Regional Hemodynamic Effects of a Digitalis Glycoside in the Conscious Dog with and without Experimental Heart Failure

By Charles B. Higgins, Stephen F. Vatner, and Eugene Braunwald

ABSTRACT

The effects of intravenous ouabain (C-strophanthin), 20 µg/kg, on the superior mesenteric, renal, and external iliac beds were studied over a 30-minute period in eight normal conscious dogs before, and in five of them after, cholinergic blockade and in six conscious dogs with heart failure produced by tricuspid avulsion and progressive pulmonary stenosis. Blood flows were measured with Doppler ultrasonic and electromagnetic flowmeters. In normal dogs, prior to cholinergic blockade, ouabain caused early increases in mesenteric, renal, and external iliac resistances but later, between 15 and 30 minutes, mesenteric resistance decreased below the control level while renal and external iliac resistances remained elevated. After cholinergic blockade, the ouabain-induced alterations in renal and iliac resistance were unaltered, but the later decline in resistance in the mesenteric bed was reversed, resulting in sustained constriction. The late decline in mesenteric resistance also was not observed in the conscious animal after the injection of ouabain, 5 µg/kg, directly into the mesenteric artery or in the majority of dogs given ouabain intravenously after having been anesthetized with pentobarbital sodium. In the conscious dogs with heart failure, ouabain resulted in increased renal and iliac resistances initially, and in vasodilatation later while mesenteric resistance fell initially and remained below control for the entire 30 minutes. Thus, in the normal conscious dog, ouabain causes vasoconstriction in the regional vascular beds, apparently by its direct vascular action, and, in addition, a cholinergically mediated vasodilatation in the mesenteric bed. In the conscious dog with heart failure, ouabain increases blood flow to all beds, reducing the compensatory vasoconstriction of the low output state.

KEY WORDS anesthesia mesenteric vascular bed heart rate ouabain arterial pressure renal vascular bed iliac vascular bed blood flow cholinergic blockade

In addition to their well-known myocardial effects (1–5), digitalis glycosides cause a direct and potent action on the peripheral circulation of man (2, 6) and experimental animals (7–12). In normal man and in normal animals, this action is manifested as a large increase in resistance in the systemic vascular bed (2, 5, 7, 10, 12) and in several regional circulatory beds (6–12). However, the extent to which the various regional beds participate in this increase in resistance has not been determined. It has also been suggested that alterations in the functional status of the circulatory system, as may occur in states of myocardial depression, can influence the overall effect of digitalis compounds upon total peripheral resistance. Thus, in the studies of Harvey et al. (13) and of Mason and Braunwald (6), total peripheral resistance...
declined when digitalis glycosides were administered to patients with congestive heart failure.

To gain a clearer understanding of the effects of digitalis glycosides on the peripheral circulation, we sought to elucidate the pattern of response of three major regional circulations in both normal healthy dogs and dogs with experimental heart failure. Since previous studies have shown that anesthesia can substantially alter both the myocardial (5) and peripheral circulatory (12) effects of digitalis glycosides, the present investigation was conducted in unsedated, conscious dogs.

**Methods**

Six adult mongrel dogs weighing between 22 and 29 kg underwent a two-stage surgical procedure for the production of congestive heart failure by a modification of the technique described by Barger et al. (14). Using general anesthesia (pentobarbital Na, 30 mg/kg) and sterile surgical technique, the tricuspid valve was avulsed and a pneumatic cuff placed around the main pulmonary artery. Partial inflation of the pneumatic cuff with saline at various intervals over 2 to 3 weeks produced progressive pulmonary stenosis and eventually congestive heart failure (Table 1). In these six dogs with heart failure and in four other normal dogs, laparotomies were performed for implantation of Doppler ultrasonic flow probes on the superior mesenteric, left renal, and left iliac arteries. In an additional six normal dogs, Doppler flow probes were implanted on the superior mesenteric, left renal, and left iliac arteries. In an additional six normal dogs, Doppler flow probes were implanted on the superior mesenteric artery only, and in three of these normal dogs a P 20 polyethylene catheter was placed in a branch of the superior mesenteric artery proximal to the flow probe. Electromagnetic flow probes were implanted on the superior mesenteric artery in four other normal dogs and, in addition, on the renal artery of one of them; pneumatic occluders were placed on the arteries in each dog just distal to the flow probes. At the time of laparotomy, catheters filled with heparin were placed in the aorta through a lumbar artery to monitor arterial pressure during the experiments.

