Neurally Mediated and Direct Effects of Acetylstrophanthidin on Canine Skeletal Muscle Vascular Resistance

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ABSTRACT
The effects of acetylstrophanthidin on the vascular resistance of the isolated canine gracilis muscle were examined in 38 anesthetized mongrel dogs. The neurally mediated effect of intravenous acetylstrophanthidin (0.5 mg) on the vascular resistance of the innervated, separately perfused (constant flow) right gracilis muscle was a transient mean fall of 4.0 ± 0.9 resistance units (RU) (P < 0.001) at 1 minute followed by a sustained rise to 4 RU (P < 0.05) above control from 8 to 18 minutes after injection. The sustained increase in resistance was blocked by intra-arterial phenoxybenzamine. The combined direct and neurally mediated effects of intravenous acetylstrophanthidin were directionally similar and greater in magnitude in the innervated, autoperfused (intact circulation) left gracilis. Following denervation of the muscle, however, the initial decrease in vascular resistance was abolished and the prolonged subsequent rise was greatly reduced. Hence the initial fall and most of the subsequent rise in vascular resistance are neurally mediated. There was no consistent change in aortic pressure to explain either of these changes in vascular resistance solely on a reflex basis. Thus, systemically administered acetylstrophanthidin produces skeletal muscle vasoconstriction through both a direct and a neurally mediated effect. The latter is mediated through alpha-receptor stimulation and appears to be the predominant mechanism whereby this drug increases muscle vascular resistance.

KEY WORDS
digitalis alpha receptors vagotony
denervation of the carotid bifurcations

Digitalis has been considered to have both a direct and a neurogenic effect on peripheral vascular resistance. The direct vasoconstrictor effect of digitalis on vascular smooth muscle is well established (1). However, controversy exists as to whether the principal neurogenic effect of digitalis is withdrawal or enhancement of sympathetic tone mediated by alpha-receptors. Ross et al. (1), Mason and Braunwald (2), and Daggett and Weisfeldt (3) have demonstrated reflex withdrawal of sympathetic activity associated with the pressor response following the administration of digitalis to experimental animals and to man. However, recent studies by Gillis (4) have shown a neurally mediated increase in the activity of the sympathetic nervous system following the systemic administration of ouabain, reflected in an increase in the frequency of discharge of cardiac sympathetic nerves.

We therefore undertook studies to examine the manner in which acetylstrophanthidin affects vascular resistance in an isolated skeletal muscle preparation. These studies permitted the independent assessment of the direct and the neurogenic effects of the drug. The purpose of this communication is to present data which will permit a characterization of the neurogenic effect of acetylstrophanthidin on skeletal muscle vascular resistance and allow an assessment of the relative
contributions of the direct and the neurogenic effects of this drug. A preliminary report of the data has been made (5).

Methods

Thirty-eight mongrel dogs of either sex weighing between 18 and 22 kg were anesthetized with pentobarbital (30 mg/kg, i.v.), intubated, and ventilated with a Harvard respirator with 95% O₂-5% CO₂. Arterial pH and PCO₂ were maintained within the physiologic range during the experiments.

The experimental preparation was based on that of Renkin and Rosell (6), and a constant-flow, oil-displacement pump was used. As shown in Figure 1, the right gracilis muscle was surgically isolated with its tendons tied at both ends, and the nerve was either left intact or cut depending on whether the neurogenic effects of acetylstrophanthidin given by systemic intravenous administration or the direct effects of the drug given by intra-arterial administration were being examined. The muscle was perfused with blood at a near constant flow. Compressed air at 30 lb/inch² displaced high viscosity oil from an oil reservoir. The flow of oil was controlled by a needle valve. The oil in turn displaced an equal volume of blood from one of three reservoirs. The blood flowed past a Statham P23db transducer and into the cannulated right gracilis artery. The left gracilis muscle and its vessels and nerve were similarly isolated, but the artery was left intact; hence the muscle was autoperfused from the dog. Venous outflow from each muscle was monitored with a drop-rate flowmeter. An on-line analog computer calculated instantaneous vascular resistance of the right gracilis muscle in resistance units (mm Hg/ml [100 g muscle]-1 min-1). Vascular resistance of the left gracilis muscle was calculated by dividing mean aortic pressure by venous outflow and normalized to 100 g of muscle (wet weight).

