real-time monitoring of CSFp. The fluid in the sealed system was a sterile, isosmotic, protein-free, balanced salt solution (16). (See Ref. 17 for complete technical details).

Baseline CSFp were recorded and, at Time 0, a 100-min infusion of artificial CSF, 2 μl/min, with or without 10 ng/min angiotensin II (ile^5-angiotensin II; Sigma Chemical Co.), was started. CSFp data were partitioned into 10-min time bins and averaged for statistical analyses. Bolus injections, 10 μl/0.5 sec, were made using a Gralab timer attached to a pump at 5 min before and at 60 and 90 min into the infusion. The first 5 sec of recording after bolus injections were bypassed for volume distribution, and the linear regression of the pressure decay curve over the next 60 sec was used to calculate Ro.

Ro was calculated according to the standard procedure described by Marmarou *et al.* (18):

$$Ro = \frac{t \cdot Po}{[V/\log(Pp/Po)]\log \{Pt/Pp[Pp - Po)/(Pt - Po)]\}} \quad [1]$$

where V is the 10-μl bolus volume, Pp is the peak pressure 5 sec after the bolus, Po is the baseline pressure before the bolus, t is a selected time after the bolus, and Pt is CSFp at that time.

CSFp and Ro data for the control and angiotensin groups were evaluated by two-way analysis of variance with time as a repeat measure. Significant ($P < 0.05$) F-ratios were evaluated with the Newman-Keuls range statistic.

Results

The effects of the artificial CSF and angiotensin infusions on CSFp are illustrated in Figure 1. The analysis of variance yielded a significant F-ratio (5.4, df = 1,17, $P < 0.05$) for treatment, which documents an overall increase in CSFp evoked by angiotensin. The F-ratio for time was also significant (F = 14, df = 11,187, $P < 0.01$). The Newman-Keuls test showed that the baseline data before the first bolus injection were similar between groups ($P > 0.05$), but CSFp rose in both groups afterward ($P < 0.05$). The interaction term of the analysis of variance was not significant (F = 2.1, df = 11,187, $P > 0.1$). It is important to note that the bolus injections delivered *additional* angiotensin or solvent into the infusion/recording system and these pulses are incorporated into the subsequent 10-min time bins. The distortion of the system, inherent in pressure/volume tests, may explain why the angiotensin group began to show a rise before the CSF group, and why the small solvent effect was observed before 2 hr (8).

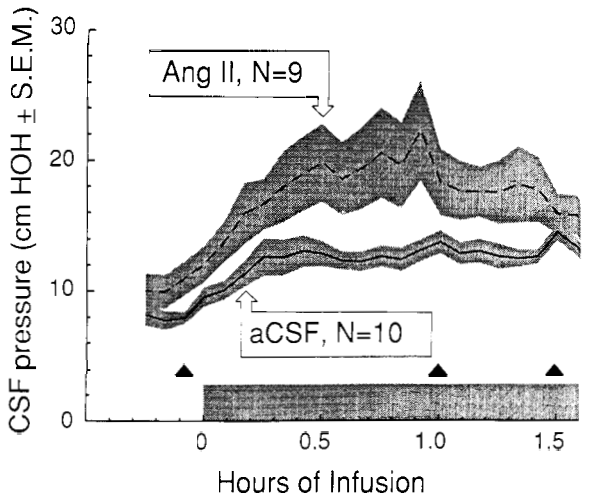

Figure 1. Cerebrospinal fluid pressure of conscious rats receiving a 100-min infusion of artificial CSF with or without angiotensin (10 ng/min). Analysis of variance showed that pressure during the infusion period was elevated ($P < 0.05$) by angiotensin compared with CSF, although CSFp rose in both groups. The preinfusion and terminal CSFp were similar ($P > 0.05$). The triangles indicate times when bolus injections were made to determine resistance to outflow.

