Contrasting Effects of Digitalis and Dobutamine on Baroreflex Sympathetic Control in Normal Humans

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Background. Digitalis glycosides augment cardiopulmonary baroreceptor mechanisms in animals. This could result from inotropic actions or from direct sensitization of cardiac mechanoreceptors.

Methods and Results. To determine if digitalis has similar actions in humans and to evaluate the mechanisms involved, we measured muscle sympathetic nerve activity (MSNA; microneurography) during unloading of cardiopulmonary baroreceptors with incremental lower body negative pressure (LBNP; 0 to -15 mm Hg) and during the cold pressor test in 22 normal subjects (age 22±1 year, mean±SEM). Arterial and central venous pressures, heart rate, and MSNA were measured during LBNP before and after intravenous digitalis (Cedilanid 0.02 mg/kg, n=8), dobutamine (2.8±0.5 μg/kg/min, n=8), or placebo (n=6). Digitalis and dobutamine produced similar increases in baseline mean arterial pressure and decreases in central venous pressure and MSNA. LBNP produced similar decreases in central venous pressure in all groups before and after drug administration. The MSNA responses to LBNP were markedly potentiated by digitalis but not by dobutamine or placebo.

Conclusions. Digitalis did not alter responses to the cold pressor test. Thus, digitalis selectively potentiated cardiopulmonary baroreflex regulation of sympathetic neural responses in normal humans, whereas dobutamine (another positive inotropic agent) did not produce this effect. We conclude that digitalis augments cardiopulmonary baroreflex control of sympathetic activity, probably by direct baroreceptor sensitization. (Circulation 1991;84:1118–1129)

Digitalis glycosides have played a prominent role in the management of patients with heart failure for over 200 years.1 Although the prevailing view relates the therapeutic effects of these agents to their inotropic action, there is increasing evidence from experimental and clinical studies that digitalis glycosides also possess important autonomic and central nervous system actions. Digitalis glycosides exert actions at different sites in the neurocardiovascular axis. In animals, digitalis directly sensitizes tonically active cardiopulmonary2,3 and arterial baroreceptors,4–6 potentiates cardiopulmonary baroreflex vagal afferents,7 and reflexly inhibits renal sympathetic nerve activity.8 Digitalis exerts central neural and efferent sympathoexcitatory actions in animals5,9–12 and direct constricting actions on vascular smooth muscle.13–15

Limited studies in humans suggest that digitalis glycosides alter autonomic mechanisms, although the exact mechanisms remain unclear. Mason and Braunwald16 observed that ouabain produces forearm arterial and venous dilation in heart failure patients and vasoconstriction in normal humans. Prior studies in our laboratory using venous plethysmography to indirectly assess sympathetically mediated vascular responses confirmed these observations and suggested that digitalis glycosides potentiate cardiopulmonary baroreflex control of vascular resistance in heart failure patients.17 Ferrari and colleagues18 have likewise demonstrated that digitalis augments the bradycardic and hypotensive responses to arterial baroreceptor activation in normotensive and hypertensive patients. More recently, studies
from our laboratory have demonstrated an early and profound attenuation of efferent muscle sympathetic nerve activity (MSNA) in heart failure patients after administration of a rapidly acting digitalis preparation (Cedilanid-D) but no such sympathoinhibition after administration of equal inotropic doses of the nondigitalis inotropic agent dobutamine.\textsuperscript{19} We hypothesized that this effect resulted from a direct sensitization of cardiopulmonary afferents by digitalis similar to that reported in animal models.

To assess more critically the autonomic actions of digitalis in humans and to define the mechanisms of these effects, we performed an entirely new series of studies in normal human subjects to test the hypothesis that acute administration of a digitalis glycoside would directly potentiate cardiopulmonary baroreflex control of efferent MSNA. We used percutaneous peroneal microneurography as a quantitative measurement of the neuroeffector limb during unloading of cardiopulmonary baroreceptors by lower body negative pressure (LBNP). To determine whether observed actions of digitalis were simply secondary to its inotropic action, we performed comparative studies with LBNP before and during administration of similar inotropic doses of dobutamine in a separate group of subjects.

\section*{Methods}

\subsection*{Subjects}

Twenty-two male subjects (age 22±1 year, mean±SEM) were studied in three treatment groups: digitalis group (n=8; subjects 1–8), dobutamine group (n=8; subjects 9–16), and placebo group (n=6; subjects 17–22). All subjects were studied without sedation in the supine, postabsorptive state and were free of cardiovascular or other systemic diseases based on medical history and physical examination. Informed written consent was obtained before the study and the protocol was approved by the human subjects review committee of the University of Iowa.

\subsection*{Measurements}

A direct writing, multichannel physiological recorder was used to simultaneously record phasic and mean arterial and central venous pressures, heart rate, respiratory activity, forearm blood flow, level of LBNP, and MSNA.

Arterial pressure was measured directly through a 4Fr polyethylene arterial catheter inserted percutaneously in the right brachial artery. Mean arterial pressure was obtained by an electrical mean signal. Arterial pulse pressure was determined as systolic pressure minus diastolic pressure. Central venous pressure was measured through an 18.5-gauge polyethylene catheter inserted percutaneously in a right median antecubital vein and advanced to an intrathoracic vein. Heart rate and rhythm were recorded continuously by electrocardiogram, and respiratory activity was recorded by a strain gauge pneumograph. Zero reference point for all hemodynamic measurements was defined at the phlebotstatic axis in the midaxillary position.

