HEMODYNAMIC EFFECTS OF INTRA-ARTERIAL INFUSION OF OUABAIN IN THE CANINE FORELIMB

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
RICHARD ARLAN NYHOF
1976

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ABSTRACT

HEMODYNAMIC EFFECTS OF INTRA-ARTERIAL INFUSION
OF OUABAIN IN THE CANINE FORELIMB

By

Richard Arlan Nyhof

There are no reports of the action of intra-arterially infused ouabain on the in-series elements of the vasculature. There are also no reports dealing with its effects in two peripheral vascular beds simultaneously. This study addresses itself to both questions. The isolated canine forelimb provided a model in which the intralumenal pressures of small arteries, small veins and large veins could be monitored in both skin and skeletal muscle beds. Venous outflows were measured and segmental resistances through the vascular tree were calculated. Ouabain (6.4 ug/min.) was infused for 60 minutes intra-arterially in both constant pressure and constant flow systems. With constant pressure, total muscle vascular resistance increased mainly due to increased arteriolar constriction. Total skin vascular resistance also increased due to arteriolar vasoconstriction but not to as high a degree as in muscle. There was no effect on the large veins. With constant flow, skin total

vascular resistance again increased but now the small artery played a greater role in the response than previously. Total muscle vascular resistance rose, peaked, and then fell showing a waning of the vasoconstrictor response of arterioles in skeletal muscle. This waning response allowed a shunt of blood flow from skin to skeletal muscle. Doubling the infusion rate after consecutive 20 minute periods (to 12.4 ug/min. and then 24.7 ug/min.) did not alter the pattern of response seen with the single rate. Again, there was no effect seen on the large veins. It is therefore evident that ouabain has a direct vasoconstrictor action on arterioles in both skin and skeletal muscle beds and a lesser effect on small arteries. The vasoconstrictor effect in the skeletal muscle bed under constant flow differs from that in skin in that the response wanes with time even though the infusion is continued. There is no effect observable in the large veins of either vascular bed under constant pressure or constant flow perfusion.

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By

Richard Arlan Nyhof

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1976

DEDICATION

To my parents, whose constant
support was felt and appreciated.

ACKNOWLEDGMENTS

I wish to acknowledge the help and support of my advisor, Dr. J. Dabney, and to the other members of my committee, Dr. J. Scott and Dr. F. Haddy. Many thanks are due each of them for their helpful suggestions and advice in preparing this thesis.

I also wish to thank Mr. B. T. Swindell and Mr. G. Gamble for their surgical and technical assistance.

Richard Nyhof

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INTRODUCTION

The cardiac glycosides have been used for medical purposes since antiquity. Present in the skin venom of certain toads, squill was used by the Chinese in the form of powdered toad skins. The ancient Egyptians also used a preparation of squill and the Romans later used it as a diuretic, heart tonic and emetic (as well as rat poison). William Withering in 1785 recorded the use of Digitalis purperea (purple foxglove plant) leaves in the treatment of dropsy and ascribed its primary action to its being a diuretic. In 1799 John Ferriar first attributed the primary action of digitalis to its effect on the heart; the diuretic action being secondary. In 1890, Sir Thomas Fraser discovered the medicinal value of Strophanthus while studying African arrow poisons.

In the twenthieth century, the glycosides became recognized as invaluable in therapeutic treatment for congestive heart failure (1, 2). During this time it was discovered that they had an effect on the peripheral vasculature as well. However, a systematic study of parallel and series resistances within an intact blood perfused vascular bed is lacking.

The goal of this study was to describe the direct vasoconstrictor action of a cardiac glycoside, ouabain, on arteries, small vessels and veins of skin and skeletal muscle vasculature in the forelimb of the anesthetized dog. Effects on veins in particular were sought.

LITERATURE SURVEY

Biochemistry

The cardiac glycosides are divided into groups on the basis of their source, although they are all commonly known as digitalis drugs. There are four major groups: those originating from the plant genera Digitalis, from Strophanthus and from Squilla, and the synthetically derived compounds. The basic structure consists of a cyclopentanoperhydophenanthrene ring to which an unsaturated lactone ring is attached (Fig. 1).

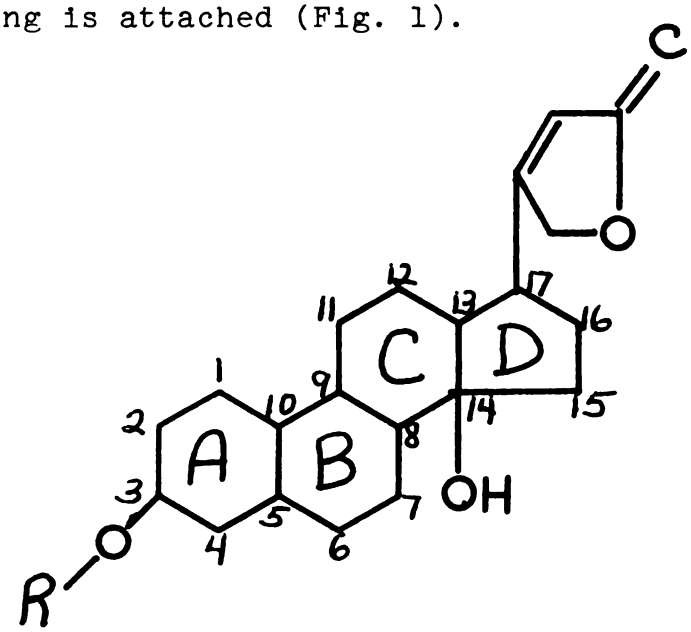

Figure 1. Cyclopentanoperhydrophenanthrene with attached lactone ring. R is a sugar group.

In this structure are four basic requirements for biological activity: 1) presence of the unsaturated lactone ring, 2) the hydroxy group attached to C_{14}, 3) cis formation of the

bond between rings C and D at C_{13} and C_{14}, and 4) presence of a sugar group(s) on C_3 (3). Ouabain, also known as Strophanthin G, is derived from the seed of Strophanthus gratus. The sugar group on ouabain is one molecule of glucose and there are hydroxy groups attached to C_1, C_5 and C_{11}, a methylhydroxy group on C_{10} and a methyl group on C_{13}. Ouabain is a strongly polar molecule due to the many hydroxy groups attached to it. Because it is so polar, it is not effective orally and must be administered parenterally. Ouabain is found largely unbound in plasma and has a biologically active half life of 21-26 hours (2, 4). Typically, the more polar the molecule, the more rapidly it is excreted from the body without being first metabolized. Ouabain, being polar, is therefore excreted largely unmetabolized by the kidney.

The exact mechanism of action of digitalis on cells is not known. It has been shown to inhibit the active transport of Na^+ and K^+ in myocardial tissue, apparently by inhibiting the Na^+-K^+ activated adenosinetriphosphatase (ATPase) (5, 6, 7, 8, 9). It also appears to increase the concentration of free intracellular Ca^{++}, permitting more forceful contractions to occur (7). Na^+-K^+ activated ATPase has also been demonstrated in vascular smooth muscle and is also inhibited by ouabain (10, 11, 12). The vasoconstrictor effect of digitalis here, too, seems to depend on increased intracellular Ca^{++} (13).

In therapeutic doses, digitalis causes an increased contractility in both the failing and non-failing heart. Its effect on cardiac output is variable, depending on pre-existing sympathetic nervous activity and on sympathetic reflex arcs (14, 15, 16). Digitalis toxicity presents itself most often in ventricular extrasystoles. Other symptoms include ventricular bradycardia (atrio-ventricular block), atrial tachycardia and atrial fibrillation (2, 17).

Arterial and Venous Studies

It has been known since at least 1911 that the effect of digitalis was not limited to the myocardial portion of the circulatory system (18). Since then, the response of both arteries and veins *in vitro* has been shown to be one of constriction (19, 20, 21). Because the dramatic effect of digitalis on the heart tended to obscure any action in the vasculature, the early work was done on isolated vessel strips, eliminating cardiac effects. Subsequent development of new techniques circumventing cardiac alterations or rendering them less important allowed for extensive *in vivo* study.

When injected intravenously, the glycoside is diluted and delivered to the entire systemic circuit in about equal concentrations. The resulting response may be a direct effect of the drug on the vasculature or may be secondary to effects on other tissues, e.g., heart or central nervous system. Several investigators have found that total systemic vascular resistance rises with intravenous glycosides

(22, 23, 24, 25), although Daggett and Weisfeldt (26) found an increased peripheral resistance only after reflexes were eliminated by ganglionic blockade. This discrepancy may be due to higher levels of pre-existing sympathetic nervous activity in the latter experiments. The direct vasoconstricting effect of ouabain might be counterbalanced by reflex withdrawal of the pre-existing sympathetic nerve impulses. It follows that after the neural responses were eliminated, the results were similar to those found by the other investigators.

In studying specific vascular beds, Mason and Braunwald monitored systemic arterial and forearm venous pressures and used a plethysmographic method to measure forearm blood flow in humans (27). In normal men, they found that following intravenous ouabain the cardiac output was unchanged; venous compliance decreased; and forearm vascular resistance increased bringing about a fall in blood flow to the forearm. Treatment with guanethidine (an inhibitor of α adrenergic responses) did not alter any of the effects. The vasoconstriction therefore appeared not to be due to increased sympathetic nerve activity in the forearm but to the direct action of ouabain on the vessels. Levinsky, et al. (28) found that intravenous digoxin caused an increased superior mesenteric vascular resistance and increased vascular resistance in an isolated gut segment. Injection of acetylstrophanthidin into the arterial side of a pulmonary bypass system showed a dose related increase

in renal vascular resistance (29). Higgins, et al. (30) found an increased vascular resistance following intravenous ouabain in the renal, iliac and mesenteric beds. The mesenteric bed later dilated but cholinergic blockade abolished the dilation. To determine the mediator of the response, Stark, et al. (31) used a pump-perfused innervated gracilis muscle preparation. Acetylstrophanthidin administered intravenously caused a short dilation initially, followed by a sustained constriction. Denervation greatly reduced the constrictor response as did phenoxybenzamine, an α adrenergic blocker. Denervation also eliminated the initial decrease in resistance. Since the majority of the response appeared neural in origin, they attempted to locate the receptor area for digitalis activity. Vagotomy reduced the initial fall in resistance but even when coupled with carotid sinus denervation did not change the sustained increase. Since these subsequent tests eliminated afferent input from receptors in the heart and carotid sinus, they postulated neural receptor activity in another area, perhaps the central nervous system (CNS) or sympathetic ganglia. Garan, et al. (32) monitored cerebrospinal fluid (CSF) concentrations of digoxin following intravenous administration and found that increased CSF levels of digoxin correlated with increased vascular resistance in the coronary bed. They also set up a circuit in which the head of one dog was perfused with blood supplied by the body of a second dog at natural pressure. Injection of digoxin into

the cisternum magnum of the first dog caused a rise in systemic resistance in the body of the first dog, even though the perfusion circuits were separate. This further indicated CNS involvement in the response.

These data indicate two possible responses of the sympathetic nervous system to systemically delivered digitalis and the opposite effects of those responses on the vasculature: 1) the increased arterial pressure following administration may trigger reflex withdrawal of sympathetic nerve activity allowing dilation to occur, and 2) a direct effect on neural receptors may cause increased sympathetic nerve activity producing constriction.

Materials administered intra-arterially to a particular vascular bed will travel through the resistance arterioles, capillaries and veins of the bed before reaching the systemic circuit. This route of administration emphasizes the direct or local effect of the drug on the vasculature because the drug reaches only the perfused bed under study in the desired concentration. The material then undergoes dilution and mixing in the veins and heart before reaching the rest of the system. The resultant lower systemic concentrations minimize other than local responses, e.g., the possible CNS response described above. Ross, et al. (32) noted an increased perfusion pressure after digitalis injection in dogs on cardiopulmonary bypass. Bilateral adrenalectomy did not alter the response, nor did treatment with hexamethonium (a ganglionic blocker) indicating no participation of

neural or catecholamine influence. They then separated the circuit into two sections: 1) the naturally perfused upper portion containing the brain, heart, viscera and skeletal muscle, and 2) the constant flow pump perfused lower portion consisting of mostly skeletal muscle. Digitalis injection into the upper portion caused an increased arterial pressure in the upper and a decreased perfusion pressure in the lower portion, the latter probably due to a baroreceptor induced reflex withdrawal of sympathetic nerve impulses. Injection into the lower portion caused a local increased perfusion pressure and no change in pressure in the upper portion of the body indicating that the constrictor response causing the increased perfusion pressure was probably a local effect.

Bloor, et al. (22) and Vatner, et al. (33) injected ouabain into a coronary artery of conscious dogs and found increased coronary resistance and decreased blood flow. Intra-renal artery injection of digitalis was reported by Waldhausen and Herendson (29) to produce increased renal resistance and therefore decrease renal blood flow. Higgens, et al. (30) noted vasoconstriction following arterial administration of glycosides in the mesenteric vascular bed, as did Treat, et al. (34). Treat, et al. also found increased vascular resistance in the head region after infusing high doses into the carotid artery. No effects were noted on the jugular vein pressure. Stark, et al. (31) studied the effect of intra-arterial acetylstrophanthidin

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on an isolated, denervated, pump perfused gracilis muscle preparation. Infusion of the drug caused an increase in vascular resistance. Glover, et al. (35) infused ouabain and digoxin into the brachial artery in humans and measured flow and venous compliance changes by plethysmography. Using the contralateral forearm as control, doses of ouabain ranging from 5-40 ug/min. for 5 minute infusion periods caused an 11%-31% decrease in blood flow to the forearm. A 20ug-80ug/min. infusion for 2-5 minutes caused a 13.5%-25% decrease in blood flow to the hand. Digoxin (10ug/min. x 10 min.) produced a mean blood flow decrease of 36%. There was no change in venous distensibility during the ouabain treatment.

The participation of the venous side of the vasculature in glycoside treatment is somewhat unclear. A selective venoconstriction, as, for example in the hepatic veins, would cause pooling upstream and therefore limit blood return to the heart and possibly decrease cardiac output (35). A generalized venoconstriction would decrease the volume of the capacitance vessels, increase the cardiac filling pressure and thus increase cardiac output (36). Most studies involving the response of the veins have been done with intravenous digitalis administration in intact circuits and concentrated mainly on the reaction of the hepatic veins. Early investigators saw an increased peripheral resistance accompanied by decreased large vein or atrial pressure and decreased cardiac output. They

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interpreted the decreased cardiac output as being due to limited venous return. The limitation was the result of hepatic venoconstriction yielding pooling in the hepatic portal and splenic vessels. This pooling caused decreased filling pressure and thus decreased cardiac output (35, 37, 38, 39). This also explained the paradox of an apparent increase in myocardial contractility and yet the decrease in cardiac output. Others have also demonstrated splanchnic pooling of blood (15, 40, 41), especially in the dog, after digitalis.

In other beds, Horsely and Eckstein (42) and Mason and Braunwald (27) found some degree of venoconstriction in man following intravenous glycoside treatment. Archer and Hinshaw (40) showed that, with high concentrations of digitalis, left atrial pressure increased in both intact dogs and in dogs which have been eviscerated to eliminate splanchnic pooling. Others (43, 44) report possible venoconstriction. Glover, et al. (35), on the other hand, report no change in venous distensibility resulting from intra-arterial glycoside infusion in the human forearm.

The present study was done in an attempt to describe more completely the relative responses to digitalis of arteries and veins in two systemic vascular beds outside the splanchnic area. It has been shown by Abdel-Sayed, et al. (55) that the quantity of vascular smooth muscle in veins draining skin is greater than in those veins draining skeletal muscle. They also showed that the veins draining

skin are more reactive to several vasoactive agents. Therefore, there was a possibility that veins in the parallel beds of skin and skeletal muscle would differ in their sensitivity to ouabain. It is also possible that the two beds would differ in their contribution to total forelimb vascular resistance. Consequently, the study was designed to describe the effects of intra-arterial infusion of ouabain on the in-series elements (arteries, small vessels and veins) in the parallel vasculature of skin and skeletal muscle in the dog forelimb.

METHODS

Mongrel dogs weighing between 17 and 23 kg were anesthetized with sodium pentobarbital (30 mg/kg intravenously) and respired on room air through an endotracheal tube with a Harvard positive pressure ventilator.

The skin 3-5 cm above the right forelimb elbow was circumferentially sectioned with electracautery. The brachial artery, brachial and cephalic veins, and the forelimb nerves (median ulnar, radial and musculo cutaneous) were isolated and the remaining skeletal muscle and connective tissue was sectioned by electrocautery. The isolated vessels to be cannulated for recording intravascular pressure were: 1) small skin artery from the third superficial volar metacarpal artery on the ventral surface of the paw, 2) small muscle artery from a vessel supplying a flexor muscle in the upper portion of the forelimb, 3) small skin vein from the second superficial dorsal metacarpal vein from a deep vessel draining a flexor muscle in the middle portion of the forelimb, and 5) median cubital vein forming an anastomosis between the brachial and the cephalic veins. The humerus was cut and the exposed marrow cavities packed with bone wax to prevent blood seepage. Blood entered the limb only through the brachial artery and exited only through the

brachial and cephalic veins. The nerves were coated with inert silicone spray to prevent drying. Sodium heparin (10,000 units) was injected intravenously to prevent clotting.

