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The Inhibition of Angiotensin Converting Enzyme in Chronic Renal Hypertension by a Synthetic Peptide[1] (39055)

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Blood pressure in chronic renal hypertension has been reduced by subcutaneous injection of a synthetic peptide SQ 20858 (Pry-Asn-Trp-Pro-His-Pro-Glu-Ile-Pro-Pro) Ondetti *et al.* (1) synthesized seven peptides found in the venom of *Bothrops jararaca* and Schaeffer *et al.* (2) showed the inhibition of the pressor effect of AT I by one of these synthetic peptides, SQ 20858 (SQ). The pressor effects of the decapeptide angiotensin I (AT I) are due to the conversion to the active octapeptide angiotensin II (AT II).

This work describes the effect of biweekly subcutaneous injections of the decapeptide SQ 20858 upon blood pressure in chronic renal hypertensive rats, and studies the conversion of angiotensin I to II by observing the effect of renin added to serum and determining the type of angiotensin produced.

Methods. Observations were made on 29 Sprague–Dawley rats initially weighing 100 g. The rats were pair fed Purina Chow and given tap water *ad lib.* Thirteen males were made renal hypertensive by ligating both poles of one kidney and performing contralateral nephrectomy one week later. Animals were weighed, and blood pressure was recorded twice weekly by the Friedman microphone method (3) in preheated, unanesthetized animals. On reaching and maintaining hypertensive systolic pressures (namely, greater than 160 mmHg) for 3 months, eleven renal hypertensive and two normotensive animals were given twice a week subcutaneous injections of SQ 20858 which was dissolved in distilled water (10 mg/100 g) for a total of five doses and blood pressures were measured the following day. Thirteen normotensive and three renal hypertensive animals were untreated and used as controls as was done in earlier work (4, 5). Tail blood samples were collected at the end of the treatment period and frozen for later use. Half of the treated and untreated animals were sacrificed after the twentieth week and the remaining after the twenty-third week.

For conversion to angiotensin, sera were thawed and most angiotensinases destroyed by bringing samples to pH 4 for 30 min at 25°, and back to pH of 7.0. Angiotensin was formed by addition of 1 ml (.3 GU) of angiotensinase free buffered hog renin to 1 ml samples of serum. The mixture was incubated at 37° for 10 min and the reaction was stopped by lowering the pH to 5.5 and boiling. The supernatant from centrifugation was collected and frozen for paper chromatography.

Decapeptide angiotensin I and octapeptide angiotensin II were separated from the filtrate by Wisenbaugh's method (6) of chromatography. Eluates were collected in siliconized tubes and frozen for rat assay. (Fig. 1). Synthetic angiotensin I and II were used to confirm that the test eluates were angiotensin I and/or II.

Assay was done on 200 g rats under sodium amobarbital anesthesia after vagotomy and injection of 500 units heparin and 20 mg/kg pentolinium chloride. Test doses of Val 5 amide angiotensin II (Ciba) were given intravenously in ascending order (.005, .01, .015 mcg) followed by 0.6 ml samples from each of the 25 tubes of eluate and again followed by ascending amounts of angiotensin.

To test if a block in converting enzyme was present, SQ and nontreated sera was studied. Those sera yielding activity corresponding only to angiotensin I (sample No. 8) were mixed with normal serum (converting enzyme) that yielded only angiotensin II (sample No. 24) and incubated at

