PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 160, 278-280 (1979)

On the Role of Angiotensin in the Cerebellum[1] (40434)

CHRISTINE B. THOMAS,[2] LANNY C. KEIL, PATRICIA A. KLASE, AND WALTER B. SEVERS

Department of Pharmacology, The Milton S. Hershey Medical Center of The Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033 and (LCK) Biomedical Research Division, NASA, Ames Research Center, Moffett Field, California 94035

A considerable amount of research has been performed to determine the effects of angiotensin on the central nervous system. The peptide is endogenous to mammalian brain (1–3), where it may act to regulate blood pressure and salt/water balance (4, 5). Recently, immunohistochemical mapping of the rat brain revealed a high density of angiotensin-positive synapses in the deep cerebellar nuclei (6). High affinity receptors for angiotensin have also been found in cerebellar tissue (7, 8). However, the cerebellum has never been implicated as a site of action of angiotensin. The present experiments were designed to determine if an angiotensin injection into the region of the dentate nuclei evoked pressor responses, drinking behavior or release of vasopressin and ACTH. These four effects are known to occur after administration of angiotensin into other central nervous structures (4, 5).

Materials and methods. Adult male Sprague-Dawley rats (310–380 g) were anesthetized with sodium pentobarbital (40 mg/kg ip) to implant a cannula (9) directed at the superior surface of the right dentate nucleus. The cannula and a small stainless steel anchor screw were cemented to the skull with dental acrylic. In these experiments the cannula location approximated plate 79 of Pellagrino and Cushman's atlas (10) at a depth of 4 mm and lateral, 3.2 mm. Injection of 1 μl fast green showed that dye penetration was less than 1 mm in all directions within an hour. Dye spread included portions of the dentate and interpositus nuclei. Dye was not detectable in the ventricular system.

Six rats with accurate cannula placements were used for blood pressure and drinking behavior experiments. Two days after cerebellar cannulation a catheter was inserted into the caudal artery under ether anesthesia (11). On the following day the rats were placed in individual cages with a calibrated water bottle; the arterial catheter was connected to a Beckman Dynograph by a Statham pressure transducer. Rats received a sequence of three injections into the cerebellar cannula with a Hamilton microsyringe fitted with a stop to prevent the needle from penetrating beyond the cannula tip. The first injection was 1 μl artificial cerebrospinal fluid (CSF, 12); angiotensin II (Hypertensin, 100 ng/1 μl CSF) was given 1 hr later, followed by a second angiotensin injection (same dose) 3 hr after the original injection.

Rats with cerebellar cannulae implanted 3 days earlier were injected with 1 μl CSF or angiotensin (100 ng/1 μl CSF). They were decapitated 1 or 5 min later and trunk blood was collected into heparinized tubes kept in ice water. Plasma was separated in a refrigerated centrifuge and frozen until assayed for vasopressin (13) and corticosterone (14).

Results. Blood pressure and drinking. The blood pressure catheter was patent in five of six rats. Their mean blood pressure was 108 ± 2.6 mmHg (mean ± SE). None of the intracerebellar injections significantly altered blood pressure ($p > 0.2$, paired t test) over the 4 hr observation period. None of the six rats drank during this interval.

Plasma vasopressin and corticosterone. The results of the hormone assays appear in Table I. Angiotensin did not affect plasma vasopressin or corticosterone levels at 1 or 5 min (2-way analysis of variance, $F < 2$, df 144). Plasma corticosterone at 1 min and 5 min were slightly, but significantly, different ($p < 0.01$, analysis of variance, $F = 7.8$, df 144).

Discussion. The role of angiotensin in the

[1] Supported by Grant NSG-2122 from the National Aeronautics and Space Administration.

[2] Present address: Department of Surgery, Yale University School of Medicine, New Haven, Connecticut 06510.

0037-9727/79/0278-03$01.00/0

TABLE I. PLASMA VASOPRESSIN AND CORTICOSTERONE CONTENT AFTER ANGIOTENSIN INJECTION INTO THE CEREBELLUM.

	Vasopressin, pg/ml ± SE		Corticosterone, μg/dl ± SE	
	1 min	5 min	1 min	5 min
Control Injection*	1.63 ±0.13	1.41 ±0.14	19.3 ±1.1	20.8 ±0.8
Angiotensin (100 ng)*	2.09 ±0.40	1.59 ±0.12	18.2 ±0.6	20.9 ±0.5

* All means represent data from 11 to 14 rats.

brain may be of considerable importance because of the marked ability of the peptide to increase blood pressure and affect salt/water balance by central nervous mechanisms. This area of research has recently been summarized in the proceedings of an International Symposium (15). A major impetus to the study of angiotensin's neuropharmacology was the discovery that renin and angiotensin are endogenous to brain tissue (1, 2). Reid (16) suggested that brain renin was Cathepsin D, a nonspecific acid protease, but recent work has separated brain renin and cathepsin activities (3).

