Effect of Digitalis on Carotid Sinus Baroreceptor Activity
By John A. Quest and Richard A. Gillis

ABSTRACT
The effect of intracarotid injections of ouabain (25.0 μg) or acetylstrophanthidin (1.56 μg) on feline carotid sinus baroreceptors was evaluated using an isolated perfused carotid sinus preparation. The effects of the drugs on baroreceptor activity were determined by monitoring carotid sinus nerve activity and systemic arterial blood pressure. Administration of either drug to the isolated carotid sinus region altered the relationship between the change in carotid sinus pressure and the fall in systemic arterial blood pressure. Raising the carotid sinus pressure produced a greater depressor response when digitalis was present (-29.6 ± 3.8 mm Hg) than it did when digitalis was absent (-15.3 ± 2.6 mm Hg). Digitalis administration also resulted in a greater increase in carotid sinus nerve discharge when carotid sinus pressure was increased. At a constant carotid sinus pressure, digitalis increased the spontaneous firing of the carotid sinus nerve; the drug-induced augmentation in nerve firing was identical to that produced by raising carotid sinus pressure. These changes in baroreceptor activity in the presence of digitalis occurred without drug-induced changes in the pressure in the isolated carotid sinus region. The results demonstrate that the digitalis preparations studied can directly alter the sensitivity of baroreceptors and produce significant changes in carotid sinus nerve activity and cardiovascular function.

KEY WORDS acetylstrophanthidin ouabain carotid sinus nerve activity cat baroreceptor accommodation blood pressure

We have recently reported results which suggest that digitalis excites the carotid sinus baroreceptors (1); dose-dependent increases in the spontaneously occurring electrical activity of the intact carotid sinus nerve are evoked by intracarotid administration of ouabain or acetylstrophanthidin in cats. Although the mechanism for the increased activity has not been investigated in detail, several observations indicate that the drug sensitizes the baroreceptors. One indication of sensitization is that the enhanced sinus nerve activity produced by intracarotid administration of ouabain or acetylstrophanthidin is associated with reductions in blood pressure and contractile force; these cardiovascular responses are identical to those produced by pressure-induced stimulation of the baroreceptors (2). Another indication of sensitization is that digitalis induces marked neural firing in preparations in which the carotid body chemoreceptors have been destroyed by acetic acid. These studies are incomplete, however, and subject to other interpretations. First, digitalis could have acted on receptors in the carotid sinus region other than the baroreceptors. In this regard, chemical stimulation of chemoreceptors which are not of the classical type (i.e., do not respond to changes in oxygen tension) but are located in the carotid body has been reported, in some instances, to produce hypotension and a decrease in contractile force in dogs (3, 4). Second, digitalis could have evoked reflex activity indirectly by raising pressure in the carotid sinus region. Digitalis is known to have a direct vasoconstrictor effect on smooth muscle (5), and Treat and co-workers (6) have recently reported that intra-arterial infusion of ouabain constricts the common carotid artery in dogs. Third, an action of digitalis on the sympathetic innervation to the carotid sinus regions (7–9) could have initiated the reflex effects of the drug. Electrical stimulation of these sympathetic fibers has been shown to alter carotid sinus baroreceptor (10, 11) and chemoreceptor (12) activity, and digitalis is known to exert excitatory effects on the sympathetic nervous system (13, 14).

In view of the number of ways in which ouabain and acetylstrophanthidin could influence carotid
sinus nerve activity, the present study was undertaken to determine the mechanism of reflex activation produced by these substances.

**Methods**

Experiments were performed on cats of either sex (1.7-3.6 kg) anesthetized with α-chloralose (60-80 mg/kg, iv). The trachea of each cat was intubated and mechanical ventilation was instituted with 98.5% O₂-1.5% CO₂; tidal volumes ranged from 30 to 35 ml and rates ranged from 30 to 35/min. Under these conditions, arterial blood pH was maintained in the range of 7.32 to 7.45 throughout the experiment. Rectal temperature was maintained at 37.0-38.0°C with a heating pad. Catheters were inserted into the right femoral vein and artery to administer drugs and record blood pressure, respectively. All cats were routinely heparinized (5 mg/kg, iv). Arterial blood pressure and lead II of the electrocardiogram were recorded continuously; heart rate determinations were made from the electrocardiogram. All recordings were made on a Beckman RM dynograph recorder.

