Effects of Acetylstrophanthidin on Isolated Veins of the Dog

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ABSTRACT

Acetylstrophanthidin, 1.0 to 10.0 μg/ml, caused contraction of strips of the lateral saphenous vein of the dog in an organ bath at 37°C. The superior mesenteric vein, which exhibited spontaneous activity in the organ bath, responded to acetylstrophanthidin with an almost immediate increase in tension and an increased frequency of contractions; this was followed by a sustained contraction which had the same time lag as the contraction of a saphenous vein strip from the same dog. In strips from both veins, the contraction was depressed by cooling and augmented by warming, an effect opposite to that seen with norepinephrine-induced contractions. A latent period of 10 to 60 minutes preceded the contraction of the saphenous vein; during this time, the vein showed increased reactivity to added norepinephrine, 5-hydroxytryptamine, acetylcholine, barium chloride, increased bath concentration of K⁺, and decreased bath concentration of Na⁺. The increased reactivity and contraction still occurred after catecholamine depletion of the veins by chronic lumbar sympathectomy and in umbilical veins, which are devoid of adrenergic nerves. In addition to its direct constrictor action on cutaneous and mesenteric veins, acetylstrophanthidin sensitized cutaneous veins to vasoactive agents and to changes in ionic environment. These actions did not depend on the presence of norepinephrine in the veins.

ADDITIONAL KEY WORDS

superior mesenteric veins catecholamines
acetylcholine barium chloride denervated vein umbilical vein
temperature temperature and acetylstrophanthidin
electric stimulation

In a previous study, Brender and associates (1) showed that acetylstrophanthidin causes contraction of strips of the dog's cutaneous veins. During the latent period of 10 to 60 minutes which preceded the contraction, the veins showed increased reactivity to adrenergic stimulation. This sensitizing action of the digitalis glycoside on the venous smooth muscle to sympathetic nerve stimulation was confirmed in intact anesthetized dogs. The present studies were done to determine if this sensitizing action was specific for the sympathetic nervous system and if other veins react to acetylstrophanthidin like the cutaneous veins.

The results demonstrate that acetylstrophanthidin potentiates the contraction of strips of dog's lateral saphenous veins caused by several vasoactive drugs and by changes in ionic composition of the organ bath. Neither this potentiating effect of acetylstrophanthidin nor its vasoconstrictor effect depends on the presence of catecholamine stores in the vessel wall. The superior mesenteric vein responds to acetylstrophanthidin with an almost immediate increase in tension and an alteration of the pattern of its rhythmic contractions, followed by a more marked contraction later.

Methods

Lateral saphenous and superior mesenteric
Veins were removed from mongrel dogs (anesthetized with thiopental, 20 mg/kg, pentoarbital sodium, 60 mg/kg, or ether) and placed in physiologic salt solution of the following composition (mM): NaCl, 119; KCl, 4.7; KH₂PO₄, 1.18; MgSO₄, 1.17; NaHCO₃, 14.9; dextrose, 5.5; sucrose, 80; CaCl₂, 1.6; and calcium disodium ethylenediaminetetraacetate (Versenate), 0.026. The Versenate was added to chelate possible trace amounts of heavy metals which catalyze the auto-oxidation of catecholamines (2).

Helical strips, approximately 10 mm long and 3 mm wide, were prepared from the saphenous veins as described previously (1). Longitudinal strips of the same diameters were cut from the superior mesenteric veins. The strips were placed in a 10-ml organ bath filled with the same physiologic salt solution, aerated with a 95% O₂ to 5% CO₂ mixture, and maintained at 37°C. The distal end of the strip was fixed in a holder at the base of the bath. The upper end was connected to a force transducer (Grass, Model FT 03), and tension of the strip was recorded on a direct pen-writing Grass recorder. The transducer was mounted on a movable support which allowed fine adjustment of the strip length. The bath temperature was altered by warming or cooling the surrounding water jacket and was measured by a thermistor probe (Yellow Springs Instrument Co.).

