Possible contribution of brain angiotensin III to ingestive behaviors in baboons

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1Department of Physiology and Medicine and 3Southwest Regional Primate Research Center, Southwest Foundation for Biomedical Research, San Antonio, Texas 78245-0549; and 2Department of Physiology and 4Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria 3010, Australia

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Blair-West, J. R., K. D. Carey, D. A. Denton, L. J. Madden, R. S. Weisinger, and R. E. Shade. Possible contribution of brain angiotensin III to ingestive behaviors in baboons. Am J Physiol Regulatory Integrative Comp Physiol 281: R1633–R1636, 2001.—Recent experiments with specific aminopeptidase inhibitors in rats have strengthened earlier proposals that ANG III may be an important regulatory peptide in the brain. Central mechanisms regulating blood pressure, ingestive behaviors, and vasopressin release could be involved. Arguments in favor of a role for ANG III depend, in part, on the efficacy of ANG III as an agonist. These first studies in primates tested whether ANG III stimulates ingestive behaviors in baboons. Intracerebroventricular (ICV) infusions of ANG III were as potent as ANG II in stimulating water drinking and intake of NaCl solution. On the basis of this criterion and consistent with findings in rats, ANG III could be a main effector peptide in the regulation of ingestive behaviors in a primate.

angiotensin II; sodium intake; water intake; intracerebroventricular infusion

OUR RECENT STUDIES (1, 10) in baboons (Papio hamadryas sensu lato) found that prolonged intracerebroventricular (ICV) infusion of ANG II increased the daily voluntary intake of water and 300 mM NaCl solution. Concurrent ICV infusions of ANG II receptor antagonists inhibited those responses and also inhibited the increased water intake caused by water restriction and the increased 300 mM NaCl intake caused by furosemide administration. These findings, consistent with results in some other nonprimate mammals, led to the proposal that brain ANG II was involved in both thirst and Na appetite in baboons (1, 10).

In peripheral ANG systems, ANG II is the main effector peptide in the systemic circulation, although exogenous ANG III can be as potent as ANG II in, for example, stimulating aldosterone secretion (2) or inhibiting renin release (3) in sheep. In the rat brain, ANG III was found to be equipotent with ANG II as a pressor agent or dipsogen (4, 7, 13–15), and then Har-
surgery. On that day, they were anesthetized or in recovery throughout the morning. For those reasons, the intakes of water, NaCl solution, and food were often reduced in that 24-h period.

**Agents used.** The vehicle for infusions was sterile 0.9% saline. Solutions were prepared in sterile syringes and Milipore filtered into osmotic pumps. 2-ML-2 or 2-ML-4 pumps were used during baseline periods, and 2-ML-1 (10 μl/h) pumps were used for 7-day infusions of agents.

ANG II (human octapeptide; Bachem, Torrance, CA) and ANG III (human 2-8 heptapeptide; Bachem) were infused intraventricularly at 5 and 4.6 μg/h (approximately equimolar), respectively. These doses were chosen because ANG II infusion at 5 μg/h had consistently and significantly increased water and NaCl solution consumption in earlier experiments in baboons (1).

**Experimental procedures.** After a baseline of 6 days, the pump containing 0.9% saline was changed at ~0900 to a pump containing ANG III (4.6 μg/10 μl). After 7 days of observations, this pump was changed at ~0900 to a pump containing saline. Observations were continued for 7 recovery days. After a rest period of at least 1 mo, the experiment was repeated with ICV infusion of ANG II (5.0 μg/10 μl).

**Statistical analysis.** Data are presented as means ± SE. A two-way repeated-measures ANOVA (2-factor repetition) was used to test for significant differences between the effects of ANG III and ANG II. A one-way repeated-measures ANOVA was used to detect significant time effects within each infusion treatment with post hoc least significant difference tests to establish significant changes from the baseline condition.

**RESULTS**

Daily intakes of fluids during baseline and ANG infusion periods varied considerably between baboons (Fig. 1). For example, in the ANG III experiments, the baboon with the lowest NaCl intake in the baseline period consumed 5.0 ± 1.2 mmol/day (mean of the baboon's 6 baseline day values), whereas the highest consumption was 230 ± 24 mmol/day. ANG III infusion increased daily NaCl intake in these two baboons to maxima of 57 and 818 mmol, respectively. One baboon had little or no increase in daily fluid intakes with ANG III infusion, whereas the other five baboons increased NaCl intake 3- to 15-fold and almost doubled water intake. Similar variability was observed in the ANG II experiments.