The carotid sinus regions were exposed by reflecting the trachea and esophagus, and the carotid sinus nerve on the right side was carefully dissected free of surrounding tissues. The internal carotid, ascending pharyngeal, occipital, and superior thyroid arteries on the right side were also identified and ligated. Sympathetic nerve fibers innervating the right carotid sinus region via projections from the superior cervical ganglion (7, 8) were routinely served from their central connections before they reached the sinus region. The right nodose ganglion was surgically ablated. The carotid sinus regions were bathed in mineral oil warmed to 37.5 ± 0.5°C with a thermoregulator.

To study the changes in systemic arterial blood pressure evoked by alterations in carotid sinus perfusion pressure, a modification of the Moissejeff preparation (15) was employed. The right external carotid artery was clamped, and a cannula was inserted into the common carotid artery and moved to the area of the carotid bifurcation. The cannula was connected to a reservoir containing heparinized saline. A sphygmomanometer bulb was connected to the reservoir and used to vary the air pressure on the saline perfusing the sinus region. Carotid sinus pressure was recorded with a Statham P23 series transducer connected to a sidearm leading from the carotid cannula. A polyethylene cannula was introduced into the lingual artery for administering drugs intra-arterially to the carotid sinus region. With this apparatus, carotid sinus perfusion was initially set at a static pressure of 50 mm Hg and then abruptly elevated to 100 mm Hg for 5-10 seconds. The changes in systemic arterial blood pressure thus produced were compared before and after intra-arterial administration of digitalis into the sinus region. In these experiments, both vagus nerves were sectioned to prevent compensatory reflex effects initiated by aortic arch and cardiac receptors from interfering with responses to carotid sinus baroreceptor activation.

To study the changes in carotid sinus nerve electrical activity evoked by alterations in carotid sinus perfusion pressure, a modification of the technique described by Price and Widdicombe (16) and Biscoe and Millar (17) was used (Fig. 1). A glass T-shaped cannula was inserted into the right common carotid artery below the carotid sinus region. One branch of the T-shaped cannula was connected by soft plastic tubing to a 100-ml blood reservoir pressure bottle, which in turn was connected to a manometer and a sphygmonanometer bulb. About 10–20 ml of blood was allowed to flow from the cat into the reservoir, and this blood was replaced by an equivalent volume of systemically administered dextran. The air pressure on the blood in the reservoir could be altered as desired. When the branch of the T-shaped cannula connected to the reservoir was clamped (at B), the carotid sinus region was exposed to the pulsatile pressure of the cat. When both the external and common carotid arteries were clamped (at A and C, respectively) and the clamp at B was opened, the carotid sinus region was exposed to the static pressure level present in the blood reservoir pressure bottle. The pressure within the carotid

![Diagram depicting the experimental setup used for applying pressures to the carotid sinus region.](http://circres.ahajournals.org/content/35/2/248.full.png)
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sinus region was recorded with a Statham P23 series transducer connected to a catheter placed in the lingual artery; a stopcock was placed proximally to the transducer for injecting drugs intra-arterially into the sinus region. The right carotid sinus nerve was isolated according to the procedure of von Euler et al. (18). The nerve was sectioned close to the point where it joins the glossopharyngeal nerve, and the peripheral end was placed on bipolar platinum electrodes. Activity was amplified using a Tektronix type 3A-9 amplifier and then displayed on an oscilloscope, taped, and subsequently photographed. The criteria used to establish that the nerve activity was of baroreceptor origin were as follows: (1) neural activity occurred in synchrony with the systolic pressure, (2) neural activity was abolished by either occluding the carotid artery below the carotid bifurcation or lowering the pressure of the isolated carotid sinus region to zero, and (3) neural activity progressively increased with pressure in the isolated carotid sinus region.

