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PERIPHERAL VASCULAR EFFECTS OF ANGIOTENSIN II AND NOREPINEPHRINE IN THE DOG, CAT, AND MONKEY

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THOMAS EDWARD EMERSON, JR.

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PERIPHERAL VASCULAR EFFECTS OF ANGIOTENSIN II AND NOREPINEPHRINE

IN THE DOG, CAT, AND MONKEY

APPROVED BY

Francis J. Haddy

Lerner B. Hinshaw

M. Jack Kyle

Cliff E. Hopla

DISSERTATION COMMITTEE

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PERIPHERAL VASCULAR EFFECTS OF ANGIOTENSIN II AND NOREPINEPHRINE IN THE DOG, CAT, AND MONKEY

CHAPTER I

HISTORY AND INTRODUCTION

In 1898 Tigerstedt and Bergman first demonstrated a pressor substance in kidney saline extracts which elevated rabbit's arterial blood pressure when injected intravenously (39). The unknown pressor substance was named renin. It was later shown by Braun-Menendez and colleagues (5) and Page and Helmer in 1940 (32) that renin possessed no vasopressor activity itself, but acted as an enzyme to release or activate an inactive plasma precursor to a polypeptide which had potent vasopressor properties. This pressor substance was called 'hypertensin' by Braun-Menendez and 'angiotonin' by Page. It was demonstrated in 1954 by Skeggs and co-workers (36) that release of the active pressor substance actually involved two steps in the plasma. A decapeptide, which is believed to come from a plasma alpha-globulin, is first released by renin and subsequently split by a plasma 'converting-enzyme' to the active octapeptide. The pure octapeptide was synthesized independently and simultaneously in 1957 by Rittel and colleagues (34) and Bumpus, Schwartz and Page (7). To avoid the confusion of having two names for the same substance, Braun-Menendez and Page in 1958 mutually suggested the term 'angiotensin' to

to replace hypertensin and angiotonin (6). The plasma precursor is now known as angiotensinogen while the inactive decapeptide and active octapeptide are called angiotensin I and angiotensin II, respectively. Renin is thought to be produced and stored in the juxtaglomerular cells of the kidney (40).

A pressor substance from the adrenal gland was first noted in 1895 by Oliver and Schafer (30). One of the substances responsible for this property, epinephrine, was identified and synthetized in 1901 by Takamine (38). In their classical study of 1910, Barger and Dale (3) delineated the basic relationship between chemical structure and adrenergic activity. However, it was not until 1945, almost 50 years after Oliver and Schafer's original observation, that norepinephrine was identified in adrenal extracts and implicated as a second adrenal pressor hormone by von Euler (13). Norepinephrine is a naturally occurring substance, a cathecholamine, produced at sympathetic nerve endings and in the medulla of the adrenal gland (14, 15). Norepinephrine has potent vasoactive properties, constricting peripheral arteries and veins as well as exhibiting other hemodynamic effects (11).

Angiotensin II is one of the most potent vasoconstrictors known, elevating systemic arterial blood pressure 5 - 10 times higher than epinephrine or norepinephrine on a weight basis (2, 16). Although considerable research has been completed concerning angiotensin's action on systemic arterial blood pressure (1, 2, 16, 28, 31) and regional blood flow (1), little has been done regarding its direct effect on veins. Unlike the catecholamines, which have been shown by several investigators to constrict veins as well as arteries (19, 20, 23, 24), available

evidence concerning angiotensin's direct effect on veins is unclear. Experimental work with animals indicates that angiotensin is not a venoconstrictor. In 1961, Folkow, Johansson and Mellander (17) studied the action of angiotensin on the 'resistance' and 'capacitance' vessels in a skin-muscle region of the cat. The hindquarters were isolated from the body except for an aortic connection. Arterial blood pressure and hindquarter flow were measured directly and volume changes were indicated plethysmographically. The authors concluded that although angiotensin was a potent arterial constrictor, it did not constrict veins. They further elaborated that while norepinephrine 'receptors' were located in both pre- and postcapillary vessels, angiotensin 'receptors' were mainly, if not totally, confined to precapillary vascular segments.

Haddy and co-workers in 1961 (22) used a natural flow dog limb preparation to investigate the local effect of vasoactive agents on pre- and postcapillary limb vessels. Blood pressures were measured directly from limb large artery and vein and small artery and small vein to segment the vascular bed. No evidence for venous constriction was obtained when angiotensin was infused in various concentrations into the brachial artery. The action of angiotensin on limb vessels was investigated further in 1962 by Haddy and associates using dog forelimbs (21). Blood flow rate was maintained constant with a pump in these studies and pressure measured from four points along the vascular bed as previously described. The authors were again unable to demonstrate small vein constriction with intra-brachial artery infusion of angiotensin.

In 1962, Rose and co-workers (35) studied the effect of angiotensin II and norepinephrine on systemic veins and systemic vascular capacity

using isolated femoral artery and vein segments and a venous return monitoring device, respectively. In these dog experiments, angiotensin did not constrict the femoral vein segment when applied topically nor did injections of angiotensin increase the venous return, indicating the absence of systemic 'capacitance' venous constriction. Norepinephrine was effective in both regards.

The pertinent work concerning angiotensin's effect on human peripheral veins has been less uniform than the animal results. In 1961 Wood (41) studied the action of angiotensin II infusion on peripheral arteries and veins in man. Arterial pressure was measured by the ausculatory method and calf or forearm venous pressure taken directly with a saline manometer. Blood flow and venous distensibility were indicated plethysmographically. Venous activity was inferred from these parameters. A decrease in venous compliance was indicated during angiotensin infusion in eight normotensive patients. The authors interpreted these changes as venous constriction.

This phenomenon was reinvestigated in 1963 by DePasquale and Burch (10), utilizing forearm veins in man. To avoid possible indirect effects and pressure changes due to blood flow alterations, an intact segment of a large forearm vein was isolated <u>in vivo</u> and the intraluminal pressure measured directly. In eighteen human subjects no venous constriction was evident when angiotensin II was injected into the isolated segments, whereas norepinephrine evoked intense venoconstriction. However, a slight rise in the isolated venous segment pressure was observed when angiotensin was infused intravenously in five subjects. Subsequent test proved this effect to be reflex in nature, as it did not occur when the segment was

denervated. The authors concluded that angiotensin II has no direct effect upon forearm veins in man.

Late in 1963 Daugherty and Haddy (8) completed a series of experiments in human subjects in which emphasis was placed on the effect of angiotensin on small veins. Small artery and small vein pressure were measured directly through indwelling needles; arm flow was unobstructed and natural. With equiweight infusion in each subject, angiotensin II caused an increase in hand small vein pressure equal to that produced by norepinephrine.

This review of the pertinent literature suggests the response of veins to angiotensin may vary between species as well as between different veins in the same species. These apparent differences and the paucity of information in general concerning angiotensin's effect on peripheral veins gave impetus to this study. Additionally, the importance of venous pressure in influencing capillary hydrostatic pressure, transcapillary fluid movement, venous return to the heart and the possible therapeutic use of angiotensin in various hypotensive states emphasizes the importance of the present investigation.

Angiotensin was compared with norepinephrine in regard to both peak pressure elevation and area response of limb brachial artery and small vein pressures and limb weight changes. Since angiotensin has a molecular weight approximately 6 times greater than norepinephrine, both equiweight and equimolar comparisons were made.

