Reflex Cardiovascular Effects of Epicardial Stimulation by Acetylstrophanthidin in Dogs

By Peter Sleight, M.D., Amrit Lall, Ph.D., and Martin Muers, B.A.

ABSTRACT

Application of 25 to 100 μg of acetylstrophanthidin to the epicardium of the left ventricle of anesthetized and unanesthetized dogs caused hypotension and bradycardia without signs of discomfort. The response developed after an average latency of 8 seconds and lasted up to 12 minutes. Cooling the cervical vagi to 8 to 10°C or prior application of 0.1% procaine hydrochloride to the epicardium of the heart blocked the response. The response was, therefore, a reflex; the sensory receptors were located in the surface layers of the left ventricle. Electrophysiological recordings from single and multifiber preparations of the right recurrent cardiac nerve showed that the receptors for this reflex were mechanoreceptors whose fibers belonged to the C group. The sinus bradycardia caused by digitalis glycosides may result partly from excitation of these receptors.

ADDITIONAL KEY WORDS
coronary chemoreflex digitalis bradycardia
cardiac depressor reflex left ventricular mechanoreceptors
C fiber afferents Bezold-Jarisch effect
epicardial chemoreflex vagal afferent fibers

Various causes have been ascribed to the bradycardia produced by therapeutic doses of digitalis. These are: (a) stimulation of the motor nuclei of the vagus (1, 2), (b) an increase in the sensitivity of the heart to motor vagal impulses (3, 4), (c) stimulation of the nodose ganglion (5), (d) sensitization of the carotid sinus (6), and (e) stimulation of the receptors responsible for the Bezold reflex (7). The last cause has been difficult to separate from other causes because of the long latency of action of digitalis glycosides available in the past.

One of us (PS) reported that a reflex bradycardia and hypotension follows local application of nicotine or veratridine to the epicardium of the left ventricle of the dog, but not to that of the right ventricle or atria (8). The receptors lie in the epicardium and myocardium of the left ventricle (9). This method of stimulating the cardiac receptors makes it possible to study effects of drugs on these receptors uncomplicated by effects on other receptors when a drug is injected into the systemic circulation. It was therefore thought worthwhile to investigate the action of local application to the epicardium of a rapidly acting glycoside, acetylstrophanthidin, to learn more about the mechanism of bradycardia caused by digitalis.

Methods

STUDY OF THE REFLEX RESPONSE

These experiments were performed on 17 dogs weighing 8.5 to 22.0 kg; 4 were intact and conscious, 13 were anesthetized by intravenous injection of 10% chloralose in polyethylene glycol 200 (80 to 100 mg/kg), or chloralose and urethane (70 mg/kg and 0.7 g/kg, respectively). Supplements of 20 mg/kg of chloralose were injected intravenously, when required, to maintain light anesthesia; no experimental procedure...
was undertaken for 15 minutes thereafter. Rectal 
temperature was maintained at 37 ± 1°C.

The trachea was intubated, positive-pressure 
artificial respiration was administered with a 
Harvard pump, and the chest was opened by a 
median sternotomy. The pericardium was opened 
longitudinally and a cradle was formed by suturing 
the edges to the chest wall. Systemic 
arterial blood pressure, sensed by a strain gauge 
transducer, and electrocardiogram (lead II) were 
recorded on a Grass Polygraph.

The conscious dogs were prepared as follows. 
They were anesthetized with sodium pentobarbi-
tal, 30 mg/kg, and were operated on under 
aseptic conditions. Two polyvinyl catheters (1.2 
mm i.d. and 1.8 mm o.d.) were sewn in the 
pericardial sac over the left ventricle, a third was 
placed in the ascending aorta by way of the left 
internal mammary artery. The catheters were 
filled with solution of heparin sodium (50 
mg/ml), and were led out of the thorax through 
the skin between the scapulas. Studies with drugs 
were done 24 hours later.

Drug solutions at room temperature (warming 
was found unnecessary) were injected into the 
pericardial sac of conscious dogs, or were squirted 
on the surface of the left ventricle of anesthetized 
dogs, and, immediately after the response to the 
drug had been recorded, were washed off by 3 
successive amounts of 10 ml of physiological 
saline. Each wash was aspirated from the 
pericardial sac (or cradle) in order to reduce 
systemic absorption of drugs.