With this procedure for studying changes in carotid sinus nerve activity evoked by changes in carotid sinus pressure, two types of experiments were conducted. In one type, the carotid sinus was first isolated by placing the clamps at A and C and removing the clamp at B (Fig. 1). A given basal level of static pressure was applied to the sinus region, and the clamp at B was replaced. The pressure in the reservoir was then elevated by 50 or 100 mm Hg, the clamp at B removed, and the sinus suddenly exposed to the elevated pressure for 4–5 seconds. The pressure was then lowered to the basal level, and the clamp at B was replaced. This procedure was carried out several times prior to and after digitalis was administered intra-arterially to the sinus region, and the changes in nerve activity were recorded. In the other type of experiment, a given basal level of static pressure was applied to the sinus region, and the change in the level of intrasinus pressure induced by the intra-arterial administration of digitalis was recorded. In several of these experiments, changes in carotid sinus neural activity were also recorded and compared with those produced by mechanically raising the level of carotid sinus pressure.

The following drugs were used in these experiments: acetylstrophanthidin,1 ouabain octahydrate (Sigma Chemical Company), heparin sodium (Fisher Scientific Company), and a-chloralose (Établissements Kuhlmann, Paris, France). The acetylstrophanthidin was received in 29% ethyl alcohol, and dilutions were made in the same solvent. Ouabain octahydrate was also dissolved in ethyl alcohol. No significant cardiovascular or neural effects were produced by the volume of solvent used in these experiments.

Data were analyzed using paired comparisons and Student's t-test for grouped data as described by Simpson and colleagues (19). The criterion for significance was P < 0.05.

Results

Studies were performed using the digitalis preparations acetylstrophanthidin and ouabain, since both drugs have been shown previously to have identical effects on carotid sinus nerve activity (1). The doses chosen were those that were found to be active by the intracarotid route used in our previous study (1). In the first series of experiments, the effect of acetylstrophanthidin on the systemic arterial blood pressure response evoked by a step increase in carotid sinus pressure (Moissejeff technique) was studied in four cats. Before the drug was administered, the level of pressure within the isolated carotid sinus region was set at 50 mm Hg. Next, intrasinus pressure was abruptly elevated to 100 mm Hg for 5 seconds and then lowered back to 50 mm Hg. This procedure was repeated several times to ensure reproducibility in the systemic arterial blood pressure response. Next, acetylstrophanthidin (1.56 μg) was administered by intracarotid injection, and the procedure was repeated 1–2 minutes later. The drug had no effect on either the intrasinus pressure or the systemic arterial blood pressure, but it did increase the systemic arterial hypotensive response evoked by doubling the intrasinus pressure. The data are summarized in Table 1; the depressor response occurring with increased carotid sinus pressure in the presence of digitalis was twice as great as that in the absence of the drug.

Figure 2 shows that the change in arterial blood pressure was greater when baroreceptor pressure was raised in the presence of digitalis. Exposure of the carotid sinus to a pressure step of 50 mm Hg resulted in a fall in arterial blood pressure (A). Intra-arterial administration of 1.56 μg of acetylstrophanthidin into the isolated sinus region maintained at 50 mm Hg did not affect either the carotid sinus pressure or the systemic arterial blood pressure (B). However, when the pressure in the

TABLE 1

<table>
<thead>
<tr>
<th>Condition</th>
<th>Initial (mm Hg)</th>
<th>Change (mm Hg)</th>
<th>Percent change</th>
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</thead>
<tbody>
<tr>
<td>Before acetylstrophanthidin</td>
<td>154 ± 5.6</td>
<td>-15.3 ± 2.6*</td>
<td>-9.8 ± 1.4*</td>
</tr>
<tr>
<td>After acetylstrophanthidin (1.56 μg, ia)</td>
<td>153 ± 5.0</td>
<td>-29.6 ± 3.8*</td>
<td>-19.2 ± 2.7*</td>
</tr>
</tbody>
</table>

Values are means ± se from four cats.

* P < 0.05 with paired comparisons (i.e., Student's t-test for the difference between paired samples).

† P < 0.05 compared with corresponding value obtained before acetylstrophanthidin administration.