In all dogs, aortic pressure was monitored, and in 12 dogs a rigid wide-bore cannula inserted through a left thoracotomy into the apex of the left ventricle was used to assess left ventricular end-diastolic pressure as previously described (7). In these animals, left ventricular end-diastolic pressure was consistently less than 10 mm Hg. Although the intravenous dose of acetylstrophanthidin resulted in little change in heart rate, in 6 dogs the heart was paced via electrodes sutured on the epicardial surface of the right ventricle at 130 stimuli/min with a Medtronics model 5800 pacemaker. In these experiments, the data were directionally similar to those from the experiments in which pacing was not employed.

Acetylstrophanthidin, 0.5 mg/ml, in 30% ethanol, was administered as a 1-ml systemic intravenous bolus. Control injections, 1 ml of 30% ethanol of the same pH and osmolality were given to eight dogs. The left gracilis muscle was denervated after the first acetylstrophanthidin injection in nine dogs and at the start of the experiment in four dogs.

In five dogs local cholinergic blockade of the innervated, separately perfused right gracilis muscle was achieved with intra-arterial injections of 0.2-0.4 ml of atropine (0.5 mg/ml). The blockade was tested with intra-arterial norepinephrine (0.1 ml of a 1-µg/ml solution). Acetylcholine produced decreases in vascular resistance of at least 10 resistance units (RU) which were consistently abolished by atropine. In five dogs, local alpha-receptor blockade of the innervated, separately perfused right gracilis muscle was achieved with intra-arterial phenoxybenzamine (0.1-0.3 ml of a 10-µg/ml solution). The blockade was tested with intra-arterial norepinephrine (0.1 ml of a 1-µg/ml solution). In two of the five dogs, the responsiveness of the gracilis muscle vascular bed...
before and after 0.3 ml of phenoxybenzamine was tested with intra-arterial angiotensin II (0.1 ml of a 1-μg/ml solution) and intra-arterial isoproterenol (0.1 ml of a 1-μg/ml solution).

Acetylstrophanthidin was administered intravenously after bilateral cervical vagotomy in five dogs, vagotomy and subsequent bilateral denervation of the carotid bifurcations in seven dogs, and denervation of the carotid bifurcations alone in two dogs. Carotid denervation abolished the transient increase in vascular resistance of the gracilis muscle produced by clamping the ipsilateral common carotid artery.

In experiments in which the interaction of the direct constrictor effect of acetylstrophanthidin and muscle exercise was examined, the drug was given intra-arterially to the isolated denervated (constant flow) right gracilis muscle in eight dogs. Acetylstrophanthidin was administered as a bolus of 0.1 mg (in 0.2 ml) or as a constant infusion of 0.5 mg/ml (0.05 ml/min), with a Braun infusion pump. Gracilis muscle exercise was achieved by gracilis nerve stimulation with a Grass SD5 stimulator at frequencies ranging from 0.2 to 1.5 contractions/sec at 2—5 v and 0.5-msec duration. Acetylstrophanthidin was given as a bolus or infused beginning 1—2 minutes before the start of exercise. The duration of exercise was 1.5—2 minutes.

Mean changes from control in the vascular resistance of the gracilus muscle in all experiments were expressed as RU ± se. Significance was determined by Student's t-test.

