Inhibition of Central Pressor Effects of Angiotensin I and II

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Abstract □ Two peptides which naturally occur in the venom of *Bothrops jararaca* were studied for their effects on the centrally mediated pressor responses of angiotensin I and II in α-chloralose-anesthetized cats utilizing the lateral ventricular perfusion technique. Intraventricular administration of either the pentapeptide or the nonapeptide attenuated the central pressor effects elicited by both angiotensin I and II. This action appeared to be specific to the CNS because administration of the pentapeptide or the nonapeptide peripherally did not antagonize angiotensin II but did inhibit angiotensin I. The inhibitors did not significantly affect ganglionic or neuronal transmission in nictitating membrane studies in cats and did not alter the parasympathetic components since there was no shift in the frequency-response curve obtained by vagal stimulation. In addition, they did not produce significant alterations in the blood pressure responses to acetylcholine, epinephrine, or bilateral carotid occlusion. Therefore, inhibition of the centrally elicited pressor effect of angiotensin II by these peptides apparently is selective to CNS receptor sites.

Keyphrases □ *Bothrops jararaca* venom peptides—effect on central pressor effects of angiotensin I and II □ Pyrrolidone carboxylic acid-L-lysyl-L-tryptophyl-L-alanyl-L-proline and pyrrolididone carboxylic acid-L-tryptophyl-L-prolyl-L-arginyl-L-prolyl-L-glutaminyl-L-isoleucyl-L-prolyl-L-proline—studied as inhibitors of central pressor effects of angiotensin I and II, anesthetized cat □ Angiotensin I and II—inhibition of central pressor effects by pentapeptide and nonapeptide of *Bothrops jararaca* venom, anesthetized cat □ Pressor responses—angiotensin I and II effects inhibited by pentapeptide and nonapeptide from *Bothrops jararaca* venom, anesthetized cat

In 1961, Bickerton and Buckley (1) presented evidence that angiotensin II exerted effects on the central nervous system (CNS). Utilizing the dog cross-circulation preparation, they showed that administration of this octapeptide into the vascularly isolated, neurally intact head of the recipient resulted in a pressor effect in the trunk. This action of angiotensin II has been confirmed (2–11) and shown to be mainly mediated *via* a central augmentation of sympathetic neuronal tone to the vasculature (1, 12). It has been suggested that this effect of angiotensin II is important in the central control of the cardiovascular system (13) as well as in the development of cardiovascular hypertensive disease (14). If this is true, then a possible mechanism for counteracting this pathological condition would be to block the renin-angiotensin system in the CNS. One way of achieving this could be to inhibit the synthesis of angiotensin II from angiotensin I.

Several peptides isolated from a pharmacologically active peptide fraction from *Bothrops jararaca* venom (15) have been reported to inhibit the conversion of angiotensin I to angiotensin II (16–20). Two of these peptides, a pentapeptide (pyrrolidone carboxylic acid-L-lysyl-L-tryptophyl-L-alanyl-L-proline[1]) and a nonapeptide (pyrrolididone carboxylic acid-L-tryptophyl-L-prolyl-L-arginyl-L-prolyl-L-glutaminyl-L-isoleucyl-L-prolyl-L-proline[2]), were studied to determine their effects on the centrally induced pressor activities of angiotensin I and II.

METHODS

Autonomic Function Studies—Adult cats, weighing 2.2–3.5 kg, were anesthetized with purified α-chloralose (70 mg/kg iv). Blood pressure was monitored from a femoral artery, and the ipsilateral femoral vein was catheterized for the administration of the test compounds. The left and right common carotid arteries were isolated so that the bilateral carotid occlusion reflex could be obtained. The right cervical vagus was isolated for supramaximal stimulation (v = 3–4.5, duration = 1 msec, frequency = 1.5–12 Hz, for 15 sec) by means of bipolar electrodes, and decreases in heart rate were recorded. The preganglionic sympathetic trunk to the nictitating membrane was also isolated for supramaximal stimulation in the same manner, and the developed nictitating membrane tension was measured with a force-displacement transducer. Test doses of angiotensin I and II (0.5 and 2.0 μg/kg), epinephrine (1.0 μg/kg), and acetylcholine (0.5 μg/kg) were administered intravenously prior to and during intravenous infusion of the pentapeptide or the nonapeptide (100 or 500 μg/kg/min). The inhibitors of the converting enzyme were infused for 15 min prior to administering the other compounds or stimulating the neurons, and infusion was continued for approximately 110 min. A minimum of 10 min was permitted between procedures. Only one inhibitor was administered per animal. The mean blood pressure equals diastolic pressure plus one-third the pulse pressure.