1 Generously supplied by Dr. G. C. Chiu, Eli Lilly and Company, Indianapolis, Indiana.
Effect of acetylstrophanthidin on changes in systemic blood pressure produced by increasing the intrasinus pressure. A: Control systemic blood pressure response obtained by raising intrasinus pressure from 50 mm Hg to 100 mm Hg. B: Systemic blood pressure response obtained by raising intrasinus pressure from 50 mm Hg to 100 mm Hg after intracarotid injection of 1.56 µg of acetylstrophanthidin. C: Effect of raising intrasinus pressure from 50 mm Hg to 100 mm Hg on systemic blood pressure after the ipsilateral carotid sinus nerve has been sectioned.

In a second series of experiments, the effect of ouabain on the carotid sinus nerve electrical activity evoked by alteration of carotid sinus perfusion pressure was studied in three cats. In each experiment, the neural responses to the increases in intrasinus pressure were greater after the administration of ouabain (Fig. 3). The usual sinus nerve discharge in response to the normal pulsatile pressure prior to isolation of the carotid sinus region is shown in Figure 3A. B illustrates the sinus nerve firing in the isolated carotid sinus preparation produced by a static pressure of 50 mm Hg; no distinct electrical discharge is evident. When the intrasinus pressure was mechanically increased from 50 mm Hg to 100 mm Hg for 4-5 seconds (C), the sinus nerve discharged. The intrasinus pressure was then mechanically lowered to 50 mm Hg, and the neural discharge disappeared (D). Ouabain (25 µg) was administered intra-arterially between D and E; it had little effect on sinus nerve activity at a pressure of 50 mm Hg and no effect on carotid sinus pressure. However, when the pressure was mechanically raised to 100 mm Hg, the neural discharge increased in frequency and amplitude compared with that seen in the absence of ouabain (E vs. C).

In one of the three experiments in this series, it was possible to count the number of spikes occurring in the sinus nerve when the intrasinus pressure was increased in steps. Control pressure was set at 50 mm Hg and raised first to 100 mm Hg then to 150 mm Hg. The relationship between intrasinus pressure and sinus nerve spike frequency was determined before and after ouabain administration. In both cases, a linear relationship was noted between intrasinus pressure and sinus nerve activity (Fig. 4). As intrasinus pressure was raised from 50 mm Hg to 100 mm Hg and then to 150 mm Hg, spike discharge frequency increased from 0 impulses/sec to 130 impulses/sec and then to 220 impulses/sec (repeated twice, Fig. 4, controls 1 and 2). After ouabain administration, the line relating pressure and impulse discharge frequency was...
shifted to the left. For pressures of 100 mm Hg and 150 mm Hg, spike discharges were 190 impulses/sec and 290 impulses/sec, respectively.

In a third series of experiments, the effect of intracarotid administration of ouabain (25 μg) on carotid sinus nerve discharge was compared with the effect of pressure on carotid sinus nerve discharge in four cats. In these experiments, the sinus neurogram, sinus pressure, systemic arterial blood pressure, and heart rate were monitored. The left of Figure 5 depicts the changes in intrasinus pressure and the right of the figure shows the simultaneous changes in the carotid sinus neurogram for a representative experiment. In A, the neural activity during a constant static pressure of 100 mm Hg is shown. B shows the neural activity produced by raising sinus pressure to 200 mm Hg. Intrasinus pressure was then mechanically lowered to 100 mm Hg, and neural activity returned to control levels (C). While the intrasinus pressure was at 100 mm Hg, 25 μg of ouabain was administered; it increased the amplitude of the electrical discharge in a manner similar to that produced by mechanically increasing the intrasinus pressure to 200 mm Hg (compare D with B). Ouabain had no effect on the intrasinus pressure (arrow at D), and in the four experiments the actual change seen with 25 μg of ouabain administered intra-arterially was 3.7 ± 1.3 mm Hg. To ensure that changes in carotid sinus pressure could be detected by intracarotid administration of drugs, two experiments were performed in which 0.25 μg of norepinephrine was used in place of ouabain. In both, an increase in pressure occurred; in one experiment pressure rose from 50 mm Hg to 65 mm Hg and in the other it rose from 50 mm Hg to 69 mm Hg.