The concentrations and volumes of solutions of 
drugs injected into the pericardial sac or cradle 
were: nicotine bitartrate (Brewer Co., Inc.) 25 to 
100 µg/ml of base in 0.9% NaCl, 0.5 to 1.0 ml; 
procaine hydrochloride 0.1%, 1 to 3 ml; acetyl-
strophanthidin,1 25 to 100 µg/ml, 0.5 to 1.0 ml in 
3% or 6% ethanol in saline. Before a drug was 
applied or injected, the response to control 
injection of saline or ethanol-saline was tested. In 
one dog, atropine sulfate, 1% solution, was used as 
a parasympathetic blocking agent.

In two dogs, acetylstrophanthidin (20 and 40 
µg) was injected directly into the anterior 
descending branch of the left coronary artery by 
means of a fine catheter passed retrograde up a 
small peripheral branch.

In five dogs, we studied the temperature at 
which conduction in afferent vagal fibers was 
blocked. For this, both cervical vagi were laid on 
thermodes through which cold water circulated. 
It took about 5 minutes for the temperature of the 
nerves, sensed by a needle thermister (Yellow 
Springs Instruments Co., Inc.), to become stable 

1Kindly supplied by Eli Lilly & Co.

at the desired level. Before and after cooling the 
vagi the response of the dog to drugs was tested 
when the nerves lay on the thermodes and water 
at 37°C circulated through them. In this way the 
possibility of nerve block due to mechanical or 
ischemic factors was excluded.

ELECTROPHYSIOLOGICAL STUDIES

Techniques for the surgical procedures and 
electrical recordings were similar to those de-
scribed in detail by Sleight and Widdicombe (9). 
These experiments were done on 16 additional 
dogs anesthetized as described earlier. Loss of 
blood due to hemorrhage was counteracted by 
continuous infusion of dextran solution (Dextra-
lan 110; dextran in 0.9% saline, Fisons Pharma-
cuticals) into a vein at the rate of 20 ml/hour 
throughout the experiment. Rectal temperature 
was maintained at 37 ± 1°C.

In one dog, a narrow metal cannula was 
inserted into the mouth of the left common carotid 
artery, or way of the left common carotid artery. 
Intracoronary injections were made through this 
cannula. Drug and control solutions were applied 
the surface of the heart as described earlier.

The arterial blood pressure, electrocardiogram, 
nerve action potentials and time-event marker 
were displayed on a dual beam oscilloscope and 
photographed on 70-mm paper.

All electrical recordings were obtained from 
fibers in the right recurrent cardiac nerve. The 
appearance of potentials in nerve fibers following 
gentle mechanical stimulation with a blunt 
instrument of discrete areas of the surface of the 
heart signified that the nerve fibers were from 
cardiac mechanoreceptors. Conduction velocities 
of these nerve fibers were determined by 
measuring the time between the electrical 
stimulation of the surface of the heart and the 
recording of action potentials in the nerve fiber 
and the distance between the points of electrical 
stimulation and recording.

The highest discharge frequency in the nerve 
fibers in response to stimulation of the epicardial 
receptors by drugs was compared with that 
preceding the stimulation. The highest discharge 
frequencies preceding and following stimulation 
of the epicardium were selected from among the 
discharge frequencies measured at 5-second 
intervals over 40 seconds. If the poststimulation 
frequency was twice, or more than twice, the 
prestimulation frequency, the drug was con-
considered to have stimulated the epicardial recep-
tors. No further analysis was performed in this 
case. If the poststimulation frequency was more 
than, but less than twice, the prestimulation 
frequency, the comparison of the two frequencies 
was done by comparing the means of frequencies 
measured at ten consecutive intervals of 2 seconds
**TABLE 1**

Effect of Application of Acetylstrophanthidin to the Epicardium of the Left Ventricle of the Dog

<table>
<thead>
<tr>
<th>Dog</th>
<th>Dose (µg)</th>
<th>Blood Pressure (mm Hg) (systolic/diastolic)</th>
<th>Heart rate (beats/min)</th>
<th>Latency of response (sec)</th>
<th>Duration of action (min)</th>
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<td>10/15</td>
<td>120</td>
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<tr>
<td>17</td>
<td>50</td>
<td>100/50</td>
<td>25/40</td>
<td>83</td>
<td>25</td>
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**MEAN ± SE**