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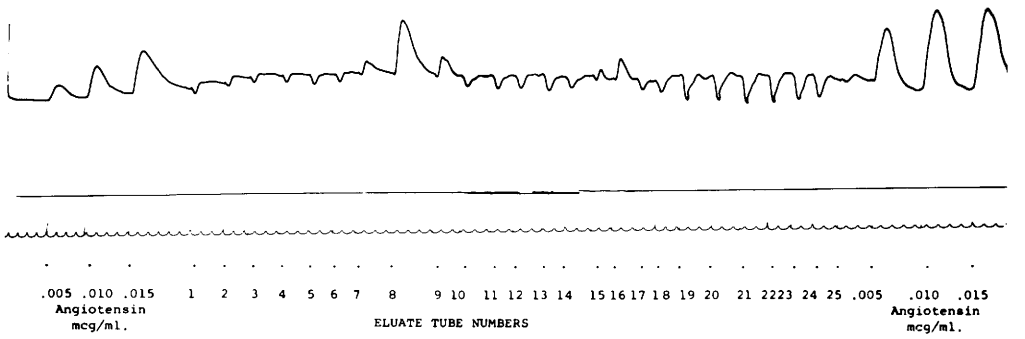

FIG. 1. Separation of angiotensin I and II by paper chromatography. Pressor responses 8–9–10 identify angiotensin I and tubes 15–16 angiotensin II.

37° for 10 min, chromatographed and frozen for assay.

Results. The average systolic blood pressure of the animals with renal hypertension rose from an initial level of 130–193 mmHg at 18 weeks (Table I). At the completion of treatment with SQ, the mean pressure had decreased to 176 ± 1.9 mmHg (SE) and using paired data was significantly less than the pressure during the hypertensive period ($P < .001$). Upon discontinuing the injections of SQ 20858, the pressure rose to 180 at the 21st week and 187 mmHg at the 22nd week period, and to 190 mmHg at the final 23rd week of study. The 18 week pressure of the normotensive rats was 142 mmHg, and after treatment with SQ injections there was no change. Animal's weights were comparable between treated and untreated animals throughout all periods.

No angiotensin II activity was demonstrated in seven of the renal groups treated (Table I) with SQ showing a complete block in converting enzyme activity. In two of this group, only a trace of angiotensin II was detected and two had amounts similar to those seen in the untreated renal group. In the normotensive treated group, primarily angiotensin I activity was found in the animals and all of the 13 untreated control group showed angiotensin II activity (Table I).

Incubation of serum containing only angiotensin I activity with that of normal serum, containing converting enzyme and only angiotensin II activity, showed angiotensin I and II, further demonstrating the block of converting enzyme in the SQ treated animals' serum. This incubation also shows that SQ does not inactivate angiotensin II.

Discussion. This experiment demonstrates a gradual reduction of blood pressure in chronic renal hypertensive rats by repeated subcutaneous injection of decapeptide SQ 20858. When the treatment was discontinued, the blood pressure gradually returned to the pretreatment hypertensive levels. In previous experiments (4, 5) the same type of renal hypertension has been maintained for similar periods of time and in no instance did hypertension abate without treatment. During treatment the blood pressure did not return to normal levels perhaps because the treatment was not long enough since only 5 doses were given due to the short supply of the drug. This explanation is suggested by our previous studies (4, 5) in which CCl_4 was used to block converting enzyme and we found that a total of 15 doses were needed to reduce the pressures to normal.

Previously, Greene *et al.* (7) had shown the reduction in blood pressure by using a pentapeptide in short term hypertensive rats. Infusions of the pentapeptide BPP_{5a} only transiently decreased blood pressure in acute and subacute hypertensive rats, however, in rats with unilateral nephrectomy and renal clip, no pressure effect was noted (7). Our contrary observations have been made on rats made hypertensive by bipolar ligation rather than renal artery clip. Engel *et al.* (9) after stat subcutaneous infusion of the peptide SQ 20858 in normotensive rats found the effect to last up to 90 minutes. Our previous pressor studies (10) in SQ

TABLE I. SYSTOLIC BLOOD PRESSURE AND TYPE ANGIOTENSIN.