After an equilibration period (30 to 60 minutes), the strips were placed at the optimal point of their length-active tension curve (3), using a standard stimulus (square waves, 10 v, 2 msec, 10 cps for 10 seconds) of transmural electrical stimulation (1, 4). In each experiment, each strip represented a vein taken from a different dog unless stated otherwise.

Umbilical veins were obtained from four pregnant dogs by hysterotomy under thiopental (20 mg/kg) anesthesia. As far as could be ascertained, the dogs were within 3 to 5 days of spontaneous delivery at the time of hysterotomy. In three dogs, the nerve supply to the left saphenous vein was interrupted by removal, under sterile conditions, of the paravertebral lumbar chain from L2 through L7. The dogs recovered from the surgical procedure, and the left and right saphenous veins were removed, under thiopental anesthesia, on the 19th, 20th, or 21st day after the sympathectomy. Strips from deservated and innervated (control) veins were mounted in the same organ bath for comparative study.

Because neither deservated veins nor umbilical veins responded to transmural electric stimulation, the optimal point of their length-active tension curve could not be determined precisely. The initial length of the strips was increased by 6% because previous studies (3) have shown that the reactivity of spiral strips of cutaneous veins reaches its optimum at about that length.

The following vasoactive agents were used: norepinephrine (Winthrop), 5-hydroxytryptamine ( serotonin creatinine sulfate, Aldrich Chemical Co., Inc.), acetylcholine chloride (Roche Laboratories), and barium chloride (Fisher Scientific Company).

Physiologic salt solutions deficient in NaCl ([Na+] = 90mM) were balanced osmotically by the addition of 11.9 g of sucrose for each 1 g of NaCl removed. To produce physiologic salt solutions containing increased concentrations of KCl, appropriate amounts of KCl were added to standard physiologic salt solutions. A stock solution was prepared by dissolving 1 mg of acetylstrophanthidin (Sandoz) in 10 ml of physiologic salt solution. Between experiments, the solution was kept at 5°C. All “concentrations” refer to final concentrations in the muscle bath and are expressed as micrograms per milliliter.

Results

Effect of Acetylstrophanthidin on Cutaneous and Superior Mesenteric Veins

Cutaneous Vein Strips.—The addition of acetylstrophanthidin to the organ bath was followed by a contraction at 40 to 60 minutes with 1 μg/ml (22 veins), 25 to 45 minutes with 5 μg/ml (5 veins), and 10 to 30 minutes with 10 μg/ml (8 veins).

Superior Mesenteric Vein Strips.—Mesenteric vein strips exhibited rhythmic activity under control conditions; they responded almost immediately to the addition of acetyl- strophanthidin at 1 μg/ml with an increase in tension and an increase in the frequency of contractions. Over this time interval, there was no effect on base-line tension of a saphenous vein strip taken from the same dog and mounted in the same organ bath. Similar results were obtained on comparison of saphenous and mesenteric vein strips from a total of 20 dogs.

An additional eight mesenteric and eight saphenous strips were exposed to larger doses of acetylstrophanthidin for a longer period (Fig. 1). A concentration of 10 μg/ml resulted in a rapid increase in tension in the superior mesenteric strip, followed by a partial relaxation. A contraction occurred in both mesenteric and saphenous strips after 20 minutes; there was no significant difference...
Response of superior mesenteric and saphenous vein strips (two dogs) to prolonged exposure to acetylstrophanthidin. Superior mesenteric vein strips respond with immediate increase in tension. Subsequently, both strips contracted simultaneously.

Norepinephrine

Acetylstrophanthidin 10 μg/ml

Washout

0.1 μg/ml

Potassium Chloride

Acetylstrophanthidin 10 μg/ml

Washout

50 μl

Potentiation by acetylstrophanthidin of responses of saphenous vein strips to added norepinephrine (top), acetycholine (middle), and potassium chloride (bottom).