Forearm blood flow was measured by venous occlusion plethysmography with a mercury-in-silastic Whitney strain gauge as previously described.\textsuperscript{17,20,21} Blood flow was measured every 15 seconds, and the average value per minute was determined. Forearm vascular resistance was derived by dividing mean arterial pressure (mm Hg) by forearm blood flow (milliliters per minute per 100 ml of forearm volume) and expressed as units.

Microneurographic recordings of postganglionic MSNA were obtained from a muscle nerve fascicle in the peroneal nerve posterior to the fibular head in all subjects. This technique has been validated and extensively described in studies from our laboratory and elsewhere.\textsuperscript{19,22–28} In brief, recordings were obtained by percutaneous insertion of tungsten microelectrodes into the peroneal nerve. The electrodes were connected to a preamplifier and the nerve signal was fed through a band pass filter and routed through an amplitude discriminator to a storage oscilloscope and loudspeaker. For recording and analysis, the filtered neurogram was fed through a resistance-capacitance integrating network to obtain a mean voltage display of the neural activity. Standard criteria for acceptance of a recording of MSNA were achieved in all subjects.\textsuperscript{19,22–28} Resting nerve activity was measured for up to 10 minutes before the study was begun to ensure that a stable baseline of nerve activity had been obtained. Sympathetic bursts were identified by inspection of the mean voltage neurogram. Individual burst frequency was determined as bursts per minute. Nerve activity was also corrected for heart rate and expressed as bursts per 100 heartbeats.\textsuperscript{19} Individual burst amplitude was measured and total integrated muscle sympathetic nerve activity was calculated as the total sum of burst amplitudes per minute and expressed as units per minute. Thus, sympathetic nerve activity was expressed in four ways: 1) bursts per minute, 2) total integrated nerve activity as units per minute, 3) heart rate–corrected activity as sympathetic bursts per 100 heartbeats, and 4) total activity corrected for heart rate and expressed as units per 100 heartbeats. Prior studies in our laboratory determined an intraobserver variability of 5% and an interobserver variability of less than 10% in this calculation of sympathetic nerve activity.\textsuperscript{24}

\subsection*{Procedures}

Orthostatic stress was simulated by the technique of LBNP using a chamber placed over the subject's body below the iliac crest.\textsuperscript{17,26,29–32} LBNP was applied for consecutive sequential 2-minute periods at levels of 0 (control), −5, −10, and −15 mm Hg to reduce cardiac filling pressures without significantly altering arterial or pulse pressure. Responses to the cold pressor test were assessed by immersion of one of the subject's hands up to the wrist in ice water for 2 minutes. Subjects were instructed to avoid isometric contraction, performance of Valsalva maneuver, or
TABLE 1. Effects of Cedilanid-D on Hemodynamic and Muscle Sympathetic Nerve Activity Responses to Cardiopulmonary Baroreceptor Deactivation (Lower Body Negative Pressure)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>LBNP (-5 mm Hg)</th>
<th>LBNP (-10 mm Hg)</th>
<th>LBNP (-15 mm Hg)</th>
<th>Control</th>
<th>LBNP (-5 mm Hg)</th>
<th>LBNP (-10 mm Hg)</th>
<th>LBNP (-15 mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAP (mm Hg)</td>
<td>131.0±4.4</td>
<td>131.1±4.8</td>
<td>129.9±4.9</td>
<td>129.5±4.7</td>
<td>148.5±5.8*</td>
<td>149.6±6.2</td>
<td>149.6±6.1</td>
<td>150.1±6.1</td>
</tr>
<tr>
<td>DAP (mm Hg)</td>
<td>72±4.0</td>
<td>71.5±2.2</td>
<td>71.5±2.2</td>
<td>71.1±2.3</td>
<td>73.3±2.5</td>
<td>73.0±3.1</td>
<td>72.1±2.9</td>
<td>72.1±2.7</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>92±4.2</td>
<td>92.8±2.6</td>
<td>92.6±2.9</td>
<td>91.3±2.7</td>
<td>98.8±3.5*</td>
<td>97.8±3.8</td>
<td>98.4±3.7</td>
<td>97.0±3.5</td>
</tr>
<tr>
<td>PPr (mm Hg)</td>
<td>58.6±3.2</td>
<td>59.6±3.0</td>
<td>58.4±3.2</td>
<td>58.4±2.9</td>
<td>75.3±3.5*</td>
<td>76.8±3.7</td>
<td>77.5±3.8</td>
<td>78.0±4.2</td>
</tr>
<tr>
<td>CVP (mm Hg)</td>
<td>5.1±0.7</td>
<td>3.6±0.5†</td>
<td>2.3±0.4†</td>
<td>1.2±0.4†</td>
<td>4.4±0.9</td>
<td>2.6±0.7†</td>
<td>1.6±0.5†</td>
<td>0.5±0.4†</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>67±5.6</td>
<td>66.8±3.9</td>
<td>67.4±4.1</td>
<td>67.2±3.8</td>
<td>61.8±5.4*</td>
<td>62.6±5.6</td>
<td>61.1±5.3</td>
<td>61.4±5.1</td>
</tr>
<tr>
<td>FBF (ml/min/100 ml)</td>
<td>5.5±0.6</td>
<td>5.1±0.6</td>
<td>4.9±0.5†</td>
<td>4.6±0.5†</td>
<td>5.4±0.7</td>
<td>4.8±0.6†</td>
<td>4.6±0.5†</td>
<td>4.3±0.5†</td>
</tr>
<tr>
<td>FVR (units)</td>
<td>18.2±2.1</td>
<td>20.1±2.3</td>
<td>21.0±2.6†</td>
<td>22.1±3.0†</td>
<td>20.8±2.9</td>
<td>22.5±3.0†</td>
<td>23.4±2.8†</td>
<td>25.1±3.0†</td>
</tr>
<tr>
<td>MSNA (bursts/min)</td>
<td>28.2±3.6</td>
<td>31.0±3.8</td>
<td>32.5±3.4†</td>
<td>36.1±4.0†</td>
<td>18.6±3.2†</td>
<td>23.8±4.0†</td>
<td>25.7±3.7†</td>
<td>28.8±3.6†</td>
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<tr>
<td>MSNA (units/min)</td>
<td>381.3±83.2</td>
<td>432.4±102.7</td>
<td>493.4±113.4†</td>
<td>568.3±126.6†</td>
<td>192.2±52.2*</td>
<td>250.6±61.6†</td>
<td>311.8±77.6†</td>
<td>382.0±103.4†</td>
</tr>
<tr>
<td>MSNA (bursts/100 h)</td>
<td>41.6±5.1</td>
<td>46.6±5.8</td>
<td>48.2±4.4†</td>
<td>53.5±5.5†</td>
<td>29.6±4.6*</td>
<td>37.6±5.8†</td>
<td>41.9±5.5†</td>
<td>46.5±5.0†</td>
</tr>
<tr>
<td>MSNA (units/100 h)</td>
<td>559.9±118.9</td>
<td>650.2±154.3</td>
<td>733.5±167.3†</td>
<td>839.3±182.2†</td>
<td>299.2±71.4*</td>
<td>393.4±89.6†</td>
<td>507.8±126.5†</td>
<td>616.4±170.7†</td>
</tr>
</tbody>
</table>