The experiments were conducted 2 to 3 weeks after laparotomy in both normal dogs and dogs with heart failure and at least 2 months after thoracotomy in the animals with heart failure. Two of the dogs in the heart failure group were studied in the normal state prior to the production of heart failure. While the dogs reclined unrestrained in the conscious and unsedated state, measurements of arterial pressure and blood flow were recorded continuously during the control period and for 30 minutes after the intravenous administration of a 20 µg/kg dose of ouabain. In five of the normal dogs, the response to ouabain was determined on a separate day after partial cholinergic blockade had been produced with 0.1 to 0.2 mg/kg of atropine. Larger doses of atropine, which induce essentially complete cholinergic blockade, produce excitement in the conscious dog, thereby interfering with accurate hemodynamic observations, and hence were avoided.

In six dogs with Doppler flow probes and in two with electromagnetic flow probes implanted on the mesenteric artery only, the response to a 20 µg/kg dose of ouabain was determined after general anesthesia had been induced with pentobarbital Na, 30 mg/kg, and respirations controlled with a Harvard pump. The standard dose of ouabain employed under the above conditions, 20 µg/kg, did not produce observable gastrointestinal or cardiac toxicity; slightly higher doses frequently did produce vomiting and occasionally arrhythmias in the conscious dogs and were avoided in this study except in terminal experiments on three anesthetized dogs. The latter, previously instrumented with Doppler flow probes on the superior mesenteric artery, had electromagnetic flow probes placed just proximal to the Doppler probes during a second laparotomy, and mesenteric flow was monitored simultaneously by the two flowmeters during the control period and for 30 minutes after the intravenous administration of ouabain.

<table>
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<th>TABLE 1</th>
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<tr>
<td><strong>Characterization of Experimental Heart Failure</strong></td>
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<td>Incidence of ascites</td>
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<td>RV/LV middle free wall thickness</td>
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<td>RV norepinephrine conc (µg/g heart)</td>
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<td>RV peak systolic pressure (mm Hg)</td>
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<td>RV end-diastolic pressure (mm Hg)</td>
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Values except incidence of ascites are means ± se.
administration of 50 \( \mu g/kg \) ouabain. In three conscious dogs the effects of a small dose of ouabain, 5 \( \mu g/kg \), injected into a catheter previously placed into a proximal branch of the superior mesenteric artery were studied. This dose of ouabain produced no observable systemic effects, as evidenced by negligible alterations in arterial pressure and heart rate.

Blood flow in the regional beds was determined as the product of blood flow velocity measured by the Doppler ultrasonic flowmeter (15) and the cross-sectional area of the vessel measured at autopsy. Because the cross-sectional area of the blood vessel at autopsy can be expected to vary from that during life, the absolute values for volume flow must be interpreted with caution. Velocity, as measured by the Doppler flowmeter, is linearly related to volume flow as long as the cross-sectional area within the transducer does not vary (15, 16). At autopsy, the vessel walls were found to be closely attached to the transducer by a fibrous shell, minimizing changes in cross-sectional area with alterations in arterial pressure. Furthermore, volume calibrations conducted in our laboratory by timed collections of blood have verified the accuracy and linearity of the flowmeter (16). Zero flow was repeatedly determined electrically and confirmed terminally by mechanical occlusion of the vessel. A prior study from our laboratory demonstrated repeatedly and consistently the accuracy and constancy of electrical zero with the Doppler flowmeter (16). In those dogs with electromagnetic flow transducers, blood flow was measured with a gated square wave electromagnetic flowmeter (Statham SP2300), and zero flow was ascertained by mechanical occlusion of the vessel by inflation of the pneumatic cuff placed distal to the flow transducer. Arterial pressure was sensed by the catheter previously placed in the central aorta and measured with a Statham P23Db strain-gauge manometer.
REGIONAL HEMODYNAMIC EFFECTS OF DIGITALIS

<table>
<thead>
<tr>
<th>Renal flow (ml/min)</th>
<th>Renal resistance (mm Hg/ml min⁻¹)</th>
<th>Iliac flow (ml/min)</th>
<th>Iliac resistance (mm Hg/ml min⁻¹)</th>
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<td>45</td>
<td>0.81</td>
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Mean arterial pressure and mean blood flows were derived by RC electronic filters with a 2-second time constant. A cardiotachometer (Beckman type 9857B) triggered by the electrical signal from the arterial pressure pulse, provided instantaneous and continuous records of heart rate. Data were recorded on a multichannel tape recorder and written out on a multichannel oscillograph. Mean regional vascular resistances were calculated as the quotient of mean arterial pressure and mean regional blood flows. Differences between the normal dogs before and after cholinergic blockade and between the normal dogs and those with heart failure were tested for statistical significance by the group t-test (17).