Results

NEUROGENIC EFFECT OF SYSTEMICALLY ADMINISTERED ACETYLSSTROPHANTHIDIN

Separately Perfused, Innervated Right Gracilis Muscle.—Following 31 intravenous injections of acetylstrophanthidin to 23 animals, there was a mean fall followed by a sustained rise in the vascular resistance of the innervated, separately perfused right gracilis muscle (Fig. 2). (Eight animals each received two injections, which were at least 45 minutes apart.) The fall in vascular resistance from a mean control level of 28.4 ± 2.4 RU was 4.0 ± 0.9 RU (P < 0.001), occurring at 1 minute after injection. The subsequent prolonged rise in vascular resistance achieved statistical significance (P < 0.05) at 2 minutes after injection (Fig. 2) and remained significantly above control until 18 minutes, at which time it returned toward control. There was an accompanying small but significant increase in mean aortic pressure beginning 20 seconds after injection, which remained greater than 5 mm Hg above control from 40 seconds to 12 minutes after injection. However, in eight of the above experiments in 5 dogs in which systemic blood pressure initially
either did not rise or decreased following acetylstrophanthidin administration, there was a mean initial fall in the vascular resistance of the gracilis muscle of 4.8 ± 0.8 RU associated with a mean decrease in mean aortic pressure of 5.6 ± 1.8 mm Hg (Fig. 3); this indicated that the initial fall in vascular resistance could not be explained solely as a reflex response to the increase in systemic blood pressure. Similarly, the sustained increase in vascular resistance could not be explained on a reflex basis related to the change in systemic blood pressure since, in addition to the increase in mean aortic pressure, systemic pulse pressure did not change significantly in the 23 animals. The mean control pulse pressure was 33.5 ± 2.8 mm Hg and the pulse pressure 10 minutes after acetylstrophanthidin injection was 33.6 ± 2.9 mm Hg.

In five dogs, the mean initial decreases in vascular resistance were not significantly different (P > 0.1) before (5.6 ± 2.0 RU) and after (2.0 ± 0.7 RU) intra-arterial administration of atropine to the separately perfused muscle. The control resistances before and after atropine were 32.0 ± 7.7 and 23.8 ± 5.1 RU, respectively. In each of five additional animals, the prolonged increase in vascular resistance was abolished by prior intra-arterial administration of phenoxybenzamine. In these animals, the mean change in vascular resistance of the gracilis muscle at 10 minutes after the intravenous administration of acetylstrophanthidin was +6.9 ± 1.3 RU before and −0.3 ± 0.2 RU after intra-arterial administration of phenoxybenzamine (P < 0.01). These changes in vascular resistance were from control resistances of 26.6 ± 6.1 and 14.3 ± 1.2 RU, respectively. In the eight animals that received two injections of acetylstrophanthidin in the absence of phenoxybenzamine, the mean changes in vascular resistance at 10 minutes after the first and second injections were +6.9 ± 2.8 RU and +10.0 ± 2.5 RU, respectively. These changes were from control resistances of 28.3 ± 5.3 RU and 25.7 ± 3.5 RU and were not significantly different from each other, thus indicating that tachyphylaxis to acetylstrophanthidin did not occur. In the phenoxybenzamine-blockade experiments, the change in vascular resistance 1 minute after the intra-arterial injection of norepinephrine was +7.3 ± 2.1 RU before and −0.3 ± 0.4 RU after intra-arterial administration of phenoxybenzamine (P < 0.05), indicating that an adequate blockade of the alpha-receptors had been achieved. In the two gracilis muscles receiving the highest amount of phenoxybenzamine, vascular reactivity to intra-arterial angiotensin and to intra-arterial isoproterenol was maintained, indicating preservation of vascular reactivity following treatment with this alpha-receptor blocking agent. Following angiotensin, the vascular resistances were 117% of control before and 138% after phenoxybenzamine in the first animal and 230% before and 200% after the drug in the second. Similar results with isoproterenol were 60% and 60%
and 42% and 36%, respectively. Figure 4 illustrates the data from one of these two animals.