(P > 0.8) in the time of onset of this contraction in mesenteric and saphenous strips from the same dog.

Increased response of cutaneous veins to vasoactive agents in presence of acetylstrophanthidin

During the latent period preceding the contraction caused by acetylstrophanthidin, the saphenous strips exhibited increased reactivity to several vasoactive agents.

Norepinephrine—After addition of acetylstrophanthidin, 10 μg/ml, the response to norepinephrine was augmented (Fig. 2). When the strip was washed free of norepinephrine with physiologic salt solution containing acetylstrophanthidin, 10 μg/ml, it relaxed.
and the tension returned to the control level. In four strips, at 3 minutes after addition of acetylstrophanthin, the mean increase in response to norepinephrine (0.01 μg/ml) was 47% (range, 43 to 55%) of the control value.

5-Hydroxytryptamine.—A similar augmentation by acetylstrophanthin of the contraction caused by 5-hydroxytryptamine (10 μg/ml) was demonstrated in eight saphenous strips. The mean increase in response at 3 minutes after addition of acetylstrophanthin to the bath was 14% (range, 6 to 25%) of the control value.

Acetylcholine.—The contraction caused by acetylcholine (0.1 μg/ml) was augmented by acetylstrophanthin (Fig. 2). In eight strips, at 3 minutes after addition of acetylstrophanthin, 5 μg/ml, the mean increase in response was 185% (range, 100 to 400%) of the control value.

Potassium Chloride.—The contraction caused by potassium chloride (50mM) was augmented by acetylstrophanthin (Fig. 2). In three strips, at 3 minutes after addition of acetylstrophanthin, 10 μg/ml, the increases in response were 133, 156, and 500% of the control value.

After the period of increased reactivity to added vasoactive agents, there was a later contraction due to the direct action of acetylstrophanthin. When this contraction was maximal, addition of these agents did not cause any further increase in tension (Fig. 2).

MODIFICATION BY VASOACTIVE AGENTS OF RESPONSE OF CUTANEOUS VEINS TO ACETYLSTROPHANTHIN

Norepinephrine.—Acetylstrophanthin (1 μg/ml) was added to seven strips partially contracted by norepinephrine (0.01 μg/ml). This resulted, almost immediately, in an additional contraction that averaged 36% (range, 25 to 50%) of the contraction produced by norepinephrine alone (Fig. 3, bottom). When the response to norepinephrine plus acetylstrophanthin was at a plateau, the strips were washed with fresh physiologic salt solution containing acetylstrophanthin. In every case the tension returned to the level present before addition of norepinephrine. An acetylstrophanthin contraction occurred after 40 to 55 minutes. A second strip cut from the same vein and mounted in a separate organ bath served as control. Addition of norepinephrine (0.01 μg/ml) to this strip resulted in a contraction that rapidly reached a plateau and then gradually diminished (Fig. 3, top).

When acetylstrophanthin (10 μg/ml) was added to six strips partially contracted by norepinephrine (0.01 μg/ml), there was an almost immediate increase in tension. A further contraction occurred after 20 minutes. Addition of acetylstrophanthin (10 μg/ml) to control strips, in the absence of exogenous norepinephrine, resulted in a contraction at 15 to 20 minutes.

Increased Bath [K+]—Acetylstrophanthin (1 μg/ml) was added to seven strips partially contracted by an increased bath concentration of potassium. This resulted in an almost immediate additional contraction that averaged 173% (range, 66 to 300%) of the contraction produced by increased [K+] alone. Addition of acetylstrophanthin, 1 μg/ml, to control strips had no effect on baseline tension during this period.

In these experiments, another strip was cut from the same vein and mounted in a separate organ bath. This strip was stretched, by raising the attached transducer, so that the baseline tension was increased to the level produced by placing the strips in physiologic salt solution (26mM, KC1). Addition of the same dose of acetylstrophanthin to the strip which was stretched did not result in early contraction.