CHAPTER II

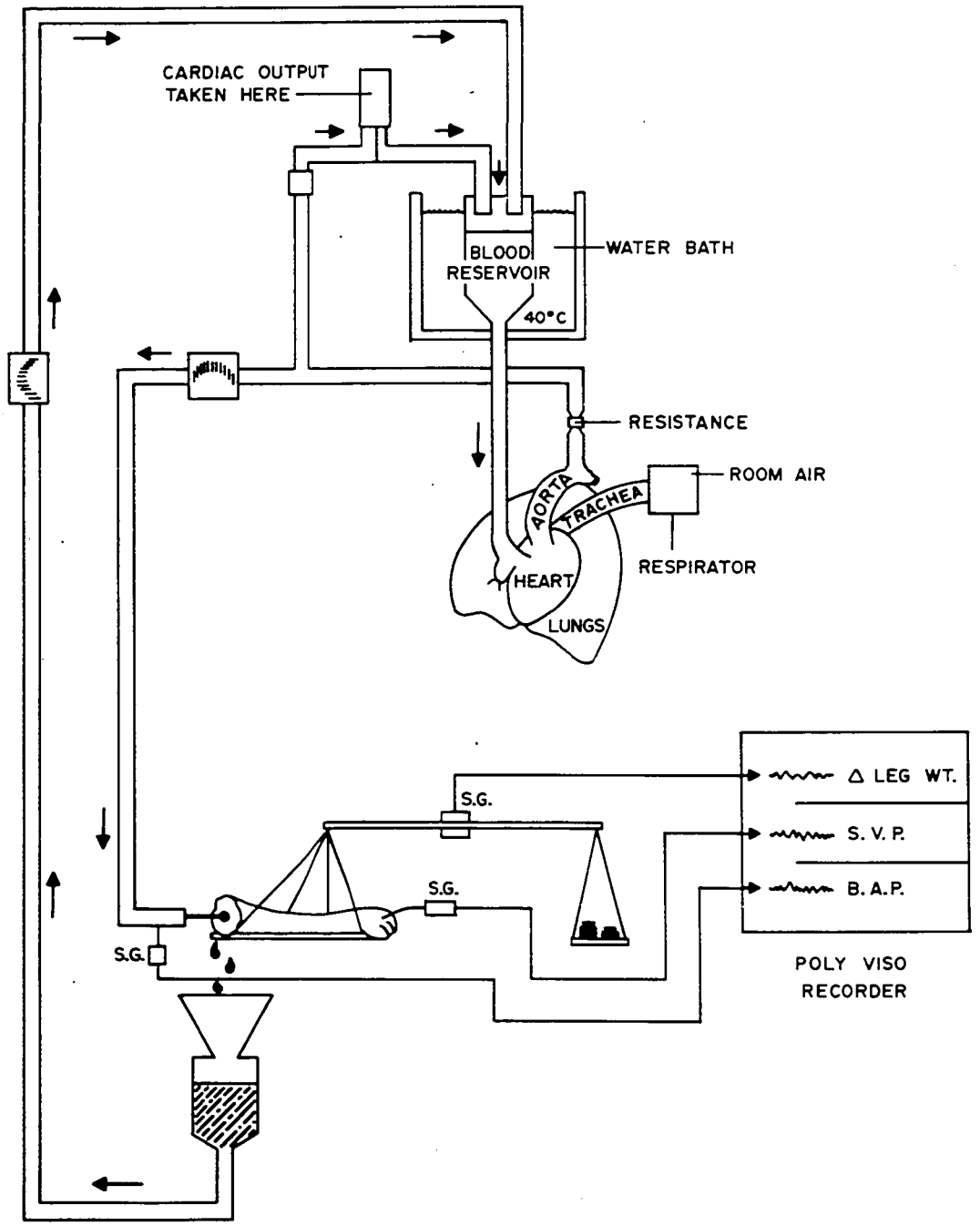
METHODS

Mongrel dogs, cats and sooty mangebey monkeys (Cerocebus torquatus) were intravenously or intraperitoneally anesthetized with sodium pentobarbital (30 mg/kgm). Heparin was used as anticoagulant. All pressures were measured with Statham pressure transducers and recorded on a Sanborn direct-writing recorder. Forty-one experiments were completed in thirty-two animal preparations as described below.

An isolated forelimb was perfused at constant flow by a Sigmamotor pump with oxygenated whole blood from an isolated Starling heart-lung system in 9 preparations. The heart-lung system, excluding the limb circuit, is a modification of Starling's original preparation (25, 26) and has been previously detailed (42). Figure 1 is a diagram of the perfusion circuit. The system was primed with about one liter of whole blood from another animal. After establishing the heart-lung system, blood was led from a point distal to the heart outflow resistor through a Sigmamotor pump to the isolated leg of a donor dog. Blood drained from the cut brachial and cephalic veins by gravity into a venous reservoir and was returned by a second pump to the elevated heart-reservoir. The heart-reservoir supplied blood at an almost constant pressure to the heart. Bleeding

Figure 1. Diagram showing experimental set-up of heart-lung dog forelimb preparation. Pump flow is constant. S. G. (weighing device) = strain gauge transducer. S. G. (pressure measurement) = strain gauge pressure transducer. Cardiac inflow is controlled with a screw-clamp. Arrows indicate the direction the blood is pumped. Δ leg weight = continuous recording of forelimb weight; SVP = limb small vein pressure; BAP = limb brachial artery pressure.

HEART-LUNG-PUMP LEG
(FLOW CONSTANT)



was prevented from cut surfaces of the leg by ligation and the bone stump was sealed with bone wax. An animal-pump-isolated limb preparation was used in the other experiments. Figure 2 shows the preparation diagramatically using a dog as an example: A pump was interposed between the femoral and brachial arteries or the animal's completely severed limb in 6 dogs, 8 cats and 4 monkeys. Bleeding from cut surfaces and the bone stumps was prevented as indicated above. The limb was perfused at constant flow compatible with the isogravimetric state, i.e., leg weight constant (33). Blood from the cut brachial and cephalic veins flowed by gravity into a reservoir and was returned to a cannulated femoral vein by a pump. The small extracorporeal system was primed with approximately 100 cc of Dextran. In all studies a small paw or hand vein was cannulated with small bore polyethylene tubing (0.61 - 0.97 mm OD), and its tip advanced retrogradely into a digital vein (19). Pressure measured by this technique is not significantly different from true lateral vein pressure (22) because of abundant venous anastomoses in this area (9, 29). The following criteria for the reliability of this method were met in each experiment: a) venous blood could be freely withdrawn and replaced with little resistance; b) a rapid, immediate return of the recording stylus to baseline following a positive pressure small vein catheter flush; and c) rapidly injecting saline through the small vein catheter resulted in an immediate increase in limb outflow. In order to compare the effect of drugs on different sized forelimb veins, two catheters were utilized in addition to the digital vein catheter in 4 heart-lung leg studies as illustrated in the upper portion of Figure 3: a) one in a superficial dorsal metacarpal vein at about mid-paw level, and b) another in the

Figure 2. Diagram showing dog-pump-isolated forelimb experimental set-up. Limb flow is constant. S. G. (weighing device) = strain gauge transducer; S. G. (pressure measurement) = strain gauge pressure transducer; △ leg weight = continuous recording of limb weight; SVP = forelimb small vein pressure; BAP = limb brachial artery pressure; SABP = animal's systemic arterial blood pressure. Arrows indicate the direction the blood is being pumped. Blood is drawn from a femoral artery and returned to a femoral vein. Forelimb blood inflow is maintained constant with a pump.

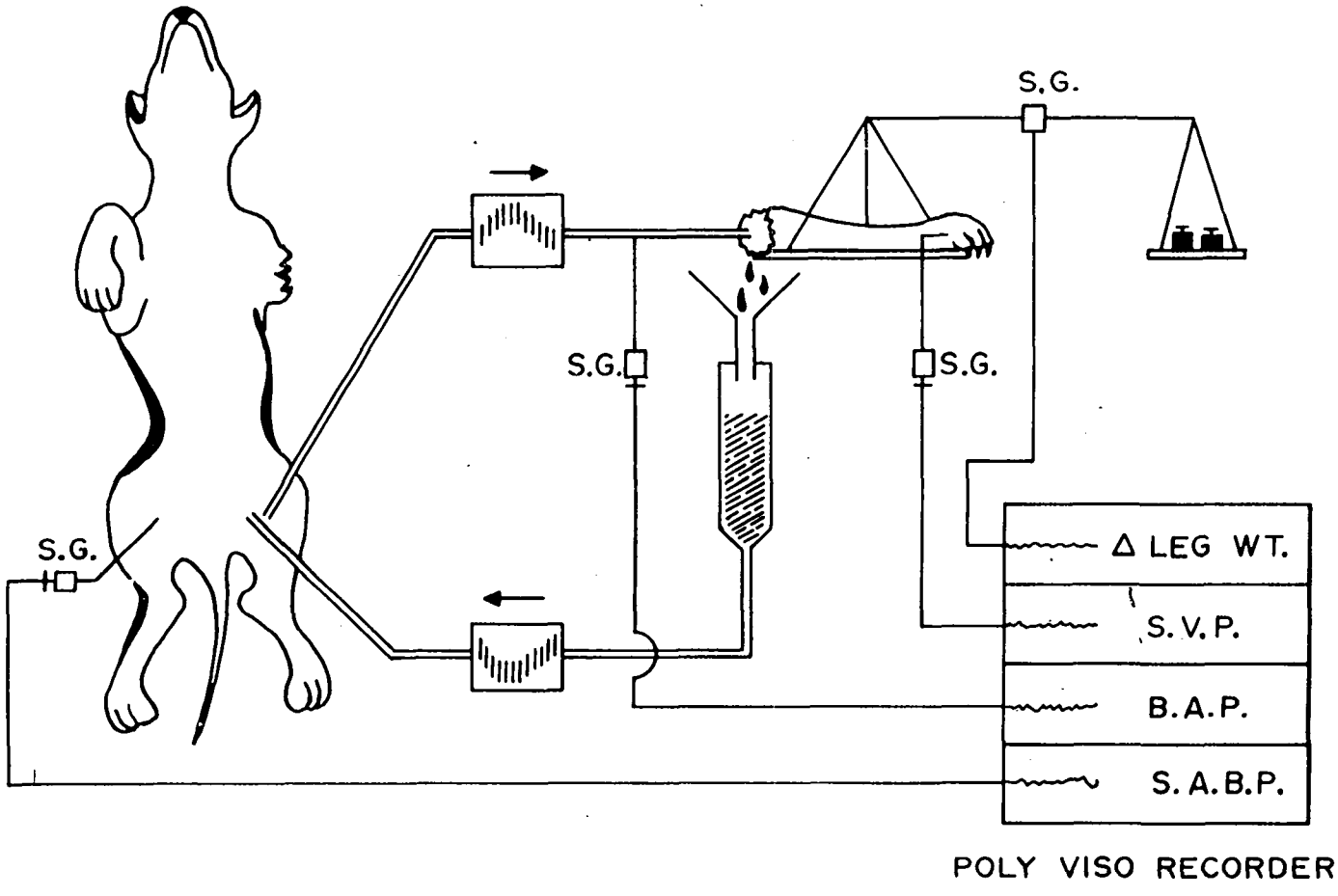
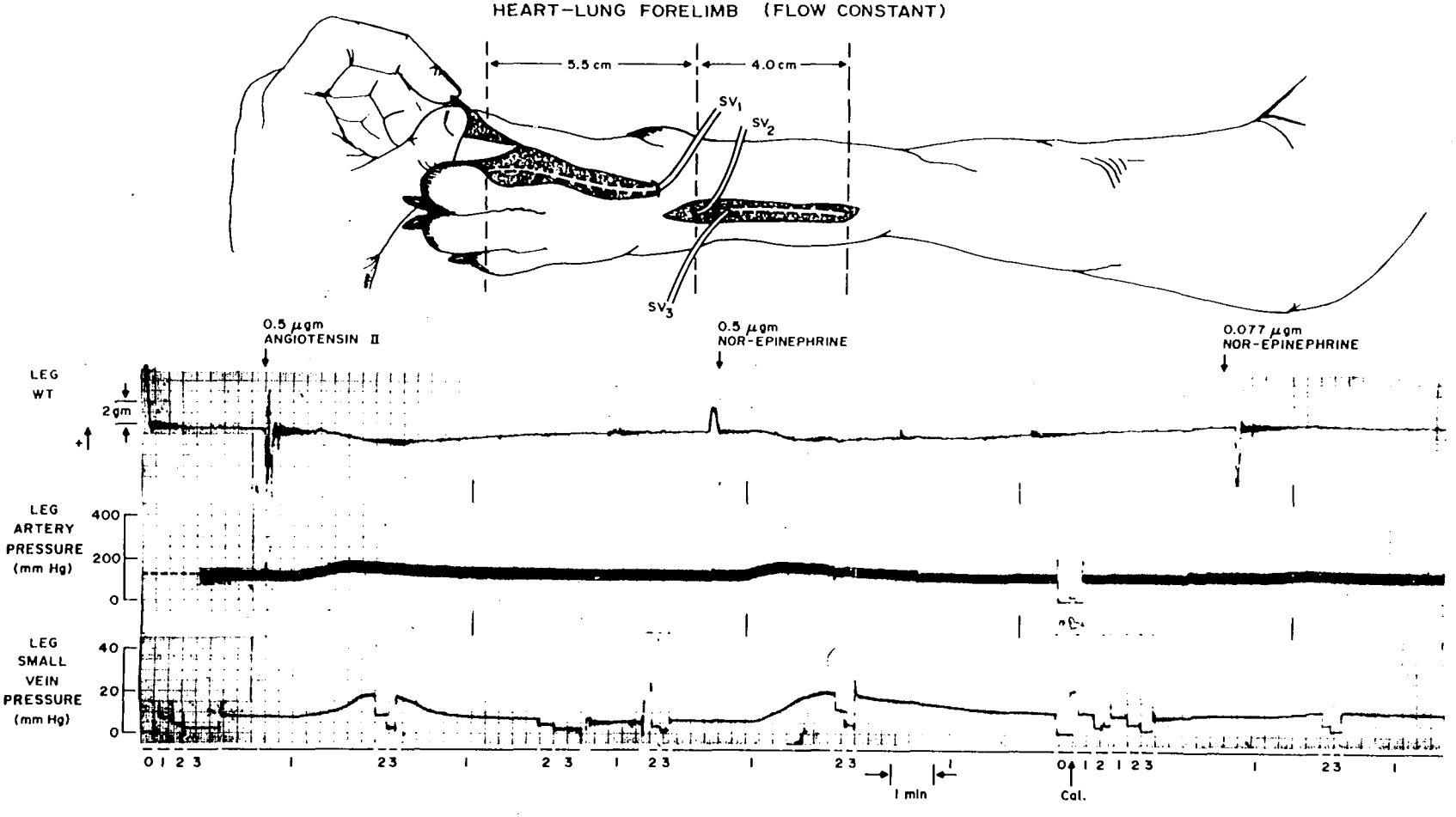