Perfused Cat Lateral Ventricle Preparation—Adult cats of either sex, weighing 2–3 kg, were anesthetized and prepared for blood pressure monitoring as already described. A tracheotomy was performed and a tracheal catheter was inserted for the establishment of artificial respiration. The animal was then affixed into a stereotaxic instrument[3], and the calvarium was surgically exposed along the sagittal suture line. A small hole was made in the skull above the right lateral ventricle and an unbeveled 22-gauge stainless steel needle, approximately 35 mm in length, was stereotaxically lowered into the ventricle according to the coordinates described by Snyder and Niemer (21). The coordinates used were: frontal, 15 mm; horizontal, 6.75 mm; and lateral, 2.5 mm. The cannula was affixed to the skull with dental acrylic cement, and the cerebral ventricles were perfused with artificial cerebral spinal fluid (22) after the method of Bhattacharya and Feldberg (23). The cerebral spinal fluid entered the lateral ventricle and passed through the third ventricle, the aqueduct of Sylvius, and the fourth ventricle and drained through a catheter inserted into the cisterna magna. The perfusion rate was kept constant at 0.1 ml/min by means of a pump[4] connected by a polyethylene tubing to a three-way stopcock placed in the stainless steel catheter. The artificial cerebral spinal fluid was maintained at 37° by passing the tubing through a heated water jacket. Injections of angioten-

[1] SQ 20,475.
[2] SQ 20,881.
[3] Trent H. Wells, Jr.
[4] Harvard Petti-pump.

Table I—Effects of the Pentapeptide and the Nonapeptide, Administered Intravenously, on Mean Blood Pressure and Blood Pressure Responses Induced by Bilateral Carotid Occlusion and Intravenously Administered Epinephrine and Acetylcholine in α-Chloralose-Anesthetized Cats[a]

	Inhibitor	Dose of Inhibitor, μg/kg/min		
		Control (n = 8)	100 (n = 4)	500 (n = 4)
Mean blood pressure, mm Hg[b]	Pentapeptide	113.3 ± 7.2	119.4 ± 6.1	112.5 ± 11.6
	Nonapeptide	106.3 ± 10.4	112.5 ± 8.6	121.6 ± 13.1
		Changes in Mean Blood Pressure, mm Hg[b]		
Epinephrine, 1.0 μg/kg	Pentapeptide	68.1 ± 7.3	61.7 ± 9.4	63.7 ± 12.5
	Nonapeptide	61.2 ± 9.1	62.0 ± 10.3	61.9 ± 8.3
Acetylcholine, 0.5 μg/kg	Pentapeptide	−44.5 ± 9.3	−46.1 ± 13.8	−45.5 ± 7.0
	Nonapeptide	−49.5 ± 5.4	−51.0 ± 5.3	−47.5 ± 6.9
Bilateral carotid occlusion	Pentapeptide	36.1 ± 5.2	35.4 ± 4.8	38.1 ± 7.3
	Nonapeptide	38.2 ± 7.1	41.4 ± 8.1	42.5 ± 10.3

[a] No significant differences. [b] $X \pm SEM$.

Table II—Mean Percent Inhibition of Pressor Responses of Angiotensin I and II by the Pentapeptide and Nonapeptide[a]

	Route of Angiotensin	Route of Pentapeptide	Dose of Pentapeptide, μg/kg/min	
			100	500
Angiotensin I	Intravenous	Intravenous	41.5	63.8
	Intraventricular	Intraventricular	58.8	74.2
Angiotensin II	Intravenous	Intravenous	4.9	9.6
	Intraventricular	Intraventricular	46.9	72.1
	Route of Angiotensin	Route of Nonapeptide	Dose of Nonapeptide, μg/kg/min	
			100	500
Angiotensin I	Intravenous	Intravenous	60.4	93.7
	Intraventricular	Intraventricular	77.6[b]	92.0[b]
Angiotensin II	Intravenous	Intravenous	3.2	11.8
	Intraventricular	Intraventricular	77.5[b]	88.2[b]

