Differential Effects of Digitalis on Chemoreflex Responses in Humans

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Abstract To investigate the effects of digitalis on chemoreflexes in humans, we measured muscle sympathetic nerve activity (microneurography), minute ventilation, oxygen saturation, end-tidal carbon dioxide, mean arterial pressure, heart rate, and central venous pressure during stimulation of peripheral chemoreceptors with hypoxia, during stimulation of central chemoreceptors with hypercapnia, and during a cold pressor test before and after digitalis and placebo in 10 healthy volunteers on two different days (randomized, double-blind, crossover design). Digitalis did not affect baseline measurements significantly. Despite similar changes in oxygen saturation and end-tidal carbon dioxide during hypoxia and hypercapnia with both placebo and digitalis, digitalis significantly potentiated overall ventilatory responses to hypoxia (+67±12% before versus +98±3% after digitalis; mean±SEM; P<0.01) but did not affect the response to hypercapnia. Sympathetic nerve activity increased by 25±9% during hypoxia before digitalis and 30±10% during hypoxia after digitalis (P=NS) and increased by 38±18% during hypercapnia before digitalis and 26±11% during hypercapnia after digitalis (P=NS). Digitalis did not significantly change responses to the cold pressor test. Placebo had no effect on ventilatory and sympathetic nerve activity responses. We conclude that digitalis selectively augments ventilatory responses to peripheral chemoreceptor stimulation by hypoxia. (Hypertension. 1994;23:302-307.)

Key Words • chemoreceptors • sympathetic nervous system • digitalis • ventilation • apnea

Digitalis glycosides have been shown to potentiate sympathoinhibitory baroreceptor reflex mechanisms. This potentiation may in part explain the reduction in central sympathetic outflow and other beneficial effects of digitalis in patients with heart failure. Studies on the effects of digitalis on sympathoexcitatory chemoreflexes are few and limited to work in animals. Quest and Gillis found that digitalis resulted in increases in carotid sinus nerve activity. Elimination of chemoreceptors with intracarotid acetic acid produced smaller increases in carotid sinus nerve discharge, suggesting that digitalis excited both chemoreceptor and baroreceptor afferents. Schmitt et al specifically examined the effects of digitalis on chemoreceptor carotid sinus afferent nerve activity and noted that intracarotid injection of digitalis increased chemoreceptor firing. McQueen and Ribeiro subsequently reported that digitalis both increased tonic carotid body discharge and augmented the neural responses to hypoxia. We are unaware of studies in humans investigating the effects of digitalis on chemoreflex function.

Using direct intraneural measurements of sympathetic efferent activity to muscle blood vessels in humans (microneurography), we have previously reported that both hypoxia (peripheral chemoreceptor stimulation) and hypercapnia (primarily central chemoreceptor stimulation) increase sympathetic nerve activity (SNA) in humans. Increased minute ventilation, as well as baroreceptor reflex activation (by raising blood pressure), inhibits these sympathetic responses. There is considerable selectivity in this inhibition, in that responses to hypoxia are inhibited more than the responses to hypercapnia.

We investigated the effects of digitalis on ventilatory, sympathetic, and hemodynamic responses to peripheral (hypoxic) and central (hypercapnic) chemoreceptor stimulation and contrasted these responses with the effects of digitalis on the response to a cold pressor stimulus (a nonspecific sympathoexcitatory stimulus).

Methods

Subjects

Ten normal volunteers (9 males, 1 female; age, 22.7±2.1 years) were studied with digitalis and placebo on two different days (range, 12 to 72 days between studies) in a double-blinded, randomized fashion. 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. All were nonsmokers and were on no medications. Informed written consent was obtained. These studies were approved by the Human Subjects Review Committee of the University of Iowa.

Measurements

Blood pressure was measured with a Physio-Control Lifesost 200 semiautomated sphygmomanometer. Heart rate (electrocardiogram), breathing pattern (pneumograph), O₂ saturation (Nellcor N-1100 C pulse oximeter), end-tidal CO₂ (47210 A capnometer, Hewlett-Packard Co), central venous pressure, and SNA to muscle were recorded on a 2800 S recorder (Gould Electronics Inc). Ventilatory rate and minute ventila-
tion were monitored with an LS-75 ventilation monitor (Bourns).