Angiotensin caused drinking behavior, mostly at the start of the infusion. Angiotensin-treated rats drank 5.2 ± 1.7 ml, compared with 0.2 ± 0.1 ml ($P < 0.05$, t test) for the solvent-treated rats. This volume is less than anticipated, but, again, the protocol incorporated pressure/volume boluses, which differs from similar studies (1, 8). No other behavioral signs or symptoms, such as ataxia or unresponsiveness, were observed, as would occur with acute cerebral edema.

The Ro data are shown in Figure 2. The analysis of variance showed that the control and angiotensin groups were similar at all times (F = 0.7, df = 1,48, $P > 0.25$), and the times were also similar (F = 0.6, df = 2,47, $P > 0.30$). There was no significant interaction between treatment and time.

Discussion

Several experiments suggested that angiotensin could affect intracranial pressure. Vertebral arterial in-

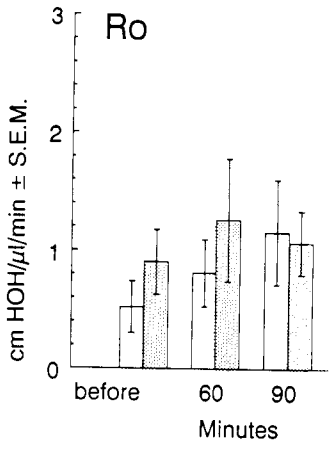

Figure 2. Resistance to outflow of cerebrospinal fluid in conscious rats. The data correspond to the times shown as the triangles in Figure 1. Open bars represent control (N = 10) and hatched bars represent angiotensin-treated rats (N = 9). Analysis of variance revealed no effects ($P > 0.05$).

fusions in anesthetized dogs raised CSFp (19), and unilateral carotid arterial infusions of the peptide produced a symmetrical reduction in the cerebral blood flow of anesthetized rabbits (20). Also, as indicated earlier, cerebroventricular administration of angiotensin elevates CSFp in rodents (1, 8). However, to our knowledge, there are no data dealing with the general mechanism by which the peptide increases CSFp. Because angiotensin increases CSFp relatively rapidly (1) and CSFp is relatively independent of volume input (11), we anticipated that the peptide might increase Ro. The data obtained reject this hypothesis because CSFp was increased by the peptide when Ro was not. There is good evidence that choroid plexuses contain an intrinsic renin-angiotensin system (14). Since the function of this tissue is to secrete CSF, it is possible that CSFp is elevated by a large increase in the rate of volume input into the cerebroventricles.

More recently (21), *topical* application of angiotensin in rats with a closed cranial window was found to exert a strong vasodilator action at micromolar concentrations. This activity was suggested to result from receptors reached from CSF (22), which would be the contact route in our experiments. Thus, the rapid action of angiotensin to increase CSFp may be explained by a centrally mediated increase in cerebral blood volume.

Many investigators have noted that cerebroventricular infusions of balanced salt solutions, after a relatively long lag time, raise CSFp (6, 8–10). The data from this study showed a solvent induced rise in CSFp after a relatively short latency. This may be due to the rapid bolus injection used to determine resistance to outflow. Nevertheless, Ro in the CSF and angiotensin groups were always similar, and the rise in CSFp in either group did not appear to be in a pathological range (15, 17). Prolonged infusions of balanced salt solutions may result in a delayed increase in CSFp by diluting an endogenous peptide(s), by stimulating the release of an endogenous peptide(s), or both. An evaluation of the mechanisms of peptidergic control of CSFp may suggest new treatment strategies to control intracranial hypertension.

Conclusions

Angiotensin II, infused into the cerebroventricles of conscious rats, raises CSF pressure compared with solvent, but it does not change resistance to outflow of CSF. Since resistance to outflow was unchanged, the angiotensin effect may be related to increased CSF synthesis. Alternately, pial arterial dilation is a possibility.