[a] All values are expressed as percent change from control. [b] Inhibitory effects significantly greater than that produced by the pentapeptide ($p < 0.05$) as determined by analysis of variance.

sin I or II (0.5–2.0 μg/kg) were made through the stopcock, without interruption of the perfusion, prior to and during the intraventricular perfusion of the pentapeptide or nonapeptide (100 or 500 μg/kg/min). The inhibitors of the converting enzyme were infused for 15 min prior to administering angiotensin I and II, and infusion was continued for approximately 90 min. A minimum of 15 min was permitted between treatments, and only one inhibitor was administered per animal.

Drugs and Chemicals—Angiotensin I[5], 94% pure; angiotensin II[6]; and the pentapeptide (pyrrolidone carboxylic acid-L-lysyl-L-tryptophyl-L-alanyl-L-proline) and the nonapeptide (pyrrolididone carboxylic acid-L-tryptophyl-L-prolyl-L-arginyl-L-prolyl-L-glutaminyl-L-isoleucyl-L-prolyl-L-proline) were dissolved in artificial cerebral spinal fluid for intraventricular administration and in saline for intravenous administration.

RESULTS

Table I summarizes the effects of intravenous administration of the enzyme inhibitors on blood pressure and the blood pressure responses induced by bilateral carotid occlusion and intravenous administration of epinephrine and acetylcholine. Neither the pentapeptide nor the nonapeptide in doses of 100 and 500 μg/kg/min altered the mean blood pressure of α-chloralose-anesthetized cats. They also had no effect on the bilateral carotid occlusion pressor reflex; the decrease in heart rate induced by vagal stimulation at frequencies of 1.5, 3.0, 6.0, and 12.0 Hz; the increased tension of the nictitating membrane due to stimulation of the preganglionic sympathetic trunk; the pressor response to epinephrine; or the depressor response to acetylcholine.

The intravenous infusion of either the pentapeptide or the nonapeptide produced significant inhibitory effects on the pressor responses to intravenously administered angiotensin I but had little effect on hypertensive responses to angiotensin II, as determined by analysis of variance. Doses of 100 and 500 μg/kg/min of the pentapeptide significantly ($p < 0.05$) attenuated the dose-response curve to angiotensin I, 41.5 and 63.8%, respectively (Fig. 1); the nonapeptide produced 60.4 and 93.7% inhibition ($p < 0.01$) in the same doses (Fig. 2). The dose required to produce approximately 60% inhibition of the angiotensin I pressor response was five times greater for the pentapeptide than for the nonapeptide. This observation is in accord with the findings reported by Schaeffer and Evans[7].

Perfused Cat Lateral Ventricle Preparation—The pentapeptide, 100 μg/kg/min, infused into the lateral ventricles did not alter the mean blood pressure significantly (control, $\bar{X} \pm SEM$, 102.5 ± 10.6 mm Hg; treated, 108.8 ± 10.2 mm Hg) but there was a significant increase ($p < 0.05$) in mean blood pressure to 123.8 ± 9.2 mm Hg with 500 μg/kg/min. Intraventricular administration of both doses of nonapeptide caused significant pressor effects ($p < 0.05$) (control, 109.4 ± 7.0; 100 μg/kg/min, 125.0 ± 7.2 mm Hg; 500 μg/kg/min, 130.6 ± 8.5 mm Hg), but these increases in mean blood pressure by the nonapeptide were not significantly greater than those resulting from intraventricular infusion of the pentapeptide.

Intraventricular administration of the converting enzyme in-

[5] Schwarz-Mann, Orangeburg, N.Y.
[6] Hypertensin, Ciba-Geigy.

[7] T. Schaeffer and D. B. Evans, Squibb Institute for Medical Research, personal communication.