The vessels previously isolated were cannulated with small bore polyethylene tubing (P.E. 10 to P.E. 60) filled with heparinized saline; small artery cannulae inserted in a downstream direction and small vein cannulae in an upstream direction. The large veins were cannulated through their respective sides of the median cubital vein. The cannulated small vessel acts as an extension of the cannula and thus the pressure measured is that of the first branching of the cannulated vessel. This pressure is a true lateral pressure as long as the cannulated vessel is patent and without valves (verified periodically throughout the experiment by the ability to withdraw blood from and to flush saline easily into the cannulated vessel and with the pressure recordings rapidly returning to previous levels). The presence of the catheter does not measurably alter the pressure in the arterial or venous system because in the canine forelimb the cannulated vessel is a negligible fraction of the total cross sectional area of the arterial or venous bed, and there are abundant artery-to-artery and vein-to-vein anastomoses (45, 46, 47). To eliminate any discrepancies minor calibration differences might cause, the small muscle vein and brachial vein were both measured on the same strain gauge by means of multiple stopcocks.

The small skin and cephalic veins were measured similarly, as were perfusion pressure, small skin and small muscle artery pressures. Intravascular pressures were measured with low volume displacement transducers (Statham P23 Gb) and recorded on a Sanborn direct-writing oscillograph.

The brachial and cephalic veins were partially transected 3-5 cm downstream from the median cubital anastomosis (and therefore downstream from the site of large veins pressure measurement) and both vessels were cannulated with short sections of large bore polyethylene tubing (P.E. 320). Outflow from both veins was directed into a reservoir maintained at constant volume with a variable speed roller pump which continuously returned blood to the animal via a cannulated femoral vein. Blood flow was determined by timed collections of the two venous outflows in graduated cylinders. In this preparation the median cubital vein represents the major anastomotic channel between the brachial and cephalic veins. This vessel was ligated in all experiments to insure that brachial venous flow was predominantly from skeletal muscle and that cephalic flow came primarily from skin. Although this approach does not accomplish complete anatomical isolation of skin and muscle blood flows, the degree of flow separation is sufficient to permit comparison of resistance changes in the two parallel coupled beds (48, 49, 50, 51).

In series I and II the brachial artery was perfused at constant flow with arterial blood from a femoral artery

by means of a Sigmamotor pump. The flow level was adjusted to bring about a perfusion pressure approximating the animal's own initial arterial pressure. The perfusion pressure was monitored via a needle inserted into a section of latex tubing located in the perfusion line just upstream from the site of cannulation. The site of drug infusion was upstream from the pump in order not to alter the flow to the limb. Arterial pressure was measured by means of a cannula introduced into the aorta via the brachial artery. In series III, the forelimb was normally perfused. A small side branch of the brachial artery was cannulated for drug infusion and normal saline was infused at the same rate (0.123 ml/min) during the control period to compensate for the possible added pressure of infusion and the volume dilution effects. Arterial pressure was measured through a cannulated femoral artery with the catheter tip located in the abdominal aorta.

Once all cannulae were in position, the flows and pressures were allowed to stabilize. This was determined by taking all pressure and flow readings repeatedly (recording both pulsatile and mean pressures) with three minutes between the end of one set of readings and the beginning of the next and by checking zero pressure baselines at the end of each set of readings. When two consecutive sets of readings were nearly identical, steady-state conditions were considered achieved.

In series I the forelimb was perfused at constant flow. Using a Harvard constant rate infusion pump, ouabain in a

concentration of 50 ug/ml was infused into the brachial artery at a rate of 0.123 ml/min. (effective dose: 6.4 ug/min.). After 20 minutes the rate was doubled to 0.247 ml/min. (effective dose: 12.4 ug/min.). Following 20 minutes at the second dose, the infusion rate was again doubled to 0.494 ml/min. (effective dose: 24.7 ug/min.). The infusion was terminated after a total of 60 minutes, each of the three infusion rates having lasted 20 minutes. At this point the total dose approximated a full therapeutic digitalizing dose of 50 ug/kg for the animal (34).

In series II, the forelimb blood flow was again held constant. The infusion rate of ouabain was 0.123 ml/min. (effective dose: 6.4 ug/min.) for the entire 60 minute period.

In series III, the forelimb was naturally perfused. Ouabain was infused at 0.123 ml/min. (effective dose: 6.4 ug/min.) for the 60 minute infusion period.

Flow and pressure readings were taken was in the pre-experimental control period. Segmental vascular resistances in both skin and skeletal muscle were calculated using the following equations:

$$R_{mt} = \frac{P_p - P_{bv}}{BV} \qquad R_{ma} = \frac{P_p - P_{sma}}{BV} \qquad R_{msv} = \frac{P_{sma} - P_{smv}}{BV}$$

$$R_{mv} = \frac{P_{smv} - P_{bv}}{BV} \qquad R_{st} = \frac{P_p - P_{cv}}{CV} \qquad R_{sa} = \frac{P_p - P_{ssa}}{CV}$$

$$R_{ssv} = \frac{P_{ssa} - P_{ssv}}{CV} \qquad R_{sv} = \frac{P_{ssv} - P_{sv}}{CV} \qquad R_t = \frac{P_p - \left(\frac{P_{bv} + P_{cv}}{2}\right)}{BV + CV}$$

R_{mt} = Total vascular resistance in muscle tissue

R_{ma} = Resistance in the muscle artery

R_{msv} = Resistance in the small vessels in muscle tissue

R_{mv} = Resistance in the muscle vein

R_{st} = Total vascular resistance in skin tissue

R_{sa} = Resistance in the skin artery

R_{ssv} = Resistance in the small vessels in skin tissue

R_{sv} = Resistance in the skin vein

R_{t} = Total vascular resistance in the forelimb

P_{p} = Forelimb perfusion pressure

P_{bv} = Pressure in the brachial vein

BV = Outflow from the brachial vein

P_{sma} = Pressure in a small artery in muscle tissue

P_{smv} = Pressure in a small vein in muscle tissue

P_{cv} = Pressure in the cephalic vein

CV = Outflow from the cephalic vein

P_{ssa} = Pressure in a small artery in skin tissue

P_{ssv} = Pressure in a small vein in skin tissue

All data were analyzed using Student's t-test modified for paired replicates. Control values were obtained by taking the mean of the two readings immediately preccding the infusion.

RESULTS

The following data were collected from 23 mongrel dogs. The data are plotted as mean values plus or minus standard error for each series and are also presented in tabular form in Appendix B.

Series I: Constant flow, three infusion rates.

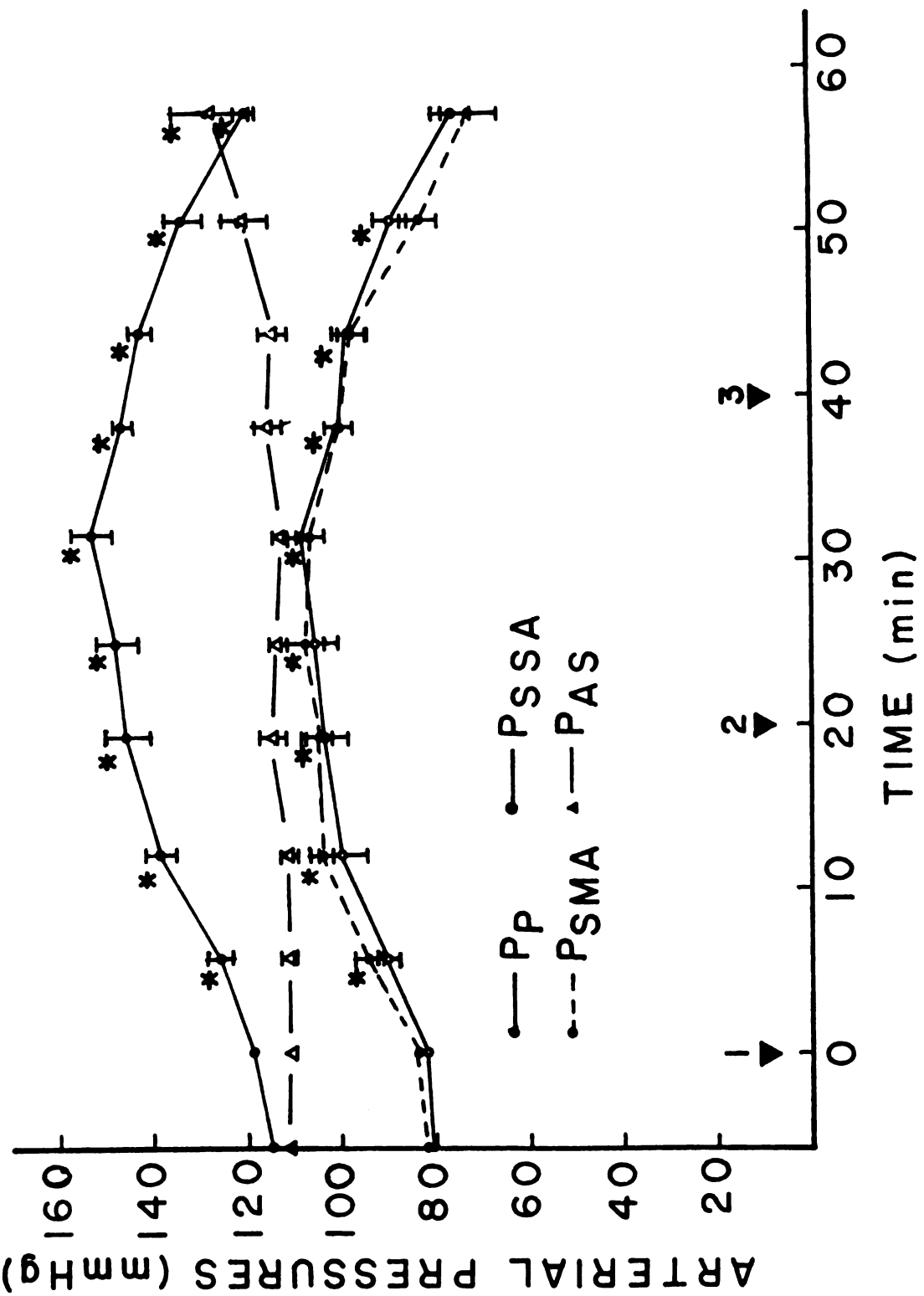
Perfusion and arterial pressures (Fig. 2).

The perfusion pressure increased almost immediately after infusion of ouabain was begun. After about thirty minutes, the pressure peaked and began to wane, although the final readings were still significantly above the control values.

Systemic arterial pressure remained constant until the final reading at 60 minutes where the value was significantly above control.

Pressure in the small arteries to skin and muscle paralleled each other, rising almost immediately after infusion was begun. They declined after reaching a peak value at about thirty minutes of infusion time. The final two readings at 49 and 57 minutes of infusion for small muscle artery and the final reading at minutes 57 for small skin artery were not different from control.

Figure 2. Mean values and standard errors of perfusion pressure (P_p), systemic arterial pressure (P_{as}), small skin artery pressure (P_{ssa}) and small muscle arterial pressure (P_{sma}) of the canine forelimb during ouabain infusion at consecutive infusion rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9, * denotes significant difference from control, $p < 0.05$.


ARTERIAL PRESSURES (mmHg)
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
TIME (min)
1
2
3
P_P
P_{SSA}
P_{SMA}
P_{AS}

Venous pressures (Fig. 3).

Pressure in the small skin vein was above control at minutes 6, 12, 25 and 31. The remaining values (minutes 18, 37, 43, 49, 57) were not significantly different from control.

Pressure in the small vein of the muscle began to increase by minute 18 and continued to increase to the end of the infusion, except for minute 49 which was not significantly different from control.

Pressures in the large veins draining muscle (brachial vein) and skin (cephalic vein) tended to follow the pattern of the pressures in their respective small veins. The pressure in the cephalic vein tended to decrease after minute 37 of infusion, but, like the pressure in the small skin vein, the difference from control was not significant. The pressure in the brachial vein rose parallel to that of the small muscle vein, the readings at minutes 43, 49 and 57 being significantly above control.

Venous outflows (Fig. 4).

The venous outflows diverged with flow through the cephalic vein falling below control by minute 12 and continuing to fall to the end of the infusion period. Flow through the brachial vein was above control by minute 18 and continued to rise for the duration of the ouabain infusion. Total forelimb flow (flow from both veins combined) remained unchanged throughout the infusion.

Figure 3. Mean values and standard errors of small muscle vein pressure (P_{smv}), small skin vein pressure (P_{ssv}), brachial vein pressure (P_{bv}) and cephalic vein pressure (P_{cv}) of the canine forelimb during ouabain infusion at consecutive rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.

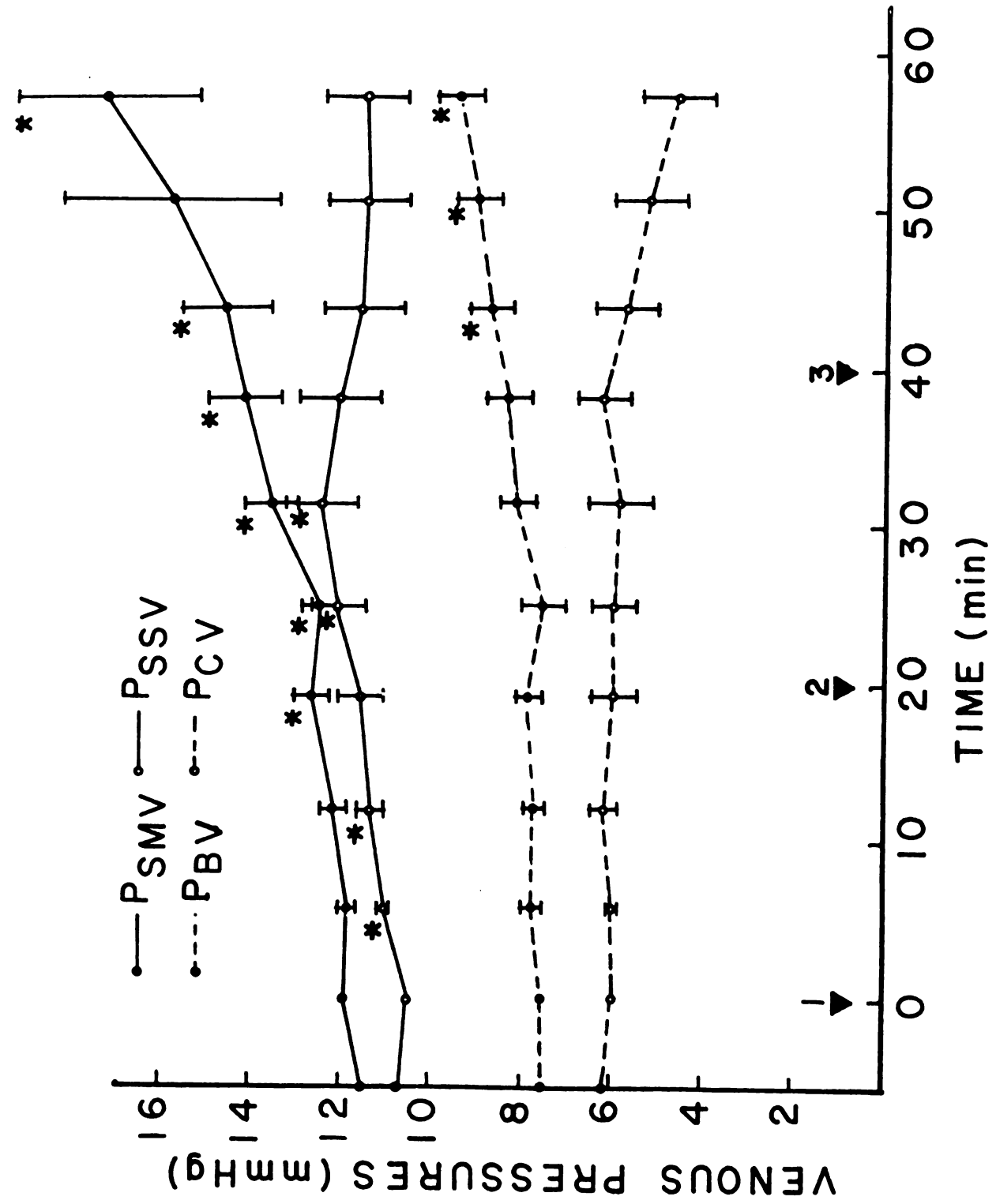
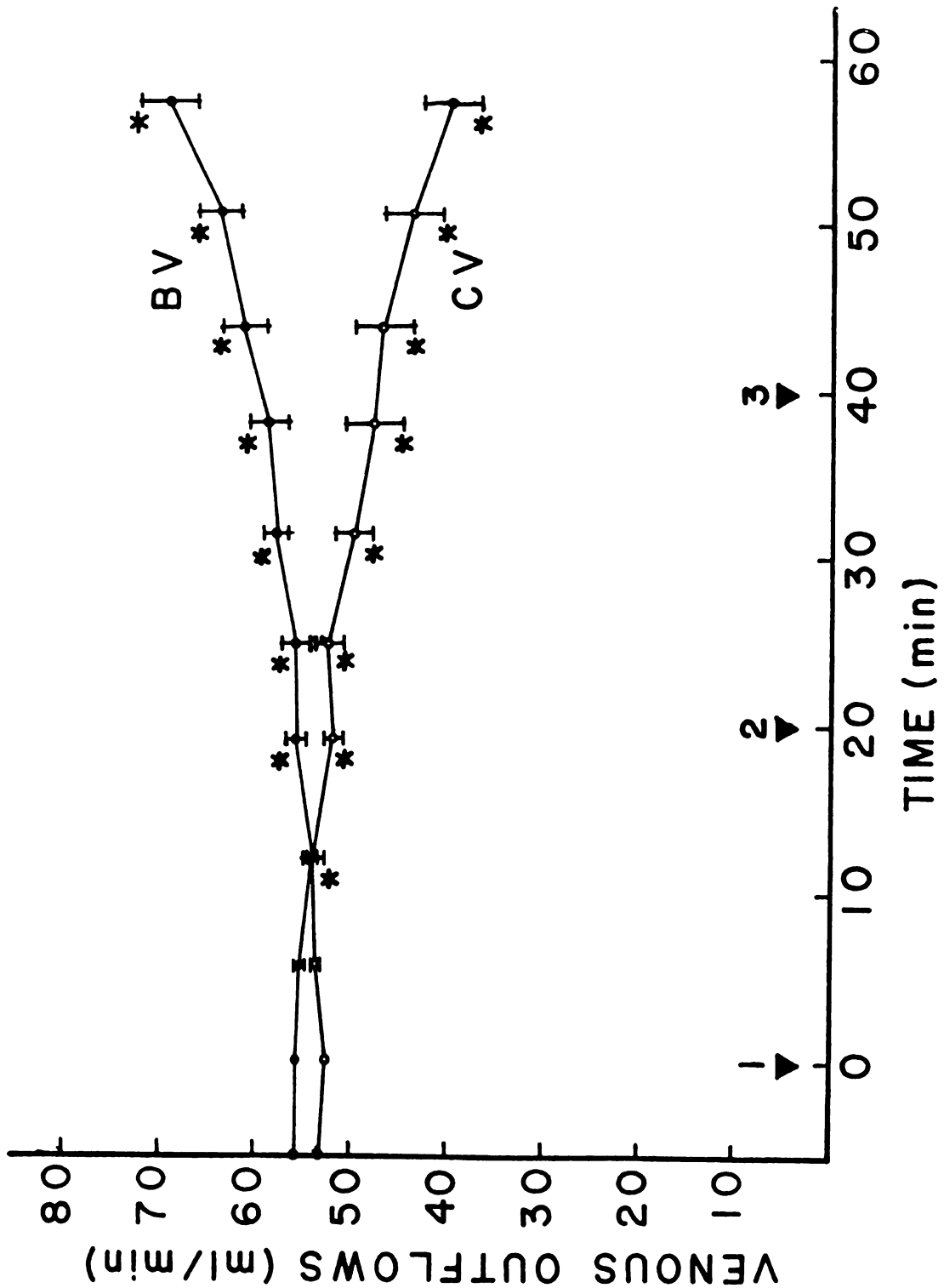

