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 \( \mu \)g 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 digitalis bradycardia
coronary chemoreflex left ventricular mechanoreceptors
cardiac depressor reflex epicardial chemoreflex vagal afferent fibers
C fiber afferents Bezold-Jarisch effect

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 pentobarbital, 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; acetylstrophanthidin, 1 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 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 described 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 (Dextran 110, dextran in 0.9% saline, Fisons Pharmaceuticals) 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 coronary artery by way of the left common carotid artery. Intracoronary injections were made through this cannula. Drug and control solutions were applied to 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 considered to have stimulated the epicardial receptors. 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

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1Kindly supplied by Eli Lilly & Co.
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>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>Fall</td>
<td>Control</td>
<td>Fall</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>25</td>
<td>105/80</td>
<td>30/35</td>
<td>132</td>
<td>32</td>
</tr>
<tr>
<td>2</td>
<td>60</td>
<td>175/110</td>
<td>65/55</td>
<td>160</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>50</td>
<td>165/135</td>
<td>00/00</td>
<td>126</td>
<td>0</td>
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<tr>
<td>4</td>
<td>50</td>
<td>180/125</td>
<td>15/15</td>
<td>160</td>
<td>18</td>
</tr>
<tr>
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<td>100</td>
<td>145/110</td>
<td>10/15</td>
<td>186</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>100</td>
<td>185/140</td>
<td>15/10</td>
<td>175</td>
<td>3</td>
</tr>
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<td>8</td>
<td>100</td>
<td>175/125</td>
<td>40/65</td>
<td>170</td>
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<td>195/150</td>
<td>20/15</td>
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<td>11</td>
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<td>10</td>
<td>100</td>
<td>125/85</td>
<td>30/40</td>
<td>170</td>
<td>65</td>
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<tr>
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<td>115/70</td>
<td>10/15</td>
<td>120</td>
<td>15</td>
</tr>
<tr>
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<td>50</td>
<td>100/50</td>
<td>25/40</td>
<td>83</td>
<td>25</td>
</tr>
</tbody>
</table>

**MEAN ± SE**

<table>
<thead>
<tr>
<th>Blood Pressure</th>
<th>Heart rate</th>
<th>Latency of response</th>
<th>Duration of action</th>
</tr>
</thead>
<tbody>
<tr>
<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>
</tr>
</tbody>
</table>

*Mean of 16 experiments.
Effect of procaine and vagal cooling on the epicardial reflex; all records from the same dog. Numbers above the blood pressure records refer to heart rate. The response to acetylstrophanthidin (intrapericardial) (records 2 and 4) was abolished when the vagi were cooled to 8°C (record 3). The response was cut short by the injection of 3 ml of 0.1% procaine into the pericardium (records 2 and 4). Neither the vagal cooling nor the procaine caused a block of the motor fibers of the vagus, since electrical vagal stimulation above the cooled area (records 3 and 4) still caused bradycardia. Ethanol (3%) (upper trace) is a solvent for acetylstrophanthidin. Time marked in seconds.

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 conducting.
In one dog, intravenous administration of 1 mg/kg of atropine sulfate 30 minutes before application of acetylstrophanthidin 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 acetylstrophanthidin blocked the action of the latter. Application of procaine after the application of acetylstrophanthidin and at a time when the action of acetylstrophanthidin 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 Acetylstrophanthidin.**—In two dogs, the effect of intracoronary injection of acetylstrophanthidin (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 acetylstrophanthidin 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 acetylstrophanthidin (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 acetylstrophanthidin were all located in the left ventricle. In one single-fiber preparation, acetylstrophanthidin, 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 acetylstrophanthidin molecule into the myocardium than that of the nicotine molecule.

The response to application of acetylstrophanthidin to the epicardium of the left ventricle was qualitatively similar to that
produced by similar application of nicotine (9) (Fig. 3), but was more delayed (several seconds) in its onset (5 of 8 preparations, \( P < 0.05 \)), and more prolonged in its duration. The peak frequency of discharge reached after application of acetylstrophanthidin was less than that after application of nicotine (9/9, \( P < 0.01 \)).

**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. Theafferent fibers of this reflex are in the vagus nerves.

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.
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 acetylstrophanthinidin 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 acetylstrophanthinidin 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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