**Autoperfused, Left Gracilis Muscle.**—As shown in Figure 5, in 18 experiments in 16 animals in the innervated, autoperfused left gracilis muscle, there was a mean initial fall in vascular resistance followed by a prolonged subsequent rise. The fall in resistance from a control of 26.9 ± 4.3 RU was 2.4 ± 1.1 RU (P < 0.05) at 20 seconds after injection. There was a subsequent rise which remained greater than 15 RU above control (P < 0.005) from 2 to 16 minutes after injection. Following subsequent denervation in 9 of these animals and denervation at the beginning of the experiment in 4 additional animals, there was no significant initial dip and a mean sustained rise only to 3 RU (P < 0.05) above control (28.0 ± 5.2 RU). The transient increase to 14 RU above control at 1 minute may have represented the direct effect of an initial bolus of high concentration of acetylstrophanthidin. There was a significant difference between the sustained increases in vascular resistance in the innervated and the denervated left gracilis from 3 to 15 minutes following injection (P < 0.05). As can be seen in Figure 5, in the autoperfused muscle most of the sustained increase in vascular resistance appeared to be related to a neurogenic effect of acetylstrophanthidin.

**Afferent Receptor Pathways.**—In a total of ten animals, control injections of acetylstrophanthidin were followed by bilateral cervical vagotomy, carotid denervation, or both. As shown in Figure 6, the initial fall in vascular resistance of the separately perfused, innervated right gracilis muscle which occurred at 1 minute was reduced but not abolished by vagotomy, but it was abolished (P < 0.025) by subsequent carotid denervation. The prolonged rise at 10 minutes was unaffected by vagotomy and was not significantly reduced.

![Figure 4](image)

*The influence of the intra-arterial administration of phenoxybenzamine on the neurogenic vasoconstriction produced by intravenous administration of acetylstrophanthidin in the innervated, separately perfused right gracilis muscle. The drugs were injected in the order depicted, beginning at the upper left and ending at the lower right. See text for details.*

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by subsequent carotid denervation. In two animals, carotid denervation alone did not appreciably alter the sustained increase in vascular resistance following intravenous acetylstrophanthidin. The increases in resistance before and after carotid denervation were 18 and 27 RU in the first animal and 7 and 7 RU in the second animal, respectively. These increases were from control resistances of 30 and 32 RU in the first animal and 28 and 35 RU in the second.

To test the influence of the solvent, intravenous injections of 1 ml of 30% ethanol of the same pH and osmolality as acetylstrophanthidin were carried out. These produced no significant initial dip or subsequent rise in the vascular resistance of the innervated right gracilis muscle (13 experiments in nine dogs), innervated left gracilis muscle (6 experiments in six dogs), or denervated left gracilis muscle (2 experiments in two dogs).

Discussion

The data in this study demonstrate that in addition to a direct vasoconstrictor effect, acetylstrophanthidin produces a sustained neurogenically mediated elevation of skeletal muscle vascular resistance. This sustained neural effect appears to act through alpha-receptor stimulation. In the present study, this effect accounted for the predominant vasoconstrictor effect of the drug.

The afferent receptor of this neurally mediated increase in vascular resistance does not appear to be located in the heart or lungs, as evidenced by the lack of influence of bilateral vagotomy on the increase in vascular resistance. Furthermore, intact baroreceptors at the carotid bifurcations are not essential for...
The influence of bilateral vagotomy (left) and of combined bilateral vagotomy and denervation of the carotid bifurcations (right) on the neurally mediated initial decrease and subsequent sustained increase in vascular resistance following intravenous administration of acetylstrophanthidin. These data are from innervated, separately perfused right gracilis muscles. The first bar of each stippled pair represents the percent change in vascular resistance at 1 minute, and the second bar, the percent change at 10 minutes following the intravenous injection of 0.5 mg of the drug. Left: The mean resistance before acetylstrophanthidin was 26.3 ± 1.6 RU for controls and 21.8 ± 5.1 RU after vagotomy. The data are from five animals. Right: The mean resistance before acetylstrophanthidin was 28.7 ± 3.5 RU for controls and 24.2 ± 2.2 RU after vagotomy and carotid denervation. The data are from seven animals, four of which were included in the group with bilateral vagotomy alone.

this effect. This suggests that acetylstrophanthidin acts elsewhere in the peripheral circulation. It is not possible from the present data to localize further the peripheral site of action—for example, to determine whether the drug has a direct central nervous system effect or acts at ganglionic synapses.