LBNP, lower body negative pressure; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure; PPr, pulse pressure; CVP, central venous pressure; HR, heart rate; FBF, forearm blood flow; FVR, forearm vascular resistance; MSNA, muscle sympathetic nerve activity; hb, heartbeats.

Values are mean±SEM, n=8. *p<0.05 control pre-Cedilanid vs. control post-Cedilanid.
†p<0.05 control vs. LBNP.

held expiration during performance of the cold pressor test.33 The order of experimental interventions was varied randomly between subjects.

Protocol

Studies were initiated after a 20-minute rest period during which all subjects were familiarized with the experimental techniques. In all subjects, measurements of hemodynamic parameters and MSNA were obtained over 2-minute periods in control state, during LBNP at -5, -10, and -15 mm Hg, and during recovery. Control, intervention, and recovery periods for the cold pressor test were also of 2-minute duration. There was a 5–10-minute rest period between interventions to permit hemodynamic and MSNA parameters to return to control levels. The average response during each period of control, intervention, and recovery was determined.

After the predrug interventions, subjects received intravenous administration of Cedilanid-D (0.02 mg/kg, n=8), dobutamine (2.8±0.5 µg/kg/min), or placebo (normal saline, n=6). Digitalis or placebo was administered randomly and subjects were blinded to which agent they received. Dobutamine studies were performed after the digitalis and placebo studies were completed to assess the specificity of digitalis effects; therefore, dobutamine subjects were not randomly assigned. The Cedilanid-D or placebo was administered intravenously over 5 minutes, whereas dobutamine was infused continuously. The dobutamine infusion was titrated to achieve the same magnitude of increase in arterial pressure as was achieved after administration of Cedilanid-D, based on responses in this study population and prior studies in human subjects from our laboratory.19 Beginning 20 minutes after onset of drug administration, the subjects underwent LBNP and the cold pressor test in the same order as in the predrug trials.

Statistical Analysis

All statistical analysis was performed in consultation with biostatisticians in the Clinical Research Center at the University of Iowa. Control hemodynamic and MSNA parameters before and after drug administration were compared within each treatment group by paired t test. Comparison of control variables between the digitalis and dobutamine groups was performed by unpaired t tests. Hemodynamic and MSNA responses to LBNP before and after drug administration were compared by repeated-measures ANOVA within each treatment group separately. Comparisons between different levels of LBNP to control predrug or postdrug were made by Dunnett’s test. Intragroup hemodynamic and MSNA responses to the cold pressor test were compared by paired t test. Statistical significance was considered as p<0.05. Values are presented in the text, figures, and tables as mean±SEM.

Results

Effects of Drug Treatment on Control Hemodynamics and MSNA

Tables 1 and 2 and Figure 1 summarize the effects of Cedilanid-D and dobutamine on control (pre-LBNP) hemodynamics and MSNA. Intravenous administration of Cedilanid-D resulted in significant increases in arterial systolic pressure (+13.4±2.4%, p<0.001), mean pressure (+6.5±2.3%, p<0.05), and pulse pressure (+29.1±4.3%, p<0.001), whereas control heart rate (-9.1±3.2%, p<0.05) and total MSNA activity (units per minute; -46.1±10.1%, p<0.05) decreased. No
significant change was observed in diastolic arterial pressure. Central venous pressure tended to decrease (−19.0±14.6%, p=0.17) and forearm vascular resistance tended to increase (+14.8±10.1%, p=0.18) after administration of the digitalis glycoside.