Results

Normal State.—In eight normal conscious dogs, mean arterial pressure rose immediately following the intravenous administration of 20 μg/kg ouabain, at 15 minutes had risen 8 ± 2% (se) above a control value of 101 ± 3 mm Hg, and remained slightly above control at 30 minutes. Heart rate decreased below the control of 83 ± 4 beats/min within 1 minute and remained decreased at 5 minutes, but by 15 minutes it had returned almost to control levels and remained near control levels for the duration of the 30-minute observation period (Fig. 1, Table 2).

Renal blood flow fell below the control value of 187 ± 25 ml/min during the first minute following the intravenous injection of ouabain, decreased by 10 ± 4% below control at 15 minutes, and remained below control for the entire 30-minute observation period. Renal vascular resistance rose in the first minute from a control value of 0.61 ± 0.10 mm Hg/ml...
Time course of the hemodynamic effects of ouabain in normal conscious dogs before cholinergic blockade. Control values are indicated on the base line.

The effect of ouabain on the mesenteric bed was biphasic. Initially, it caused mesenteric flow to decrease below a control value of 273 ± 27 ml/min, but by 15 minutes the flow increased to 20 ± 5% above control and remained elevated for the remainder of the 30-minute observation period. Mesenteric vascular resistance increased transiently above a control value of 0.40 ± 0.04 mm Hg/ml min⁻¹ during the first 5 minutes after ouabain; it decreased 15 ± 3% below control at 15 minutes, and was still below control at 30 minutes (Fig. 1). The same dose of ouabain administered intravenously to eight normal anesthetized dogs again caused an initial profound decrease in mesenteric flow below the control value of 259 ± 38 ml/min and an increase in resistance above the control value of 0.45 ± 0.03 mm Hg/ml min⁻¹, but under these circumstances the later vasodilatation was observed in only two of the eight animals, and the mean value for the group as a whole between 15 to 30 minutes was not significantly different from the control value (Table 3). On the other hand, 50 µg/kg ouabain administered intravenously to three of these dogs anesthetized on another day, caused large increases in calculated mesenteric vascular resistance for the duration of the 30-minute observation period, both when mesenteric flow was measured with the Doppler and electromagnetic flowmeters (Table 3). The sustained increase in mesenteric resistance for the entire 30 minutes was also observed in these three dogs in the conscious state when a dose of ouabain too small to elicit observable systemic effects was administered directly into the mesenteric artery (Table 3).

Cholinergic Blockade.—In five normal conscious dogs after cholinergic blockade, the ouabain-induced elevations of arterial pressure were similar to those observed in the dogs not treated with atropine. After cholinergic blockade, heart rate progressively decreased from the level of 212 ± 16 beats/min, presumably reflecting to some extent both the action of the glycoside and the gradual dissipation over this time of the effects of atropine. The changes induced by this dose of ouabain on
Heart Failure.—In six conscious dogs with experimentally produced heart failure, arterial pressure increased slightly above the control level of $99 \pm 3$ mm Hg during the first 5 minutes, but returned to control levels by 15 minutes and was still at control levels at 30 minutes. The differences between the alterations in arterial pressure in the normal state and in heart failure were statistically significant at 15 minutes ($P<0.02$). Heart rate decreased immediately below the control level of $114 \pm 14$ beats/min, was decreased $14 \pm 2\%$ below control at 15 minutes, and remained below control for the duration of the 30-minute observation period. Both absolute and relative reductions in heart rate were significantly greater in the dogs with heart failure than in the normal dogs at 15 and 30 minutes ($P<0.01$) (Fig. 3, Table 2).

