Inhibition of Pressor Effects of Angiotensin I and Augmentation of Depressor Effects of Bradykinin by Synthetic Peptides[1] (36433)

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In 1965 Ferreira (1) described the isolation and properties of bradykinin-potentiating factor (BPF), a crude peptidic extract from the venom of the South American snake *Bothrops jararaca*. Three years later Bakhle (2) demonstrated that BPF also prevented the conversion of angiotensin I to angiotensin II *in vitro* and similar effects were seen in the perfused lung (3) and in intact animals (4).

These dual activities of BPF were also exhibited by the first peptide isolated and identified from BPF, Pyr-Lys-Trp-Ala-Pro, originally called BPP_{5a} and herein referred to as SQ 20,475 (5). At least 8 other peptides from the venom of *B. jararaca* have been isolated by Ferreira, Bartelt, and Greene (6) and by Ondetti *et al.* (7). The latter investigators also determined the amino acid sequence of 6 of these.

We have investigated the dual activities *in vivo* of these 6 peptides and of SQ 20,475, all of which were synthesized by Ondetti *et al.* (8). Structures of the 7 test peptides are shown in Table I.

Materials and Methods. Male rats of the Sprague-Dawley strain, 250 to 450 g, were anesthetized with urethane, 1.5 g/kg, im, and then tracheotomized. The trachea and the arteries and veins mentioned below were cannulated with polyethylene tubing of appropriate size. Blood pressure was monitored from a femoral artery via a Statham P23GB pressure transducer coupled to a Beckman dynograph. The rats were given atropine sulfate (1 mg/kg, iv) and heparin (250 units/kg, iv). Each test peptide, except the less soluble SQ 20,475, was dissolved in physiological saline and injected *stat* iv, 0.1 ml/dose, followed by 0.4 ml saline "wash-in" within the next 30 sec. SQ 20,475 was first dissolved in 0.1 *N* NaOH, adjusted with 0.1 *N* HCl to about pH 8.5 and injected *stat* iv, 0.1 ml/dose, "washed-in" with 0.4 ml saline. Other injections of some of the test peptides were made im or sc, 0.1 ml/injection. Rats employed in experiments involving angiotensions I and II received a continuous infusion of pentolinium bitartrate (0.2 mg/kg/min) into a femoral vein. In experiments involving bradykinin, pentolinium was not infused.

[Ile^5]-angiotensin I was purchased from Schwartz/Mann, [Asn^1, Val^5]-angiotensin II was kindly supplied by Dr. A. J. Plummer of Ciba Pharmaceutical Company, and bradykinin was purchased from Cyclo Chemical and Nutritional Biochemicals. These challenge peptides were administered *stat* iv, 0.1 ml/dose, in physiological saline, followed by 0.4 ml saline wash-in, in all experiments.

Blood pressure responses to control doses of the challenging agent(s), *i.e.*, to 0.10 or 0.31 μg/kg of angiotensin I and to 0.031 or 0.10 μg/kg of angiotensin II or to 1, 3, or 10 μg/kg of bradykinin, were first determined for each rat. The test peptide was then administered and the challenging agent(s) were injected repeatedly at 2- to 10-min intervals. Not until responses to the challenges were approximately equal to those seen in the control period was another injection of the test peptide given. At least a fourfold dose range was used with each test peptide and only one test peptide at 1 to 4 dose levels was given to a single rat. For each test peptide, 4 or 5 rats were used vs the angiotensins and another group of 4 or 5 rats vs bradykinin. Three

[1] Preliminary reports of parts of this work have been presented at the XXV Int. Congr. Physiol. Sci., Munich, Germany, July, 1971, and at the Joint Meet. Amer. Soc. Pharmacol. Exp. Ther. and Div. Med. Chem., ACS, Burlington, VT, Aug., 1971.