Intravenous infusion of dobutamine resulted in nearly identical increases in control values of arterial systolic pressure (13.7±1.8%, p<0.001 versus pre-dobutamine control), mean pressure (+6.3±1.3%, p<0.01), and pulse pressure (+31.5±5.1%, p<0.001). The change in total integrated MSNA activity (units per minute; −45.3±8.5%, p<0.01) was nearly identical to that observed after digitalis. Central venous pressure also decreased (−16.4±8.8%, p<0.05) during dobutamine infusion whereas the changes in diastolic arterial pressure, heart rate, and forearm vascular resistance did not achieve statistical significance. Thus, as demonstrated in Figure 1, dobutamine produced nearly identical responses in hemodynamic and MSNA parameters compared with Cedilanid-D. The only significant difference in effect on control parameters was a slight increase in heart rate during dobutamine infusion and a decrease in heart rate after Cedilanid-D. There was also a tendency for an increase in forearm vascular resistance after digitalis compared with dobutamine, but this did not achieve statistical significance. Intravenous administration of placebo had no statistically significant effect on resting hemodynamics or MSNA.

Effects of Digitalis on Responses to Cardiopulmonary Baroreceptor Deactivation (LBNP)

The hemodynamic and MSNA responses of subjects 1–8 during application of incremental levels of LBNP at 0 (control), −5, −10, and −15 mm Hg, before and after administration of Cedilanid-D, are summarized in Figure 1.
Table 1 and Figures 2 and 3. During the predrug trials, increasing levels of LBNP resulted in proportionally decreasing levels of central venous pressure, which were significantly less than control at each level of LBNP. LBNP did not alter arterial systolic, diastolic, mean, or pulse pressures or heart rates in these subjects. After Cedilanid-D, graded application of identical levels of LBNP caused nearly identical reductions in central venous pressure compared with the predrug trials, and there were again no changes in arterial pressure parameters or heart rate during LBNP.

Despite similar hemodynamic effects of LBNP before and after Cedilanid-D, the MSNA responses to cardiopulmonary baroreceptor deactivation were markedly potentiated after administration of this digitalis glycoside (Figure 3). During baseline (predrug) trials, total integrated MSNA activity tended to increase during LBNP −5 mm Hg (+13.5±6.4%, p=NS versus control) and increased significantly during LBNP −10 mm Hg (+33.7±9.6%, p<0.05) and LBNP −15 mm Hg (+52.8±6.9%, p<0.05). After administration of Cedilanid-D, total MSNA tended to increase during LBNP −5 mm Hg (+32.8±11.1%, p=NS) and did increase during LBNP −10 mm Hg (+72.9±14.3%, p<0.05) and LBNP −15 mm Hg (+110.9±17.2%, p<0.05). Despite almost identical changes in central venous pressure during LBNP before and after Cedilanid-D, the sympathetic responses to LBNP over the range of 0 to −15 mm Hg were significantly augmented (p<0.01 for MSNA expressed as units per minute; p<0.05 for MSNA expressed as bursts per minute; p<0.01 for MSNA expressed as units per 100 heartbeats; repeated-measures ANOVA) by this digitalis glycoside (Figure 3). This potentiation of reflex responses after digitalis was confirmed by examining both relative as well as absolute changes in MSNA.

Figure 4 presents portions of experimental recordings of hemodynamics and MSNA from a normal subject during LBNP performed before and after administration of Cedilanid-D. During baseline studies, LBNP produced a dose-dependent decrease in cardiac filling pressures with a resultant increase in MSNA. After administration of the digitalis glycoside, identical levels of LBNP produced very similar decreases in cardiac filling pressures. However, the sympathetic response to cardiopulmonary baroreceptor deactivation was markedly potentiated after digitalis.

Effects of Dobutamine on Responses to Cardiopulmonary Baroreceptor Deactivation (LBNP)

The responses of subjects 9–16 during cardiopulmonary baroreceptor unloading with graded LBNP before and during infusions of dobutamine are summarized in Table 2 and Figures 2 and 3. During the predrug trials, increasing levels of LBNP resulted in
FIGURE 3. Graphs show comparison of effects of digitalis (left panels), dobutamine (middle vertical panels), and placebo (right panels) on muscle sympathetic nerve activity (MSNA) responses to cardiopulmonary baroreceptor unloading with lower body negative pressure (LBNP). The sympathoexcitatory responses to cardiopulmonary baroreceptor deactivation, expressed as MSNA burst frequency (upper panels), as percent change in total integrated MSNA (middle horizontal panels), and as percent change in total MSNA corrected for heart rate (lower panels) were potentiated by digitalis but were not altered by dobutamine or placebo (repeated-measures ANOVA). Values are mean±SEM. LBNP −5, LBNP at −5 mm Hg; Hb, heartbeats.