S.G.
S.G.
S.G.
S.G.
Δ LEG WT.
S.V.P.
B.A.P.
S.A.B.P.
POLY VISO RECORDER

Figure 3. Upper portion shows drawing of dog forelimb detailing the relative position of three venous catheters used to compare the response of different sized veins to angiotensin and norepinephrine. Equiweight and equimolar comparisons; 0.5 μgm angiotensin equimolar to 0.077 μgm norepinephrine. Pressures were obtained alternately from a pressure transducer through a multiple stopcock arrangement. Bottom half shows record obtained from limb diagrammed below. Numbers 1, 2, and 3 indicate portion of the small vein pressure tracing corresponding to catheters SV_1, SV_2, and SV_3 above. Constant limb flow = 4 cc/min/100 gms limb; limb weight = 364 gms.


HEART–LUNG FORELIMB (FLOW CONSTANT)
5.5 cm
4.0 cm
SV1
SV2
SV3
0.5 μgm ANGIOTENSIN II
0.5 μgm NOR-EPINEPHRINE
0.077 μgm NOR-EPINEPHRINE
LEG WT
2gm
LEG ARTERY PRESSURE (mm Hg)
400
200
0
LEG SMALL VEIN PRESSURE (mm Hg)
40
20
0
1 min
Cal.

cephalic vein, its tip pointing centrally and resting at about wrist level. Although the catheter pointing centrally would admittedly register a lower pressure than if pointing in the reverse direction, it should nevertheless be valid to compare changes of pressure in this region produced by the drugs. All three catheters were connected to a single pressure transducer, pressure being recorded alternately from different stopcocks. Brachial artery (perfusion) pressure, obtained from a needle in the inflow tubing distal to the pump, small vein pressure and the animal's aortic blood pressure (except in the heart-lung studies) were recorded continuously. Leg weight was registered continuously with a strain gauge weighing device which measured weight changes accurately to 0.1 gm (37). Limb vascular responsiveness was tested comparing angiotensin II (Hypertensin, Ciba; 0.25 - 1.0 μgm) and norepinephrine (Levophed, Wintrop; 0.039 - 1.0 μgm of free base) on an equiweight and equimolar basis in the dog and cat and on an equiweight basis in the monkey. The drug dose used for any particular limb depended on limb weight and blood flow rate, and was selected to give an easily measured response. The amount of angiotensin and norepinephrine tested for any given limb was always the same and limb flow was always constant and equal for each comparison. The order of drug administration was randomized. The molecular weight of norepinephrine is 169 compared to approximately 1091 for angiotensin II, thereby making 0.039 μgm norepinephrine the mole equivalent of 0.25 μgm angiotensin. Concentrations were such that only 0.039 - 0.1 cc of solution was injected per test. Saline blank injections were also given. Drugs were injected rapidly into the brachial artery inflow tubing just proximal to the pump. With flow constant, changes in brachial artery and

small vein pressure directly reflect vascular resistance changes in like direction. In this preparation, essentially all resistance changes should be due to active vessel radius alteration. Thus, an increase in pressure above baseline indicates vasoconstriction and vice versa. Although most of a change in brachial artery pressure is probably due to small artery and especially arteriolar radius change, postcapillary vascular resistance also can influence the pressure measured in the brachial artery. Pressure measured in the digital vein is due almost entirely to resistance downstream from the measuring point; resistance upstream to the catheter tip has little effect on the recorded pressure.[1] An immediate rapid loss of leg weight should be due mainly to active vascular constriction, while a secondary, more gradual decrease probably results from a net loss of interstitial fluid due to altered capillary hydrostatic pressure. Changes in capillary pressure are largely passive, resulting from active changes in postcapillary vascular pressure and resistance.

Five dogs were infused separately with angiotensin and norepinephrine for 20-25 minutes intra-arterially. Infusion rates were 0.1 μgm/min (0.2 cc solution/min) in four and 0.5 μgm/min (0.5 cc solution/min) in one experiment. Saline blank infusions were completed in each limb. The effect of these drugs on brachial artery and small (digital) vein pressures and leg weight was compared on an equiweight infusion basis. Infusion rates were such that the dog's aortic blood pressure was not appreciably altered.

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[1] An experiment was carried out to demonstrate this phenomenon: Water was pumped at constant flow through rigid tubing and two screwclamps placed on the tubing distal to the pump. Pressure was measured on both sides of the upstream clamp. Large pressure changes proximal to the upstream clamp, produced by adjusting the upstream screwclamp, had no effect on pressure distal to that clamp. Whereas any change in the downstream clamp resulted in identical pressure changes proximal to both the downstream and upstream clamps.

In a group of five dogs, weighing from 14 - 20 kgm, angiotensin and norepinephrine were injected directly into a small paw vein and small vein pressure was recorded in intact, innervated, natural flow dog limbs as follows: Approximately one centimeter of a metacarpal vein was exposed through a small skin incision. In four animals two small bore polyethylene catheters (0.61 mm OD) were inserted side by side into a metacarpal vein and advanced retrogradely into a digital vein. In one animal the two catheters were placed in adjacent vessels. Drugs were injected through one catheter and pressure recorded continuously through the other. Equiweight comparisons of angiotensin and norepinephrine (0.5 - 1.0 μgm) were completed in each limb. Saline blank injections were given in each case. In this preparation only small vein pressure was measured. Limb flow rate was unknown and not necessarily constant and small artery pressure was not measured. Even though the drugs were injected directly into a digital vein it is not possible to calculate or make any definite statement about small vein resistance changes. It is possible, however, that an immediate increase in small vein pressure is due to active venoconstriction.

Drug responses were evaluated as both peak increase in blood pressure above baseline and as a composite of height and duration of response (change in area under the pressure curve) above control. Per cent change in pressure was calculated as change in pressure above baseline. For any particular comparison test, the per cent differences of response between the drugs was used.

CHAPTER III

RESULTS

Table I shows mean data of leg artery and small vein pressure responses and leg weight changes from 15 dog, 8 cat and 4 monkey forelimb perfusion studies. Dog forelimb data is given in Table I-A. On an equiweight basis norepinephrine increased brachial artery pressure (BAP) 31% more (25 mm Hg) than angiotensin while the area response (composite of height and duration of response above baseline) was not significantly different. Norepinephrine increased small vein pressure (SVP) 87% more (8 mm Hg) than angiotensin with a 197 mm^2 greater area response. However, when compared on an equimolar basis, angiotensin increased BAP 36% (25 mm Hg) more than norepinephrine did, and the area response was 268 mm^2 greater. Angiotensin elevated SVP 56% (5 mm Hg) higher with a larger area duration of 165 mm^2. Although these mean data represent the overall picture, extremes were noted in either direction. In some cases norepinephrine, on an equiweight basis, produced a substantially greater small vein constriction than indicated by the mean data, whereas in other limbs, angiotensin was an equally potent venoconstrictor. It should also be mentioned that although the norepinephrine response could usually be consistently repeated in the same forelimb, angiotensin induced vasoconstriction was often appreciably diminished with a second injection, particularly on the venous side. Although loss of leg weight was approximately equal with

Table I. Mean data ± standard errors from dog, cat, and monkey forelimb drug injection experiments. Responsiveness of limb arteries and small veins to angiotensin and norepinephrine is compared as peak change in pressure from baseline and the area response (composite of height and duration of response). The effect of angiotensin and norepinephrine on limb weight is also shown.