Discussion

The purpose of our study was to extend our previous observations (1) on the reflex effect of digitalis by focusing on the question of whether the drug alters the sensitivity of carotid sinus baroreceptors or whether other mechanisms are involved in this effect. The question was explored by exam-
ining the relationship between the change in carotid sinus pressure and the fall in arterial blood pressure before and after digitalis administration. It was found that for a given change in baroreceptor pressure there was a significantly greater change in arterial blood pressure when digitalis was present. This result is consistent with the idea that digitalis enhances the sensitivity of receptors in the carotid sinus that are baroreceptors of the classical type (i.e., receptors that respond in a specific manner to pressure).

To substantiate further that digitalis was acting on receptors that respond specifically to pressure, experiments were performed to examine the relationship between the changes in carotid sinus pressure and neural activity before and after digitalis administration. It was found that in the presence of digitalis a shift in this relationship occurred; that is, for a given change in carotid sinus pressure there was a greater increase in neural activity when digitalis was present (Fig. 4). Furthermore, the effects of changes in carotid sinus pressure on carotid sinus nerve discharge were mimicked by the administration of digitalis (Fig. 5).

Influence of intrasinus pressure and intracarotid injection of ouabain on electrical activity of the centrally cut carotid sinus nerve (right), heart rate, and systemic mean blood pressure (B.P.) (left) of an anesthetized cat. A and C: Control recordings obtained at an intrasinus pressure of 100 mm Hg. B: Recording obtained after raising intrasinus pressure to 200 mm Hg. D: Recording obtained at an intrasinus pressure of 100 mm Hg after administration of 25.0 μg of ouabain.

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In summary, three findings strongly indicate that carotid sinus baroreceptors per se are activated by digitalis drugs. (1) Intracarotid injections of digitalis result in a greater fall in systemic arterial blood pressure when carotid sinus pressure is increased. (2) Intracarotid injections of digitalis result in a greater increase in carotid sinus nerve discharge when carotid sinus pressure is increased. (3) Intracarotid injections of digitalis produce carotid sinus neural effects identical to those produced by raising the carotid sinus pressure.

These results can be explained either as a change in the threshold of baroreceptor excitation such that for a given pressure (between threshold and suprathreshold levels) the baroreceptors are more active or as a direct depolarizing action of the drug on the sensory nerve endings. The latter explanation appears untenable since digitalis had little effect on baroreceptor nerve firing when no activity was present in the nerve (Fig. 3). Furthermore, we are not aware of any studies which show that digitalis can initiate depolarization of neural membranes in quiescent structures. The postulation that a change in the threshold for excitation of sensory receptors occurred with digitalis is consistent with the reported actions of these drugs. It is well known that ouabain can inhibit the active transport of K\(^+\) and Na\(^+\) by the membrane (20, 21). Inhibition of this transport system abolishes post-stimulation hyperpolarization of unmyelinated fibers and thus changes the level of the membrane potential so that it is closer to the threshold potential. This change in effect would enable a given stimulus to become suprathreshold for a greater number of fibers and result in an increased frequency of discharge. Consistent with this explanation is the finding that the carotid sinus nerve contains a preponderance of unmyelinated afferent fibers (22).

We also found that the gradual reduction in neural activity (i.e., adaptation) that occurred after a step increase in carotid sinus pressure was reduced in the presence of ouabain (Fig. 3). This effect can be interpreted as an action of digitalis which involves modification of the accommodative properties of the baroreceptors.

Other possible ways in which sensitization of sensory receptors can occur with drugs have been pointed out by Paintal (23). One involves the ability of a drug to increase the magnitude of the generator potential of the sensory nerve endings. There is, as shown by Katz (24), a linear relationship between the magnitude of the generator potential and the frequency of the afferent discharge in sensory endings. Another mechanism for sensitization involves the ability of a drug to enhance the recovery of sensory nerve endings in the absence of a change in threshold (i.e., a given natural stimulus can produce an impulse after a shorter delay following another stimulus). This effect would enable threshold excitability of the nerve ending to be attained after a shorter interval following a stimulus and thus would allow for an increased frequency of discharge. These phenomena can also account for the effects observed with digitalis in this study.