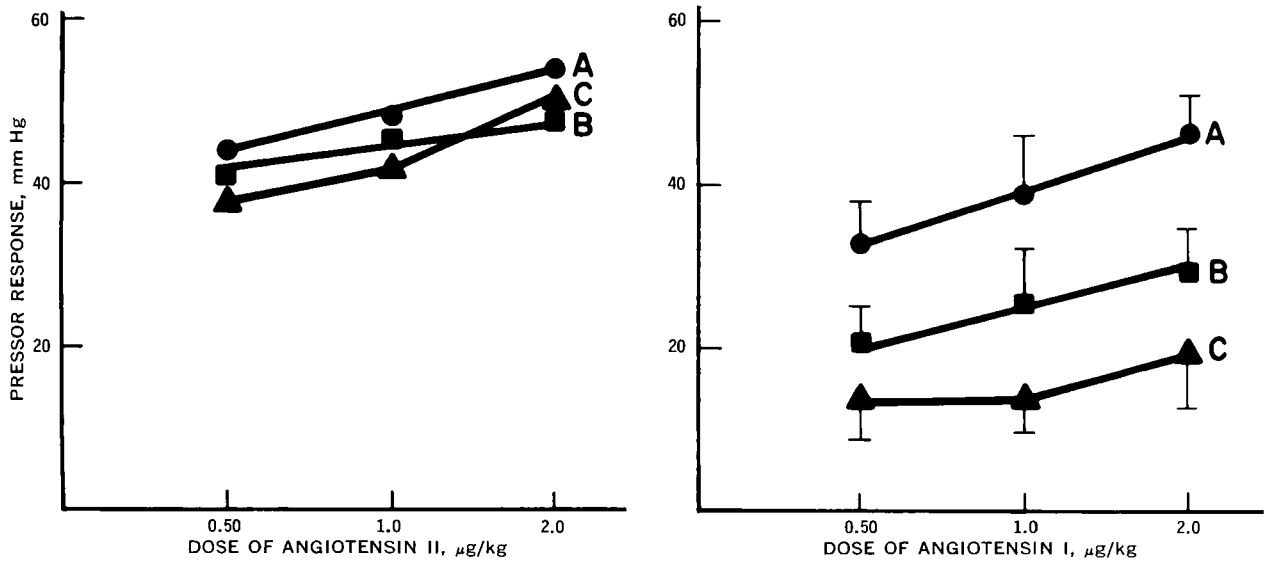

Figure 1—*Effects of the pentapeptide on the pressor response to angiotensin I and II, administered intravenously to an α-chloralose-anesthetized cat. Key: A, control,* n = *8; B, pentapeptide, intravenous, 100 μg/kg/min,* n = *4; and C, pentapeptide intravenous, 500 μg/kg/min,* n = *4. Significant differences by analysis of variance: angiotensin I, A and B,* $p < 0.05$; *A and C,* $p < 0.05$; *and angiotensin II, A and B, not significant; A and C, not significant. Vertical bars = standard error of the mean.*