proportionally decreasing levels of central venous pressure that were significantly less than control at each level of LBNP. LBNP did not alter arterial systolic, mean, or diastolic pressure, but there was a small decrease in pulse pressure during LBNP −15 mm Hg (from 60.1±4.1 mm Hg control to 57.3±4.6 mm Hg at LBNP −15 mm Hg, p<0.05). There were no observed changes in heart rates in these subjects during LBNP. During dobutamine infusion, graded application of identical levels of LBNP caused similar reductions in central venous pressure compared with the predrug trials. When LBNP −5 mm Hg was performed during dobutamine infusion, there was a small increase in arterial systolic (from 151.0±5.3 mm Hg control to 154.0±5.8 mm Hg during LBNP −5 mm Hg, p<0.05) and pulse (from 78.9±6.2 mm Hg control to 81.1±6.6 mm Hg during LBNP −5 mm Hg, p<0.05) pressures, but no change in arterial mean or diastolic pressures. With LBNP −10 and −15 mm Hg during dobutamine infusion, there were no significant changes in arterial pressure parameters or heart rate except for a small increase in diastolic arterial pressure during LBNP −15 mm Hg (from 72.1±3.3 to 74.5±3.4 mm Hg, p<0.05).

In contrast with the augmentation of sympathetic responses to unloading of cardiopulmonary baroreceptors with LBNP observed during digitalis administration, the MSNA responses to LBNP were not potentiated during dobutamine infusion. The sympathetic responses to LBNP over the range 0−15 mm Hg were not significantly different before versus during dobutamine infusion (p=NS predopamine versus during dobutamine infusion for all MSNA parameters; repeated-measures ANOVA; Figure 3). During predobutamine trials, total integrated MSNA activity tended to increase during LBNP −5 mm Hg (+46.3±13.0%, p=NS versus control) and increased significantly during LBNP −10 mm Hg (+97.5±19.4%, p<0.05) and LBNP −15 mm Hg (+142.3±28.9%, p<0.05) during infusion of intravenous digitalis, total integrated MSNA tended to increase during LBNP −5 mm Hg (+24.4±21.6%, p=NS) and LBNP −10 mm Hg (+110.6±48.3%, p=NS) and increased significantly during LBNP −15 mm Hg (+210.8±67.9%, p<0.05). No evidence of augmented response to LBNP was observed when comparing either relative or absolute responses with LBNP before versus during dobutamine infusion.

Effects of Placebo on Responses to Cardiopulmonary Baroreceptor Deactivation (LBNP)

Subjects 17–22 underwent graded LBNP before and after administration of placebo to assess the potential effects of time, repeated intervention, and placebo on
hemodynamic and MSNA responses during unloading of cardiopulmonary baroreceptors with LBNP. No differences in responses were observed before compared with after administration of placebo.

Graded application of LBNP before placebo resulted in significant decreases in central venous pressure at each level of LBNP (control, 5.8±0.5 mm Hg; LBNP -5 mm Hg, 4.3±0.6 mm Hg, p<0.05 versus control; LBNP -10 mm Hg, 3.1±0.7 mm Hg, p<0.05 versus control; LBNP -15 mm Hg, 2.2±0.6 mm Hg, p<0.05 versus control) without alteration in arterial pressure parameters or heart rate. The decrease in cardiac filling pressures was accompanied by a tendency for increase in total MSNA at LBNP -5 mm Hg (+14.9±6.1%, p=NS versus control) and increases in total MSNA at LBNP -10 mm Hg (+37.8±8.9%, p<0.05 versus control) and LBNP -15 mm Hg (+63.2±17.8%, p<0.05 versus control).

After administration of placebo, graded levels of LBNP resulted in similar reductions of central venous pressures at LBNP -5 mm Hg (from control level of 6.1±0.6 mm Hg to 4.2±0.6 mm Hg, p<0.05), at LBNP -10 mm Hg (to 3.1±0.6 mm Hg, p<0.05 versus control), and at LBNP -15 mm Hg (to 2.3±0.7 mm Hg, p<0.05 versus control). Again, there were no changes in arterial pressure parameters or heart rate during LBNP performed after placebo administration. Increases in MSNA during LBNP tended to be somewhat attenuated after placebo with an increase of +7.3±6.4% during LBNP -5 mm Hg (p=NS versus control), +21.5±11.5% during LBNP -10 mm Hg (p=NS versus control), and +43.2±19.0% during LBNP -15 mm Hg (p<0.05 versus control). The sympathetic responses to LBNP over the range of 0 to -15 mm Hg, however, were not significantly different before and after placebo (p=NS preplacebo versus postplacebo for all MSNA parameters; repeated-measures ANOVA).

Effects of Digitalis on Responses to Cold Pressor Stimulus

To assess for specificity of effects of digitalis on reflex responses, responses to the cold pressor stimulus were examined in six of the eight digitalis treatment group subjects before and after administration of Cedilanid-D. In the predrug trials, the cold pressor stimulus resulted in significant increases in mean arterial pressure (89.3±2.1–105.5±4.3 mm Hg, p<0.005), heart rate (67.3±5.3–71.9±5.9 beats/min, p<0.01), and total integrated MSNA activity.
(282.2 ± 72.4–366.2 ± 61.0 units, p < 0.01). Central venous pressure was not altered during the cold pressor stimulus. After administration of Cedilanid-D, the cold pressor stimulus resulted in similar increases in mean arterial pressure (95.0 ± 3.3–109.3 ± 3.3 mm Hg, p < 0.001) and total integrated MSNA (230.5 ± 66.3–382.7 ± 75.0 units, p < 0.05). Heart rate and central venous pressure were not altered during the cold pressor test performed after digitalis administration. Thus, the MSNA responses to the cold pressor test were not different before (+52 ± 19% increase in total integrated MSNA) versus after (+111 ± 64%, p = NS) administration of digitalis.