TABLE I. Effects of Venom Peptides on Angiotensin I- and Bradykinin-Induced Changes in the Blood Pressure of Anesthetized Rats.[a]

Compound		Dose	vs pressor responses, angiotensin I[b]			vs depressor responses, bradykinin		
SQ No.	Structure	(mg/kg, iv)	N	Max inhib (% ± SE)	Approx 50% recov time[c] (min)	N	Max augmen (% ± SE)	Approx 50% recov time[c] (min)
20475	Pyr-Lys-Trp-Ala-Pro	0.0313		—	—	4	21.3 ± 12.3	1.5
		0.125		—	—	4	38.0 ± 21.7	2.0
		0.5		—	—	4	57.2 ± 18.4	1.5
		2.0	4	28.3 ± 3.6	4	4	105.1 ± 24.2	2.5
		8.0	4	85.4 ± 5.5	4		—	—
20661	Pyr-Trp-Pro-Arg-Pro-Thr-Pro-Gln-Ile-Pro-Pro	0.5	4	53.2 ± 4.5	11		—	—
		2.0	4	82.2 ± 6.1	30		—	—
		8.0	3	87.5 ± 8.2	33		—	—
20718	Pyr-Gly-Gly-Trp-Pro-Arg-Pro-Gly-Pro-Glu-Ile-Pro-Pro	2.0	4	41.5 ± 9.8	6		—	—
		8.0	4	73.3 ± 10.6	9.5		—	—
20858	Pyr-Asn-Trp-Pro-His-Pro-Gln-Ile-Pro-Pro	0.0078		—	—	4	34.4 ± 11.3	—
		0.0313		—	—	4	71.7 ± 10.0	8
		0.125		—	—	5	71.7 ± 11.7	25
		0.5	4	60.5 ± 3.6	10.5	5	81.3 ± 17.8	58
		2.0	4	79.8 ± 8.8	> 90		—	—
20859	Pyr-Ser-Trp-Pro-Gly-Pro-Asn-Ile-Pro-Pro	8.0	4	41.9 ± 10.5	4.5		—	—
		32.0	4	78.3 ± 3.0	9		—	—
20861	Pyr-Asn-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	0.5	3	55.7 ± 13.3	4.5		—	—
		2.0	4	87.4 ± 3.6	19.5		—	—
		8.0	4	89.1 ± 4.7	45		—	—
20881	Pyr-Trp-Pro-Arg-Pro-Gln-Ile-Pro-Pro	0.0078		—	—	4	38.6 ± 17.0	13
		0.0313		—	—	4	73.0 ± 16.0	9
		0.125		—	—	5	81.2 ± 25.5	68
		0.5	4	80.0 ± 3.5	9	4	154.2 ± 26.5	163
		2.0	4	87.7 ± 2.4	53		—	—

[a] inhib = inhibition; augmen = augmentation; recov = recovery. (mean responses).

[b] During the iv infusion of pentolinium bitartrate 0.2 mg/kg/min.

[c] Graphical estimation.