VENOUS PRESSURES (mmHg)
16
14
12
10
8
6
4
2
0
10
20
30
40
50
60
TIME (min)
1
2
3
P_{SMV}
P_{SSV}
P_{BV}
P_{CV}

Figure 4. Mean venous outflows, with standard errors, from brachial vein (BV) and cephalic vein (CV) in canine forelimbs during ouabain infusion at consecutive rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.


VENOUS OUTFLOWS (ml/min)
80
70
60
50
40
30
20
10
BV
CV
1
2
3
0
10
20
30
40
50
60
TIME (min)

Calculated total forelimb vascular resistance (Fig. 5).

Total forelimb vascular resistance paralleled the perfusion pressure. It rose almost immediately upon introduction of ouabain, peaked by minute 30 and fell thereafter until the final value at minute 57 was not different from control.

Calculated muscle vascular resistance (Fig. 6).

Total muscle vascular resistance and muscle small vessel resistance paralleled each other, rising by minute 6, plateauing and then falling after minute 30. The decrease continued to a point below control by minute 57.

Muscle artery resistance decreased by minute 6, then increased slightly and remained significantly elevated through the remainder of the infusion period.

Calculated skin vascular resistance (Fig. 7).

Total skin vascular resistance and skin artery resistance increased for the entire 60 minute period. Skin small vessel resistance initially rose similarly but plateaued after about 30 minutes of infusion.

Calculated large vein resistance (Fig. 8).

Large vein resistance represents the calculated resistance to flow between sites of pressure measurement in the small and large vein. Both muscle and skin large vein resistances tended to increase, but the only significant difference was a rise in large skin vein resistance at the 6th minute.

Figure 3. Means and standard errors of total forelimb vascular resistance (R_t) in mm Hg/ml/min during ouabain infusion at consecutive rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.

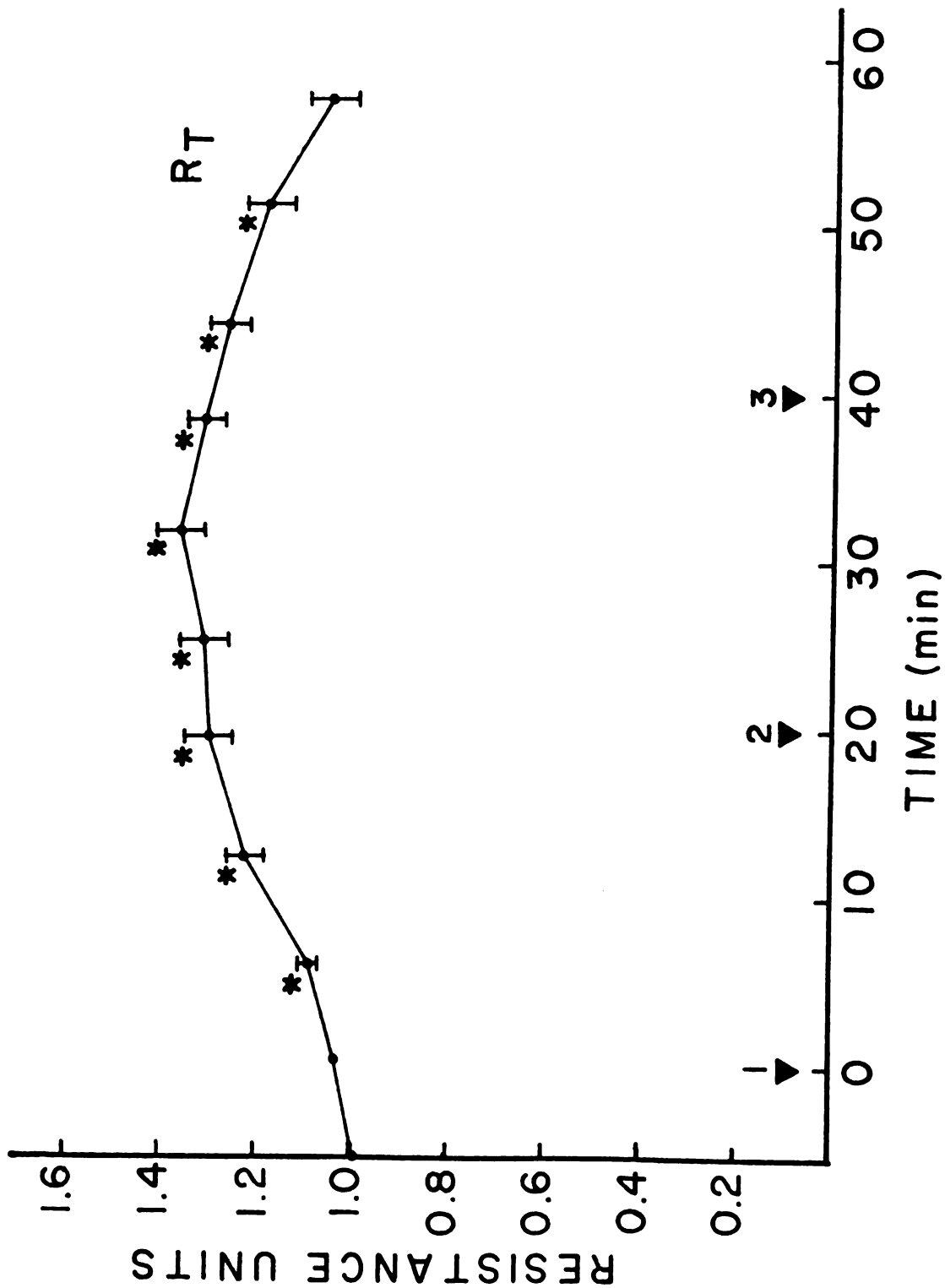

RESISTANCE UNITS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
RT
0
10
20
30
40
50
60
TIME (min)
1
2
3

Figure 6. Means and standard errors of total muscle vascular resistance (R_{mt}), muscle small vessel resistance (R_{msv}) and muscle artery resistance (R_{ma}) in mm Hg/ml/min during ouabain infusion at consecutive rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.

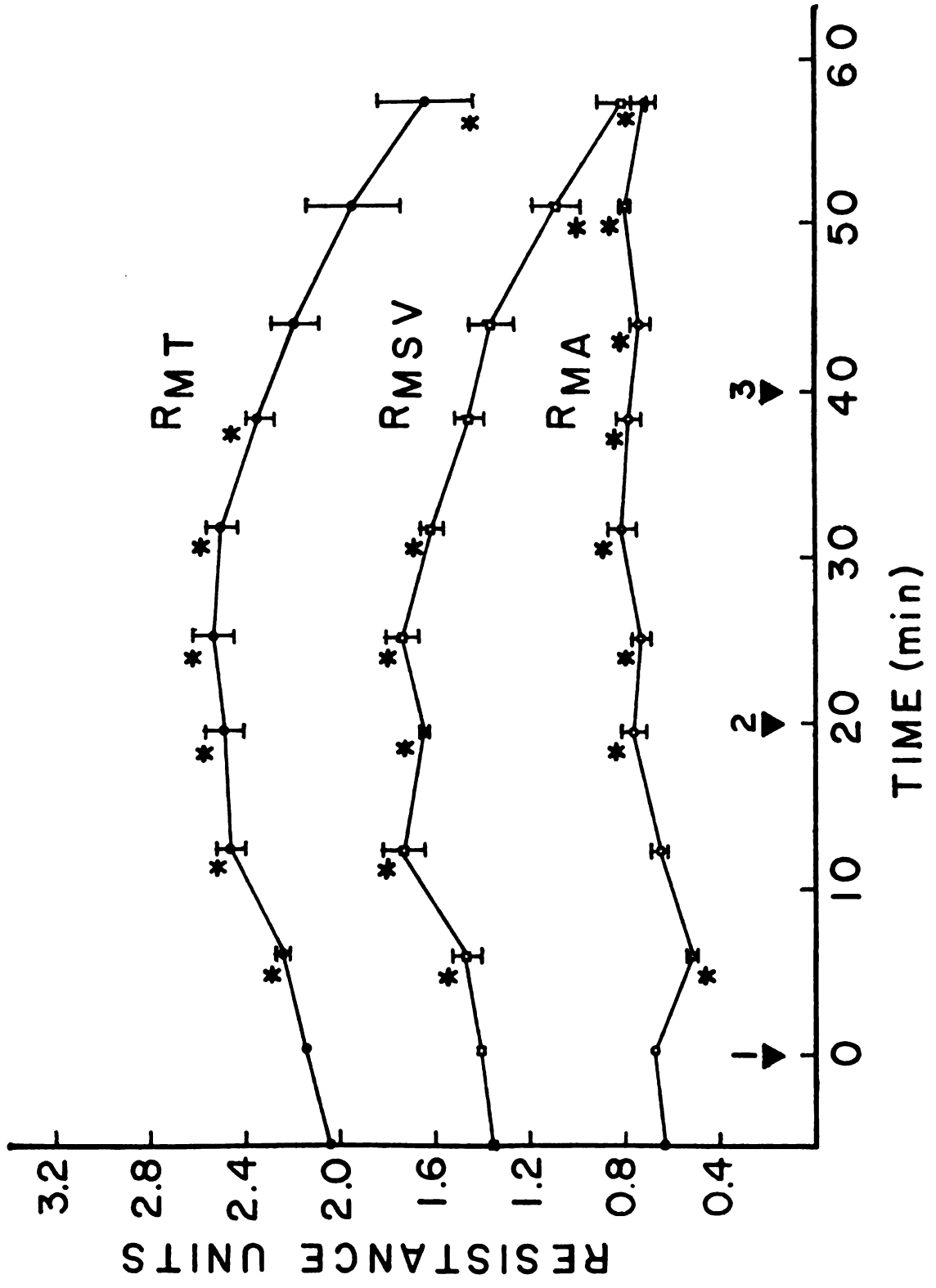

RESISTANCE UNITS
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
RMT
RMSV
RMA
1
2
3
0
10
20
30
40
50
60
TIME (min)

Figure 7. Means and standard errors of total skin vascular resistance (R_{st}), skin small vessel resistance (R_{ssv}) and skin artery resistance (R_{sa}) in mm Hg/ml/min during ouabain infusion at consecutive rates of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.

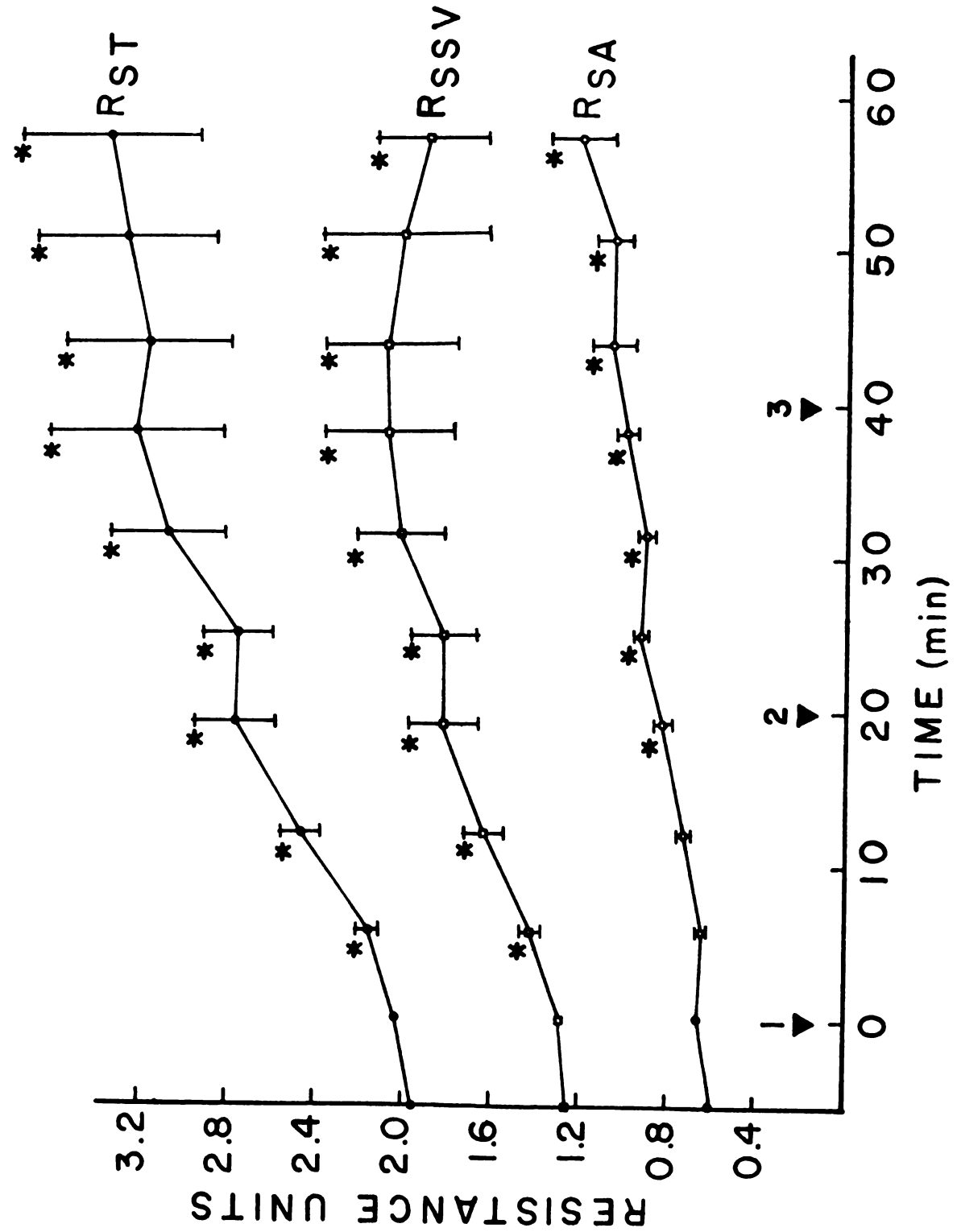
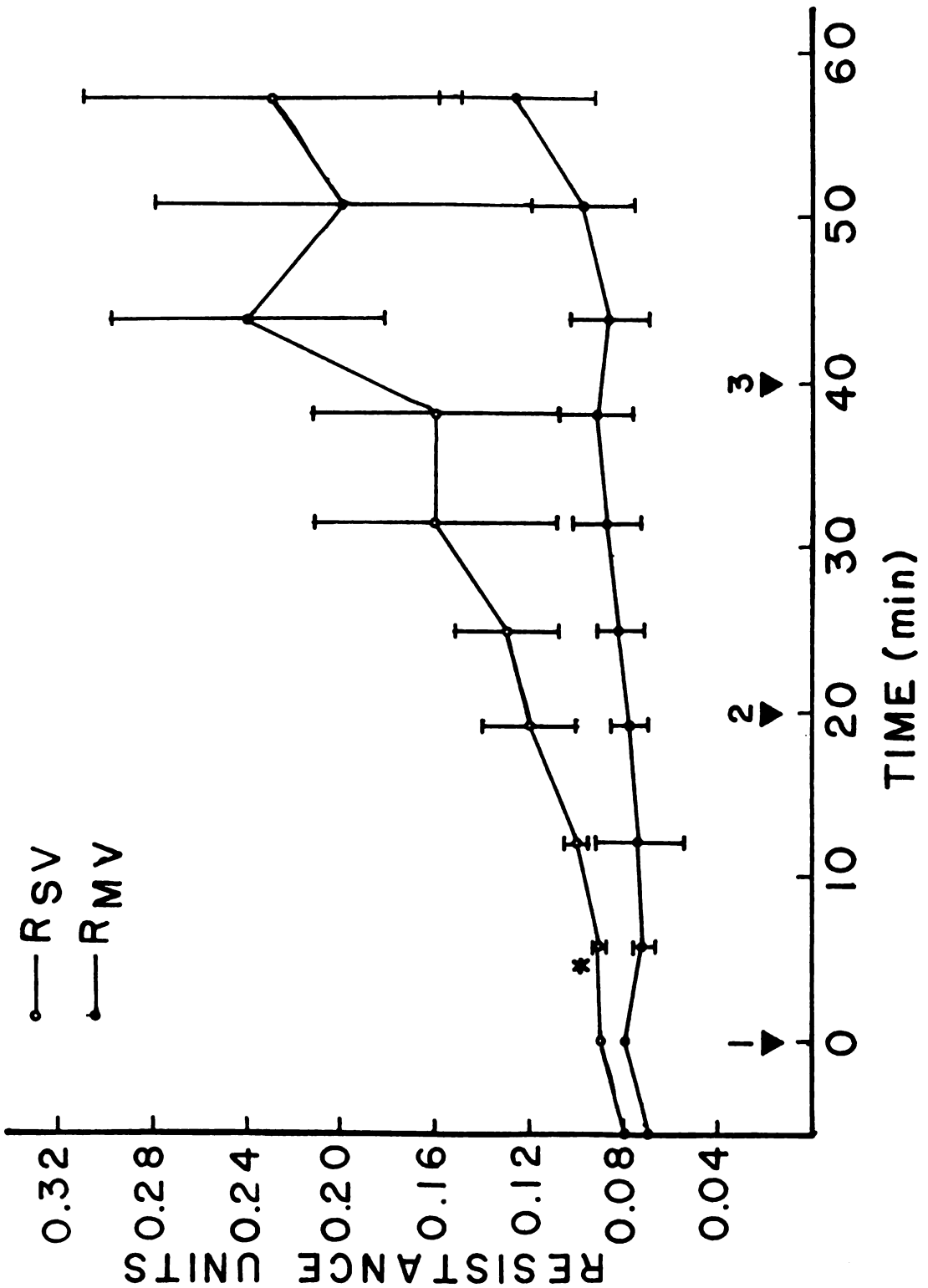