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<td></td>
<td>150 ± 7.5/104 ± 7.7</td>
<td>25 ± 4.5/26 ± 4.8</td>
<td>144 ± 7.2</td>
<td>24 ± 4.5</td>
<td>8 ± 0.7*</td>
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*Mean of 16 experiments.
each preceding and following stimulation of cardiac mechanoreceptors by drugs. The means of
the two sets of frequencies were compared by application of Student's t-test. Differences
were considered significant when $P < 0.05$.

The difference in the responses of cardiac mechanoreceptors to nicotine and acetylstrophanthidin was evaluated by application of Student's t-test for paired comparisons.

**Results**

**REFLEX RESPONSE**

The effects of acetylstrophanthidin are shown in Table 1. Except in dog 3, it caused a
decrease in blood pressure and heart rate in both conscious and anesthetized dogs (Fig.
1). Similar effects of nicotine have been reported earlier by Sleight (8). The response
to acetylstrophanthidin began in an average of 8 seconds and lasted several minutes. The full
duration of the response was studied in only two dogs. The response in one of these dogs is
illustrated in Figure 2. Transient cardiac arrhythmias or ectopic beats occurred in 7 of
17 dogs studied. In these dogs the response was terminated by washing the pericardial sac
free of the glycoside. The response to acetylstrophanthidin could be repeated every
30 to 60 minutes, but no sooner, which means that tachyphylaxis developed. During this
time, responses to nicotine, which could otherwise be elicited every 10 minutes, were
also absent. In the conscious dogs, no signs of pain or discomfort were noticed as a result of
intrapericardial injection of acetylstrophanthidin.

**Effect of Vagal Cooling and Atropine.**—The hypotension and bradycardia caused by application of acetylstrophanthidin to the epicardium of the left ventricle was completely blocked in all five dogs tested when their cervical vagi were cooled to 8 to 10°C (Fig.
1). At this temperature, electrical stimulation of the vagus above the site of cooling caused a
slowing of the heart rate indicating that efferent fibers in the vagus were still conduct-
In one dog, intravenous administration of 1 mg/kg of atropine sulfate 30 minutes before application of acetylstrophanthinidin to the epicardium of the left ventricle blocked the bradycardia but not the hypotension.

Effect of Intrapericardial Procaine.—In all four dogs tested, 1 to 3 ml of 0.1% procaine hydrochloride injected into the pericardial sac 60 seconds before injection of acetylstrophanthinidin blocked the action of the latter. Application of procaine after the application of acetylstrophanthinidin and at a time when the action of acetylstrophanthinidin appeared to be maximal, restored the blood pressure and heart rate to control levels in 40 to 50 seconds (Fig. 1), very much earlier than would have occurred spontaneously. Procaine in the quantities tested produced no effect on the blood pressure or the heart rate, nor did it affect the bradycardia resulting from peripheral vagal stimulation (Fig. 1).

Effect of Intracoronary Acetylstrophanthinidin.—In two dogs, the effect of intracoronary injection of acetylstrophanthinidin (20 and 40 μg) was compared with that of similar doses applied to the epicardium. The responses were similar in character, time of onset, and duration of action (Fig. 2).

Electrophysiological Studies

The effect of intrapericardial application of acetylstrophanthinidin was tested in 26 preparations (12 single-fiber preparations and 14 multifiber [2- to 3-fiber] preparations). Each fiber was connected to a receptor area in the ventricles. The locations of these areas were: right ventricle, 3; anterolateral surface of the left ventricle, 5; left ventricular surface near the interventricular groove, 7. The receptor areas of five preparations were more diffusely located in the entire surface of the left ventricle. The receptors of six preparations were stimulated by vigorous deformation of the left ventricular surface, but not by gentle stroking.

The resting discharge of the fibers was sparse, often less than one impulse per cardiac cycle, and was not related to the cardiac or respiratory cycles. The mean resting peak discharge was 1.4 impulses/sec. Conduction velocity, measured in six fibers, was 0.5 to 2.1 m/sec.