Immunohistochemical techniques have shown that the cerebellum contains angiotensin immunoprecipitate in the fastigial and dentate nuclei (6), as well as purkinje cells and fiber tracts (6, 17). The presence of high affinity receptors for angiotensin in cerebellar tissue (7, 8) has also been established. These investigations suggest that the cerebellum may be important in mediating central effects of angiotensin but this brain structure has never been implicated as a site of action.

The experiments reported here were designed to determine whether angiotensin injection into the dentate-interpositus nuclear region would produce any or all of four known effects of the peptide, namely: a rise in blood pressure, drinking behavior, release of vasopressin or elevation of plasma corticosterone. Blood pressure and drinking behavior were observed for several hours after angiotensin but no changes occurred. Only occasional bilateral facial tremors were observed in some of the rats. Cerebroventricular injection of 100 ng angiotensin elevated plasma vasopressin markedly within 1 min (18), but the cerebellar injection of the same dose did not alter the level of this hormone in plasma after 1 or 5 min. Plasma corticosterone rises within 5 min of known stimuli (13), but again, no effect of angiotensin was observed in this study. Thus, the role of angiotensin in the region of the cerebellar dentate-interpositus nuclei may be different from the known cardiovascular and hydration effects of the peptide.

Summary. Deep cerebellar nuclei contain angiotensin-positive synapses and high affinity receptors for angiotensin have been found in the cerebellum. Four known central effects of angiotensin were examined after injection of the peptide into the region of the dentate-interpositus nuclei: blood pressure elevation, drinking behavior, vasopressin release and ACTH release. None of these effects could be demonstrated, although the dose of angiotensin was known to produce effects when injected at other sites. The results suggest that angiotensin actions in this cerebellar region may be distinct from its known cardiovascular and hydration effects.

1. Fischer-Ferraro, C., Nahmod, V. E., Goldstein, D. J., and Finkielman, S., J. Exp. Med. **133,** 353 (1971).
2. Ganten, D., Marquez-Julio, A., Granger, P., Hayduk, K., Karsunky, K. P., Boucher, R., and Genest, J., Am. J. Physiol. **221,** 1733 (1971).
3. Yokosawa, H., Hirose, S., and Inagami, T., Fed. Proc. **37,** 1385 (1978).
4. Severs, W. B., and Daniels-Severs, A. E., Pharmacol. Rev. **25,** 415 (1973).
5. Ganten, D., Hutchinson, J. S., Schelling, P., Ganten, U., and Fischer, H., Clin. Exp. Pharmacol. Physiol. **3,** 103 (1976).
6. Changaris, D. G., Keil, L. C., and Severs, W. B., Neuroendocrinology **25,** 257 (1978).
7. Bennett, J. P., and Snyder, S. H., J. Biol. Chem. **251,** 7423 (1976).
8. Sirett, N. E., McLean, A. S., Bray, J. J., and Hubbard, J. I., Brain Res. **122,** 299 (1977).
9. Severs, W. B., Summy-Long, J., Taylor, J. S., and Connor, J. D., J. Pharmacol. Exp. Ther. **174,** 27 (1970).
10. Pellagrino, L. J., and Cushman, A. J., "A stereotaxic Atlas of the Rat Brain," Appleton-Century-Crofts, New York (1967).
11. Fujita, T., and Tedeschi, D. H., Life Sci. **7,** 673 (1968).
12. Merlis, J. K., Amer. J. Physiol. **131,** 67 (1940).
13. Keil, L. C., and Severs, W. B., Endocrinology **100,** 30 (1977).
14. Vernikos-Danellis, J., Anderson, E., and Trigg, L., Endocrinology **79,** 624 (1966).
15. Buckley, J. P., and Ferrario, C. M., "Central Actions

of Angiotensin and Related Hormones," Pergamon Press, New York (1977).
16. Reid, I. A., Circ. Res. **41**, 147 (1977).
17. Nahmod, V. E., Finkielman, S., de Gorodner, O. S., and Goldstein, D. J., *in* "Central Actions of Angiotensin and Related Hormones" (J. P. Buckley and C. M. Ferrario, eds.), p. 573. Pergamon Press, New York (1977).
18. Kapsha, J. M., Keil, L. C., Klase, P. A., and Severs, W. B., Pharmacology in press (1978).

Received July 17, 1978. P.S.E.B.M. 1979, Vol. 160.