RST
RSSV
RSA
RESISTANCE UNITS
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
10
20
30
40
50
60
TIME (min)
1
2
3

Figure 8. Means and standard errors of large skin vein resistance (R_{sv}) and large muscle vein resistance in mm Hg/ml/min during ouabain infusion at doses of 6.4 ug/min (1), 12.4 ug/min (2) and 24.7 ug/min (3). The forelimb was perfused at constant flow. n=9. * denotes significant difference from control, $p < 0.05$.


RSV
RMV
RESISTANCE UNITS
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0
10
20
30
40
50
60
TIME (min)
1
2
3
*

Series II: Constant flow, single infusion rate.

Perfusion and arterial pressures (Fig. 9).

Perfusion pressure, pressure in the small muscle artery and pressure in the small skin artery rose almost immediately on starting ouabain infusion, plateaued by minute 12 and tended to return to control by minute 49. The pressure in the small muscle artery was not significantly different from control by the last reading at minute 57. Systemic arterial pressure remained unchanged.

Venous pressures (Fig. 10).

There were no significant changes in small skin vein, cephalic vein or brachial vein pressures throughout the 60 minutes of infusion. Pressure in the small muscle vein, however, was significantly increased from minute 30 to minute 60.

Venous outflows (Fig. 11).

Flow through the cephalic vein began to decrease by minute 25 and fell steadily through the end of infusion. Brachial vein flow decreased at minute 6, returned to control and then was increased from minute 31 to minute 60. Total forelimb flow remained unchanged except at minute 31 when the flow was decreased.

Calculated total forelimb vascular resistance (Fig. 12).

Total vascular resistance rose almost immediately with ouabain administration, leveled and tended to return to

Figure 9. Means and standard errors of perfusion pressure (P_p), systemic arterial pressure (P_{as}), small skin artery pressure (P_{ssa}) and small muscle artery pressure (P_{sma}) in the canine forelimb during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.

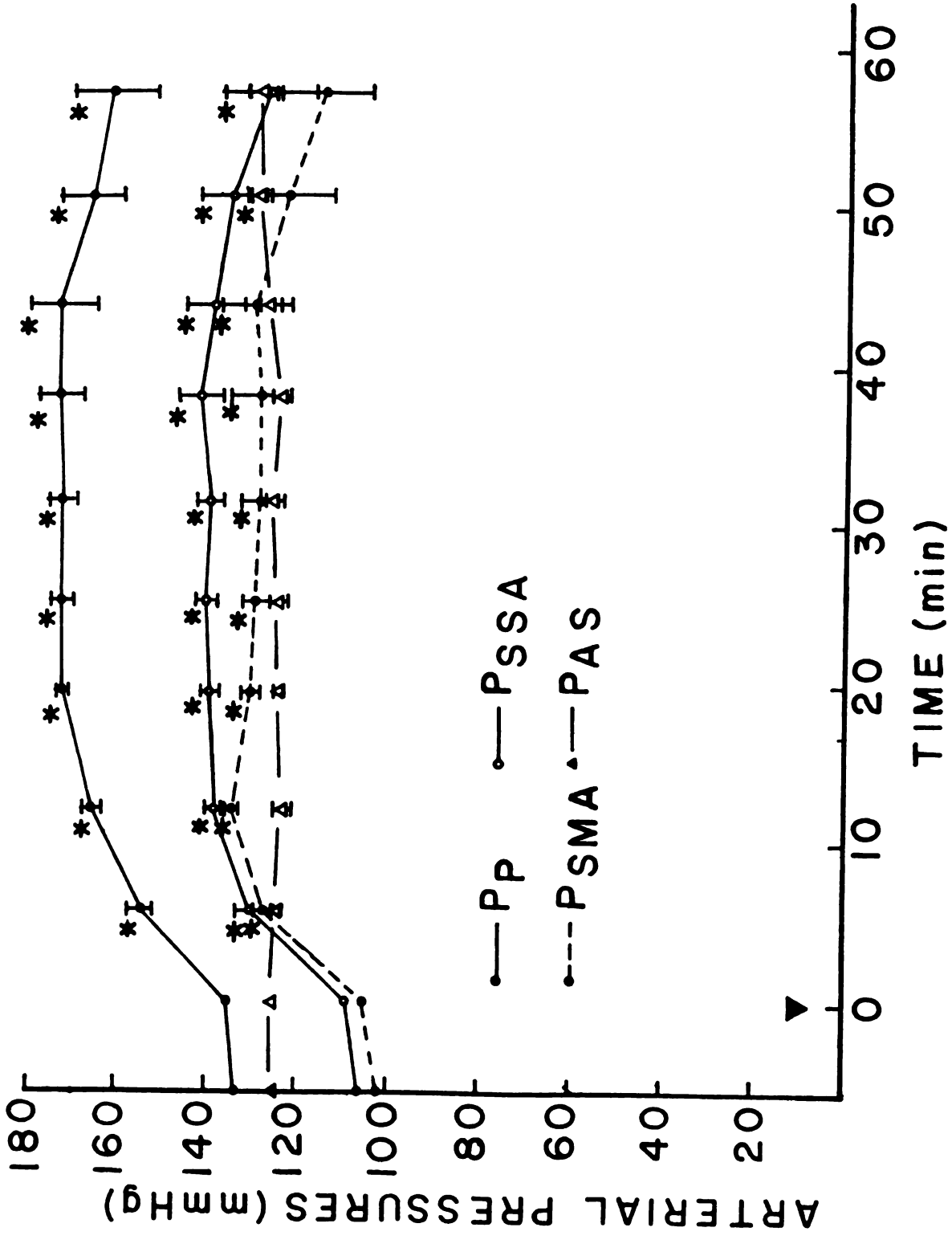

ARTERIAL PRESSURES (mmHg)
180
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
TIME (min)
P_P
P_{SSA}
P_{SMA}
P_{AS}

Figure 10. Means and standard errors of small muscle vein pressure (P_{smv}), small skin vein pressure (P_{ssv}), brachial vein pressure (P_{bv}) and cephalic vein pressure (P_{cv}) in the canine forelimb during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.

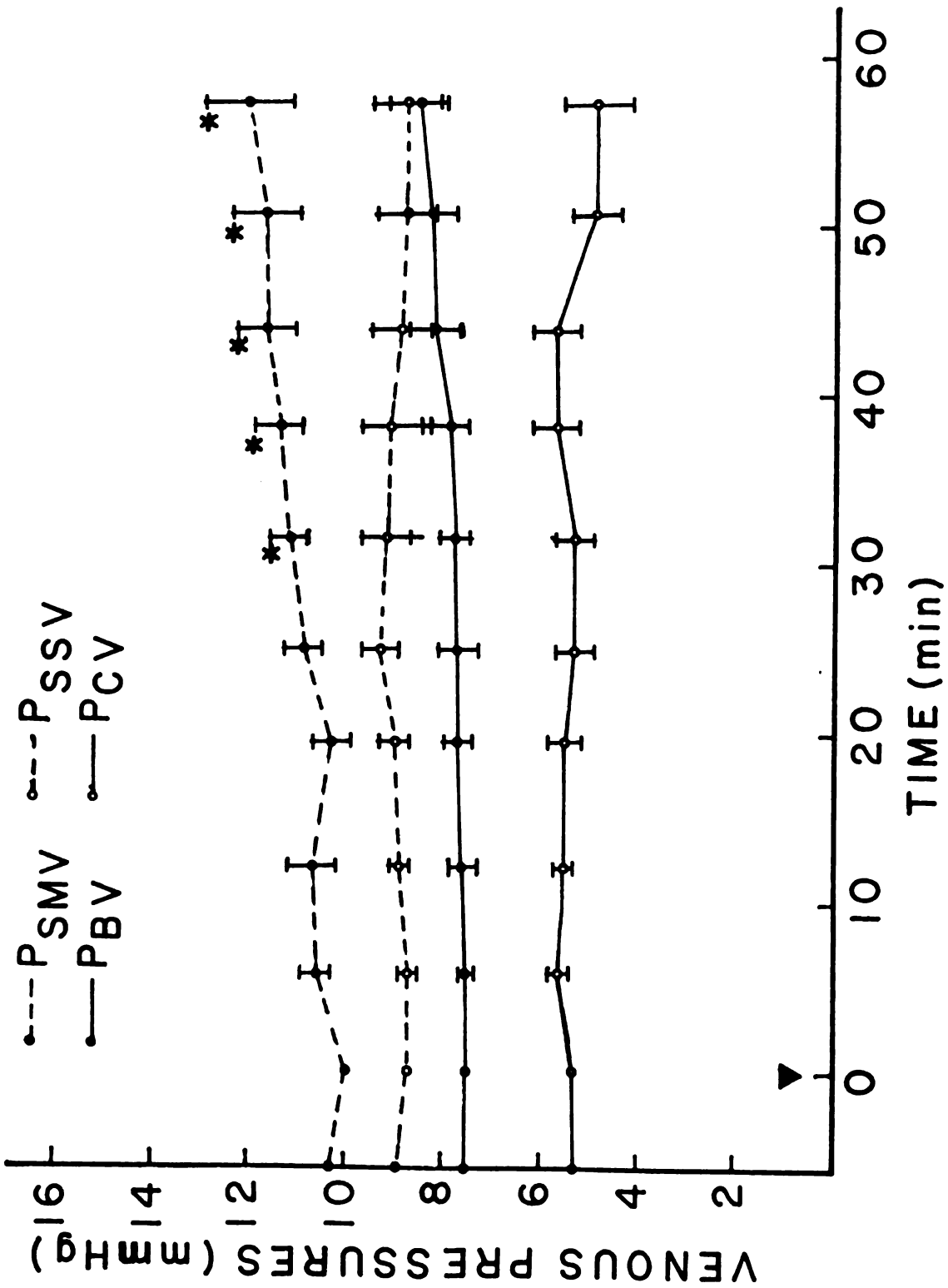

VENOUS PRESSURES (mmHg)
16
14
12
10
8
6
4
2
0
10
20
30
40
50
60
TIME (min)
P_{SMV}
P_{BV}
P_{SSV}
P_{CV}

Figure 11. Mean venous outflows, with standard errors, from brachial vein (BV) and cephalic vein (CV) in canine forelimbs during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.

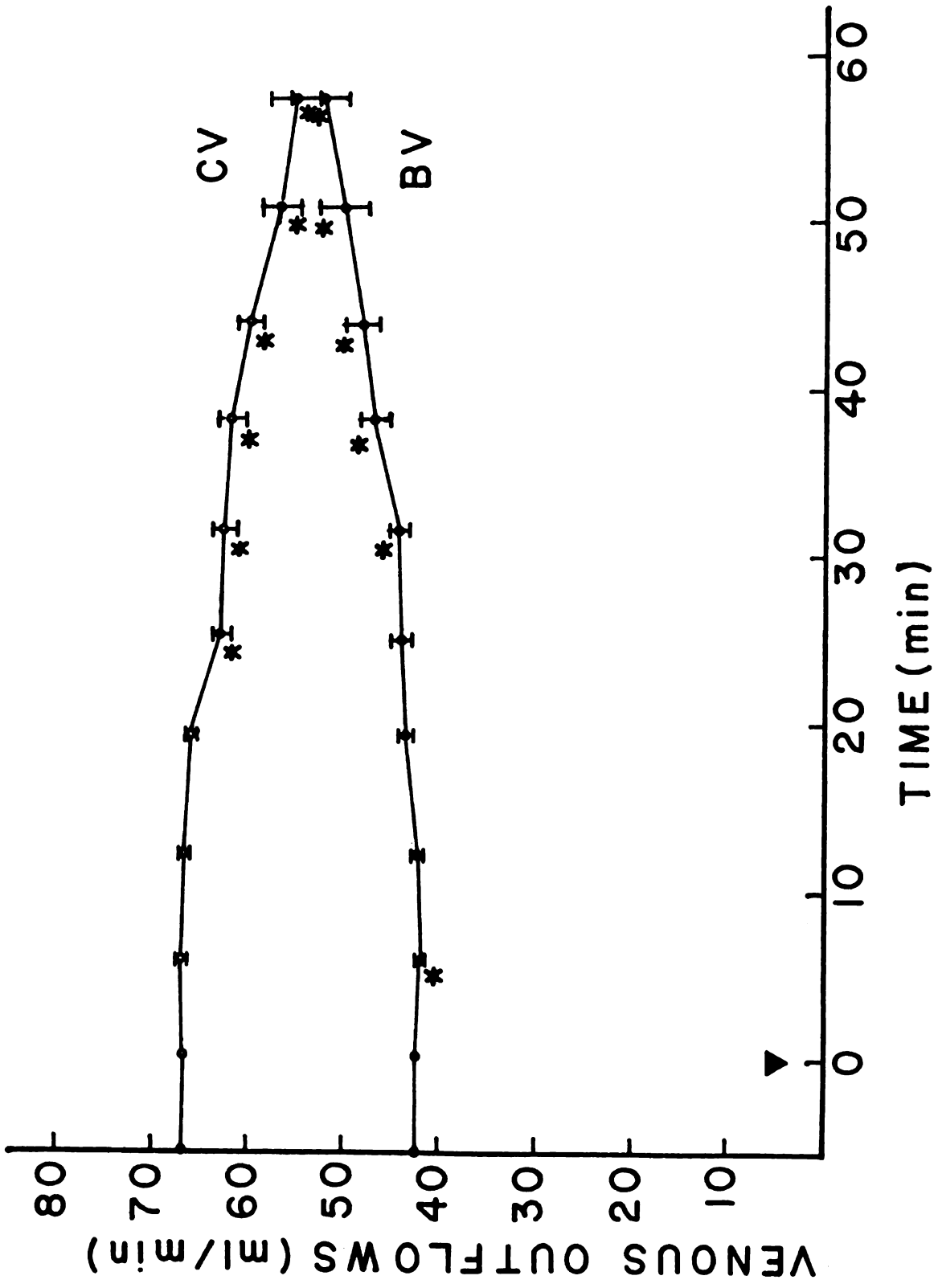
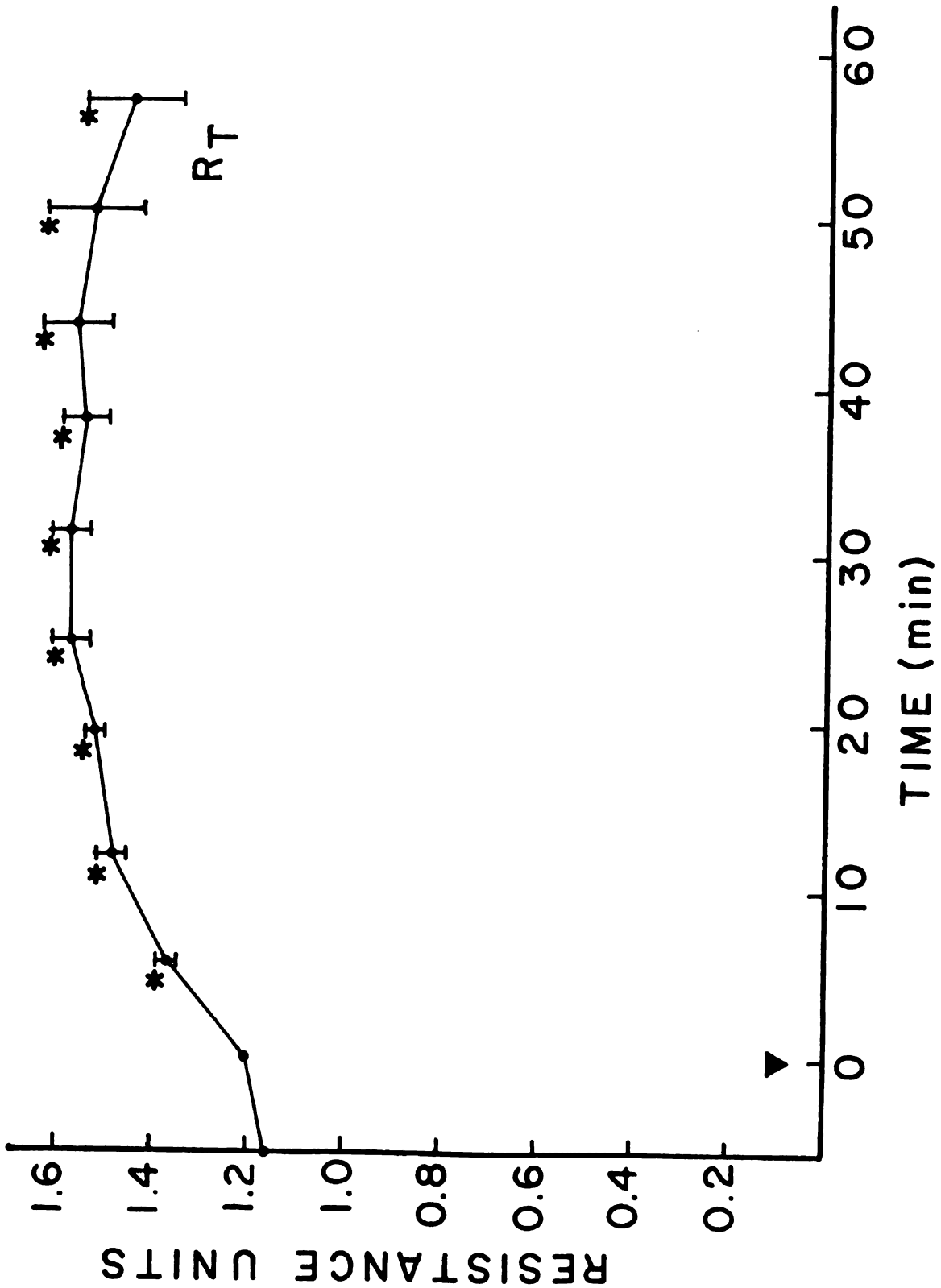