**Discussion**

Using direct recordings of efferent MSNA, the present studies demonstrate that acute administration of a digitalis glycoside to conscious normal human subjects markedly potentiates cardiopulmonary baroreflex regulation of sympathetic neuroeffector responses. Despite similar hemodynamic effects in a comparable group of subjects, dobutamine did not significantly alter cardiopulmonary baroreflex control of MSNA, suggesting that the effect of digitalis is not due solely to its inotropic action. In addition, the effect of digitalis did not appear to be a nonspecific potentiation of reflex responsiveness because sympathetic neuroeffector responses of subjects to the cold pressor stimulus were not altered by administration of digitalis glycoside. These studies support the hypothesis based on detailed studies in animals\(^2\)\(^3\)\(^7\)\(^8\) that administration of clinically relevant doses of digitalis augments cardiopulmonary baroreflex mechanisms via a direct sensitization of cardiopulmonary baroreceptors with vagal afferents.

**Comparison With Previous Studies of Effects of Digitalis on Autonomic Mechanisms**

Prior extensive studies in animals and limited studies in humans have suggested that the digitalis glycosides have important autonomic actions. Direct epicardial application\(^2\)\(^3\)\(^8\) intracoronary infusion\(^3\)\(^8\) and intravenous administration\(^7\) of rapidly acting digitalis preparations have been demonstrated to directly sensitize cardiopulmonary baroreceptors with vagal afferents and result in inhibition of efferent sympathetic responses.\(^2\)\(^3\)\(^8\) Similarly, intracarotid infusion of rapidly acting digitalis preparation in normal animals,\(^4\)\(^5\) in animals with heart failure,\(^6\) and bathing the isolated aortic arch in normal rats\(^34\) has been shown to directly sensitize arterial baroreceptors and/or produce peripheral vascular vasodilation. In addition to these acute effects of digitalis, recent studies have suggested that chronic administration of digitalis produces sustained potentiation of vagally mediated cardiopulmonary baroreflex mechanisms in normal dogs.\(^35\) These actions of digitalis on cardiopulmonary and arterial mechanoreceptors are consistent with the known membrane-sensitizing action of digitalis caused by blockade of Na-K ATPase activity at the level of the cellular membrane. Such an effect results in a lowering of pressure threshold for both transient and steady-state discharge of isolated baroreceptor nerve preparations.\(^34\)\(^36\)

One of the earliest suggestions that digitalis exerts autonomic actions in humans was the observation by Mason and Braunwald\(^36\) that intravenous ouabain produces vasoconstriction in normal humans compared with vasodilation in patients with heart failure. Subsequently, numerous studies in patients with heart failure have suggested that this disorder is characterized by impairment of autonomic mechanisms, especially those involving the cardiopulmonary baroreflex.\(^37\) Prior studies in our laboratory using forearm blood flow responses as indirect indexes of neuroeffector responses during unloading of cardiopulmonary baroreceptors with LBNP indicated that patients with moderate to severe heart failure demonstrate impairment of forearm vasoconstrictor responses to orthostatic stress.\(^17\) These impaired cardiopulmonary baroreflex responses were acutely normalized after intravenous administration of clinical doses of a rapidly acting digitalis preparation. Similarly, intravenous administration of Cedilanid-D has been demonstrated to augment the sinoaortic baroreflex-mediated bradycardic and hypotensive response to arterial baroreceptor activation in normal and hypertensive human subjects.\(^18\)

Recently, studies in our laboratory directly examined resting hemodynamics and efferent MSNA in patients with moderate to severe heart failure before and after administration of Cedilanid-D.\(^19\) Administration of this rapidly acting digitalis preparation produced an early, marked, and sustained inhibition of resting MSNA that preceded any observed hemodynamic effect of the agent and was accompanied by forearm vasodilation and a modest increase in cardiac output and decrease in cardiac filling pressures. However, in a separate but similar group of heart failure patients, administration of equal inotropic doses of dobutamine failed to alter MSNA, suggesting that the sympathoinhibitory action of digitalis was not due simply to its inotropic properties. The studies outlined in the present report were designed to further investigate the mechanism of action of digitalis in human subjects by directly examining cardiopulmonary baroreflex control of efferent sympathetic nerve activity.

**Potential Mechanisms of Action of Digitalis in Present Studies**

Although mechanistic studies of autonomic reflex mechanisms in awake, intact human subjects have certain inherent limitations, the present studies were designed to evaluate possible mechanisms of action of digitalis on autonomic mechanisms in human subjects based on previous studies from our laboratory.\(^17\)\(^19\) We considered the following as possible mechanisms for the observed actions of Cedilanid-D in the present studies: 1) activation of arterial baroreflex mechanisms resulting from increases in arterial pressure after administration of Cedilanid-D gener-
alized, 2) nonspecific central nervous system potentiation of all sympathoexcitatory reflexes, 3) augmentation of cardiopulmonary baroreflex responses caused by mechanoreceptor stimulation by the inotropic action of digitalis, and 4) specific action of digitalis on afferent cardiopulmonary baroreflex pathways unrelated to the inotropic action of the agent.