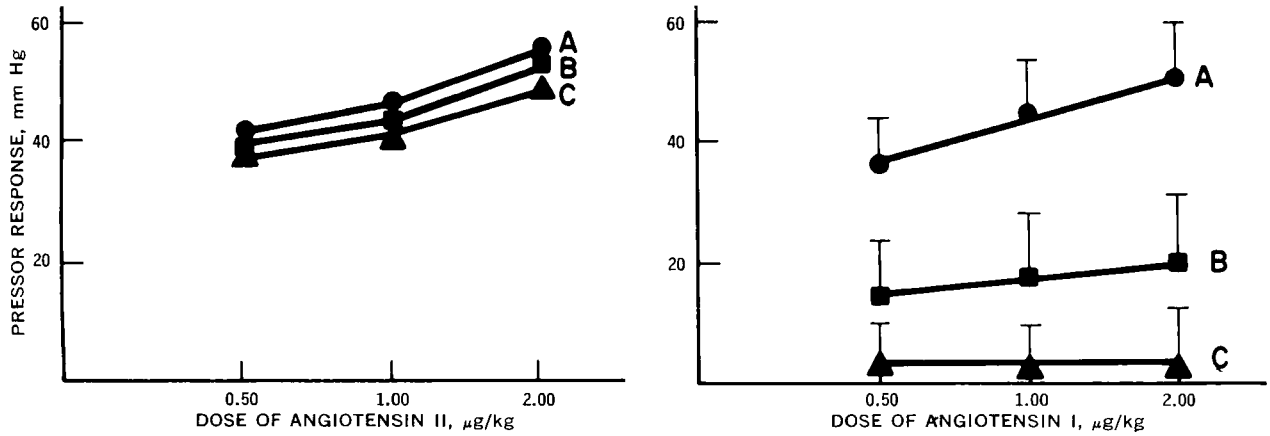

Figure 2—*Effects of the nonapeptide on the pressor response of angiotensin I and II, administered intravenously to an α-chloralose-anesthetized cat. Key: A, control,* n = *8; B, nonapeptide, intravenous, 100 μg/kg/min,* n = *4; and C, nonapeptide, intravenous, 500 μg/kg/min,* n = *4. Significant differences by analysis of variance: angiotensin I, A and B,* $p < 0.01$; *A and C,* $p < 0.01$; *and angiotensin II, A and B, not significant; A and C, not significant. Vertical bars = standard error of the mean.*

hibitors produced qualitatively similar effects on the pressor activity of angiotensin I observed in the peripheral studies. However, inhibition of angiotensin II, which was not seen on intravenous administration, was also observed. Figure 3 summarizes the effects of the pentapeptide and nonapeptide on the centrally mediated pressor response of angiotensin I. As in the peripheral studies, the attenuation of the nonapeptide was greater than that produced by the pentapeptide. The intraventricular infusion of 100 μg/kg/min of the nonapeptide inhibited the angiotensin I pressor response an average of 77.6% while the pentapeptide attenuated this response by only 58.8%; the 500-μg/kg/min dose produced a 92.0 and 74.2% inhibition, respectively. When each pair of means was subjected to an unpaired *t* test for the difference between two means, they were shown to be significant at the $p < 0.05$ level (Table II).

The centrally induced hypertensive effect of angiotensin II was also antagonized by the intraventricular perfusion of the converting enzyme inhibitors (Fig. 4). The pentapeptide decreased the activity of angiotensin II by an average of 46.9% at a dose of 100 μg/kg/min and of 72.1% at 500 μg/kg/min. Intraventricular administration of the nonapeptide had greater inhibitory effects than the pentapeptide, which is similar to the data obtained in the peripheral studies. The lower dose of the nonapeptide attenuated the pressor effect to intraventricularly administered angiotensin II 77.5% while the higher dose decreased this response by 88.2%. The inhibition produced by the nonapeptide was significantly greater than the pentapeptide at both dose levels ($p < 0.05$).

DISCUSSION

The two peptides, which have been reported to inhibit the angiotensin-converting enzyme (16–20), demonstrated a selective activity in the CNS. The data obtained with the pentapeptide and the nonapeptide intravenously agree with those reported previously (16–20); however, both peptides also inhibited the pressor response to centrally administered angiotensin II. The pressor activity induced by the intraventricular infusion of angiotensin II was antagonized to approximately the same degree as that produced by angiotensin I. Although there was a quantitative difference in the inhibition of angiotensin I by the peptides depending on the route of administration, it seems unlikely that this could account for the fact that the activity of angiotensin II is also attenuated by the converting enzyme inhibitors. Therefore, although the converting enzyme inhibitors have not been reported to have any effects other than blocking conversion of angiotensin I to angiotensin II, the data obtained in these experiments indicate that the activity of these compounds may be more complex. If angiotensin I is an active peptide and possibly important in the development of essential hypertension (24), then the two inhibi-