A. Dog limb data. Equiweight and equimolar comparison.

B. Cat limb data. Equiweight and equimolar comparison.

C. Monkey limb data. Equiweight comparison.

Comparison of angiotensin II and nor-epinephrine vasoactivity on forelimb arteries and small (digital) veins in the dog, cat and monkey*.

	Animal	Drug	Dose (μgm)	Initial BAP±SE (mm Hg)	Δ BAP±SE (mm Hg)	Δ Area (BAP)±SE (mm^2)	Initial SVP±SE (mm Hg)	Δ SVP±SE (mm Hg)	Δ Area (SVP)±SE (mm^2)	Δ Leg Wt.±SE (gms)
A	Dog Leg (N=15)	Angiotensin	0.25-1.0	80±3	54±4	322±41	11±1	8±1	240±35	-1.5±0
		Nor-epi.	0.25-1.0	80±5	79±7	379±71	10±1	16±2	437±72	-1.3±0.1
	(N=8)	Angiotensin	0.25-0.50	81±4	54±6	357±50	9±1	7±1	205±46	-1.3±0
		Nor-epi.	0.039-0.077	93±8	29±5	97±40	9±1	2±0	40±12	-0.3±0
B	Cat Leg (N=8)	Angiotensin	0.25-0.50	101±8	127±22	880±180	7±1	11±4	404±161	-0.31±0.1
		Nor-epi.	0.25-0.50	100±10	55±2	327±71	7±1	7±2	211±51	-0.41±0.1
		Nor-epi.	0.039-0.077	103±9	23±3	69±4	7±1	2±1	48±16	-0.17±0.1
C	Monkey Leg (N=4)	Angiotensin	0.25-0.50	113±9	34±6	229±58	8±1	14±3	586±195	-0.43±0
		Nor epi.	0.25-0.50	109±2	40±3	149±38	10±3	6±2	104±35	-0.26±0

Animal or Heart-Lung pump-leg (isolated) preparation, flow constant; mean dog leg flow = 13 cc/min/100 gms leg (mean leg wt. = 311 gms); mean cat leg flow = 5 cc/min/100 gms (mean leg wt. = 127 gms); mean monkey leg flow = 5 cc/min/100 gms (mean leg wt. = 165 gms). N = number of experiments; ± SE = ± standard error; initial BAP and initial SVP = mean initial leg brachial artery and small vein pressures (control); Δ BAP and Δ SVP = mean peak change in brachial artery and small vein pressures from control; Δ Area (BAP) and (SVP) = mean change in area under the brachial artery and small vein pressure curves from control; Δ leg wt. = mean peak change in leg weight from control, negative sign indicates weight loss. Angiotensin II (octapeptide) and nor-epinephrine (free base) are compared on an equiweight* and equimolar basis (0.25 μgm Angiotensin equimolar with 0.039 μgm nor-epinephrine).

either drug with equiweight doses, this was not a uniform finding. However, angiotensin was consistently more effective in this regard on a molar basis. Figure 4 shows an experimental tracing from one study in which angiotensin constricted small veins almost as much as norepinephrine with equiweight injections. Also note that norepinephrine induced a more rapid weight loss which was typical.

Table I-B represents a mean data from the cat forelimb studies. Angiotensin, with equiweight injections, exhibited more vasoactivity in both peak increase in BAP (71%; 72 mm Hg greater) and SVP (57%; 4 mm Hg greater) and area response (553 mm^2 and 193 mm^2 greater, respectively). The greater vasoconstrictor ability of angiotensin over norepinephrine is further exemplified with equimolar injections; angiotensin increased leg BAP an average of 104% (104 mm Hg) more for a greater area response of 811 mm^2 while elevating paw SVP 128% (9 mm Hg) higher with a 356 mm^2 greater area response. Angiotensin and norepinephrine (equiweight) both caused approximately the same loss of leg weight while angiotensin greatly surpassed norepinephrine on a molar basis. Decreased leg weight resulted from both drugs in all cat legs except one in which a slight increase occurred. The individual response variations from the mean were reversed to those observed in the dog. Angiotensin usually produced a greater small vein constriction but in some cases the response to either drug was about equal. Figure 5 illustrates the more powerful vasoconstriction produced by angiotensin in the cat forelimb.

The monkey forelimb data are summarized in Table I-C. Following equiweight injections norepinephrine produced a slightly greater increase in leg BAP (4%; 6 mm Hg greater), however, the area response was 80 mm^2

Figure 4. Record from isolated dog limb experiment showing vascular and limb weight effects of intrabrachial artery injected angiotensin II (octapeptide) and norepinephrine (free base). Equiweight and equimolar comparisons; 0.25 μgm angiotensin equimolar to 0.039 μgm norepinephrine.

DOG — PUMP — LEG
(FLOW CONSTANT)

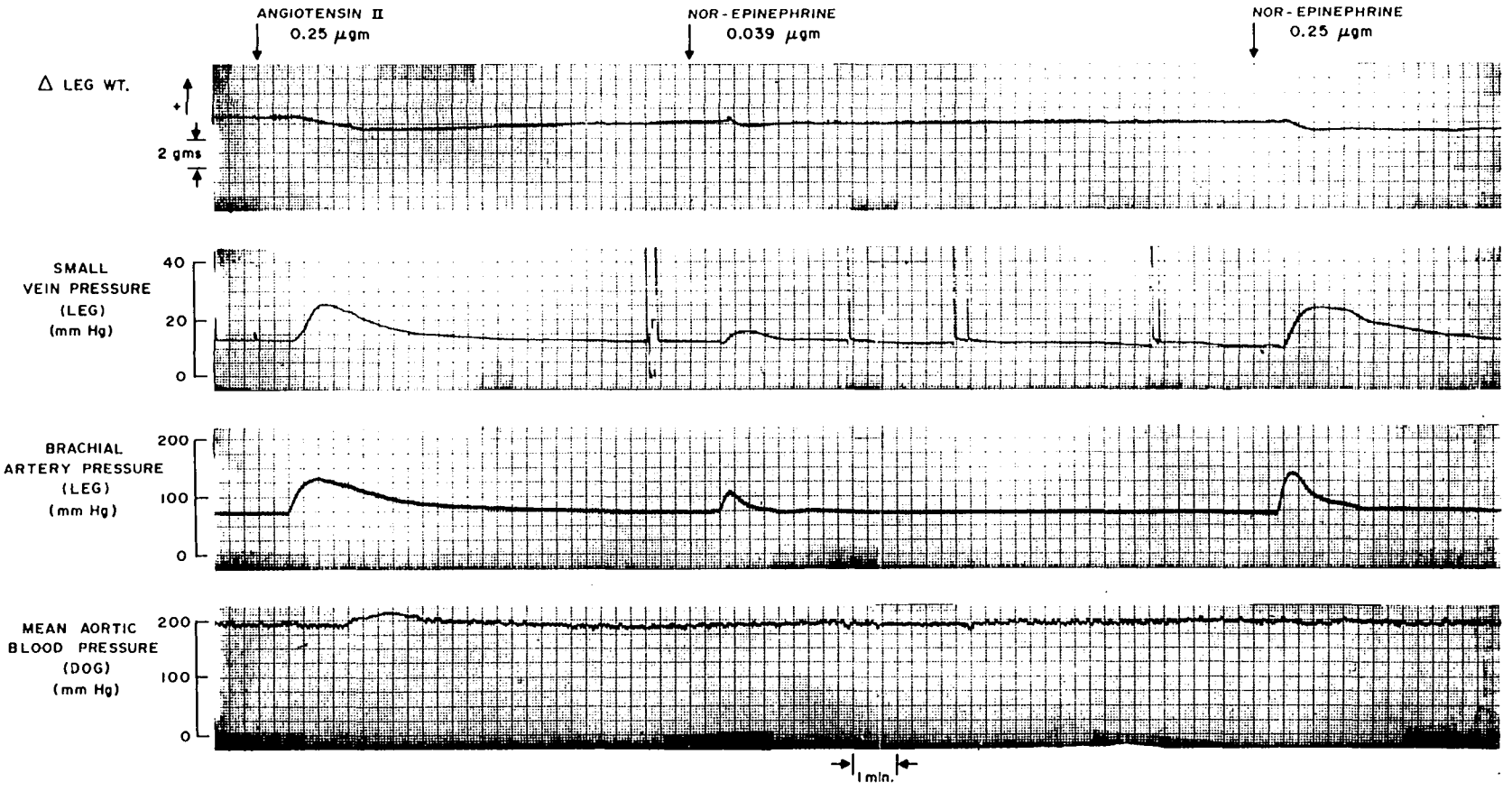
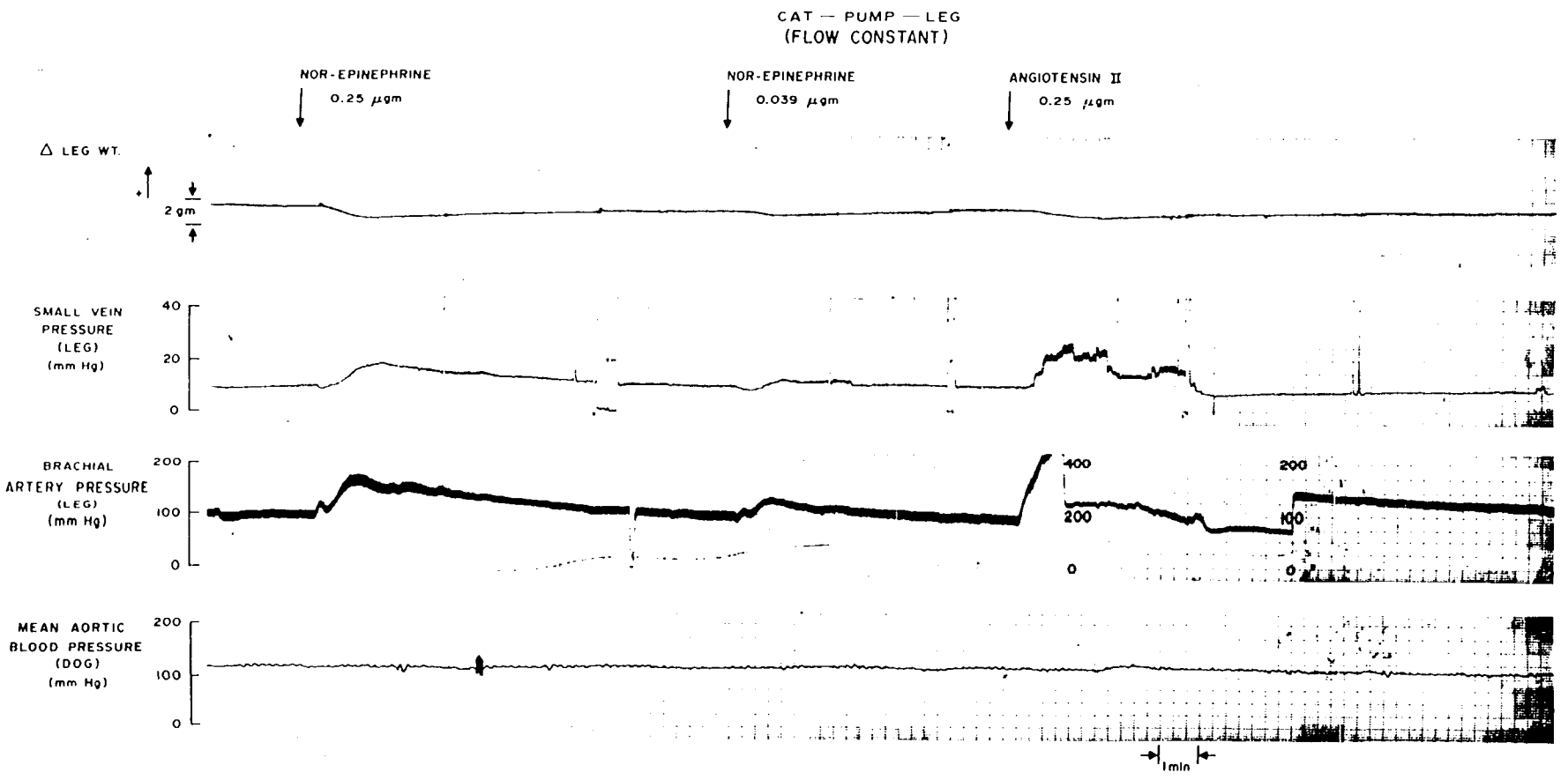