CV
BV
VENOUS OUTFLOWS (ml/min)
80
70
60
50
40
30
20
10
0
10
20
30
40
50
60
TIME (min)

Figure 12. Means and standard errors of total forelimb vascular resistance (R_t) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.


RESISTANCE UNITS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
TIME (min)
R_T

control by minute 57. All experimental values are significantly above control.

Calculated muscle vascular resistances (Fig. 13).

Total muscle vascular resistance and muscle small vessel resistance paralleled each other with an initial rise followed by a return toward control. The readings at minutes 49 and 57 for total muscle resistance and the readings at 43, 49 and 57 minutes for muscle small vessel resistance were not different from control.

Muscle artery resistance decreased significantly by minute 6, then rose by minute 18 and remained above control to minute 57, except for minute 37 which was not different from control.

Calculated skin vascular resistances (Fig. 14).

Total skin vascular resistance and skin small vessel resistance rose almost immediately and continued to rise to minute 57 where they leveled off. All values during the experimental period were significantly above control.

Skin artery resistance decreased by minute 6, rose by minute 18 and remained elevated except for the final value at minute 57 which was not different from control.

Calculated large vein resistance (Fig. 15).

There were no significant differences in either large skin vein or large muscle vein resistance throughout the infusion.

Figure 13. Means and standard errors of total muscle vascular resistance (R_{mt}), muscle small vessel resistance (R_{msv}) and muscle artery resistance (R_{ma}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.

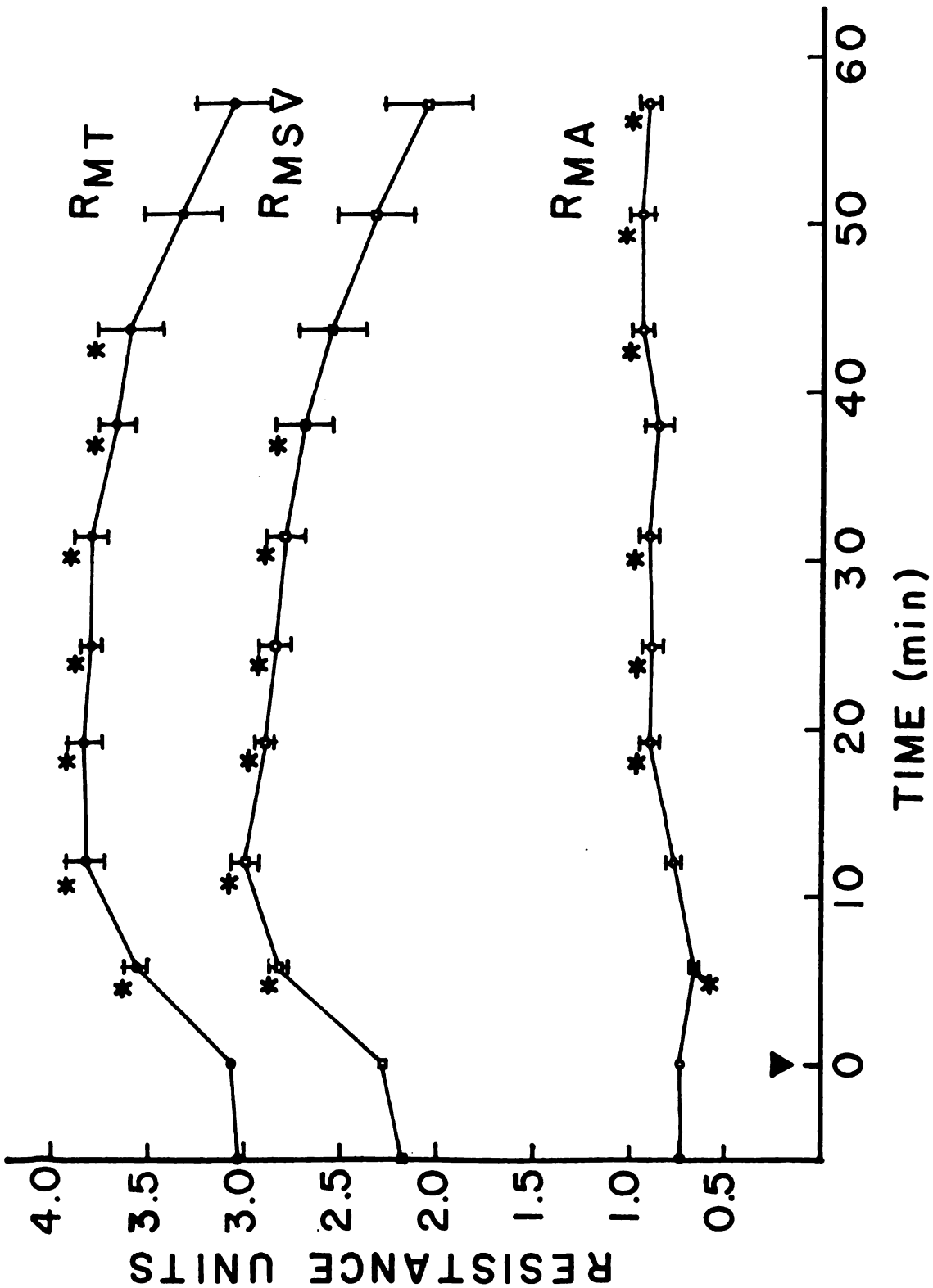

RMT
RMSV
RMA
RESISTANCE UNITS
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
TIME (min)

Figure 14. Means and standard errors of total skin vascular resistance (R_{st}), skin small vessel resistance (R_{ssv}) and skin artery resistance (R_{sa}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n=8. * denotes significant difference from control, $p < 0.05$.

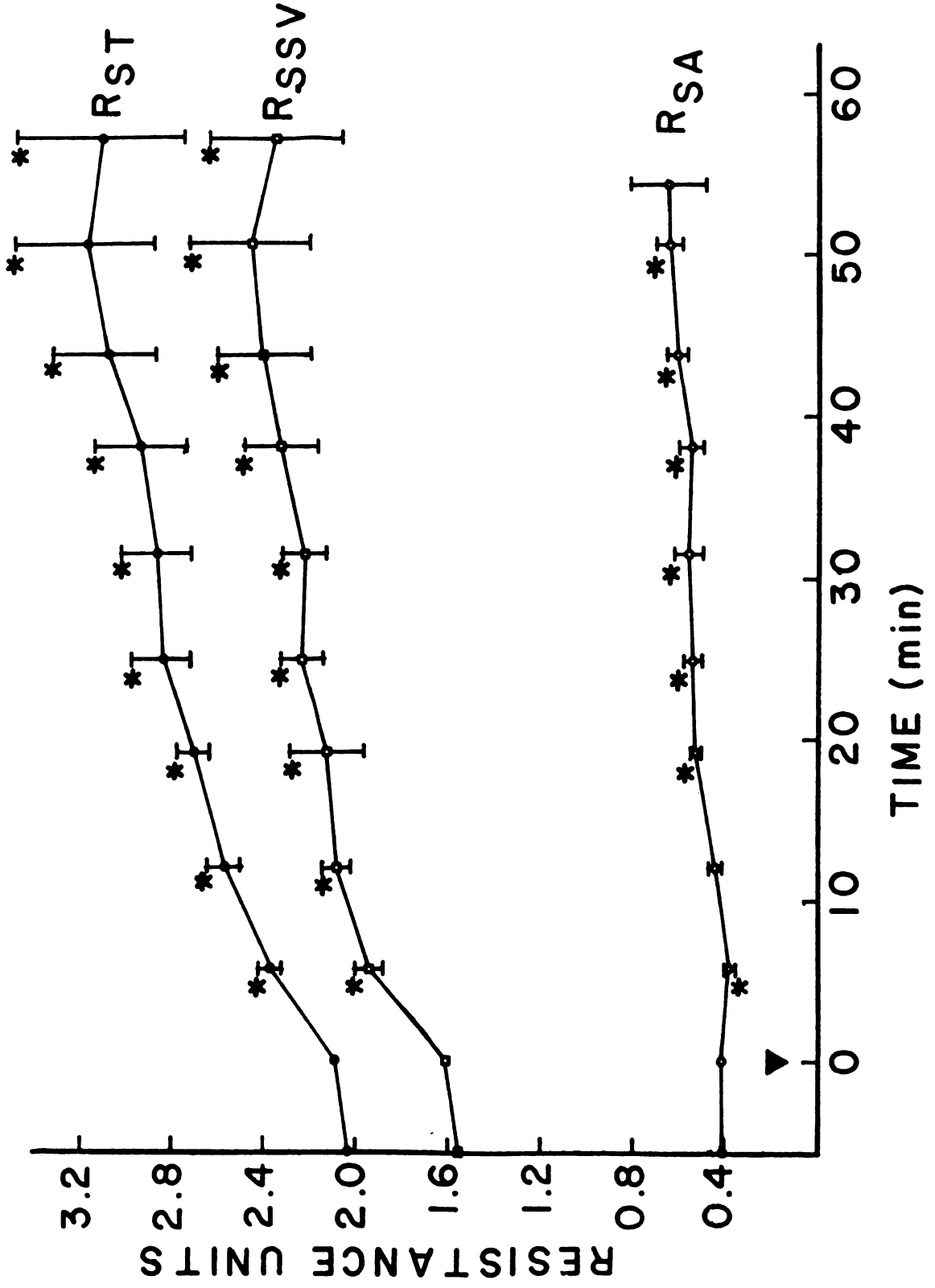
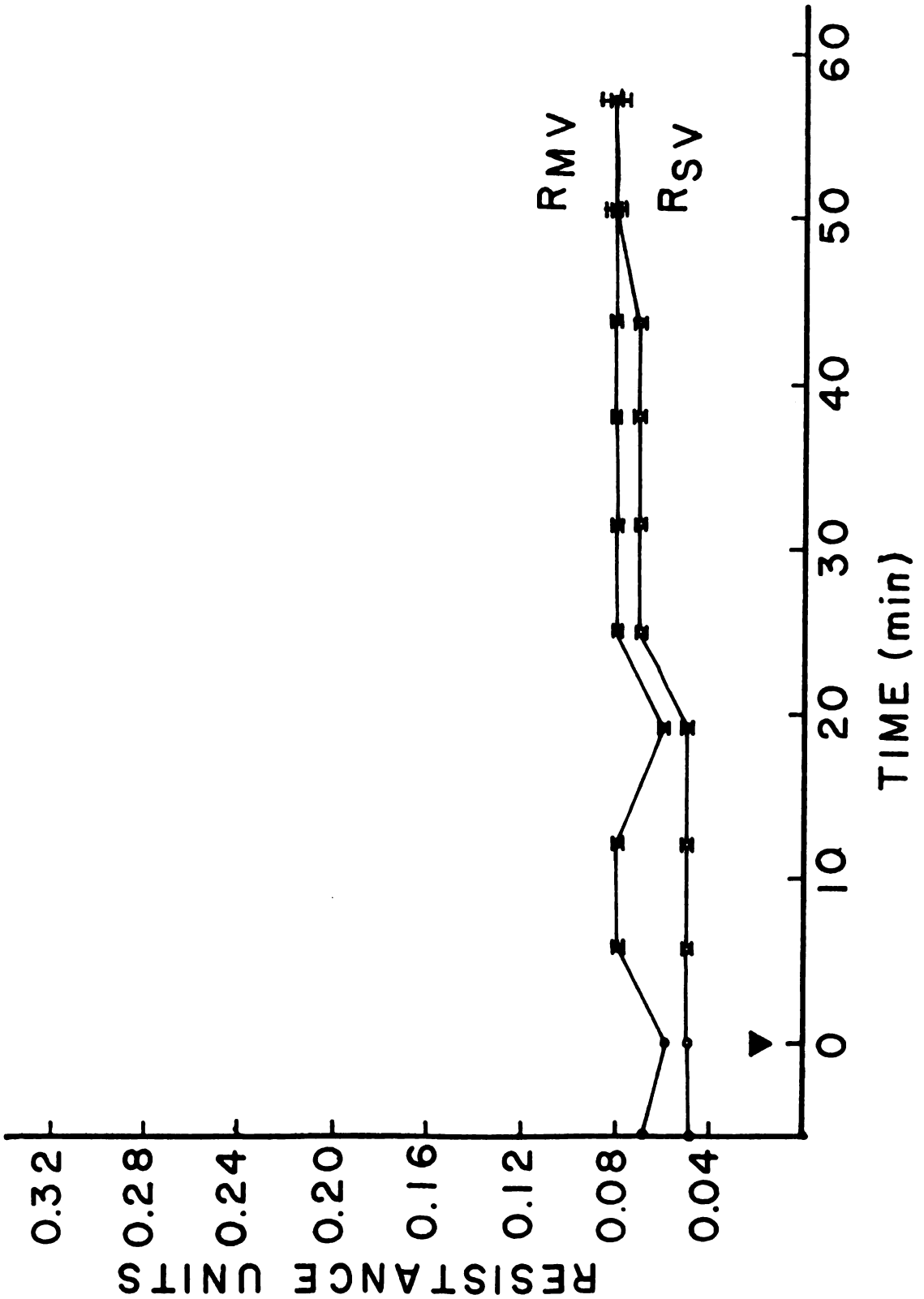

RST
RSSV
RSA
RESISTANCE UNITS
3.2
2.8
2.4
2.0
1.6
1.2
0.8
0.4
0
10
20
30
40
50
60
TIME (min)

Figure 15. Means and standard errors of muscle large vein resistance (R_{mv}) and skin large vein resistance (R_{sv}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was perfused at constant flow. n = 8.


RESISTANCE UNITS
0.32
0.28
0.24
0.20
0.16
0.12
0.08
0.04
0
10
20
30
40
50
60
TIME (min)
RMV
RSV

Series III: Natural flow, one infusion rate.

Arterial pressures (Fig. 16).

Systemic arterial pressure, the functional perfusion pressure in this series, decreased significantly by minute 6 of infusion but was not different from control during the remainder of the infusion.

Pressure in small skin and small muscle arteries showed no change for the entire 60 minutes of ouabain infusion.

Venous pressures (Fig. 17).

Pressure in the small muscle vein fell during minutes 6 and 15, returned to control by minute 24 and fell again at minutes 44 and 57 of infusion. Pressure in the small skin vein was below control at minutes 15 and 24 but was not different from control during the remainder of the infusion.

Pressure in the brachial vein was decreased at minutes 6, 15, 24 and 44 but not different from control at minutes 35 and 57. Pressure in the cephalic vein fell immediately and remained down until minute 57 at which time it was not different from control.