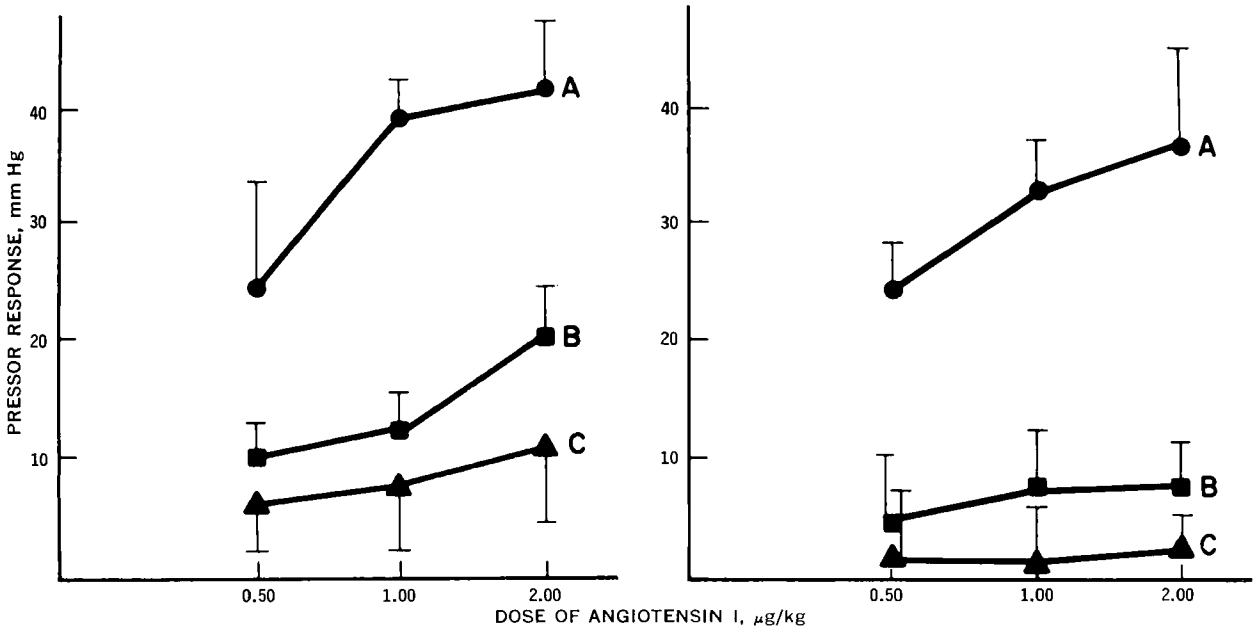

Figure 3—*Effects of intraventricular administration of the pentapeptide (left) and the nonapeptide (right) on the pressor response to intraventricular administration of angiotensin I in α-chloralose-anesthetized cats. Key: A, control,* n = *8; B, inhibitor, 100 μg/kg/min,* n = *4; and C, inhibitor, 500 μg/kg/min,* n = *4. Significant differences by analysis of variance: pentapeptide, A and B,* p < *0.01; A and C,* p < *0.01; and nonapeptide, A and B,* p < *0.05, A and C,* p < *0.01. Vertical bars = standard error of the mean.*