Figure 5. Record from an isolated cat limb experiment showing vascular and limb weight effects of intrabrachial artery injected angiotensin II (octapeptide) and norepinephrine (free base). Equiweight and equimolar comparison; 0.25 μgm angiotensin equimolar to 0.039 μgm norepinephrine.


CAT — PUMP — LEG
(FLOW CONSTANT)
NOR-EPINEPHRINE
0.25 μgm
NOR-EPINEPHRINE
0.039 μgm
ANGIOTENSIN II
0.25 μgm
Δ LEG WT.
2 gm
SMALL VEIN PRESSURE (LEG) (mm Hg)
40
20
0
BRACHIAL ARTERY PRESSURE (LEG) (mm Hg)
200
100
0
400
200
0
200
100
0
MEAN AORTIC BLOOD PRESSURE (DOG) (mm Hg)
200
100
0
1 min

less than with angiotensin. Conversely, angiotensin increased forelimb SVP 116% (8 mm Hg) more for a greater area response of 482 mm^2. Each monkey leg responded qualitatively similar to the mean data presented above. Angiotensin always produced a greater small vein constriction for a longer period than norepinephrine. Contrary to leg weight responses in the dog and cat, angiotensin produced a greater loss of weight than norepinephrine. Figure 6 shows an experimental record.

Table II shows mean values from four heart-lung leg experiments in which venous pressure was measured from the digital, dorsal superficial metacarpal, and cephalic veins. On the average, equiweight injection of norepinephrine increased digital and metacarpal vein pressure 46% (5 and 6 mm Hg, respectively) more than angiotensin, whereas it was 28% (1.7 mm Hg) more effective in the cephalic vein area. The lower portion of Figure 3 shows a record obtained from the limb illustrated in the upper half.

The responsiveness of limb vessels sometimes varied appreciably between different limbs. However, if hyper- or hyporesponsiveness to a particular dose was evident with angiotensin, the vessels usually showed the same characteristic to norepinephrine.

Figure 7 shows mean data from five dog-pump-leg preparations where angiotensin and norepinephrine were infused separately into the brachial artery inflow tubing for an average of 21 minutes. The effect of drug infusion on BAP, SVP, and leg weight was compared on an equiweight infusion basis (0.1 - 0.5 μgm/min). Angiotensin infusion resulted in an immediate 100% (9 mm Hg) increase in small vein pressure above control. However, SVP immediately began to diminish and was almost back to control pressure at 10 minutes; complete tachyphylaxis developed by the 21st minute of

Figure 6. Record from isolated monkey limb experiment showing vascular and limb weight effects of intrabrachial artery injected angiotensin II (octapeptide) and norepinephrine (free base). Equiweight comparison. Constant flow = 7 cc/min/100 gms limb; limb weight = 188 gms. SVP = limb small vein pressure; SAP = limb brachial artery pressure; SABP = systemic arterial blood pressure. Three large squares on record abscissa = 1 minute.

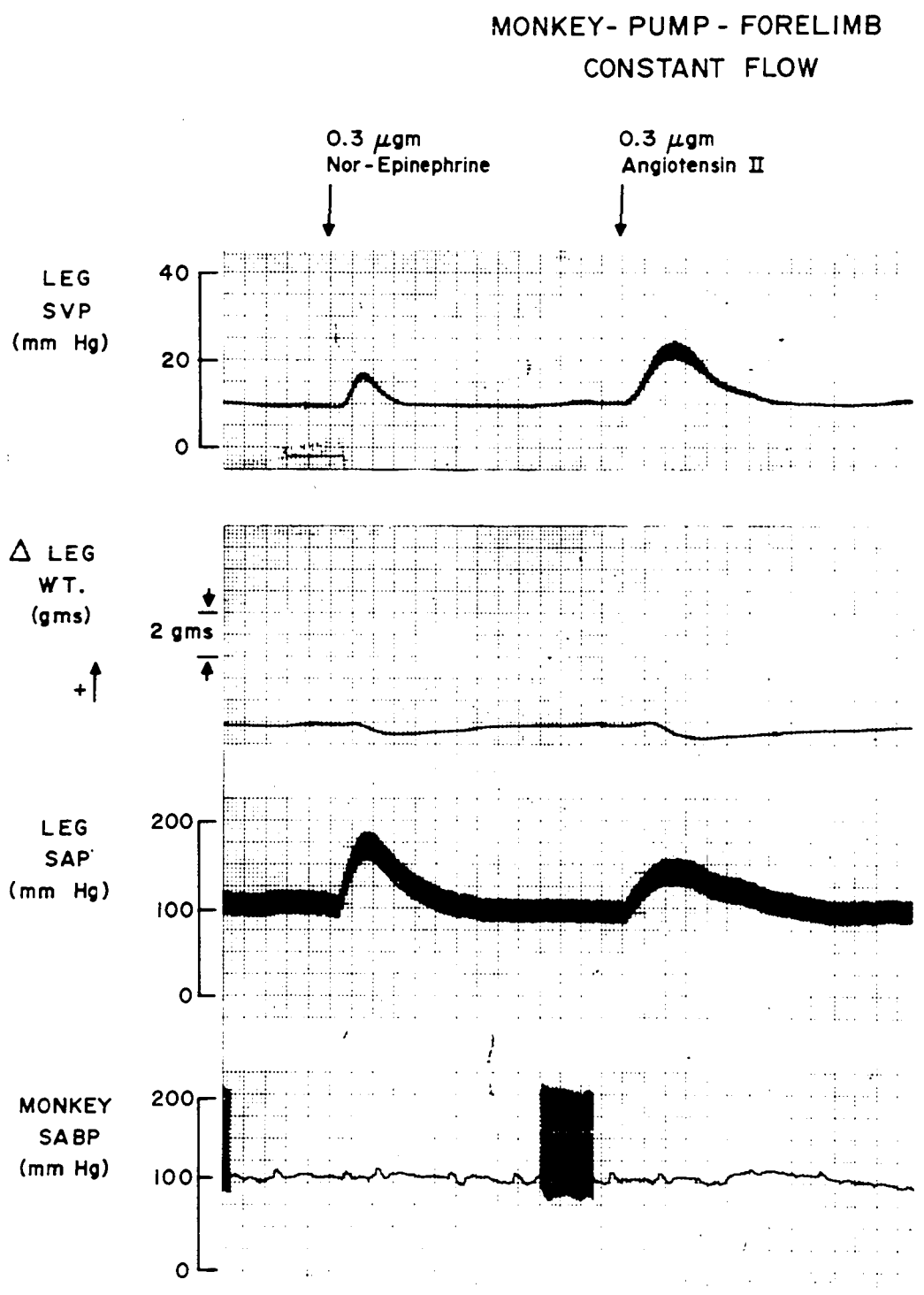

MONKEY- PUMP - FORELIMB
CONSTANT FLOW
0.3 μgm
Nor-Epinephrine
0.3 μgm
Angiotensin II
LEG
SVP
(mm Hg)
40
20
0
Δ LEG
WT.
(gms)
2 gms
+
LEG
SAP
(mm Hg)
200
100
0
MONKEY
SABP
(mm Hg)
200
100
0