The neurally mediated initial decrease in vascular resistance following intravenous administration of acetylstrophanthidin in the present studies is probably in part secondary to vasodilation which occurs reflexly to the pressor effect of the drug. However, as demonstrated in five animals, an increase in blood pressure following intravenous administration of acetylstrophanthidin is not necessary for the occurrence of the initial decrease in vascular resistance of the gracilis muscle. The finding that this decrease in gracilis resistance was completely abolished following bilateral vagotomy and subsequent denervation of the carotid bifurcations suggests that acetylstrophanthidin may, at least in part, act directly on afferent receptors in the heart and lungs or at the carotid artery bifurcations to stimulate the afferent limb of a reflex arc which results in a transient decrease in gracilis muscle resistance. This hypothesis is consistent with the recent report of Quest and Gillis (8) which demonstrated an increase in the rate of firing of afferent nerve fibers from carotid baroreceptors secondary to a direct stimulant effect of small amounts of acetylstrophanthidin on these receptors.

The observations on the neurally mediated vascular resistance changes in the present study are consonant with the efferent cardiac sympathetic nerve data of Gillis (4) in the decerebrate cat. Gillis found that the intravenous injection of ouabain first produces an
inhibition and then a stimulation of the spontaneous activity of the preganglionic sympathetic nerves to the cat heart. In his studies, denervation of the reflexogenic areas in the carotid sinuses and aortic arch prevented the inhibitory but not the stimulatory effect. There is a qualitative similarity between Gillis's cardiac sympathetic nerve data and our data concerning the alpha-receptor effect of acetylstrophanthidin on skeletal muscle vascular resistance. In light of the recent findings by Vatner and co-workers (9) that systemically administered ouabain is associated with an elevation of coronary vascular resistance in conscious dogs, it is possible, considering the present data obtained from skeletal muscle, that this elevation of coronary vascular resistance may in large part be neurally mediated.

Ross et al. (1) demonstrated vasodilatation in the separately perfused, innervated lower half of a dog following the intravenous administration of acetylstrophanthidin to the intact upper half of the dog. It is possible that the neurally mediated decreases in vascular resistance noted by these authors may have been only transient, as described in our data. Furthermore, the use of larger doses of acetylstrophanthidin by these authors resulted in a vasoconstrictor effect in the upper half of their preparations which was greater, and which therefore may have provided a greater stimulus to reflex withdrawal of sympathetic tone in the lower half of the animals than that in the experiments described in the present studies.

Under conditions of constant pressure perfusion, the sum of the direct and the neurogenic effects of acetylstrophanthidin in the present studies was to double vascular resistance. In view of the substantial net vasoconstrictor effect of digitalis on skeletal muscle, it is important to consider whether, in the presence of this drug, blood supply remains appropriate to the metabolic demands of the muscle. It has been well established that skeletal muscle exercise will overcome the vasoconstriction induced by stimulation of
sympathetic nerve fibers activating alpha receptors (10). In addition, the present data indicate that graded levels of exercise can progressively abolish the direct vasoconstrictor effect of acetylstrophanthidin on skeletal muscle, thereby allowing a blood flow which is appropriate to the level of metabolism of the muscle.

Thus, in the absence of muscle exercise, acetylstrophanthidin produces skeletal muscle vasoconstriction through both a direct and a neurally mediated effect. The latter is mediated through alpha-receptor stimulation and appears to be the predominant mechanism whereby this drug increases muscle vascular resistance.

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