Microneurographic recordings of SNA to muscle were obtained from a sympathetic nerve fascicle in the peroneal nerve posterior to the fibular head. This technique has been validated and extensively described in studies from our laboratory and elsewhere.16-18 In brief, recordings were obtained by percutaneous insertion of tungsten microelectrodes into sympathetic fascicles in 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 muscle SNA were achieved in all subjects.16-18 Sympathetic bursts were identified by inspection of the mean voltage neurogram, and sympathetic activity was calculated as bursts per minute × mean burst amplitude and expressed in arbitrary units. Prior studies in our laboratory determined an intraobserver variability of 5% and interobserver variability less than 10% in this calculation of SNA.18

**Procedures**

Measurements were taken before and during exposure to the following gas mixtures via a mouth piece; a nose clip was used to ensure exclusive mouth breathing: 10% O2 in nitrogen with added CO2 to maintain isocapnia (isocapnic hypoxia), and 7% CO2 in oxygen (hypercapnic hypercapnia). At least 20 minutes was allowed between exposures to the different gas mixtures. A cold pressor test (CPT) was performed as a nonspecific sympathoexcitatory stimulus to serve as an internal control. The CPT consisted of immersing the subject's hand into ice water for 2 minutes. Ventilatory responses to the CPT were not measured. Subjects were randomly allocated to receive digitalis or placebo first in a balanced design so that five subjects received digitalis on the first study day and placebo on the subsequent study day, and five subjects received placebo on the first study day and digitalis on the subsequent study day. Data analysis was carried out before unblinding. The order of experimental interventions was randomized between subjects, but the same order was performed before and after drug administration on both study days.

**Protocol**

Studies were initiated after a 20-minute rest period during which all subjects were familiarized with the experimental techniques. The standard protocol used for chemoreflex studies in our laboratory was adhered to.13-14 Subjects underwent measurement of baseline variables for 3 minutes while breathing room air. Then, with the use of a three-way valve, the subjects were exposed to the gas mixture for 5 minutes. All subjects tolerated the 5-minute exposure to hypoxia, and all but one subject tolerated the 5-minute exposure to hypercapnia. Thus, effects of digitalis on responses to hypercapnia were examined in nine subjects. Average values of the 3-minute control period were compared with average values for the 5-minute period of gas exposure. Immediately before the end of each of the 5-minute gas exposures, the subjects underwent a period of voluntary end-expiratory apnea maintained for as long as possible followed by a 2-minute recovery period. Measurements during the apnea after 5 minutes of exposure to the gas were compared with measurements taken during an identical period of apnea on room air. Apnea responses were assessed to minimize the inhibitory influence of ventilation on sympathetic responses to hypoxia and hypercapnia.14 Control, intervention, and recovery periods for the CPT were 2 minutes long. Responses to the CPT were obtained in nine subjects. After the predrug interventions, subjects received intravenous administration of digitalis (0.02 mg/kg Cedilanid-D, three subjects; 0.01 mg/kg digoxin, seven subjects) or placebo (identical volumes of normal saline) in a randomized, double-blinded fashion. Dosage was based on previous studies of the effects of digitalis on reflex function in humans.14 The change was made from Cedilanid-D to an equivalent dose of digoxin because of the commercial unavailability of Cedilanid-D part way through the study. Digitalis and placebo were administered over 5 minutes. Twenty to 30 minutes after drug administration, subjects underwent repeat interventions in the same order as in the predrug sessions.

**Statistical Analysis**

Statistical analysis was conducted with a repeated-measures ANOVA comparing the responses before and after digitalis versus placebo over time (ie, two-within-factor, one-between-factor). Significant interaction between the digitalis and placebo treatments for normoxic/hypoxic and normocapnic/hypercapnic conditions was considered at a value of P < 0.05. Comparisons for each of the gas mixtures were between baseline recordings and recordings during exposure to the gas mixture averaged per minute. For the apneic periods, comparisons were between the period of apnea during exposure to the gas mixture and a period of apnea of identical duration on room air (paired two-tailed t test).13,14 Values are presented as mean ± SEM.