In the present studies, we used three levels of LBNP (−5, −10, and −15 mm Hg) to produce a graded decrease in cardiac filling pressures and thus a dose-dependent deactivation of cardiopulmonary baroreceptors. Observations were therefore determined over a range of alteration in cardiac filling pressures known to unload primarily the cardiopulmonary baroreceptors with little (if any) alteration in arterial pressure that would produce deactivation of arterial baroreceptors.17,21,26,30,31,38 As indicated in Tables 1 and 2, we achieved the desired hemodynamic responses with LBNP before and after drug administration without altering the determinants of sinoaortic baroreceptor activity. These levels of LBNP produced a stimulus-dependent unloading of cardiopulmonary receptors (decrease in cardiac filling pressure) that was accompanied by a stimulus-dependent increase in efferent MSNA (Tables 1 and 2, Figure 3). The results in the present study are comparable with those observed in prior human studies.26,38 Of particular note, the magnitude of the LBNP unloading was nearly identical in trials before and after administration of digitalis, dobutamine, and placebo. Thus, we believe these observations permit us to conclude that any observed actions of digitalis on responses to LBNP were not due to actions on arterial baroreflex pathways.

Animal studies suggest that digitalis glycosides may exert central neural actions although these effects are most commonly sympathoexcitatory in nature.5,10,11 As indicated in Table 1, control (pre-LBNP) MSNA was significantly decreased after administration of Cedilanid-D. This is consistent with prior observations from our laboratory in patients with heart failure19 and could result from sensitization of afferent cardiopulmonary baroreflex mechanisms with a resultant inhibition of central sympathetic neural outflow. Additional evidence against a central neural effect as the mechanism for the effect of digitalis on responses to LBNP was the failure of digitalis to alter responses of subjects to the cold pressor stimulus, as might be expected if this agent produced a central neural action on reflex responses.

The left ventricle is believed to be the primary location for mechanoreceptors responsible for regulating autonomic adjustments to low-level orthostatic stress.21,39–41 Although the primary determinant of the activity of these mechanoreceptors is cardiac preload, an increase in ventricular contractility is known to augment firing of these left ventricular receptors.42 In both animals40,42,43 and humans,17,21 negative inotropic agents have been demonstrated to attenuate cardiopulmonary baroreflex responses, whereas positive inotropic agents appear to augment these responses. Thus, we considered the possibility that digitalis could be exerting its effect on cardiopulmonary baroreflexes via a positive inotropic action. To assess this possibility, we examined cardiopulmonary baroreflex responses of subjects before and during administration of the nondigitalis inotropic agent dobutamine. Dobutamine was administered in doses designed to replicate the inotropic action of Cedilanid-D based on prior detailed studies in our laboratory.19 As noted in Figure 1, the hemodynamic responses to dobutamine were very similar to those observed after digitalis with the exception that digitalis produced an expected decrease in heart rate (direct vagal effect). The failure of dobutamine to potentiate cardiopulmonary baroreflex-mediated MSNA responses during LBNP argues that an inotropic effect alone cannot explain the actions of digitalis in the present studies. Similarly, previous studies from our laboratory demonstrated a marked sympathoinhibitory action of digitalis with no such effect after administration of dobutamine19 in heart failure patients. Thus, we do not believe the potentiation of cardiopulmonary baroreflex responses observed after acute digitalization in the present studies are explained by the modest inotropic action of digitalis.

Although the levels of LBNP used in the present studies altered cardiac filling pressures (and thereby cardiopulmonary baroreceptor activity) without altering arterial pressures (and thereby arterial baroreceptors), we recognize the possibility that the altered responses to LBNP after digitalis might be due to effects of the agent on the determinants of arterial baroreflex activity and secondary alteration of cardiopulmonary and arterial baroreflex interactions. As shown in Table 1 and Figure 1, administration of digitalis significantly increased resting arterial pressure parameters and decreased heart rate and MSNA. This would be consistent with an activation of sinoaortic baroreceptors via the increase in blood pressure and would be expected to inhibit efferent MSNA. The fact that we observed an augmentation of MSNA responses to LBNP after digitalis, even in the presence of presumed sinoaortic baroreflex-mediated sympathoinhibition, suggests that the potentiation of cardiopulmonary baroreflexes was of such a sufficient degree to override arterial baroreflex actions. In addition, despite similar effects of dobutamine on control blood pressure, we observed no such potentiation of cardiopulmonary baroreflexes after administration of this agent.

