Effect of a Converting Enzyme Inhibitor (SQ 20, 881) on Angiotensin-Induced Drinking[1] (36988)

WALTER B. SEVERS, JOAN SUMMY-LONG, AND ANNE DANIELS-SEVERS
(Introduced by Elliot S. Vesell)

Department of Pharmacology, College of Medicine, The Milton S. Hershey Medical Center of the Pennsylvania State University, Hershey, Pennsylvania 17033

The venom of the snake *Bothrops jararaca* contains a nonapeptide which is a potent inhibitor of angiotensin converting enzyme (ACE) (1–3). SQ 20,881 is a synthetic nonapeptide (Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro) chemically and biologically identical to the natural peptide (3, 4). ACE is found both in the periphery (5) and brain (5, 6). *In vitro* studies have shown that SQ 20,881 inhibits both peripheral (7) and brain (6) ACE. When administered *in vivo,* SQ 20,881 inhibits the cardiovascular activity of angiotensin I but not angiotensin II suggesting that ACE inhibition also occurs *in vivo* (4, 8). The purpose of the present study was to determine if SQ 20,881 could inhibit angiotensin effects on the central nervous system *in vivo.* The dipsogenic response to centrally administered angiotensin was used as the test system.

Methods. Under pentobarbital anesthesia, adult male Sprague-Dawley rats (325–375 g) were implanted with lateral cerebroventricular cannulas (9). The animals were allowed to recover for 48 hr in individual metabolism cages with food and water available *ad libitum.* On the experimental day rats received intracerebroventricular (ivt) injections of angiotensin I (Schwartz/Mann) or angiotensin II (Hypertensin, Ciba). The dose of the peptides was 0.1 μg contained in 5 μl of artificial cerebrospinal fluid (CSF) (10). The volume of water consumed in the next 15 min was recorded. Forty-five minutes after the initial angiotensin injection SQ 20,881 was administered either ivt (20 μg in 5 μl) or im (2 mg/kg). Fifteen minutes later the second dose of angiotensin was given ivt and the volume of water consumed during the next 15 min was recorded. The drinking responses to the two angiotensin doses were compared by the paired Student's t test (two-tailed).

Results. The effects of SQ 20,881 on the drinking response to angiotensins I and II are summarized in Table I. Angiotensin II drinking was not altered by either im or ivt treatmet with SQ 20,881. Angiotensin I-induced drinking was significantly ($p < .02$) inhibited by ivt, but not im, treatment with SQ 20,881. The inhibition of angiotensin I drinking behavior after ivt SQ 20,881 was not due to angiotensin I tachyphylaxis. Two ivt injections of angiotensin I at an interval of 1 hr gave drinking responses of 5.1 $\pm$ 0.7 and 4.0 $\pm$ 0.8 ml $\pm$ SE, respectively ($p > .05$, $n = 7$).

Discussion. Most of the biological effects of angiotensin I have been ascribed to its conversion to angiotensin II (11). Therefore, modification of ACE activity represents one approach to regulation of the biological effects of the renin–angiotensin system. This effect has been demonstrated in the periphery, since SQ 20,881 blocks the cardiovascular response to angiotensin I, but not to angiotensin II (4, 8).

The renin–angiotensin system may also exert effects on the central nervous system (CNS) since the brain contains renin, renin substrate, angiotensin I, ACE, and angiotensin II (6, 12, 13). It is clear that exogenous angiotensin administered into the CNS produces marked effects including pressor responses, water ingestion and release of antidiuretic hormone (9, 14). Because SQ 20,881 inhibits brain ACE *in vitro* (6), it was of interest to determine if the compound altered

[1] Supported in part by Grant No. HE-13063 from the National Heart and Lung Institute.

TABLE I. Effects of SQ 20,881 on Angiotensin-Induced Drinking.

Dipsogen	SQ 20,881 treatment[a]	Drinking response (ml ± SE)		No. rats	p
		Pretreatment	Posttreatment		
Angiotensin I	20 μg, ivt	4.8 ± 0.8	0.8 ± 0.6	6	<.02
Angiotensin II	20 μg, ivt	8.3 ± 0.8	6.7 ± 0.8	8	NS
Angiotensin I	2 mg/kg, im	5.3 ± 0.9	4.0 ± 0.7	7	NS
Angiotensin II	2 mg/kg, im	6.2 ± 1.5	7.2 ± 1.4	7	NS

[a] SQ 20,881 was administered 15 min prior to the second dose of angiotensin.

central angiotensin activity *in vivo*. The dipsogenic response to centrally administered angiotensin was used as the test system. Since ivt SQ 20,881 treatment inhibited angiotensin I but not angiotensin II drinking behavior, it appears likely that ACE in brain was inhibited. Failure of im SQ 20,881 to inhibit angiotensin I-induced drinking suggests that the compound does not easily reach brain ACE. The dose of SQ 20,881 used in this study appears to be optimal for inhibition of peripheral ACE activity *in vivo* (4). It is possible, however, that larger doses and/or different pretreatment times with peripherally administered SQ 20,881 could produce inhibition of brain ACE.

Summary. The effects of an ACE inhibitor, SQ 20,881, on the dipsogenic response to centrally administered angiotensins I and II were investigated. Central pretreatment with the inhibitor antagonized drinking behavior induced by angiotensin I, but not by angiotensin II. Peripheral treatment with 20,881 did not affect drinking responses to either angiotensin I or II. SQ 20,881 most likely inhibits brain ACE after central but not peripheral administration.

1. Ferreira, S. H., Bartelt, D. C., and Greene, L. J., Biochemistry **9**, 2583 (1970).

2. Ferreira, S. H., Greene, L. J., Alabaster, V. A., Bakhle, Y. S., and Vane, J. R., Nature (London) **225**, 379 (1970).

3. Ondetti, M. A., Williams, N. J., Sabo, E. F., Pluscec, J., Weaver, E. R., and Kocy, O., Biochemistry **10**, 4033 (1971).

4. Engel, S. L., Schaeffer, T. R., Gold, B. I., and Rubin, B., Proc. Soc. Exp. Biol. Med. **140**, 240 (1972).

5. Roth, M., Weitzman, A. F., and Piquilloud, Y., Experentia **25**, 1247 (1969).

6. Yang, H. Y. T., and Neff, N. H., J. Neurochem., in press.

7. Cushman, D. W., and Cheung, H. S., *in* "Hypertension-72" (J. Genest and E. Kow,-eds.), p. 532. Springer-Verlag, New York (1972).

8. Keim, G. R., Kirpan, J., Peterson, A. E., Murphy, B. F., Hassert, G. L., and Poutsiaka, J. W., Proc. Soc. Exp. Biol. Med. **140**, 149 (1972).

9. Severs, W. B., Summy-Long, J., Taylor, J. S., and Connor, J. D., J. Pharmacol. Exp. Ther. **174**, 27 (1970).

10. Merlis, J. K., Amer. J. Physiol. **131**, 67 (1940).

11. Aiken, J. W., and Vane, J. R., Nature (London) **228**, 30 (1970).

12. Fischer-Ferraro, C., Nahmod, V. E., Goldstein, D. J., and Finkielman, S., J. Exp. Med. **133**, 353 (1971).

13. Ganten, D., Marquez-Julio, A., Granger, P., Hayduk, K., Karsunky, K. P., Boucher, R., and Genest, J., Amer. J. Physiol. **221**, 1733 (1971).

14. Severs, W. B., Daniels-Severs, A., Summy-Long, J., and Radio, G. J., Pharmacology **6**, 242 (1971).

Received Sept. 14, 1972. P.S.E.B.M., 1973, Vol. 142.