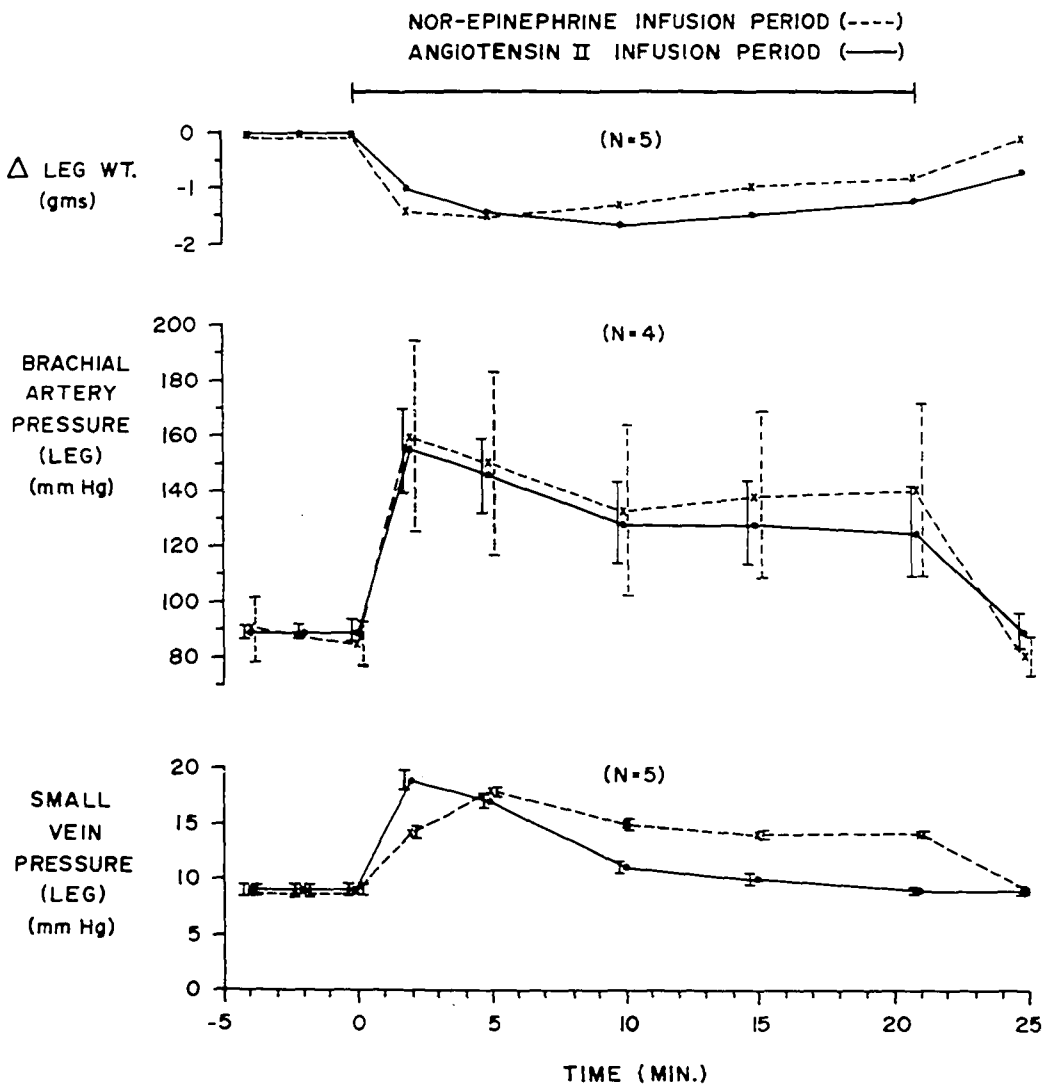
Table II. Mean data ± standard errors from four heart-lung dog limb experiments in which the responsiveness of different sized forelimb veins to equiweight and injections of angiotensin and norepinephrine was compared.

Comparison of angiotensin II and nor-epinephrine vasoactivity on small, medium, and large forelimb veins.

DRUG	DIGITAL VEIN PRESSURE		METACARPAL VEIN PRESSURE		CEPHALIC VEIN PRESSURE	
	Initial ±SE (mm Hg)	Δ ±SE (mm Hg)	Initial ±SE (mm Hg)	Δ ±SE (mm Hg)	Initial ±SE (mm Hg)	Δ ±SE (mm Hg)
Angiotensin II (0.5 μgm)	11 ±3	11 ±2	10 ±3	9 ±3	6 ±2	0.3 ±0
Nor-epinephrine (0.5 μgm)	11 ±3	16 ±3	11 ±3	15 ±3	6 ±2	2.0 ±1

Mean data ±standard error (±SE) from 4 Heart-Lung isolated limb perfusion studies. Blood flow is constant. Mean leg flow = 9 cc/min/100 gms leg. Mean leg weight = 396 gms.

Figure 7. Mean values ± standard errors from five isolated dog-pump-limb experiments in which angiotensin and norepinephrine were infused separately into the limb inflow tubing at 0.1 - 0.5 μgm/min. Equiweight infusions were compared in each limb. Standard errors for limb weight changes were all less than 0.1 gms. Mean flow (constant) = 6 cc/min/100 gms limb; mean limb weight = 229 gms.


NOR-EPINEPHRINE INFUSION PERIOD (----)
ANGIOTENSIN II INFUSION PERIOD (——)
Δ LEG WT. (gms)
0
-1
-2
(N=5)
BRACHIAL ARTERY PRESSURE (LEG) (mm Hg)
200
180
160
140
120
100
80
(N=4)
SMALL VEIN PRESSURE (LEG) (mm Hg)
20
15
10
5
0
(N=5)
-5
0
5
10
15
20
25
TIME (MIN.)

infusion. Stopping the infusion resulted in no further change in SVP. Figure 8 is a record showing the effects of angiotensin infusion in one experiment.

With norepinephrine infusion peak elevation of SVP (89%; 8 mm Hg increase) was more gradually attained but it diminished only moderately compared to angiotensin, and stabilized at a value well above control. Small vein pressure returned to baseline after the infusion was stopped. A record from one experiment, showing the effects of norepinephrine infusion, is presented in Figure 9.

On the average, both drugs produced an immediate and similar increase in BAP which stabilized at a level below the peak elevation. Brachial artery pressure returned to control after cessation of infusion with both drugs. Although the angiotensin curve continued to decrease slightly while the norepinephrine curve rose, they were not significantly different.

Angiotensin caused an initial fall in leg weight during the first two minutes of infusion which was followed by a more gradual decrease during the next eight minutes. From this point on, leg weight increased gradually for the remainder of the infusion period at which time a further weight gain occurred, but control value was not attained during the next 4 minutes. In some limbs BAP fell gradually to near control level during the infusion period. Norepinephrine infusion produced an initial loss of weight, the decrease being 0.4 gms greater than with angiotensin at 2 minutes and approximately the same by the 5th minute of infusion. No further loss occurred and the leg weight began to increase. In addition to the early increase in leg weight, the rate of increase was greater than with angiotensin. Following cessation of norepinephrine infusion

Figure 8. Record showing effect of angiotensin II infusion on dog forelimb artery and small vein pressures and limb weight. Pump perfused forelimb; Constant flow = 7 cc/min/100 gms limb; limb weight = 195 gms. Record is from the same limb experiment shown in Figure 9.