There were no significant differences (2-factor ANOVA, repeated measures) between the effects of ANG III and ANG II (Fig. 1) on daily NaCl intake, Na balance (Na intake from NaCl solution and food minus Na loss in urine), water intake, or food intake (not shown in Fig. 1). For the combined ANG III and ANG II data, 1) NaCl intake (Fig. 1, top) was significantly increased compared with baseline on all infusion days except day 1, 2) water intake (Fig. 1, bottom) was significantly increased on infusion days 3–6, and 3) Na balance was not significantly changed (Fig. 1, middle).

When the ANG III and ANG II data were tested separately (1-factor ANOVA, repeated measures), both of the peptides significantly increased NaCl intake on infusion days 3–7 compared with baseline. Both peptides significantly increased water intake on infusion day 3. The increase continued for 4 days with ANG III and for 3 days with ANG II infusion.

The mean daily Na balances were all small positive values throughout the baseline period (consistent with the unmeasured daily loss of Na in feces) except on baseline day 6, when pre- and postsurgical procedures reduced NaCl and water intakes (Fig. 1). There was no evidence that the baboons went into negative Na balance before the increases in NaCl intake caused by peptide infusion. Mean daily Na balances varied more widely during the infusion period than in the baseline period. Baseline values were almost restored by the end of the recovery period.

Three of six baboons reduced daily food intake during the ANG II infusion; two of those baboons had also eaten less during the ANG III infusion. Those three baboons were the first to increase daily NaCl intake in response to ANG infusion, and their intakes rose to the higher levels.

**DISCUSSION**

If ANG III were the main effector peptide of the brain ANG system, then it ought to be as potent as ANG II in mimicking the central actions of this peptide system. The close similarity of the actions of ANG III and ANG II on water and NaCl intake in these baboons suggests that the role of ANG III in stimulating these ingestive behaviors could be analogous with the major role proposed for ANG III in regulating pressor and vasopressin release systems in the rat brain (8, 9, 16).

The comparison of ANG II and ANG III in the present experiments was limited to a single equimolar dose level. The 5-μg/h level of ANG II was shown to be consistently effective in stimulating NaCl and water intake in earlier experiments (1, 10), and the same responses were observed here. Evidence presented here, and earlier with respect to ANG II (1, 10), indicates that the increases of NaCl intake beginning on day 1 or day 2 of peptide infusion were not secondary to urinary loss of Na caused by possible central and cardiovascular actions of exogenous angiotensins.

Conclusions based on a single large equimolar dose level of the peptides must be treated with some caution because studies with large doses in rats and other species tend to favor the argument that ANG II is a more potent dipsogen than ANG III (for review, see Ref. 5). There is also uncertainty arising from the unknown relative stability of these peptides in cerebrospinal fluid and brain tissue in baboons.

The high NaCl and water intakes reached by days 3–4 of the two ANG infusions were not sustained through the remainder of the infusion period. Late declines were observed in most baboons, but the greatest impact on mean intake values was due to the large declines that occurred in the baboons with the largest increases in water and NaCl intakes. For those baboons, the declines may be explained by feedback from cardiovascular and gastrointestinal systems, including malaise. Such reactions may also explain the transient reduction in daily food intake in some baboons.
In summary, the equipotency of ICV infusions of ANG III and ANG II in evoking water and salt ingestions in baboons satisfies one of the prerequisites for establishing a role for brain ANG III in the regulation of these behaviors. However, proof of that role, up to the level of proving that the conversion of ANG II to ANG III is a requirement for this function of the brain ANG system, must wait on the availability of specific aminopeptidase inhibitors.

**Perspectives**

Brain angiotensinergic mechanisms are implicated in regulating blood pressure, vasopressin release, ingestive behaviors, and renal function. The octapeptide ANG II has been accepted as the major biologically active form of this peptide in the past, but evidence continues to accumulate showing that smaller fragments have significant and specific functions. The present experiments extend this evidence into a primate species. The heptapeptide ANG III may have a specific central role in thirst and sodium appetite in baboons. Reservations about this conclusion are imposed by the use of only one equimolar dose of ANG II and ANG III and by possible differences in the stability of the two peptides in cerebral spinal fluid and brain tissue. Further experiments need to be done with wa-
ter restriction and sodium depletion to prove the effector role of ANG III in physiological situations. These experiments will require the availability of highly specific aminopeptidase inhibitors. They should also include monitoring of arterial blood pressure and the secretion of hypophyseal hormones.

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