Epicardial application of acetylstrophanthinidin (100 μg) increased the frequency of discharge of 7 of 12 single-fiber preparations, and of 7 of 14 multifiber preparations. The receptors that responded to acetylstrophanthinidin were all located in the left ventricle. In one single-fiber preparation, acetylstrophanthinidin, injected into the left coronary artery, also evoked a response. A rather higher proportion of preparations (18/23) responded to nicotine. This may indicate a less easy diffusion of the large acetylstrophanthinidin molecule into the myocardium than that of the nicotine molecule.

The response to application of acetylstrophanthinidin to the epicardium of the left ventricle was qualitatively similar to that
Responses of a small multifiber (one large and one small spike) preparation to intrapericardial injections of (A) nicotine, and (B) acetylstrophanthidin. Traces in all panels from above down are: electrocardiogram, electroneurogram, brachial arterial blood pressure, event marker. The panels in B were taken just before, and 20, 60, and 180 seconds after injection of acetylstrophanthidin.

It is unlikely that the bradycardia is secondary to a positive inotropic effect of the drug because (1) we did not observe any increase in systolic arterial pressure immediately after application of acetylstrophanthidin to the epicardium, and (2) the continuous electrical discharge of the cardiac mechanoreceptors in response to application of acetylstrophanthidin or nicotine is unlike the striking rhythmic discharge caused by intravenous injection of epinephrine (9). Intrapericardial injection of acetylstrophanthidin in conscious dogs elicited no signs of discomfort. Pain is therefore not the stimulus for this reflex.

Application of 0.1% procaine hydrochloride solution to the epicardium of the heart blocked the response of the receptors. Procaine is a relatively poor anesthetic when applied to the cornea or mucous membranes (10), and is also rapidly destroyed by the blood (11). Furthermore, dilute solutions of procaine take several hours to block conduction in the desheathed sciatic nerve of the frog (12). It is therefore unlikely that procaine blocks the action of epicardial application of acetylstrophanthidin by impairing conduction.

Discussion

These experiments demonstrate that small amounts of acetylstrophanthidin when locally applied to the epicardium of the left ventricle of the dog cause hypotension and bradycardia. This response is similar to that caused by injection of similar doses of acetylstrophanthidin into the left coronary artery. Cooling the cervical vagi to 8°C blocked the response. At this temperature the efferent vagal fibers were still conducting. Therefore, even though acetylstrophanthidin in appropriate concentrations may excite receptors elsewhere, in our experiments the hypotension and bradycardia caused by application of acetylstrophanthidin to the epicardium of the left ventricle are due to reflexes arising in the heart. The afferent fibers of this reflex are in the vagus nerves.
in deeply located fibers. Its most likely site of action is on nerve endings or fine axons near their terminations, located close to the surface of the left ventricle. This interpretation is in agreement with that expressed by Paintal (13) for other receptor sites. The failure of intrapericardial procaine to affect either the blood pressure or the heart rate suggests that, in the dog under resting conditions, few tonic impulses originate in the receptors in the epicardium of the left ventricle.

The failure of atropine to block the hypotension caused by epicardial application of acetylstrophanthidin suggests that the hypotension was not due solely to bradycardia but probably also due to diminution of cardiac output, peripheral resistance, or both. This agrees with similar findings on the failure of atropine to block the hypotension caused by veratridine (14) and nicotine (8). The reflex hypotension caused by application of nicotine to the surface of the dog's heart has been reported to involve cholinergic sympathetic fibers to the skeletal muscle; these fibers need large doses of atropine to block them (15). A reflex withdrawal of sympathetic tone could also be the cause of the hypotension as preliminary findings (Muers and Sleight, unpublished observations) suggest.

We have shown that the left ventricular mechanoreceptors are stimulated by both acetylstrophanthidin and nicotine (Fig. 3). Cross tachyphylaxis develops to both drugs and is similar to that reported for other sites (5). We infer from these findings that the same receptors are involved in the response to both drugs. The properties of these receptors have been reported by Sleight (9). They are mechanoreceptors, sensitive to the rate of change of ventricular pressure and volume. Their fibers are slowly conducting and belong to the C group. It is possible that the sinus bradycardia resulting from digitalis therapy is at least partly caused by excitation of these mechanoreceptors.

Acknowledgment

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References

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