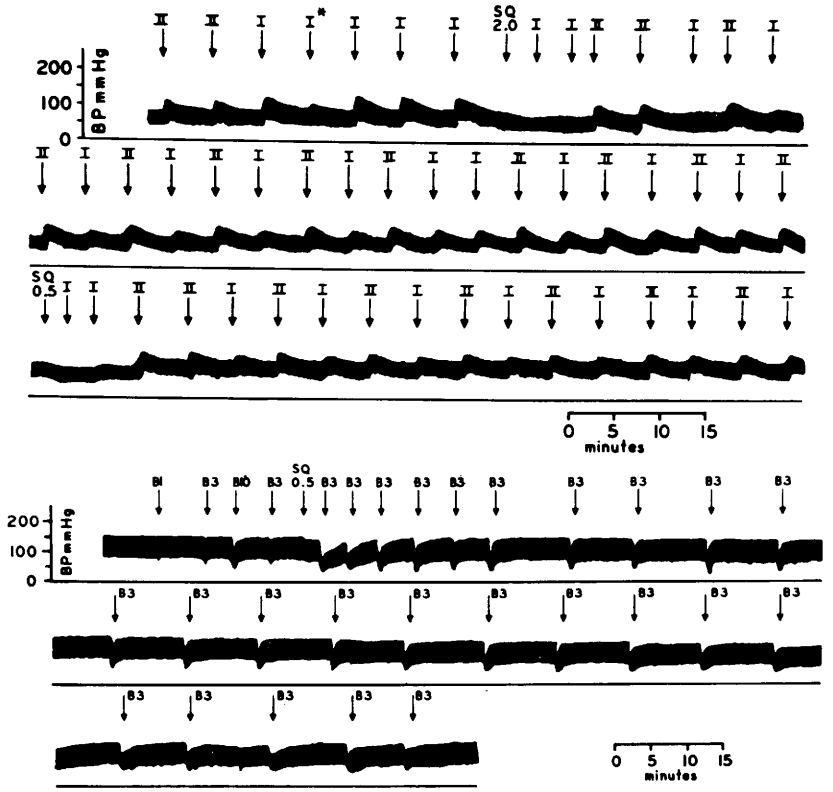

FIG. 1. Representative continuous blood pressure tracings in 2 urethanized rats; all injections iv. (upper tracing) Pentolinium-infused rat. Injections at I and I* of 0.31 and 0.10 μg/kg of angiotensin I, respectively, at II of 0.10 μg/kg of angiotensin II, and at SQ 2.0 and SQ 0.5 of 2.0 and 0.5 mg/kg of SQ 20,881 respectively. (lower tracing) Injections at B1, B3, and B10 of 1, 3 and 10 μg/kg of bradykinin, respectively, and at SQ 0.5 of 0.5 mg/kg of SQ 20,881.

of the test peptides with long durations of action after iv dosage were also tested im or sc.

Results. Representative blood pressure tracings from the iv tests conducted with SQ 20,881 vs angiotensins I and II and bradykinin are shown in Fig. 1. The results obtained with the 7 test peptides are given in Table I. All of the test peptides inhibited the pressor effects of angiotensin I, but they appeared to differ in both potency and duration of action. None of the test peptides altered the pressor effects produced by angiotensin II (9, 10). The apparent descending order of activity (potency and duration) vs angiotensin I was SQ 20,881 $\geqq$ SQ 20,858 $>$ SQ 20,861 $\geqq$ SQ 20,661 $>$ SQ 20,718 $>$ SQ 20,475 $>$ SQ 20,859.

Effective absorption of the 3 most active peptides (SQ 20,861, SQ 20,858, and SQ 20,881) after im and sc administration was noted in tests vs angiotensin I, as shown in Table II. For all of the test peptides, peak effects occurred about 2 min after iv dosage and about 5 or 10 min after im or sc dosage. Time–effect curves for SQ 20,881, at 2 mg/kg, iv, im and sc, were very similar to each other after the first few minutes as shown in Fig. 2.

Three peptides (SQ 20,475, SQ 20,858 and SQ 20,881) tested for augmentation of the depressor effect of bradykinin were all active (Table I); this activity, in the urethanized rat, occurred at doses lower than those required to produce inhibition of angiotensin I-induced pressor responses (10).

None of the 7 test peptides produced any appreciable changes in "resting" blood pres-

TABLE II. Inhibition of Angiotensin I-Induced Pressor Responses.[a]

Peptide	Dose (mg/kg)	Mean max inhibition (% ± SE) iv	im	sc	Mean half recovery time[b] (min) iv	im	sc
SQ 20861	8	89.1 ± 4.7	78.5 ± 4.1	69.4 ± 3.1	45.0	69.0	55.0
	2	87.4 ± 3.6	31.8 ± 5.8	48.7 ± 5.6	19.5	—	31.0
	0.5	55.7 ± 13.3	—	—	4.5	—	—
SQ 20881	2	87.7 ± 2.4	72.1 ± 7.3	65.7 ± 8.7	53.0	46.0	64.0
	0.5	80.0 ± 3.5	25.9 ± 8.1	36.0 ± 9.9	9.0	—	30.0
SQ 20858	2	79.8 ± 8.8	52.2 ± 3.9	50.4 ± 6.3	>90.0	68.0	24.0
	0.5	60.5 ± 3.6	34.3 ± 6.4	19.6 ± 3.3	10.5	19.0	15.0

[a] Mean responses from groups of 3 to 5 anesthetized rats infused with pentolinium.
[b] Graphically estimated.

sure in the urethanized rat, in the absence or presence of an infusion of pentolinium.