In addition to a direct effect of a drug on sensory nerve endings, sensitization can also result from an indirect action of a drug. The drug could either contract the vascular tissues in which the sensory endings lie or increase the activity of the sympathetic fibers supplying the sinus region. It has been shown that certain drugs such as epinephrine, applied topically to the wall of the carotid sinus region, can produce excitation of the carotid baroreceptors (25). This effect results from contraction of the smooth muscle in the vicinity of the baroreceptor endings, which causes distortion of the nerve endings and results in an enhancement of neural activity. This possibility was ruled out in our study; it was shown that digitalis could activate the reflex mechanism without producing a rise in the pressure in the isolated sinus.

Several investigators have indicated that the carotid sinus receives adrenergic innervation from the superior cervical ganglion (7, 8). Furthermore, stimulation of these sympathetic fibers to the sinus region results in increases in carotid sinus baroreceptor activity (11). Digitalis increases sympathetic nervous activity in the cat (13), and therefore a similar action on these sympathetic fibers could result in an apparent sensitization of the baroreceptors. However, in our studies these sympathetic fibers were routinely sectioned when the nodose ganglion regions were removed.

Other investigators have also suggested that digitalis can activate baroreceptors. Heymans and his colleagues (26) have reported that denervation of the carotid sinus and aortic baroreceptors prevents the cardiac slowing seen with intravenous administration of ouabain in dogs. In a later study (27), they investigated the effects of strophanthin on canine carotid sinus receptors with the cross-circulation perfusion technique. When this agent was administered into the cross-circulation circuit and allowed to perfuse the carotid sinus region of a
recipient dog, bradycardia was produced. The bradycardia could be prevented by not allowing the drug to reach the carotid sinus region. The conclusion of both studies was that digitalis-induced bradycardia resulted from an action on the baroreceptors. These findings are important, but they do not rule out the possibility that digitalis produces its effects by chemoreceptor stimulation. In contrast, Zipf and Ehrlicher (28) have reported that carotid sinus denervation does not influence bradycardia produced by intravenous administration of K-strophanthidin in rabbits and cats but that subsequent ligation of the aortic depressor nerves does abolish the fall in heart rate. They concluded that aortic arch reflexes are more important than carotid sinus reflexes in the action of digitalis. In agreement with Zipf and Ehrlicher, Chai and colleagues (29) also failed to demonstrate an important role for carotid sinus reflexes in the bradycardia produced by acetylstrophanthidin in cats. Instead, they concluded from studies using intracarotid injections of drug that receptors located within the nodose ganglia represent the principal site of the reflex action of digitalis. In our study, the nodose ganglia were denervated; therefore, these receptors did not participate in our responses. Furthermore, we have obtained preliminary data (30) indicating that nodose ganglia receptors play no role in the bradycardia produced by intravenous administration of acetylstrophanthidin; however, a major role for carotid sinus receptors has been documented. In regard to the findings of Zipf and Ehrlicher (28), if they had denervated the aortic arch before sectioning the carotid sinus nerves, they would have observed no effect of this procedure (31). Subsequent denervation of the carotid sinus regions would have abolished the digitalis-induced cardiac slowing (31). The reason for this effect is that both baroreceptor regions are involved in the response and one reflex arc can assume the function of the denervated one (30).

In summary, the sensitizing action of digitalis on baroreceptor activity appears to result from a direct action on sensory elements in the sinus region. The exact mechanism has not been elucidated, but available evidence indicates that both a reduction in threshold for excitation by pressure and a reduction in adaptive properties of the baroreceptor endings are probably involved.

References


7. GERARD MW, BILLINGSLEY PR: Innervations of the carotid body. Anat Rec 26:391-400, 1923


20. RITCHIE JM, STRAUB RW: Hyperpolarization which follows activity in mammalian non-medullated fibers. J Physiol (Lond) 136:90-97, 1957


23. PAINTAL AS: Effects of drugs on vertebrate mechanorecep-


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