![FIGURE 3](http://circres.ahajournals.org/)

**FIGURE 3**

Potentiation by acetylstrophanthin of contractile response of saphenous strip to added norepinephrine.
ACETYLPATROPHANTHIDIN AND VEINS

When acetylstrophanthidin, 5 μg/ml, was added to six strips partially contracted by an increased bath [K⁺], there was an almost immediate increase in tension. A further contraction occurred at about 25 to 30 minutes. Addition of acetylstrophanthidin, 5 μg/ml, to the control strip, in the absence of increased [K⁺], resulted in a contraction at about 25 to 30 minutes.

Decreased Bath [Na⁺].—Acetylstrophanthidin, 1 μg/ml, was added to six strips partially contracted by a [Na⁺], 90mM. This resulted in an almost immediate additional contraction that averaged 234% (range, 37 to 500%) of the contraction produced by decreased [Na⁺] alone.

Barium Chloride.—Acetylstrophanthidin, 1 μg/ml, was added to five strips in the presence of 1mM BaCl₂. These strips responded with an almost immediate increase in tension averaging 300% (range, 30 to 700%) of the contraction produced by BaCl₂ alone.

When the response to acetylstrophanthidin was at a plateau in the strips partially contracted by increased [K⁺], decreased [Na⁺], or addition of BaCl₂, the strips were washed with fresh physiologic salt solution containing acetylstrophanthidin. In every case, the tension returned to the level present before alteration of the ionic composition of the bath, and an acetylstrophanthidin-induced contraction occurred after 45 to 60 minutes. In another series of experiments, one strip was cut from saphenous veins of seven dogs and each was compared with a strip of mesenteric vein of the same dog but mounted in a separate bath. When 1mM BaCl₂ was added to the saphenous strip, there was a contraction in six; the remaining strip showed no change in baseline tension. Addition of acetylstrophanthidin, 5 μg/ml, to both baths resulted, in all experiments, in an almost immediate increase in tension followed, after about 30 minutes, by a more marked contraction in both saphenous and mesenteric strips.

EFFECT OF ACETYLPATROPHANTHIDIN ON DENERVATED SAPHENOUS VEINS AND UMBILICAL VEINS

Experiments were performed on saphenous veins from three dogs which had been subjected to chronic unilateral lumbar sympathectomy and on umbilical veins from four dogs.

Figure 4 illustrates the responses 19 days after unilateral lumbar sympathectomy. Strips from the denervated vein failed to contract with electric stimulation or with added tyramine, and there was an enhanced response to added norepinephrine. Addition of acetylstrophanthidin resulted in contraction in the denervated vein after 43 minutes. The contraction in the innervated vein occurred, in this experiment, after 55 minutes. During the latent period which preceded the contraction of the innervated vein, there was an increased reactivity to electric stimulation. Acetylstrophanthidin caused contraction of strips from all three denervated veins as well as strips from all umbilical veins.

Acetylstrophanthidin (1 μg/ml) was added to strips of all innervated and denervated saphenous and umbilical veins partially contracted by addition of physiologic salt solution with KCl, 26mM. This resulted in an almost immediate tension increase in all veins but had no immediate effect on tension of these strips bathed in physiologic salt solution.

**Figure 4**

Responses of strip from denervated saphenous vein (19 days after unilateral lumbar sympathectomy) compared to responses of strip from control (innervated) vein. Denervation is demonstrated by absence of response to electric stimulation and added tyramine and increased response to added norepinephrine.
EFFECT OF TEMPERATURE ON RESPONSE OF CUTANEOUS VEINS TO ACETYLSYROphanTHIDIN

Temperature and Latent Period.—Increasing the bath temperature shortened and decreasing the temperature lengthened the latent period preceding the contraction of saphenous strips. Three strips were cut from the same vein and mounted in separate baths. One bath was left at 37°C, one was increased to 42°C, and one was decreased to 25°C. Increasing the temperature had no effect on base-line tension; decreasing the temperature caused either no change or a slight decrease in base-line tension. Addition of acetylsrophanthidin, 5 μg/ml, to each bath caused contraction in 15 ± 4 (SE) minutes at 42°C and in 33 ± 8 minutes at 37°C (P < 0.05, n = 6). In every case the strip at 25°C failed to contract over a period equal to three times the latent period at 37°C.