The observation that digitalis acutely augmented cardiopulmonary baroreflex control of MSNA whereas similar inotropic doses of dobutamine failed to exert such an effect suggests that the sympathetic potentiation does not result from an inotropic action of digitalis alone. As noted in Table 1, Cedilanid-D produced a marked inhibition of control MSNA. This observation is consistent with prior studies from our laboratory in patients with heart failure19 and would
be consistent with a potentiation of cardiopulmonary baroreceptors. Such an action would be expected to increase the tonic inhibitory action of these mechanoreceptors on brainstem vasomotor centers and thereby decrease resting efferent vasoconstrictor responses. We believe that these observations, in combination with the acute potentiation of cardiopulmonary baroreflex sympathetic responses, provide the strongest data yet obtained in humans to support the concept that digitalis functions as a neurotropic agent that includes sensitization of afferent cardiopulmonary baroreceptors. We suggest that this results from the known membrane-sensitizing actions of digitalis on cardiopulmonary baroreceptors, as has been observed in animal models.12,3,7,8,34–36

Discrepancy Between Vascular and Sympathetic Neural Responses to Digitalis

In the present study involving normal human subjects, digitalis potentiated sympathetic neural responses to simulated orthostatic stress, whereas forearm vasoconstrictor responses were not altered (Table 1). In contrast, a previous study from our laboratory in heart failure patients17 demonstrated that digitalis glycosides potentiated (thus tending to normalize) impaired forearm vasoconstrictor responses to similar levels of LBNP. This previous study did not have the benefit of simultaneous direct examination of sympathetic neural responses in heart failure patients. We believe that the apparent discrepancy between sympathetic neural and vascular responses in the present study of normal subjects and the prior finding of augmented vasoconstrictor responses to orthostatic stress in heart failure patients is most likely explained by differing actions of digitalis on baseline vascular resistance in these two different groups of human subjects.

In the present study, digitalis tended to increase baseline forearm vascular resistance of these normal subjects (Table 1) from 18.2±2.1 to 20.8±2.9 units, although this did not achieve statistical significance. This reflects the known direct vasoconstrictor effects of digitalis glycosides.13 Similar effects of digitalis on arterial resistance and venous capacitance vessels in normal subjects have been previously reported by Mason and Braunwald.16 This likely prevented us from observing potentiated vasoconstrictor responses during LBNP in these normal subjects because the direct vasoconstrictor actions of digitalis could obscure the baroreflex-mediated sympathoinhibiting actions in these subjects. This possibility is supported by the finding of potentiated sympathetic neural responses to LBNP, whereas forearm vasoconstrictor responses were unchanged after administration of digitalis.

Thus, the present study provides important new observations in normal subjects by combining both direct (efferent sympathetic nerve activity) and indirect (forearm blood flow) assessments of sympathetically mediated responses to orthostatic stress. The indirect assessment by measuring forearm blood flow responses is limited by the potential direct vasoconstrictor action of digitalis on arterial smooth muscle, and such observations alone may obscure actual potentiation of baroreflex responses. However, the clear evidence of potentiated sympathetic neural responses to LBNP supports the hypothesis of digitalis-induced potentiation of cardiopulmonary baroreflex mechanisms in normal subjects. The present observations are therefore important in understanding reflex autonomic actions of digitalis that would have been missed had indirect vasoconstrictor responses alone been examined.

In our previous study of heart failure patients, digitalis resulted in a reduction in baseline vasoconstriction (vasodilation) in heart failure patients similar to that previously reported by Mason and Braunwald.16 After digitalis administration, these heart failure patients exhibited a potentiated vasoconstrictor response to LBNP consistent with potentiation and normalization of impaired baroreflex responses.

Potential Limitations of Present Studies

We recognize several potential limitations in the design and interpretation of the present studies. First, these studies were acute in nature and do not necessarily predict long-term effects of digitalis glycosides in humans. However, recent studies in normal dogs suggest that long-term administration of digitalis produces a sustained potentiation of vagally mediated cardiopulmonary baroreflex inhibition of renal sympathetic activity.35 Second, the present studies were performed in normal human subjects, and caution must be exercised in applying these observations to patients with clinical disorders in whom digitalis is used therapeutically. However, the sympathoinhibitory effects observed here are similar to those recently reported from our laboratory in patients with heart failure.19 The present studies extend these earlier observations and suggest a definite mechanism of action of digitalis on the afferent limb of the cardiopulmonary baroreflex. Third, we have attempted to examine the cardiopulmonary baroreflex during maneuvers that deactivated these mechanoreceptors (LBNP). It is possible that other effects of digitalis would be observed under conditions of activation (loading) of such receptors. However, by examining efferent sympathetic responses over an extended and physiologically relevant range of cardiac filling pressures, we believe the current studies are functionally important. A fourth concern is that the techniques used in the present studies required observations to be made with the subjects in a supine position. It is possible that other effects would be observed in subjects studied in the upright position. In the performance of these studies, we used recordings of efferent sympathetic nerve activity from only one site, the peroneal nerve. It is known that there are important differences in the control of sympathetic nerve activity to various tissues and vascular beds.23,44 Nevertheless, in normal subjects, spontaneously occurring fluctuations in MSNA are
similar in different extremity nerves as are responses during isometric exercise. In these studies, all MSNA responses were compared with a stable control recording of MSNA and each subject served as his own control for both effects of interventions and drug. Thus, while we cannot absolutely generalize to total or other organ-specific sympathetic responses, the intraindividual comparisons of peroneal MSNA responses remain valid.

**Potential Clinical Implications**

Digitalis glycosides are important therapeutic agents in the treatment of patients with heart failure. This clinical disorder is known to be associated with abnormalities of cardiopulmonary baroreflex control mechanisms. Although digitalis appears to be a modest inotropic agent in such patients when administered orally, recent studies from our laboratory suggest that this agent may also acutely exert a sympathoinhibitory action in these patients. The present study provides strong evidence that the mechanism of this effect in humans relates to actions of digitalis on afferent cardiopulmonary baroreflex mechanisms.

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