Discussion. Since most, if not all, of the biological activity associated with angiotensin I may be ascribed to its conversion to angiotensin II (11), and since the venom peptides inhibit angiotensin-converting enzyme *in vitro* (12, 13), the effects described here are most likely the results of similar activity *in vivo*. Other factors, such as absorption, distribution and metabolism, may also have been involved since SQ 20,475, the test peptide most active in a purified enzyme system (13), was less active *in vivo* than most of the longer-chain test peptides. Bakhle (14) has recently described studies with 3 of the venom peptides, SQ 20,475, SQ 20,881 and SQ 20,859, on the inhibition of angiotensin-converting enzyme in both a cell-free system and in perfused lungs of the guinea pig. Further evidence for specificity of this effect by SQ 20,881 on rat colon and guinea pig ileum has also been reported recently (15). Structural similarities among the venom peptides suggest that they may possess the same mode(s) of action.

The augmentation of bradykinin's effects is due, at least in part, to inhibition of the kininase(s) responsible for the inactivation of bradykinin (1). Potentiation of the effects of bradykinin by the venom peptides *in vitro* and *in vivo* has been described (16). The specificity of this effect with SQ 20,881 has been shown on guinea pig ileum *in vitro* (15).

Infusions of the pentapeptide, SQ 20,475, have transiently decreased blood pressure in certain types of acute and subacute renal-hypertensive rats (17). Current studies in our laboratory suggest that SQ 20,881, iv, reduces the acute pressor effect that occurs when the clamp is released in the 6-hr renal pedicle-clamped rat. These effects could be ascribed to inhibition of both angiotensin I-converting enzyme and of kininase.

Other effects of the long-acting venom peptides, such as SQ 20,881, in experimental renal hypertension remain to be ascertained, as does their potential value as diagnostic or therapeutic agents in renal and other forms of hypertension. It is of interest in this connection that Catt *et al.* (18) have recently described elevated angiotensin II blood levels

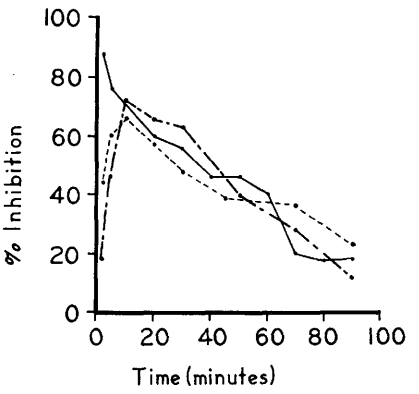

FIG. 2. The percentage inhibition of angiotensin I-induced pressor responses in urethanized rats by SQ 20,881, 2 mg/kg, injected at time 0: iv (—), im (— - —) or sc (- - -). Each point is an average of 3 to 5 determinations in as many rats.

in patients with severe essential, renal or malignant hypertension.

Summary. Seven synthetic peptides that correspond to compounds found in the venom of *Bothrops jararaca* were studied in normotensive, urethanized rats infused with pentolinium. When administered *stat* iv, the peptides inhibited pressor responses induced by angiotensin I but not those induced by angiotensin II. This activity was most likely the result of inhibition of angiotensin-converting enzyme. The peptides appeared to differ from each other with respect to potency and duration of action. The 3 test peptides injected im and sc displayed rapid, effective absorption by those routes.

Three peptides tested *stat* iv in normotensive, urethanized rats augmented the vasodepressor effect of bradykinin to different degrees.

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