Temperature and Contraction.—Two strips

![Figure 5](image1.png)

**FIGURE 5**
Effect of changes in bath temperature on response of saphenous strips to electric stimulation and acetylsrophanthidin.

![Figure 6](image2.png)

**FIGURE 6**
Effect of changes in bath temperature on response of saphenous strips to acetylsrophanthidin, increased bath [K⁺], and barium chloride.

of a saphenous vein were mounted in different baths: one was contracted by electric stimulation and the other, by acetylstrophanthidin. During electric stimulation, warming to 42°C depressed and cooling to 29°C augmented the contraction. The acetylstrophanthidin contraction (which was of comparable magnitude) was augmented by warming and depressed by cooling.

The data from 11 saphenous veins are summarized in Figure 5. At each temperature, the response is expressed as percent of the difference between the baseline tension and the highest value observed during electric stimulation or contraction by acetylstrophanthidin (these maximal values were obtained at 29°C for electric stimulation and at 42°C for acetylstrophanthidin). Similar results were obtained with mesenteric veins from four additional dogs.

Two strips were cut from each of six additional saphenous veins and mounted in separate baths. One strip was contracted by acetylstrophanthidin and the other, by an increased bath [K⁺]. Warming augmented and cooling depressed the contractions of both strips (Fig. 6). Strips from six additional dogs were contracted by barium chloride. Warming augmented and cooling depressed the contraction (Fig. 6, right).

Discussion

Previous studies (1) have shown that digitalis glycosides sensitize the smooth muscle of the dog's cutaneous vein to sympathetic nerve stimulation, as well as having a direct constrictor effect. The present in-vitro experiments extend these observations on the reactivity of cutaneous veins and compare the responses to acetylstrophanthidin in cutaneous and mesenteric veins. Because all strips were first placed at the optimal point of their length-active tension curve (3), valid comparisons could be made between the reactions of strips taken from the same or different veins of the same dog or between veins from different dogs.

During the latent period which precedes the contraction of saphenous strips by acetylstrophanthidin, there is a period of increased reactivity to different vasoactive agents. These agents cause contraction by a variety of mechanisms, all of which lead to an increase in ionized calcium in the vicinity of the contractile protein of the cell. Norepinephrine is thought to produce a contraction both by liberation of intracellular membrane-bound calcium and by an action on the cell membrane via the action potential mechanism (5). Acetylcholine acts by depolarization of the cell membrane with an increase in permeability to sodium, potassium, and possibly calcium ions as well (6). Briggs (7) and Waugh (6) have shown that in vascular smooth muscle the presence of a high external potassium ion concentration allows rapid entry of extracellular calcium ion into the interior of the cell. A similar effect on calcium flux has been demonstrated in the presence of a decreased external sodium ion concentration by Niedergerke (9) in the frog ventricle. Barium ions are thought to act both by liberating intracellular membrane-bound calcium and by having a direct effect on the contractile proteins (10, 11). The mechanism of the potentiation by acetylstrophanthidin may be related to an increased availability and mobilization of intracellular calcium, secondary to inhibition by the glycoside of membrane-bound sodium- and potassium-activated adenosine triphosphatase (12). Bohr (13) has suggested that in arterial smooth muscle the increased reactivity to catecholamines seen in the presence of desoxycorticosterone occurs by a loosening of intracellular calcium ion bonds, thus permitting more calcium ions to be released during excitation. Because we have not measured sodium, potassium, or calcium ion fluxes directly, we have no data either for or against these possibilities.