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1. Barbella YR, Keil LC, Wurpel JND, Severs WB. Cerebrospinal fluid pressure during cerebroventricular infusion of angiotensin and vasopressin. Exp Neurol **82**:325–334, 1983.
2. Senay LC, Tolbert DL. Effect of arginine vasopressin, acetazolamide and angiotensin II on CSF pressure at simulated altitude. Aviat Space Environ Med **55**:370–376, 1984.
3. Seckl J, Lightman S. Cerebrospinal fluid neurohypophysial peptides in benign essential hypertension. J Neurol Neurosurg Psychiatry **51**:1538–1541, 1988.
4. Reeder RF, Nattie EE, North WG. Effect of vasopressin on cold-induced brain edema in cats. J Neurosurg **64**:941–950, 1986.
5. Stepien H, Stanowy A, Cieslak D, Brzezinski J, Pawlikowski M. Increased CSF levels of somatostatin in patients with brain tumors and intracranial hypertension. Horm Metab Res **18**:555–557, 1986.
6. Leavitt ML, Loesch D, Maroon JC. Effect of intraventricular atrial natriuretic factor on intracranial pressure in the awake rat. Physiologist **33**:A39, 1990.
7. Steardo L, Nathanson JA. Brain barrier tissues: End organs for atriopeptins. Science **235**:470–473, 1987.
8. Severs WB, Keil LC, Wurpel JND, Dundore RL. Cerebrospinal fluid pressure of conscious rats: Effects of artificial CSF, angiotensin and vasopressin infusions. In: Buckley JP, Ferrario CM, Eds. Brain Peptides and Catecholamines in Cardiovascular Regulation. New York: Raven Press, p403, 1987.
9. Mouw DR, Vander AJ, Landis C, Kutschinski S, Mathias N, Zimmerman D. Dose-response relation of CSF sodium and renal excretion, and its absence in homozygous Brattleboro rats. Neuroendocrinology **30**:206–212, 1980.
10. Nakamura K, Osborn JW, Cowley AW. Pressor response to small elevations of cerebroventricular pressure in conscious rats. Hypertension **10**:635–641, 1987.
11. Betz AL, Goldstein GW, Katzman R. Blood-brain-CSF barriers. In: Siegel G, Agranoff B, Albers RW, Molinoff P, Eds. Basic Neurochemistry, 4th ed. New York: Raven Press, p591, 1989.
12. Katzman R, Hussey F. A simple constant-infusion manometric test for measurement of CSF absorption. Neurology **20**:535–544, 1970.
13. Cserr HF. Physiology of the choroid plexus. Physiol Rev **51**:273–311, 1971.
14. Hirose S, Ohsawa T, Murakami K, Inagami T. Pituitary renin:

Purification and characterization. In: Ganten D, Printz M, Phillips MI, Scholkens BA, Eds. The Renin-Angiotensin System in the Brain. New York: Springer-Verlag, p53, 1982.
15. Starcevic VP, Morrow BA, Farner LA, Keil LC, Severs WB. Long-term recording of cerebrospinal fluid pressure in freely behaving rats. Brain Res **462**:112–117, 1988.
16. Merlis JK. The effect of changes in the calcium content of the cerebrospinal fluid on spinal reflex activity in the dog. Am J Physiol **131**:67–72, 1940.
17. Morrow BA, Starcevic VP, Keil LC, Severs WB. Intracranial hypertension after cerebroventricular infusions in rats. Am J Physiol **258**:R1170–R1176, 1990.
18. Marmarou A, Shulman K, LaMorgese J. Compartmental analysis of compliance and outflow resistance of the cerebrospinal fluid system. J Neurosurg **43**:523–534, 1975.
19. Berti G, Ferrario CM. Discussion. In: Buckley JP, Ferrario CM, Eds. Central Actions of Angiotensin and Related Hormones. New York: Pergamon Press, p169, 1977.
20. Reynier-Rebuffel A, Pinard E, Aubineau P, Meric P, Seylaz J. Generalized cerebral vasconstriction induced by intracarotid infusion of angiotensin in the rabbit. Brain Res **269**:91–98, 1983.
21. Haberl RL, Anneser F, Villringer A, Einhaupl KM. Angiotensin II induces endothelium-dependent vasodilation of rat cerebral arterioles. Am J Physiol **258**:H1840–H1846, 1990.
22. Haberl RL, Decker PJ, Einhaupl KM. Stimulation of brain angiotensin generation increases cerebral blood flow. Fed J **5**:A741, 1991.