Venous outflows (Fig. 18).

Flow from the cephalic vein was decreased by minute 6 and leveled off after minute 15. It remained below control to the end of the 60 minute infusion period. Brachial vein flow also decreased immediately after ouabain infusion

Figure 16. Means and standard errors of systemic arterial pressure (P_{as}), small skin artery pressure (P_{ssa}) and small muscle artery pressure (P_{sma}) of the canine forelimb during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control, $p < 0.05$.

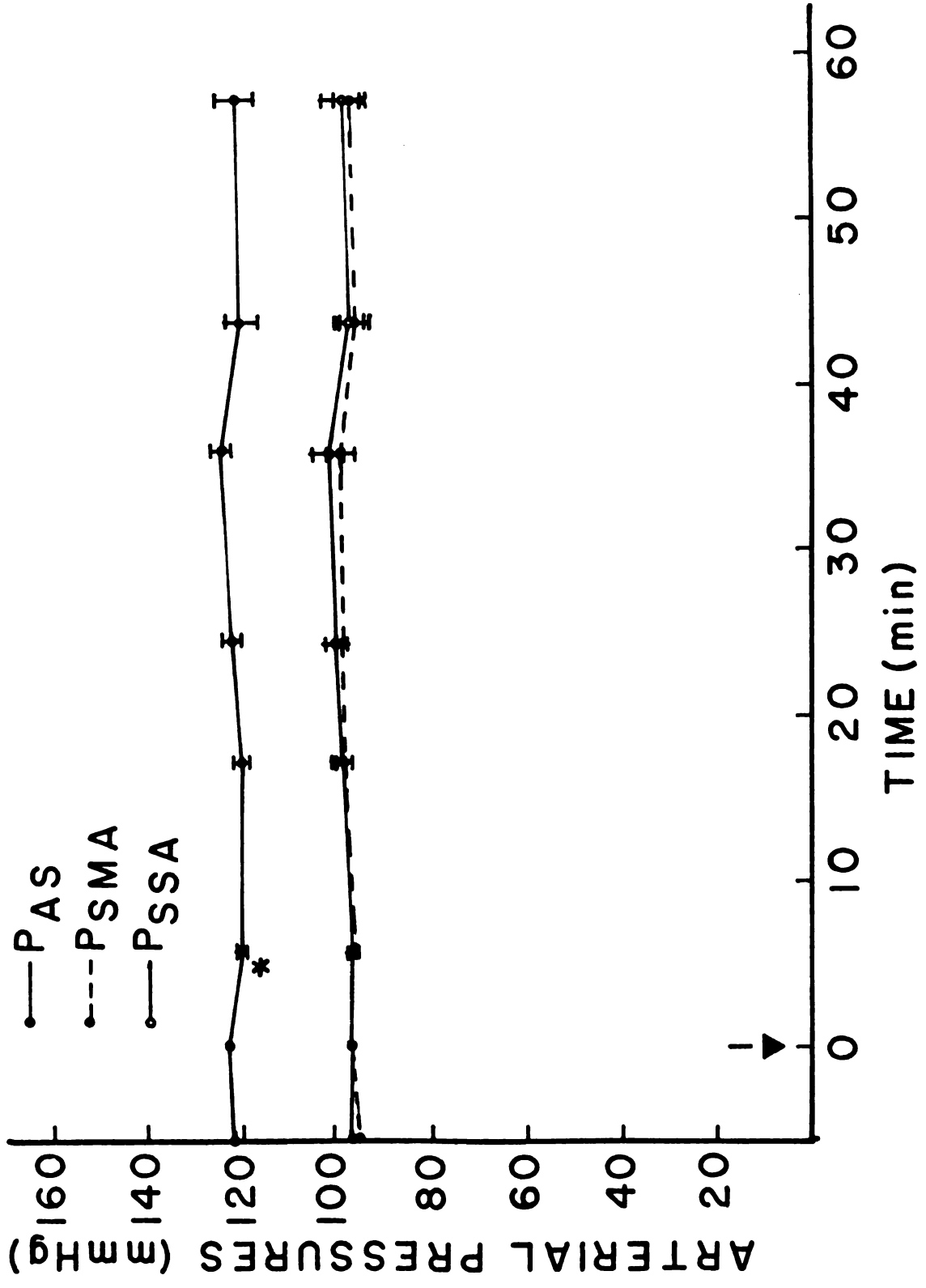

ARTERIAL PRESSURES (mmHg)
160
140
120
100
80
60
40
20
0
10
20
30
40
50
60
TIME (min)
PAS
PSMA
PSSA
*

Figure 17. Means and standard errors of small skin vein pressure (P_{ssv}), small muscle vein pressure (P_{smv}), brachial vein pressure (P_{bv}) and cephalic vein pressure (P_{cv}) in the canine forelimb during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control, $p < 0.05$.

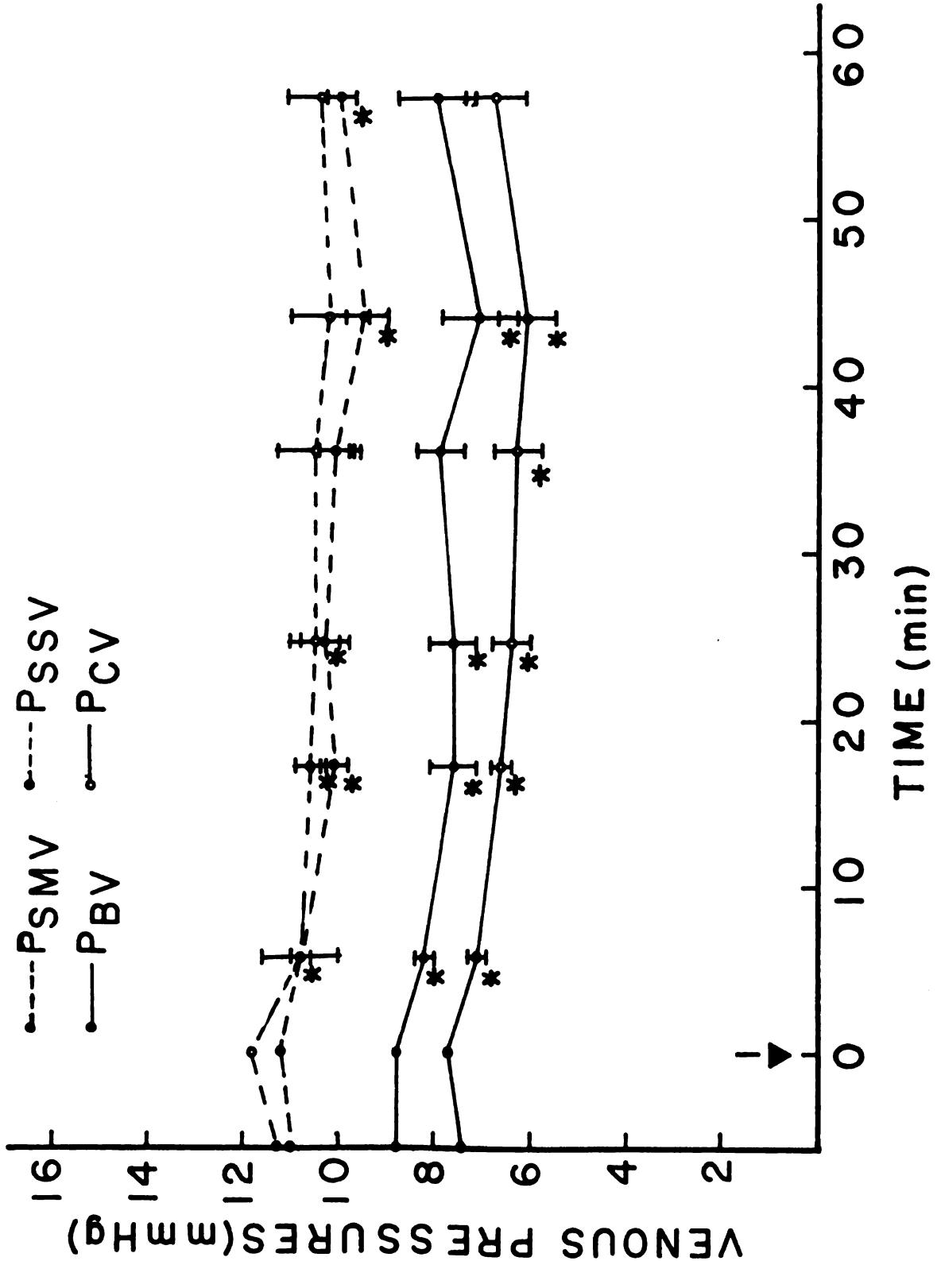
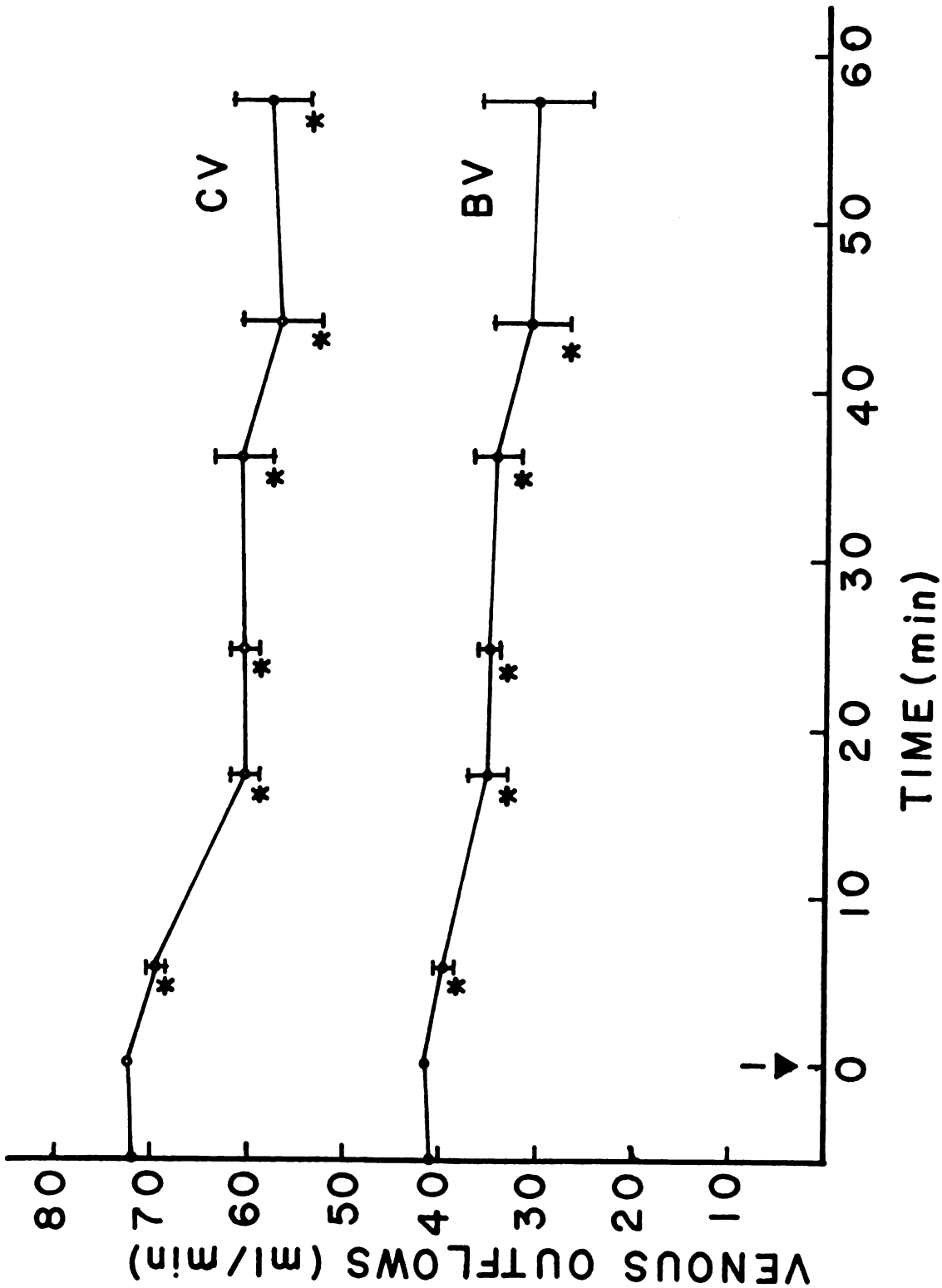

VENOUS PRESSURES(mmHg)
16
14
12
10
8
6
4
2
0
10
20
30
40
50
60
TIME (min)
PSMV
PSSV
PBV
PCV

Figure 18. Mean venous outflows, with standard errors, from cephalic veins (CV) and brachial veins (BV) of canine forelimbs during ouabain infusion at 6.4 mg/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control, $p < 0.05$.


VENOUS OUTFLOWS (ml/min)
80
70
60
50
40
30
20
10
CV
BV
0
10
20
30
40
50
60
TIME (min)

began. The flow leveled off by minute 15 and remained low until minute 57 when it was not different from control.

Total blood flow to the forelimb (Appendix B, table 3) fell immediately and remained decreased throughout the 60 minutes of ouabain infusion.

Calculated total forelimb vascular resistance (Fig. 19).

Total vascular resistance began to rise by minute 15 and continued to increase at a slow rate for the remainder of the infusion period.

Calculated muscle vascular resistance (Fig. 20).

Total muscle vascular resistance began to increase by minute 15. The rise continued throughout the remainder of the infusion period.

Muscle small vessel resistance paralleled the rise in total muscle resistance, reaching significance by minute 15 and remaining elevated through the rest of the infusion period. Muscle artery resistance remained at control levels until minute 44 when it rose slightly above control and remained there through minute 57 of infusion.

Calculated skin vascular resistance (Fig. 21).

Total skin vascular resistance and skin small vessel resistance paralleled each other, rising almost immediately above control and then leveling off by minute 15. Both values remained above control to the end of the 60 minute infusion period.

Figure 19. Means and standard errors of total forelimb vascular resistance (R_t) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control.

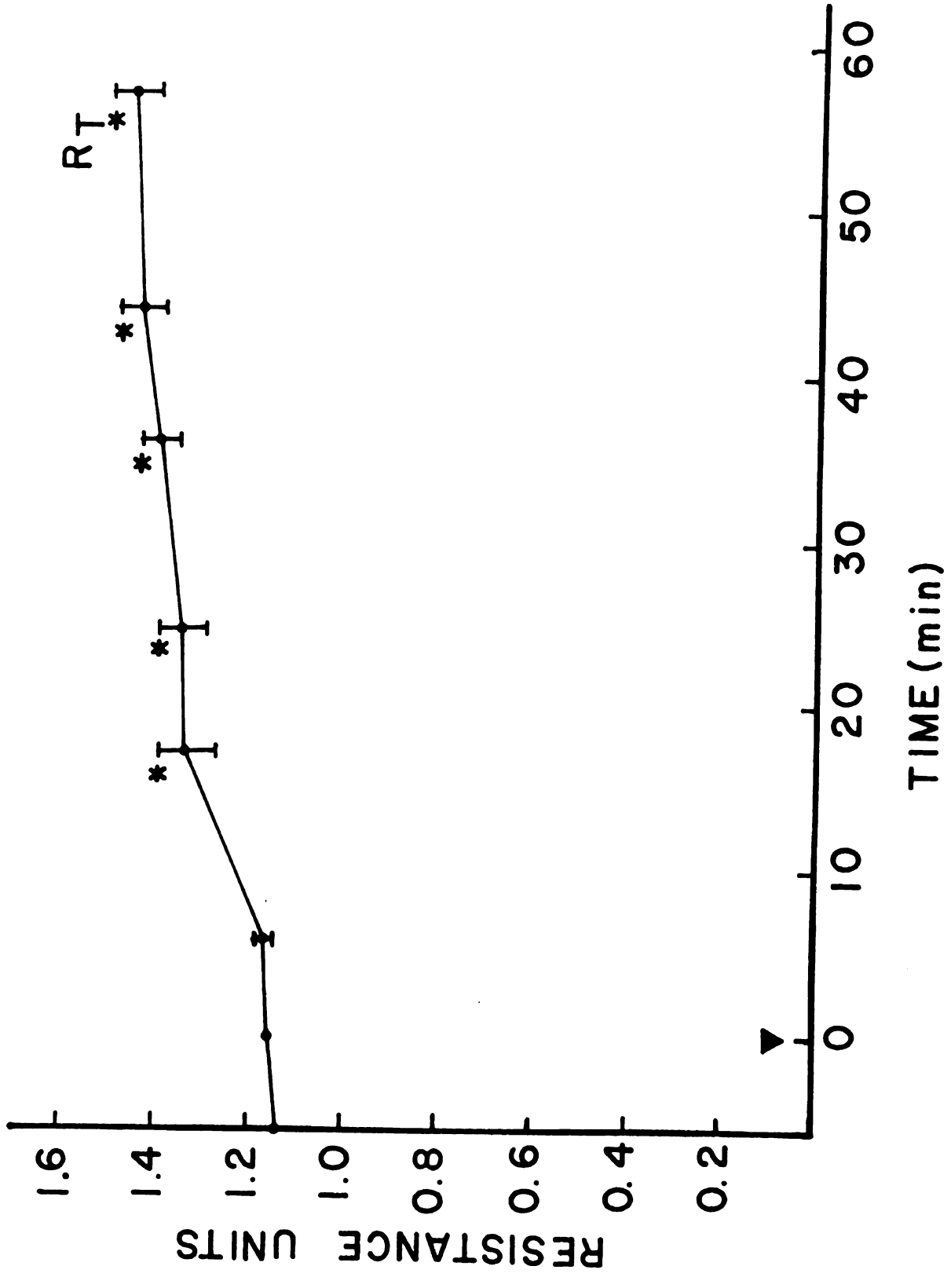

RESISTANCE UNITS
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
10
20
30
40
50
60
TIME (min)
R
T
*

Figure 20. Means and standard errors of total muscle vascular resistance (R_{mt}), muscle small vessel resistance (R_{msv}) and muscle artery resistance (R_{ma}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control, $p < 0.05$.