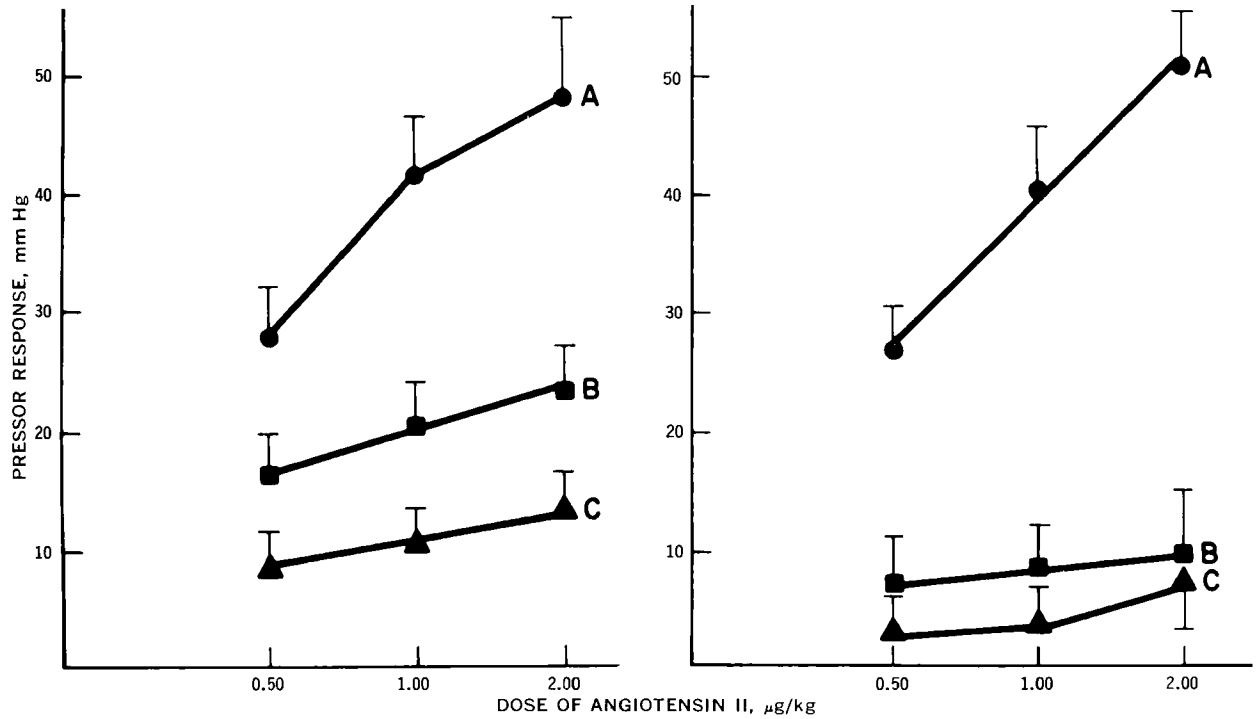

Figure 4—*Effects of intraventricular administration of the pentapeptide (left) and the nonapeptide (right) on the pressor response to intraventricular administration of angiotensin II in α-chloralose-anesthetized cats. Key: A, control,* n = *8; B, inhibitor, 100 μg/kg/min,* n = *4; and C, inhibitor, 500 μg/kg/min,* n = *4. Significant differences by analysis of variance: pentapeptide, A and B,* p < *0.05; A and C,* p < *0.01; and nonapeptide, A and B,* p < *0.01; A and C,* p < *0.01. Vertical bars = standard error of the mean.*

tors of the converting enzyme could be acting by inhibition of the "receptors" for angiotensin I and angiotensin II. If angiotensin I is, in fact, inactive, then they could be inhibiting the converting enzyme plus blocking the receptor site for angiotensin II. The antagonists had no effect on mean blood pressure, vagal stimulation, stimulation of the innervation to the nictitating membrane, the depressor response to acetylcholine, or the pressor response to bilateral carotid occlusion or epinephrine.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 17, 1973, from the *Cardiovascular Research Laboratories, Department of Pharmacology, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA 15261*

Accepted for publication December 6, 1973.

Supported in part by the National Institutes of Health Training Grant GM-1217-09 from the National Institutes of General Medical Sciences.

The pentapeptide, SQ 20,475, and the nonapeptide, SQ 20,881, were supplied by Dr. Zola Horowitz, the Squibb Institute for Medical Research, Princeton, N.J. Angiotensin II (Hypertensin) was supplied by Dr. William Wagner, Ciba-Geigy, Summit, N.J.

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Controlled Drug Release from Polymeric Delivery Devices II: Differentiation between Partition-Controlled and Matrix-Controlled Drug Release Mechanisms

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Abstract □ The drug release pattern of micronized ethynodiol diacetate from silicone devices was thoroughly investigated in polyethylene glycol-containing elution media with a wide range of solubility and partition properties. When high drug solubility was maintained, the drug release pattern followed a $Q - t^{1/2}$ relationship (matrix controlled). Under this matrix-controlled process, the drug release profiles were independent of the variation in partition coefficient magnitude and insensitive to the change in solubility parameters. As the drug solubility in the elution medium was decreased, the drug release process shifted from matrix controlled to partition controlled, and a $Q - t$ (zero-order) relationship was observed. The drug release profile was then a function of the partition coefficient of drug from the polymer matrix to the elution medium. A transition phase was also seen between these two processes. Matrix-controlled and partition-controlled drug release processes were analyzed theoretically. The experimental rates of drug release were in perfect agreement with the values calculated from the theoretical model.

Keyphrases □ Drug release, controlled—differentiation between partition-controlled and matrix-controlled release mechanisms, ethynodiol diacetate from silicone devices in polyethylene glycol 400 media □ Permeation, drug—ethynodiol diacetate from silicone devices in polyethylene glycol 400 media, differentiation between partition- and matrix-controlled release mechanisms □ Ethynodiol diacetate—release from silicone devices in polyethylene glycol 400 media, matrix- and partition-controlled release mechanisms □ Silicone devices—release of ethynodiol diacetate

An *in vitro* drug release system, which is simple in construction and allows rapid characterization of the drug release mechanism, was introduced previously (1). The rate of drug release from silicone devices measured in such a system was found to follow current theoretical models (2–9). The application of such methodology allowed characterization of the mechanism and rate of drug release. In the studies