		BP mmHg Week								Type angiotensin	
	Animal No.	1	2 (Lig)[a]	3 (Neph)[b]	18	19	20	21	23	I	II
Renal	1	120	*[c]	*	200	185	170	—	—	+[d]	+
Hypertensive	2	135	*	*	195	185	175	—	—	+	0[e]
Treated with	3	135	*	*	190	185	175	—	—	+	tr[f]
SQ 20858	4	135	*	*	190	175	180	—	—	+	0
19–20 week	5	130	*	*	190	185	175	180	190	+	+
	6	135	*	*	200	190	175	180	185	+	tr
	7	125	*	*	200	195	180	180	190	+	0
	8	130	*	*	195	185	180	—	—	+	0
	9	135	*	*	190	190	180	180	190	+	0
	10	120	*	*	190	190	170	185	200	+	0
	11	130	*	*	190	190	180	175	185	+	0
Mean	11	130			193	187	176	180	190		
Renal	12	135	*	*	195	190	195	—	—	+	+
Hypertensive	13	125	*	*	195	200	200	195	200	—	—
	14	125	*	*	190	195	200	190	195	tr	+
Untreated mean	3	128			193	193	198	193	197		
Normotensive	15	125	—	—	140	165	150	—	—	+	tr
SQ 20858 treated	16	120	—	—	150	150	150	150	140	+	+
19–20 week mean	2	123			145	154	150	150	140		
Normotensive	17	140	—	—	145	150	140	140	135	+	+
Untreated	18	130	—	—	140	145	140	140	140	tr	tr
	19	135	—	—	140	145	140	140	145	+	+
	20	130	—	—	135	140	145	—	—	+	+
	21	120	—	—	140	135	140	140	145	+	+
	22	130	—	—	140	130	140	—	—	+	+
	23	135	—	—	140	155	145	—	—	+	+
	24	130	—	—	135	140	140	—	—	0	+
	25	135	—	—	140	145	145	145	140	tr	+
	26	130	—	—	140	145	145	—	—	+	+
	27	130	—	—	135	140	140	145	145	+	+
	28	125	—	—	140	135	135	—	—	0	+
	29	130	—	—	140	135	135	—	—	+	+
Mean	13	130			140	142	141	142	142		

[a] Lig. = ligature.
[b] Neph. = nephrectomy.
[c] * = performed.
[d] + = present.
[e] 0 = absent.
[f] tr = trace amount.

treated chronic renal animals showed an immediate blocking effect of the drug after subcutaneous injection and remained effective up to 120 min when the observation was terminated. The apparent persistent effect of the peptide in this experiment may also be due to the chronic administration of SQ decapeptide. The normotensive SQ treated group show only a partial block of converting enzyme, possibly because the

drug was partially inactivated by normal animals.

Renin added to the serum of the SQ treated renal animals showed a complete block in the activity of converting enzyme in 7 of 11 as judged by the presence of only angiotensin I. A possible explanation for the failure to show a complete block of converting enzyme activity in all of the animals is that the drug was not given daily and/or enough was not present in these animals at the time of testing.

Finally, potentiating effects of bradykinin by the venom peptides *in vitro* and *in vivo* has been described (11). This synthetic peptide potentiates pulmonary bradykinin activity along with inhibition of the conversion of AT I to II as shown by Stewart *et al.* (12). Bianchi *et al.* (13) using a peptide similar to SQ 20858, suggest that the inhibition of angiotensin I induced vasopressor responses and the augmentation of bradykinin-induced vasodepression by this peptide, is due to an inhibitory effect on the same enzyme. Further study is necessary in order to clarify the mechanism(s) involved.

Summary. Renal hypertension was produced in 14 Sprague–Dawley rats by ligating both poles of one kidney followed in one week by unilateral nephrectomy. Biweekly subcutaneous injections of SQ 20858 reduced the blood pressure in the chronic renal hypertensive animals. Upon discontinuing the injections the blood pressure rose to pretreatment levels. No angiotensin II activity was seen in seven of the renal group treated with SQ indicating a complete block in serum converting enzyme activity. Likewise, serum with only angiotensin I activity when added to normal serum containing converting enzyme, continued to show angiotensin I activity. It is concluded that SQ 20858 is effective in lowering blood pressure in chronic renal hypertensive rats presumably by partially inhibiting converting enzyme.

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