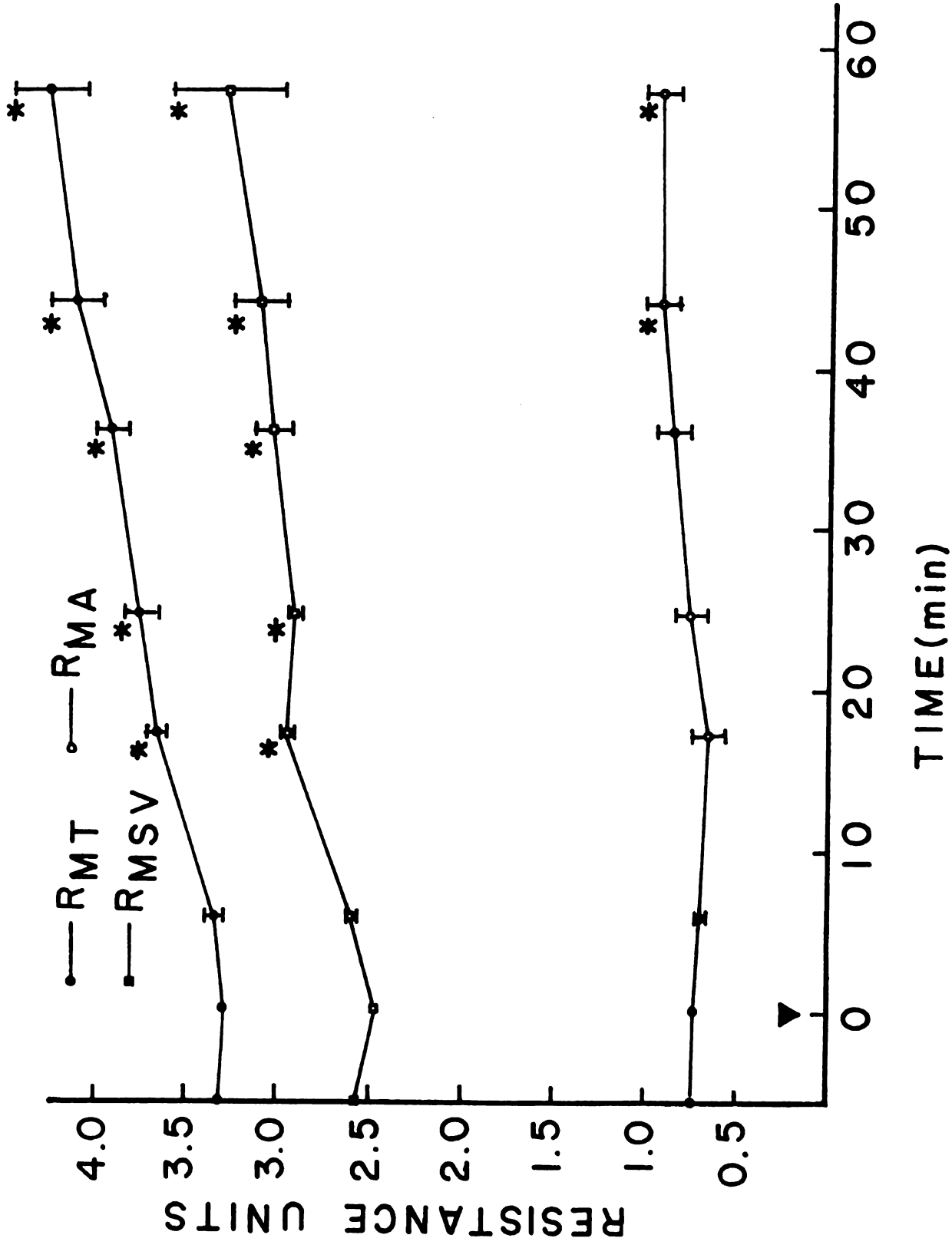
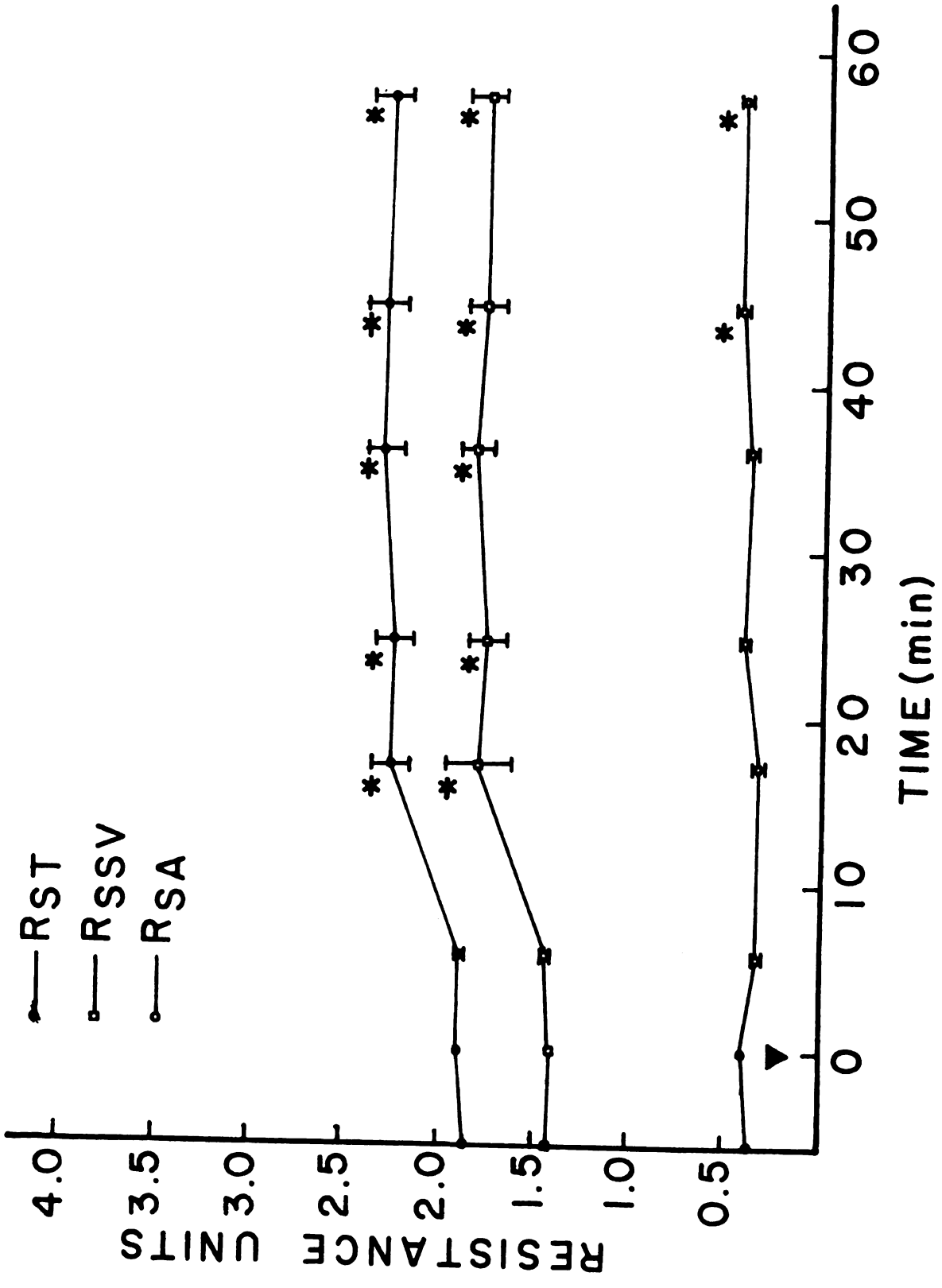

RESISTANCE UNITS
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
TIME(min)
RMT
RMA
RMSV

Figure 21. Means and standard errors of total skin vascular resistance (R_{st}), skin small vessel resistance (R_{ssv}) and skin artery resistance (R_{sa}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6. * denotes significant difference from control, $p < 0.05$.

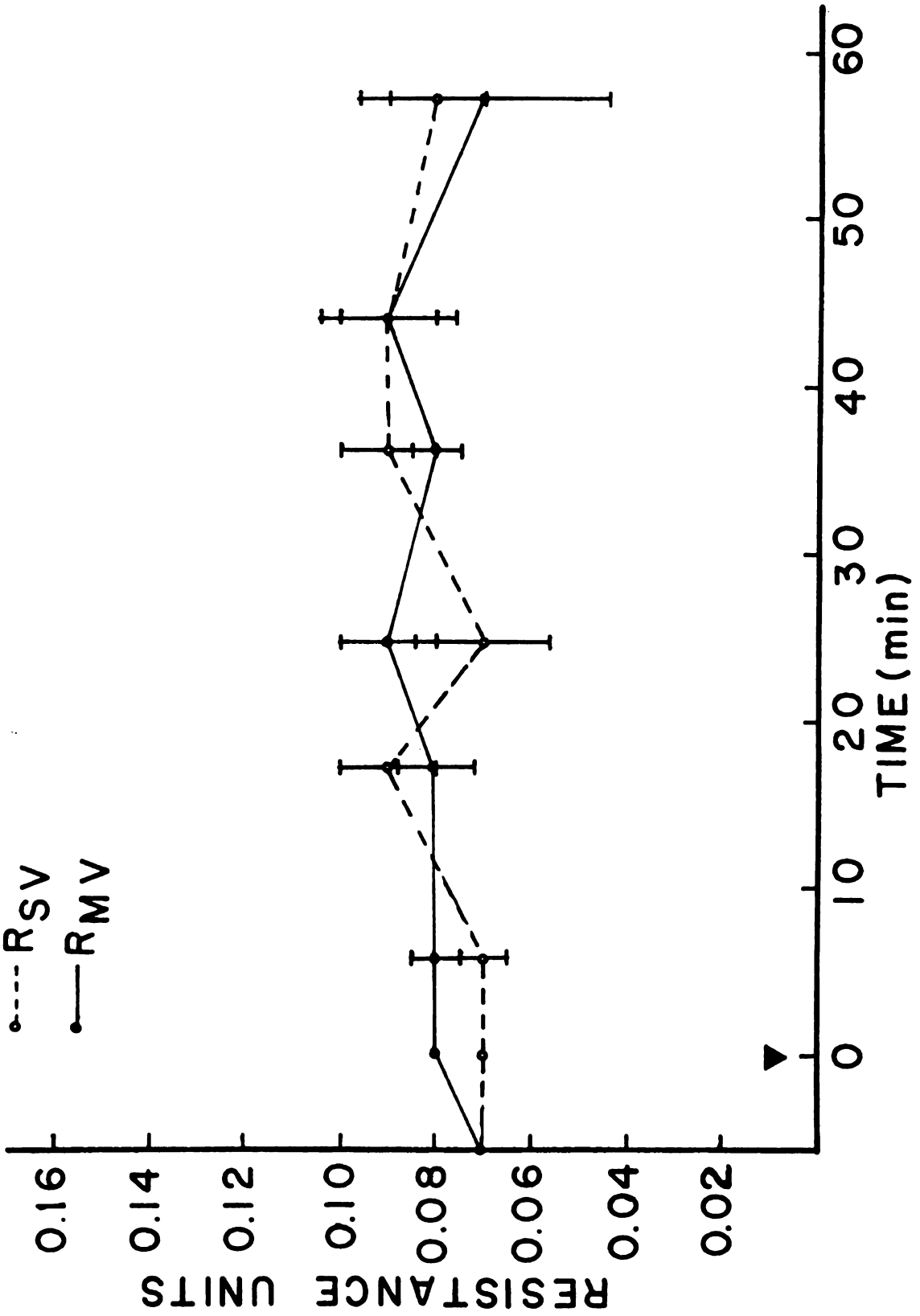

RST
RSSV
RSA
RESISTANCE UNITS
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
10
20
30
40
50
60
TIME (min)

Skin artery resistance was not different from control until the 44 and 57 minute readings when it rose slightly.

Calculated large vein resistances (Fig. 22).

Neither large muscle vein nor large skin vein was different from control at any time.

Figure 22. Means and standard errors of large skin vein resistance (R_{sv}) and large muscle vein resistance (R_{mv}) in mm Hg/ml/min during ouabain infusion at 6.4 ug/min. The forelimb was naturally perfused. n=6.


RESISTANCE UNITS
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
TIME (min)
0
10
20
30
40
50
60
R_{SV}
R_{MV}

DISCUSSION

Intra-arterial infusion of ouabain in the canine forelimb initially caused an increased total vascular resistance in both the constant flow and constant pressure systems. Resistance rose about equally in both skin and muscle tissue. This rise in resistance was mainly due to constriction in the small vessel segment of the vasculature, presumably the arterioles. The large arteries also contributed to the increased resistance.

After 30 minutes of ouabain infusion in the constant flow studies, the muscle small vessel resistance began to fall. By the end of the 60 minute infusion period, muscle small vessel resistance had fallen to control levels in the single infusion rate series and had even fallen below control values in series I in which the infusion rate was doubled after 20 and 40 minutes. The muscle total vascular resistance followed this rise and subsequent fall seen in small vessel resistance. The skin total vascular resistance continued to rise in the meantime. Since a constant flow system was being used and the resistance in muscle fell after a period of time while skin resistance remained high, the blood flow was diverted away from skin toward muscle. Doubling the infusion rate after 20 and 40 minutes did not

change the pattern of response. There was no sudden change in the response following the increase in ouabain infusion. The only difference noted was that the muscle small vessel resistance, and therefore muscle total vascular resistance, fell to a greater degree in the final 30 minutes of infusion than with the single rate of infusion. Apparently either the receptor sites for ouabain were saturated before the change in infusion rate and hence the increase had no effect or the effect of the increased ouabain had a long onset of action and the response was so spread out in time that it was hidden.

In the constant flow studies, the large veins draining both skin and muscle tissue showed no change in resistance. Even though the flow through the cephalic vein decreased and vessel caliber might be expected to passively decrease (54), no change was noted. A possible explanation for this apparent lack of response is due to the sensitivity of measurement. The minimum pressure change discernable was about 0.5 mm Hg. At the low pressures encountered in the large veins (averaging 6 to 9 mm Hg), this would allow for up to 8% error. In measuring flow, the minimum flow change which could be reliably measured was about 2 ml/min. At flows in the 40 to 70 ml/min. range, this could account for up to 5% error. It is unlikely, however, that changes below the level of sensitivity of measurement would lead consistently to errors in low resistance only.

With constant pressure, the response of the muscle small vessels differed in that the increased resistance was maintained for the entire 60 minute infusion period. As in the constant flow series, skin resistance rose and remained at an increased level. The increase in these two resistances brought about a decrease in blood flow to both skin and muscle tissue. With the decreased blood flow and corresponding decreased transmural pressure, a passive decrease in vessel caliber might be expected in the large veins. As in the constant flow series, however, no change in the large vein resistance in either skin or muscle was noted. Thus the large veins draining skin and skeletal muscle under both constant flow and natural pressure showed no vasoconstrictor response to ouabain. In fact it might be argued that they actively dilated.

It is difficult to explain the difference between the responses of muscle and skin small vessel resistance in the constant flow series. The waning response seen in the muscle small vessel resistance is not likely to be due to rate of delivery or concentration of ouabain reaching it because when the infusion rate was doubled at two different points in the infusion period it produced no observable differences. It becomes even more difficult to explain when the response was not seen under constant pressure infusion. The waning of the vasoconstrictor response of ouabain has been noted before by Chen, et al. (52) in an isolated gracilis muscle preparation. Anderson (56) reviewed the literature suggesting

that the Na+-K+ pump in vascular smooth muscle is electrogenic, i.e., causes net movement of positive charges across the cell membrane. Ouabain, by slowing down or stopping this active Na+-K+ pump diminishes its electrogenic influence. The ions then passively move down their electrochemical gradients, depending on their permeabilities, to achieve a new pseudo-steady state condition with the membrane potential partially depolarized. This partial depolarization causes contraction of the cells leading to vasoconstriction. Hendrickx and Casteels (11) found the threshold of membrane potential change leading to contraction to be 10 mv. A possible explanation for the waning of the ouabain induced vasoconstrictor response is that the intracellular Na+ concentration passively increases following the shutdown of the Na+-K+ pump. The intracellular Na+ concentration increases until it reaches a level where it overcomes the ouabain inhibition and stimulates the pump again. Once stimulated, the pump tends to return the membrane potential towards its resting potential. This repolarization ends the contraction (53). Another possibility is that the contractile elements become uncoupled from the membrane potential following sustained contraction (53). This relaxation following prolonged depolarization was noted by Hendrickx and Casteels (11) in isolated ear artery strips bathed in K+ free solution (conditions in which the Na+-K+ pump activity is decreased). These possibilities would still not explain why only the muscle resistance waned and not the skin. They

would also not explain why the response was not seen in the constant pressure study. Perhaps the waning response is due to something entirely different affecting the system. Release of a humoral agent such as serotonin in the constant flow studies may explain it. Serotonin has been shown to have a dilating effect in muscle vessels and a constricting effect in skin vessels (57). The possibility of sympathetic nerve activity may also play a role in the response. Repeating the procedure using a ganglionic blocker might clarify the problem.