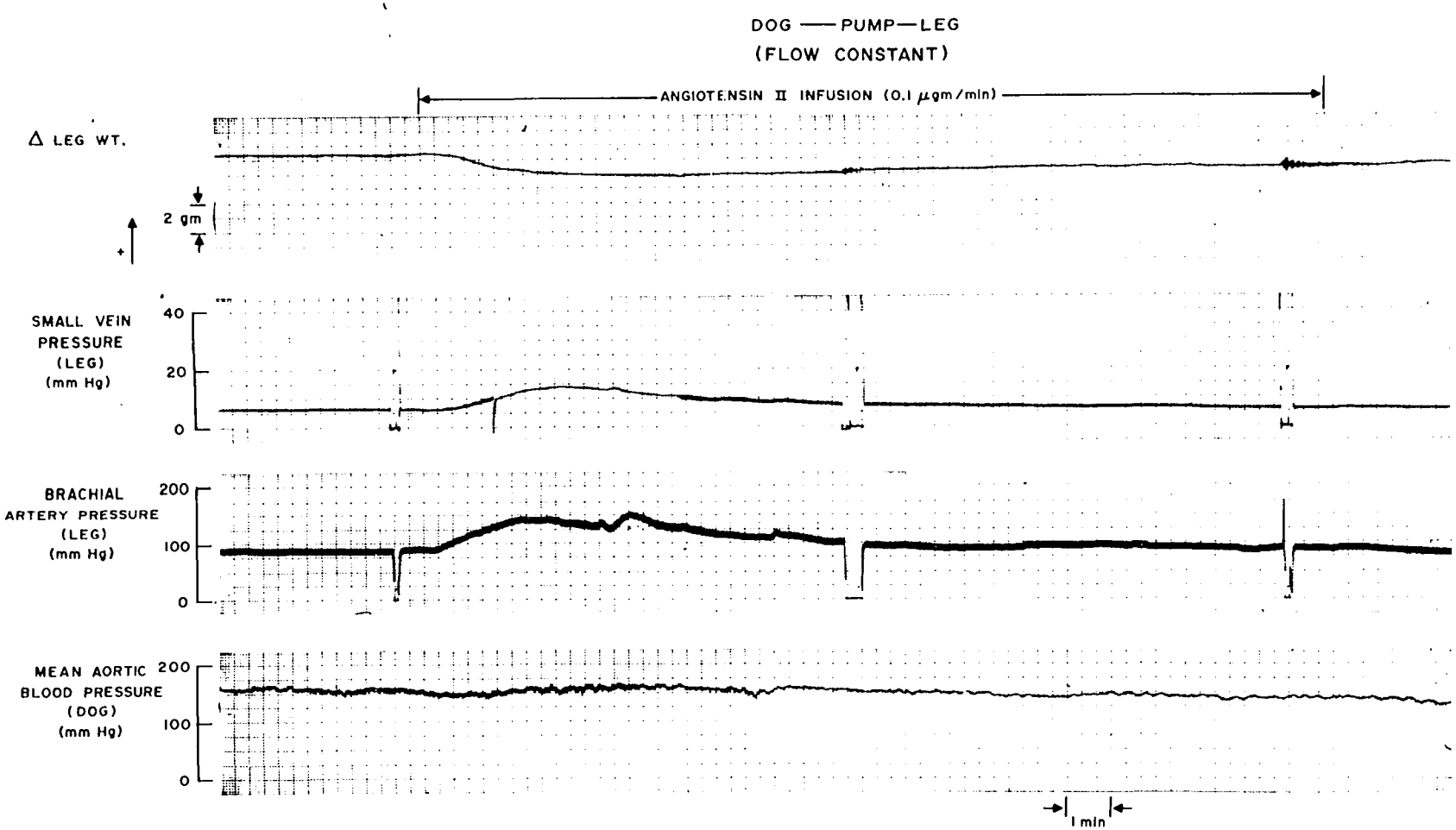
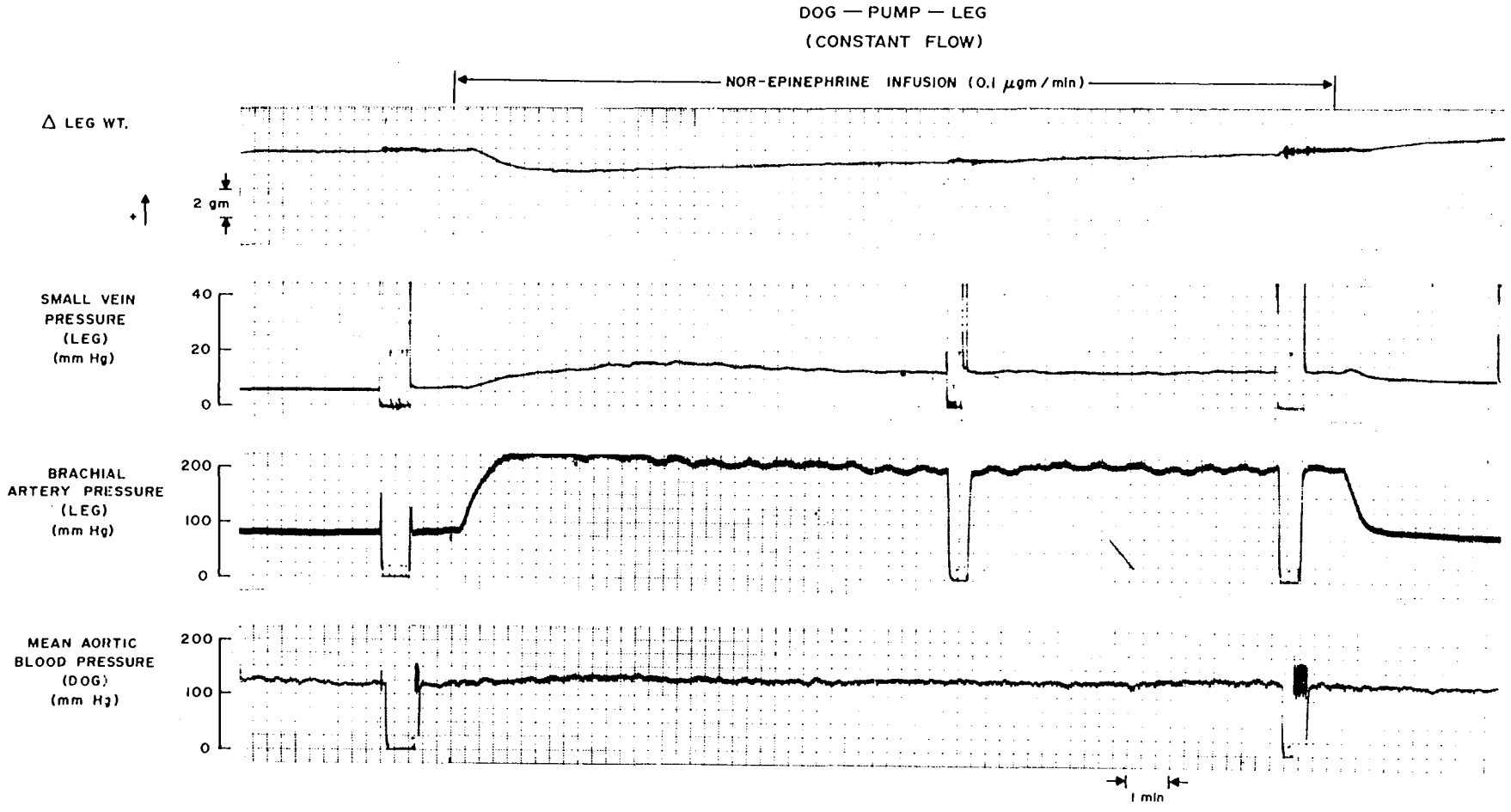

DOG — PUMP — LEG
(FLOW CONSTANT)
ANGIOTENSIN II INFUSION (0.1 μgm/min)
Δ LEG WT.
2 gm
SMALL VEIN PRESSURE (LEG) (mm Hg)
40
20
0
BRACHIAL ARTERY PRESSURE (LEG) (mm Hg)
200
100
0
MEAN AORTIC BLOOD PRESSURE (DOG) (mm Hg)
200
100
0
1 min

Figure 9. Record showing effect of norepinephrine infusion on dog forelimb artery and small vein pressures and limb weight. Pump perfused forelimb; Constant flow = 7 cc/min/100 gms limb; limb weight = 195 gms. Record is from same forelimb experiment as shown in Figure 8.


DOG — PUMP — LEG
(CONSTANT FLOW)
NOR-EPINEPHRINE INFUSION (0.1 μgm / min)
Δ LEG WT.
2 gm
SMALL VEIN PRESSURE (LEG) (mm Hg)
40
20
0
BRACHIAL ARTERY PRESSURE (LEG) (mm Hg)
200
100
0
MEAN AORTIC BLOOD PRESSURE (DOG) (mm Hg)
200
100
0
1 min

leg weight increased rapidly to near control during the next four minutes.

Mean and individual data from the intact, natural flow dog forelimb experiments are shown in Table III. On the average, norepinephrine increased SVP approximately twice as high as an equivalent amount of angiotensin. However, comparison of the individual responses illustrates the extreme variability of responses in this preparation. The SVP response to angiotensin ranges from zero pressure rise to an increase equivalent to that caused by norepinephrine. Figure 10 shows a record of one drug comparison test in a natural flow limb. Note the immediate and similar increase in SVP following the injection artifact produced when angiotensin and norepinephrine were injected into a digital vein. Injection of an identical volume of saline (0.3 cc) produced nothing but the transient injection artifact.

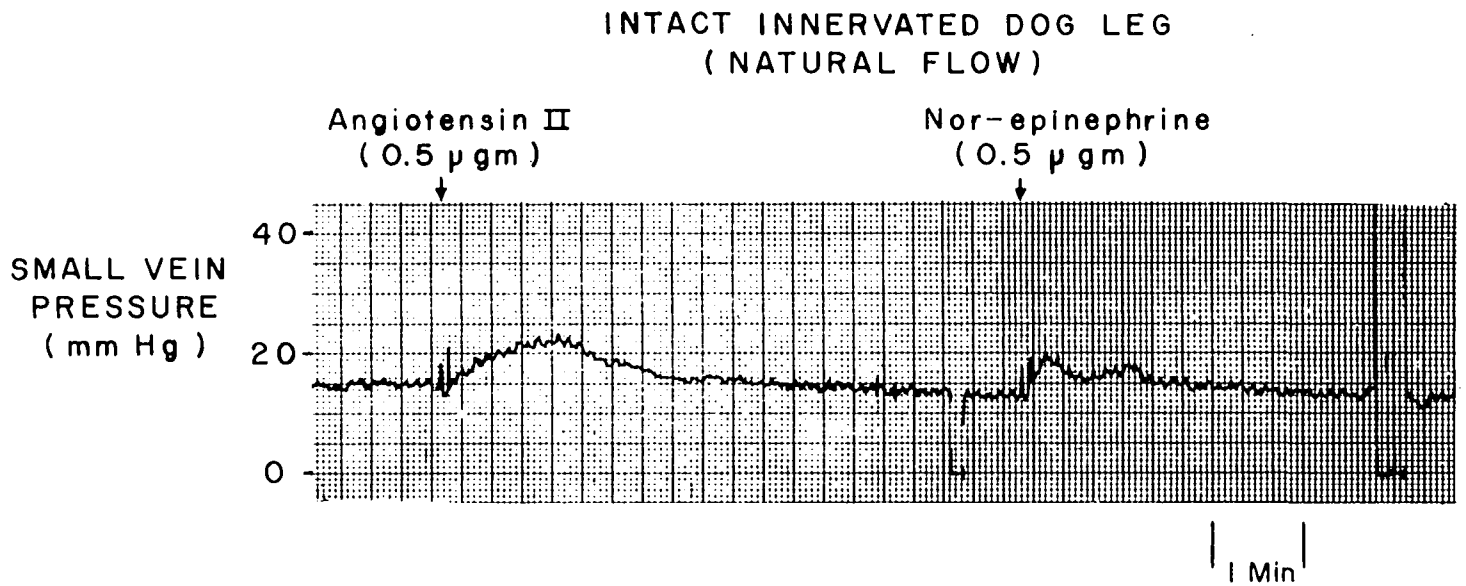
Table III

Comparison of small vein pressure change to angiotensin II and norepinephrine in the intact, innervated, natural flow, dog forelimb.