Ouabain resulted in an initial transient decrease in renal blood flow below the control value of $162 \pm 45$ ml/min, but by 15 minutes flow had increased to $10 \pm 4\%$ above control and was still elevated at 30 minutes. Although renal vascular resistance increased initially above the control value of $0.81 \pm 0.22$ mm Hg/ml min$^{-1}$, by 15 minutes resistance had decreased $12 \pm 3\%$ below control and was still below control at 30 minutes (Fig. 3, Table 2). The differences in the effects of ouabain on renal resistance between normal animals and animals with heart failure were statistically significant at 5, 10, and 15 minutes ($P<0.01$). The qualitatively different effects of ouabain on the renal bed of the same dog studied in the normal state and later after heart failure had been produced are depicted in Figure 4.

Iliac flow behaved in a similar manner; although it decreased initially, below the control value of $65 \pm 9$ ml/min, it increased to $13 \pm 4\%$ above control at 15 minutes and reached a maximum at 30 minutes. Iliac resistance increased transiently at 1 minute but by 5 minutes was below the control level of $1.6$ mm Hg/ml min$^{-1}$, decreased by $9 \pm 1\%$ below control at 15 minutes and reached its lowest level at 30 minutes. The differences in the changes in iliac resistance between the normal animals and the animals with heart failure.
Time course of the hemodynamic effects of ouabain in normal conscious dogs after cholinergic blockade. Control values are indicated on the base line.

Time course of the hemodynamic effects of ouabain in conscious dogs with heart failure. Control values are indicated on the base line.

Failure were statistically significant at 5, 15, and 30 minutes (P < 0.01).

The increase in mesenteric flow above the control level of 193 ± 35 ml/min was evident by 1 minute, increased by 32 ± 6% above control at 15 minutes and was still elevated at 30 minutes. Mesenteric vascular resistance was essentially unchanged at 1 minute, decreased to 24 ± 4% below control at 15 minutes, and was still below control at 30 minutes. The
differences in the ouabain-induced changes in mesenteric vascular resistance between normal animals and animals with heart failure were significant at 1 (P < 0.05) and 5 minutes (P < 0.01).

Discussion

The alterations in regional resistance observed in this study appear to be the result of the interaction of at least two separate known actions of digitalis glycosides. Previous studies on the in vitro effects of digitalis glycosides on vascular smooth muscle (18–21), on the effects of glycosides directly infused into the beds of anesthetized dogs (7), and on the total systemic resistance of experimental animals (7–9) and man (2, 6) have shown a potent direct vasoconstricting effect of digitalis compounds. On the other hand, these agents have been demonstrated repeatedly to exert an inotropic effect on the normal (1–5, 22, 23) and failing heart (13, 23–25), and when this action produces an increase in cardiac output it would tend to antagonize the direct vasoconstricting effect since an increase in flow tends to reduce resistance.

The time sequence of these two effects is such that after the intravenous administration of a cardiac glycoside the maximal direct vasoconstricting effect clearly precedes the maximal inotropic action (2, 5, 12, 16). Specifically, in the normal conscious dog, the peripheral vasoconstricting effect reaches a maximum during the first 15 minutes and then declines while the inotropic effect reaches a peak later, between 15 and 30 minutes after the intravenous administration of the drug (5). It is therefore likely that the early increase in regional vascular resistances, i.e., during the first 5 minutes, observed both in the normal animals and in those with heart failure, is due to a direct effect of ouabain on vascular smooth muscle.

Although modest inotropic effects of ouabain can be discerned in the normal conscious dog (5) and man (2, 3, 23), substantial evidence indicates that this inotropic effect is not accompanied by any significant changes in cardiac output (6, 12, 23, 26–28), a rise in which would tend to lower systemic vascular resistance. Consequently, in the absence of heart failure, the direct vasoconstrictor action
of the glycoside is unopposed by an increase in cardiac output; thus an increase in vascular resistance was the predominant effect of digitalis in the renal and iliac beds for the entire 30-minute observation period. These results, utilizing direct measurements of blood flow in the regional circulations of a healthy conscious animal, an experimental model which closely approximates a physiologic milieu, are consonant with those of prior studies on the isolated perfused circulations of the hind limb (7) and kidney (8) of anesthetized dogs on cardiopulmonary bypass.