It has been suggested (14) that intact cardiac stores of norepinephrine are necessary for the positive inotropic effect of digitalis glycosides. However, using papillary muscles from hearts subjected to chronic total cardiac denervation, Spann and associates (15) showed that "cardiac norepinephrine stores..."
The present study shows that neither intact vascular stores of norepinephrine nor an intact adrenergic innervation is essential for the increased venous reactivity or contractile response of saphenous strips to acetylstrophanthidin. The norepinephrine content of the denervated veins was not measured directly, but the failure of these vessels to respond to electric stimulation and tyramine and their supersensitivity to exogenous norepinephrine suggest that their catecholamine stores were considerably depleted. Transmural electric stimulation of the cutaneous vein, as applied in our experiments, has been shown (16-18) to cause contraction by a release of catecholamines from the sympathetic nerve terminals remaining in the vascular wall. The results of the studies with denervated veins were confirmed in the experiments with umbilical veins, which also failed to respond to electric stimulation and tyramine. Their norepinephrine content was not measured directly. Previous studies (19) have shown that human umbilical vessels contain less than 0.005 μg of norepinephrine per gram of wet tissue and have no histochemically demonstrable adrenergic nerves (20).

The differences observed between the responses of saphenous and superior mesenteric vein strips to acetylstrophanthidin reflect the different nature of the smooth muscle from each of these veins. The cutaneous venous smooth muscle falls into the "multiunit" category as defined by Bozler (21), whereas that of the superior mesenteric vein, which exhibits spontaneous rhythmic contractions in an organ bath, is an example of the "single-unit" type. The rhythmic contractions, evidence of cell-to-cell propagation, reflect a partially depolarized, more excitable cell membrane (22) with an increased concentration of ionized calcium in the vicinity of the contractile proteins. This results in an increase in intrinsic or myogenic tone (23-25). In the presence of exogenous norepinephrine, or potassium chloride, or barium chloride, a state of increased "venous tone" was produced in the saphenous strips and, in this situation, the saphenous strips responded to acetylstrophanthidin in a manner similar to that of the mesenteric strips.

Analysis of the early response to acetylstrophanthidin of saphenous strips in the presence of an increased venous tone indicated that, at a concentration which did not in itself produce a contraction, acetylstrophanthidin potentiated the contraction produced by an alteration of the external concentration of sodium or potassium, as well as the contraction produced by vasoactive agents. This suggested that the early response seen in the mesenteric vein reflects only an augmentation of the existing myogenic tone. The late contraction to acetylstrophanthidin, which occurred at the same time in both veins, reflects a direct effect of the drug independent of the presence of increased tone.

Vanhoutte and Shepherd (26) have shown that when excised segments of dog's saphenous veins are constricted with potassium or barium chloride, cooling the perfusate from 37°C depresses the constriction and warming augments it. By contrast, when the vein is constricted by electric stimulation, norepinephrine, acetylcholine, 5-hydroxytryptamine, or adenosine triphosphate, the vein constricts further with cooling and relaxes with warming. Thus, alteration of temperature allows classification of vasoactive agents into two broad groups. Our results show that the thermosensitivity of an acetylstrophanthidin-constricted vein resembles that of veins constricted by potassium or barium chloride. This suggests that acetylstrophanthidin acts at a site different from the sites of action of catecholamines and acetylcholine.

The sensitization of cutaneous veins to adrenergic stimulation (1) and vasoactive agents and the augmentation of intrinsic tone in the superior mesenteric vein may play roles in the overall hemodynamic effect of digitalis glycosides by mobilizing blood from the capacity system and increasing venous return, thus maintaining cardiac output in states of heart failure. Further in-vivo studies are required to correlate the relationship of these
in-vitro findings with the overall effects of digitalis glycosides on different components of the peripheral vascular system.

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References
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