	ANGIOTENSIN II		NOREPINEPHRINE		
Exp. No.	Initial SVP (mm Hg)	Δ SVP (mm Hg)	Initial SVP (mm Hg)	Δ SVP (mm Hg)	Dose (μgm)
1	20	1.5	19	5	1.0
2	19	2.0	17	2	1.0
3	15	7.0	13	7	0.5
4	8	0.0	8	3	1.0
5	10	0.0	10	4	1.0
Mean	14	2.0	13	4	

Initial SVP = control small vein pressure; Δ SVP = peak increase of small vein pressure over control. Drugs were injected directly into the limb small veins. Equiweight drug doses were given in each experiment.

Figure 10. Record showing the effect of angiotensin and norepinephrine on small vein pressure in an intact, natural flow dog forelimb. Equiweight doses injected directly into a small vein. The pressure fluctuations at the point of injection are injection artifacts. Three large squares on record abscissa = 1 minute; dog weight = 14 kgm.


INTACT INNERVATED DOG LEG
(NATURAL FLOW)
Angiotensin II
(0.5 µgm)
Nor-epinephrine
(0.5 µgm)
SMALL VEIN
PRESSURE
(mm Hg)
40
20
0
1 Min

CHAPTER IV

DISCUSSION

Results from the present study demonstrate that angiotensin, compared to norepinephrine on an equimolar injection basis, causes a greater increase of both brachial artery pressure (BAP) and small vein pressure (SVP), and the evoked area response is greater in all species examined. Loss of leg weight is also greater with angiotensin. However, when compared on an equiweight basis, angiotensin increases cat BAP and SVP and monkey SVP more than norepinephrine, but causes less elevation of dog forelimb BAP and SVP and monkey BAP than norepinephrine. Surprisingly, on the average, both drugs cause approximately equal loss of leg weight in the dog and cat, while angiotensin's effect predominates in the monkey. The initial maximum SVP increase resulting from angiotensin infusion in dog limbs was approximately equal to that caused by norepinephrine infusion, but angiotensin was unable to maintain the pressure elevation. Although angiotensin tachyphylaxis has generally not been observed (31), systemic vascular tachyphylaxis has been indicated by one group with high infusion concentrations (over 5.0 μgm/min/kg) but not with lesser rates (4, 18). However, this observation has not been confirmed by others (27).

The intact natural flow experiments show that angiotensin can cause an increase in SVP as great as norepinephrine in some limbs. Since both

drugs were injected directly into the small veins the inference is that the initial, rapid rise in SVP following an injection is due to active venoconstriction. However, interpretation of these data is uncertain due to uncontrolled and unmeasured variables.

The importance of direct comparison of angiotensin and norepinephrine under almost identical conditions during each experiment is emphasized from the results of this study. The response of one limb to a particular dose of angiotensin might be very small when compared to the same dose in another limb. But as a rule the limb vessels responding with a minimal pressure increase to angiotensin will show a similarly small response to norepinephrine. The observed differences between limbs are no doubt related to (a) differences in limb flow rate and thus different drug concentrations actually reaching the vessels, (b) differences in limb size, and (c) absolute differences in limb vascular responsiveness.

Earlier studies in which angiotensin II was infused into dog forelimbs in natural flow (22) and constant flow (21) limb preparations show no small vein constriction. It is difficult to compare single bolus injection data to results obtained from steady state infusion studies. The apparent differences between drug infusion experiments of the present study and earlier ones are not readily explainable, however, the limb flow rates were appreciably greater and the limbs were not completely separated from the animal's body in the earlier studies (21, 22). In experiments on cats suggesting lack of small vein constriction to angiotensin (17), large peripheral areas were involved and a different preparation used. Possible discrepancies between the present investigation and others showing no venoconstriction to angiotensin might lie in the preparation

employed, state of the vessels at the time of testing, drug concentration used for a particular vascular region in a given species of animal, and possibly early venous tachyphylaxis to angiotensin as observed in the present study.

Directly measured small vein pressure in the constant flow cat limb shows that angiotensin can cause an increase in SVP as great as or greater than norepinephrine with equiweight injections and angiotensin raises SVP considerably more effectively on a molar basis. However, variations from the mean were noted in some cat limbs with equiweight test, but not to the extent seen in the dogs. The most uniform small vein response was obtained in the monkey series, where angiotensin increased monkey hand SVP as well as or considerably more than norepinephrine with equiweight injection tests. This phenomenon has also been observed in human hand small veins (8). It is noteworthy that angiotensin apparently does not constrict large forearm veins in man (10), but one report indicates decreased venous compliance in human arm veins when drug levels which elevate aortic blood pressure are used (41). Lack of local peripheral large vein responsiveness to angiotensin apparently holds for dogs as well (35). Data from the present study agrees well with these observations in that angiotensin increased digital and metacarpal vein pressures well above baseline, but had almost no effect on larger limb veins. From these data it seems that angiotensin can constrict both pre- and postcapillary resistance vessels while having little effect on larger peripheral veins in animals or man.

Most of the weight change produced by single drug injections appears to have resulted from active vascular constriction and the subsequent

"squeezing out" of blood from intravascular space. If capillary filtration played a role, this was not apparent from the weight loss slope and if net filtration did occur, it should have been into the tissue compartment (weight gain) when SVP was elevated (19, 33). When angiotensin was infused, limb weight fell rapidly during the first few minutes of infusion and then decreased gradually until the 10th minute. After the initial peak elevation, small vein pressure began to decrease toward preinfusion level and reached control by the end of the infusion period. The secondary weight changes observed during angiotensin infusion were probably resultant to both changing vascular tone and filtration. Since a steady state of venous pressure was never completely achieved with angiotensin infusion further interpretation is difficult. Leg weight changes observed with norepinephrine infusion are in agreement with earlier reports demonstrating that this agent can constrict veins out of proportion to arteries, resulting in loss of vascular fluid in the dog limb (22) and gut (12). Following an initial drop, probably due to active intravascular volume displacement, leg weight began to increase with norepinephrine infusion. Some of this weight gain could have been associated with passive intravascular volume increase. However, net filtration of capillary fluid into the tissue space must have occurred to account for the further weight increase with both pressures stabilized during the latter part of infusion.

CHAPTER V

SUMMARY AND CONCLUSIONS

The present investigation was undertaken to delineate the direct vascular responsiveness of forelimb arteries and especially veins in the dog, cat and monkey to angiotensin and norepinephrine. Contrary to the established action of norepinephrine, the effect of angiotensin on peripheral veins is unclear and the few reports in literature indicate that angiotensin does not constrict veins in animals, however, both venoconstriction and lack of venoconstriction to angiotensin have been reported in humans.

The importance of this study is emphasized by the influence of venous pressure and 'tone' on venous return to the heart, capillary hydrostatic pressure, and transcapillary fluid movement.

Comparisons of the drugs were made regarding both peak elevation and area response of limb brachial artery pressure (BAP) and small vein pressure (SVP) and limb weight change. Equiweight and equimolar injections and in some instances equiweight infusions were carried out. BAP and SVP were measured directly in constant flow, pump perfused, isolated forelimb preparations. Limb weight was monitored continuously. In several experiments venous pressure was measured from three points along the forelimb, viz, a) from a digital vein; b) a metacarpal vein and c) the cephalic vein.

Experiments were also completed in intact, natural flow, dog limbs. Drugs were injected directly into a small vein and SVP was measured.

With equimolar injections in the constant flow experiments, angiotensin increased BAP and SVP more than norepinephrine and caused a greater loss of limb weight in all species examined. Angiotensin, with equiweight doses, caused a greater rise of cat forelimb BAP and SVP and monkey limb SVP than norepinephrine; whereas norepinephrine caused a greater increase of dog limb BAP and SVP and of monkey limb BAP. Limb weight loss was approximately equal in dog and cat, but angiotensin's weight loss effect predominated in the monkey. When different sized dog forelimb veins were compared, norepinephrine constricted the larger forelimb veins to a measurable degree while angiotensin's effect was negligible. The initial SVP elevation produced by angiotensin infusion was equal to that caused by norepinephrine, but the small veins became unresponsive to angiotensin after several minutes of infusion.

The dog natural limb flow experiments show that angiotensin can increase limb SVP as much as norepinephrine does, however, on the average, norepinephrine was approximately twice as effective in this regard.

It is concluded that: a) angiotensin is a more potent small vein constrictor than norepinephrine on an equimolar basis, but its comparative venous effect is variable with equiweight doses; b) loss of small vein responsiveness to angiotensin may readily develop, particulary with continuous infusion; and c) limb small veins of the monkey are consistently more responsive to angiotensin while those of the dog are the least responsive and most variable.

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