The response to ouabain is not uniform in the different regional circulatory beds of the normal conscious animal. Although an initial transient vasoconstriction occurred in the mesenteric vascular bed, which resembled that seen in the renal and iliac beds, resistance had returned to control levels by 5 minutes and for the rest of the observation period the predominant effect observed in the mesenteric bed was vasodilatation. The mesenteric vasodilatation observed in the present study differs from the predominant vasoconstriction reported when digitalis glycosides were administered directly into the mesenteric artery (29) and intravenously in anesthetized dogs (11) and rhesus monkeys (30). In the present study, predominant and sustained mesenteric vasoconstriction was observed in the conscious dog when ouabain was infused directly into the mesenteric artery, presumably reflecting its direct vascular effect alone. In the anesthetized dogs, sustained mesenteric vasoconstriction was also observed after the intravenous administration of 50 μg/kg ouabain.

Factors inherent in earlier studies may have precluded vasodilatation in the mesenteric bed from being manifest. In these earlier studies reporting sustained mesenteric vasoconstriction, anesthetized animals that had been recently operated on were studied (11, 29, 30). Besides altering basal tone of regional vessels (31–33), these influences have been shown to depress cardiovascular reflexes (33, 34–36) and parasympathetic activity (32, 33, 37). In this regard, although cholinergic blockade had little qualitative or quantitative influence on the responses to ouabain in the renal and iliac beds in the present study, it caused a totally different response in the mesenteric bed, i.e., it reversed the vasodilatation seen in the normal conscious dog, and sustained mesenteric vasoconstriction was observed for the entire observation period. This suggests that the delayed mesenteric dilatation was mediated by parasympathetic stimuli. In addition, the dose of digitalis that was used in earlier investigations with anesthetized animals was substantially greater than the subtoxic amounts administered in the present study. Therefore, the larger dose of ouabain, exerting a more intense and protracted direct vasoconstriction combined with the alterations in the circulation induced by anesthetic agents, particularly the attenuation of parasympathetic influences, may have prevented the ouabain-induced mesenteric vasodilatation from being observed.

In an earlier study from our laboratory (12), the systemic responses to ouabain of the conscious and anesthetized dog were shown to differ in several respects, including a greater and more prolonged increase in total systemic resistance in the anesthetized state. This difference was most apparent 15 to 30 minutes after the administration of ouabain, precisely the time at which mesenteric vasodilatation was apparent in the conscious animal. It is likely that the opposite directional changes in mesenteric resistance in response to ouabain in the conscious and anesthetized states is responsible to some extent for the greater and more sustained rise in total systemic resistance in the anesthetized than in the conscious state.

In the animals with heart failure, control levels of regional vascular resistance were higher than in the normal animals, a finding in agreement with that of previous investigators (6, 38–41). In spite of these higher control levels of regional vascular resistance, the initial vasoconstriction in the renal and iliac beds was still evident and was indicative of the early direct vascular effects of ouabain. However, in contrast to the predominant and sustained vasoconstriction observed in normal
animals, regional vasodilatation was the predominant manifestation of intravenously administered ouabain in the dogs with heart failure.

Digitalis glycosides have been shown to exert a greater inotropic effect on the depressed than the normal myocardium (5) and this inotropism is accompanied by a distinct increase in cardiac output (13, 23–25). The latter was reflected in the present study by the increase in blood flow to each of the regional beds studied, which was associated with dilatation in each of these beds. Although no attempt was made in this investigation to define precisely the mechanism responsible for the different effects of ouabain on the regional beds in the normal state and in heart failure, it seems likely that two effects were involved: (1) a ouabain-induced increase in cardiac output in the dogs with heart failure promoted a diminution in the compensatory vasoconstriction and heightened sympathetic activity (42–44) characteristic of the heart failure state, and (2) the increased systemic blood flow lowered resistance passively by recruiting additional vessels (45).

The findings in this study of divergent regional hemodynamic effects of ouabain in the normal state and in heart failure are of clinical interest since they indicate that the salutary increase in regional blood flow and decrease in regional resistance due to the direct inotropic effects of digitalis glycosides can be anticipated when used in the therapy of congestive heart failure, while the peripheral vasoconstricting effect which can be detrimental in certain situations, is relatively brief. However, the intravenous administration of rapidly acting glycosides in patients without congestive heart failure can be expected to produce a predominant vasoconstrictor effect, which could be hazardous in certain situations, particularly when ischemia already exists in the distribution of a vascular bed.

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