SUMMARY

Local infusion of ouabain (6.4 ug/min. for 60 minutes) into the constant pressure perfused canine forelimb raises the total forelimb vascular resistance. The resistance increased in both skin and muscle beds. Total muscle vascular resistance increased mainly due to increased resistance in the small vessels. Total skin vascular resistance was increased also due to increased small vessel resistance, but to a lesser degree than in muscle. The increase in small vessel resistance is probably due to artereolar vasoconstriction.

When the forelimb was perfused under constant flow, skin total vascular resistance again increased but now the artery resistance played a greater role. Total muscle vascular resistance also rose. However, about midway through the sixty minute infusion period, the vasoconstriction in the muscle bed waned so that the resistance returned to control. This waning response shifted blood flow toward the muscle bed because the resistance remained high in the skin vasculature. Doubling the infusion rate after consecutive 20 minute intervals (to 12.4 ug/min. and then to 24.7 ug/min.) did not alter the pattern of response set with the single rate infusion.

In neither the constant pressure system nor the constant flow system were any effects noticed on large vein resistance in skin or skeletal muscle tissue.

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APPENDIX

APPENDIX A

Tables of Mean Values ± S.E.

With Significance Indicated

TABLE 1

Forelimb arterial and venous pressures (mm Hg) and venous outflows (ml/min) during ouabain infusion at three consecutive doses: (1) = 6.4 ug/min, (2) = 12.4 ug/min, (3) = 24.7 ug/min. The forelimb was perfused at constant flow. n = 9. * denotes the value is significantly different from control, $p < 0.05$.

P_p = perfusion pressure
P_{as} = systemic arterial pressure
P_{ssa} = small skin artery pressure
P_{sma} = small muscle artery pressure
P_{ssv} = small skin vein pressure
P_{smv} = small muscle vein pressure
P_{cv} = cephalic (large skin) vein pressure
P_{bv} = brachial (large muscle) vein pressure
F_{bv} = brachial vein outflow
F_{cv} = cephalic vein outflow
F_{total} = total outflow from the forelimb

TABLE 1

Time(min)	P_p	P_{as}	P_{ssa}	P_{sma}	P_{ssv}	P_{smv}	P_{cv}	P_{bv}	F_{bv}	F_{cv}	F_{total}
-6	115	111	81	82	10.8	11.6	6.2	7.6	53.2	56.1	109.3
(1) → 0	119	111	82	84	10.6	12.0	6.0	7.6	52.8	55.8	108.5
6	126*	111	91*	95*	11.1*	11.9	6.0	7.8	53.9	55.6	109.5
	± 3	± 2	± 3	± 3	±0.1	±0.2	±0.1	±0.2	±0.5	±0.4	± 0.5
12	139*	112	100*	104*	11.4	12.2	6.2	7.8	54.1	54.5*	108.6
	± 4	± 1	± 5	± 2	±0.3	±0.3	±0.3	±0.2	±0.6	±0.5	± 0.8
18	147*.	115	104*	105*	11.6	12.7	6.0	7.9	56.0*	52.1*	108.1
(2) →	± 5	± 3	± 5	± 3	±0.5	±0.4	±0.5	±0.3	±1.2	±1.3	± 0.8
25	148*	114	106*	108*	12.1*	12.5	6.0	7.6	55.9*	52.7*	108.5
	± 4	± 2	± 5	± 4	±0.6	±0.4	±0.5	±0.5	±1.6	±1.5	± 1.0
31	153*	114	109*	107*	12.5*	13.6*	5.9	8.2	58.3*	50.2*	108.4
	± 4	± 2	± 6	± 3	±0.8	±0.6	±0.7	±0.4	±1.7	±2.2	± 0.9
37	147*	116	101*	101*	12.1	14.2*	6.3	8.4	59.1*	48.0*	107.0
(3) →	± 2	± 3	± 4	± 2	±0.9	±0.8	±0.6	±0.5	±2.1	±2.9	± 1.3
43	143*	115	99*	98*	11.6	14.6*	5.8	8.8	61.6*	47.2*	108.8
	± 3	± 3	± 3	± 3	±0.9	±1.0	±0.7	±0.5	±2.3	±3	± 1.4
49	134*	121	90*	84*	11.5	15.8	5.3	9.1*	64.0*	44.3*	108.8
	± 4	± 5	± 4	± 4	±1.0	±2.4	±0.8	±0.5	±2.4	±2.9	± 2.3
57	122*	128*	77	73	11.5	17.3*	4.7	9.5*	69.5*	40.0*	109.4
	± 2	± 8	± 4	± 6	±1.0	±2.0	±0.8	±0.5	±3.0	±3.1	± 1.1

TABLE 2

Forelimb arterial and venous pressures (mm Hg) and venous outflows (ml/min) during ouabain infusion at one dose: (1) = 6.4 ug/min. The forelimb was perfused at constant flow.

n = 8.

abbreviations are explained in TABLE 1.

* denotes a value significantly differnt from control, $p < 0.05$.

TABLE 2

Time(min)	P_p	P_{as}	P_{ssa}	P_{sma}	P_{ssv}	P_{smv}	P_{cv}	P_{bv}	F_{bv}	F_{cv}	F_{total}
-6	134	126	107	103	9.0	10.4	5.4	7.6	42.7	67.0	109.7
(1) → 0	136	126	110	106	8.8	10.1	5.4	7.6	42.6	66.7	109.3
6	155*	125	131*	128*	8.8	10.7	5.7	7.6	42.2*	66.9	109.1
	± 3	± 1	± 3	± 2	±0.2	±0.3	±0.2	±0.2	±0.2	±0.3	± 0.4
12	166*	124	319*	135*	9.0	10.8	5.6	7.7	42.6	66.7	109.3
	± 2	± 2	± 2	± 2	±0.2	±0.5	±0.2	±0.3	±0.4	±0.4	± 0.2
18	173*	125	140*	136*	9.1	10.4	5.6	7.8	43.8	66.0	109.8
	± 1	± 1	± 2	± 2	±0.3	±0.4	±0.4	±0.3	±0.7	±0.6	± 0.7
25	173*	125	141*	135*	9.4	11.0	5.4	7.8	44.2	63.2*	107.3
	± 3	± 2	± 3	± 3	±0.4	±0.4	±0.4	±0.4	±0.8	±1.3	± 1.5
31	173*	126	140*	134*	9.3	11.3*	5.4	7.9	44.5*	62.9*	107.4*
	± 3	± 2	± 3	± 4	±0.5	±0.4	±0.4	±0.3	±0.9	±1.3	± 1.1
37	173*	124	142*	134*	9.2	11.5*	5.8	8.0	47.0*	62.3*	109.2
	± 5	± 2	± 5	± 7	±0.6	±0.5	±0.5	±0.4	±1.6	±1.6	± 1.1
43	173*	127	139*	130*	9.0	11.8*	5.8	8.3	48.4*	59.4*	107.7
	± 7	± 2	± 7	± 8	±0.6	±0.6	±0.5	±0.5	±2.0	±1.2	± 1.6
49	166*	129	135*	123*	8.9	11.8*	5.0	8.4	50.4*	57.0*	107.4
	± 8	± 2	± 8	± 9	±0.6	±0.7	±0.5	±0.5	±2.7	±2.1	± 1.6
57	161*	129	127*	115	8.9	13.2*	5.0	8.7	52.6*	55.6*	108.2
	± 9	± 3	±10	±10	±0.7	±1.4	±0.7	±0.6	±2.9	±2.5	± 1.2

TABLE 3

Forelimb arterial and venous pressures (mm Hg) and venous outflows (ml/min) during ouabain infusion at one dose: (1) = 6.4 ug/min. The forelimb was naturally perfused. n = 6. Abbreviations are explained in Table 1.

* denotes a value significantly different from control, $p < 0.05$.

1. The sixth value was obtained by using the mean of the first five experiments. Statistics were then performed using all six values.

TABLE 3

Time(min)	P_{as}	P_{ssa}	P_{sma}	P_{ssv}	P_{smv}	P_{cv}	P_{bv}	F_{bv}	F_{cv}	F_{total}
-6	122	97	96	11.3	11.0	7.4	8.8	40.8	72.0	112.8
(1) → 0	123	97	97	11.8	11.2	7.7	8.8	41.5	72.3	113.8
6	120*	97	97	10.8	10.8*	7.1*	8.2*	39.4*	69.5*	108.9
	± 1	± 1	± 1	±0.8	±0.2	±0.2	±0.2	±0.8	±1.2	± 1.9
15	120	99	99	10.6*	10.1*	6.6*	7.6*	35.0*	60.1*	95.1*
	± 2	± 2	± 2	±0.5	±0.5	±0.2	±0.5	±2.0	±3.2	± 5.1
24	122	100	99	10.5*	10.3	6.4*	7.6*	34.9*	60.1*	95.0*
	± 2	± 2	± 2	±0.5	±0.5	±0.4	±0.5	±2.2	±3.6	± 5.7
35	124	102	100	10.5	10.1	6.3*	7.9	34.1*	60.5*	94.6*
	± 2	± 3	± 3	±0.8	±0.5	±0.5	±0.5	±2.4	±2.7	± 4.8
44	120	97	96	10.2	9.5*	6.1*	7.1*	30.7	56.6*	87.3*
	± 3	± 3	± 3	±0.8	±0.4	±0.6	±0.8	±4.2	±3.8	± 7.8
57	121	99	97	10.4	10.0*	6.8	8.0	29.9	57.4	87.3*
	± 4	± 4	± 3	±0.7	±0.3	±0.6	±0.8	±5.9	±4.4	±10.0

TABLE 4

Calculated segmental vascular resistances during ouabain infusion at three consecutive doses: (1) = 6.4 ug/min, (2) = 12.4 ug/min, (3) = 24.7 ug/min. The forelimb was perfused at constant flow. n = 9.

* denotes a value significantly different from control, $p < 0.05$.

R_{mt} = total muscle vascular resistance

R_{ma} = muscle artery resistance

R_{msu} = muscle small vessel resistance

R_{mv} = muscle large vein resistance

R_{st} = total skin vascular resistance

R_{sa} = skin artery resistance

R_{ssv} = skin small vessel resistance

R_{sv} = skin large vein resistance

R_{t} = total forelimb vascular resistance

TABLE 4

Time(min)	R_{mt}	R_{ma}	R_{msv}	R_{mv}	R_{st}	R_{sa}	R_{ssv}	R_{sv}	R_t
-6	2.03	0.62	1.34	0.07	1.96	0.61	1.26	0.08	0.99
(1) → 0	2.13	0.66	1.39	0.08	2.04	0.67	1.29	0.09	1.03
6	2.22*	0.50*	1.45*	0.07	2.17*	0.65	1.43*	0.09	1.09*
	±0.04	±0.02	±0.06	±0.01	±0.05	±0.01	±0.05	±0.00	±0.02
12	2.45*	0.64	1.72*	0.07	2.48*	0.73*	1.64	0.10*	1.22*
	±0.06	±0.03	±0.09	±0.02	±0.09	±0.03	±0.09	±0.00	±0.04
18	2.48*	0.75*	1.64*	0.08	2.78*	0.83*	1.83*	0.12	1.30*
(2) →	±0.09	±0.05	±0.02	±0.01	±0.18	±0.04	±0.16	±0.01	±0.05
25	2.53*	0.72*	1.73*	0.08	2.77*	0.93*	1.83*	0.13	1.31*
	±0.09	±0.04	±0.07	±0.01	±0.16	±0.02	±0.15	±0.01	±0.05
31	2.50*	0.80*	1.61*	0.09	3.09*	0.91*	2.03*	0.16	1.36*
	±0.07	±0.06	±0.05	±0.02	±0.27	±0.04	±0.21	±0.03	±0.05
37	2.34*	0.78*	1.45	0.09	3.24*	1.00*	2.08*	0.16	1.31*
(3) →	±0.06	±0.05	±0.06	±0.02	±0.37	±0.05	±0.29	±0.03	±0.04
43	2.19	0.74*	1.36	0.09	3.18*	1.07*	2.09*	0.24	1.26*
	±0.10	±0.04	±0.09	±0.02	±0.38	±0.10	±0.29	±0.03	±0.04
49	1.95	0.80*	1.09*	0.10	3.29*	1.07*	2.02*	0.20	1.18*
	±0.23	±0.02	±0.12	±0.02	±0.43	±0.08	±0.38	±0.06	±0.05
57	1.64*	0.72	0.81*	0.13	3.36*	1.22*	1.91*	0.23	1.05
	±0.18	±0.05	±0.13	±0.03	±0.44	±0.15	±0.25	±0.06	±0.06

TABLE 5

Calculated segmental vascular resistances during ouabain infusion at one dose: (1) 6.4 ug/min. The forelimb was perfused at constant flow. n = 8.

* denotes a value significantly different from control, $p < 0.05$.

Abbreviations are explained in Table 4.

TABLE 5

Time(min)	R_{mt}	R_{ma}	R_{msv}	R_{mv}	R_{st}	R_{sa}	R_{ssv}	R_{sv}	R_t
-6	3.03	0.75	2.21	0.07	2.04	0.42	1.57	0.05	1.18
(1) → 0	3.10	0.75	2.29	0.06	2.10	0.42	1.62	0.05	1.21
6	3.59*	0.68*	2.84*	0.08	2.38*	0.38*	1.95*	0.05	1.38*
	±0.06	±0.03	±0.05	±0.01	±0.05	±0.01	±0.06	±0.01	±0.02
12	3.86*	0.77	3.01*	0.08	2.58	0.44	2.09*	0.05	1.49*
	±0.11	±0.04	±0.07	±0.02	±0.07	±0.02	±0.06	±0.01	±0.03
18	3.89*	0.91*	2.92*	0.06	2.71*	0.52*	2.13*	0.05	1.53*
	±0.09	±0.06	±0.05	±0.01	±0.07	±0.02	±0.16	±0.01	±0.02
25	3.82*	0.89*	2.86*	0.08	2.85*	0.54*	2.24*	0.07	1.58*
	±0.06	±0.05	±0.08	±0.01	±0.13	±0.03	±0.09	±0.01	±0.04
31	3.81*	0.91*	2.81*	0.08	2.87*	0.56*	2.23*	0.07	1.58*
	±0.08	±0.05	±0.10	±0.01	±0.13	±0.06	±0.09	±0.01	±0.04
37	3.65*	0.86*	2.71*	0.08	2.94*	0.54*	2.33*	0.07	1.55*
	±0.12	±0.07	±0.15	±0.01	±0.21	±0.05	±0.16	±0.02	±0.05
43	3.57*	0.93*	2.56	0.08	3.08*	0.61*	2.40*	0.07	1.57*
	±0.17	±0.05	±0.18	±0.01	±0.23	±0.04	±0.40*	±0.02	±0.07
49	3.33	0.93*	2.32	0.07	3.17*	0.64*	2.46*	0.08	1.53*
	±0.19	±0.06	±0.19	±0.01	±0.32	±0.06	±0.26	±0.02	±0.10
57	3.06	0.91*	2.05	0.07	3.10*	0.67*	2.35*	0.08	1.45*
	±0.21	±0.06	±0.22	±0.01	±0.36	±0.07	±0.29	±0.03	±0.10

TABLE 6

Calculated segmental vascular resistnaces during ouabain infusion at one dose: (1) 6.4 ug/min. The forelimb was naturally perfused. n = 6.

* denotes a value significantly different from control, $p < 0.05$.

Abbreviations are explained in Table 4.

TABLE 6

Time(min)	R_{mt}	R_{ma}	R_{msv}	R_{mv}	R_{st}	R_{sa}	R_{ssv}	R_{sv}	R_t
-6	3.34	0.75	2.53	0.07	1.86	0.37	1.42	0.07	1.14
(1)→0	3.30	0.74	2.48	0.08	1.90	0.41	1.42	0.07	1.16
6	3.38	0.70	2.60	0.08	1.90	0.38	1.45	0.07	1.17
	±0.06	±0.04	±0.05	±0.01	±0.04	±0.01	±0.03	±0.00	±0.02
15	3.68*	0.65	2.95*	0.08	2.28*	0.38	1.81*	0.09	1.34*
	±0.06	±0.08	±0.06	±0.01	±0.18	±0.03	±0.17	±0.01	±0.06
24	3.76*	0.75	2.91	0.09	2.28*	0.41	1.78*	0.07	1.35*
	±0.09	±0.07	±0.06	±0.01	±0.10	±0.01	±0.10	±0.01	±0.05
35	3.91*	0.80	3.03*	0.08	2.33*	0.40	1.84*	0.09	1.40*
	±0.08	±0.07	±0.11	±0.01	±0.09	±0.03	±0.10	±0.01	±0.04
44	4.10*	0.91*	3.10*	0.09	2.35*	0.46*	1.80*	0.09	1.44*
	±0 16	±0.07	±0.14	±0.01	±0.09	±0.01	±0.09	±0.01	±0.05
57	4.25*	0.91*	3.27*	0.07	2.31*	0.44*	1.79*	0.08	1.46*
	±0.39	±0.07	±0.33	±0.03	±0.09	±0.02	±0.09	±0.01	±0.05