**Results**

**Effects of Digitalis on Baseline Variables and Responses to Hypoxia**

During hypoxia before digitalis, O2 saturation fell from 99±0.3% to 83±1.4% (P < 0.01), and heart rate,
mean arterial pressure, minute ventilation, and SNA increased significantly. Central venous pressure and end-tidal CO2 did not change (Table 1, Fig 1).

Administration of digitalis did not significantly affect baseline values (Table 1). Hypoxia after digitalis produced a similar decrease in O2 saturation, from 99±0.3% to 85±0.8% (P<.01), and similar increases in heart rate. SNA increased by 30±10% compared with an increase of 25±9% before administration of digitalis (P=NS) (Fig 1). The increase in minute ventilation was significantly augmented after digitalis (98±13%) compared with before (67±12%, P<.01) (Fig 1). Blood pressure during hypoxia after digitalis was 84.4±1.2 compared with 81.7±1.4 mm Hg with hypoxia before digitalis (P=NS). End-tidal CO2 and central venous pressure did not change significantly during postdigitalis hypoxia.

Hypoxia produced similar changes in O2 saturation, end-tidal CO2, SNA, minute ventilation, heart rate, and mean arterial pressure before and after placebo (Table 1, Fig 1). Except for a slight increase in mean arterial pressure, placebo had no effect on baseline variables (Table 1).

**Effects of Digitalis on Responses to Hypercapnia**

During hypercapnia before digitalis, end-tidal CO2 increased from 41±0.3 to 54±0.3 mm Hg (P<.01), and mean arterial pressure, central venous pressure, heart rate, O2 saturation, minute ventilation, and SNA increased significantly (Table 2, Fig 2).

Administration of digitalis did not significantly affect baseline values (Table 2). After digitalis, hypercapnia resulted in an increase in end-tidal CO2, from 41±0.3 to 53±0.3 mm Hg (P<.01), with increases in mean arterial pressure, heart rate, and O2 saturation similar to those before digitalis. Central venous pressure did not change significantly during hypercapnia after administration of digitalis. SNA increased by 26±11%, compared with an increase of 38±18% before administration of digitalis (P=NS) (Fig 2). The response of minute ventilation with an increase of 232±32% after digitalis was not different, compared with an increase of 242±54% before the drug (Fig 2).

Placebo had no significant effect on baseline variables and did not alter the responses of hemodynamics, minute ventilation, and SNA during hypercapnia (Table 2, Fig 2).

**Effects of Digitalis on Responses to Apnea During Hypoxia and Hypercapnia**

To minimize the sympathetic inhibitory effect of ventilation on sympathetic responses to hypoxia and hypercapnia, we examined the effect of a brief period of apnea on SNA during both hypoxia and hypercapnia before and after digitalis. During hypoxia before digitalis, apnea increased SNA by 329±89% compared with apnea on room air. After administration of digitalis, apnea during hypoxia increased SNA by 508±200% compared with apnea on room air (P=.15). During hypercapnia the SNA response to apnea was 184±45%

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**TABLE 2. Effects of Digitalis and Placebo on Hemodynamic, Ventilatory, and Sympathetic Nerve Responses to Hypercapnia**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Before Digitalis</th>
<th>After Digitalis</th>
<th>Before Placebo</th>
<th>After Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>81.0±1.5</td>
<td>86.2±1.7*</td>
<td>81.8±2.8</td>
<td>87.1±3.0*</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>4.6±0.3</td>
<td>5.6±0.4*</td>
<td>5.1±0.5</td>
<td>5.6±0.6</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>65.8±4.4</td>
<td>72.1±4.1*</td>
<td>64.8±3.1</td>
<td>70.6±3.1*</td>
</tr>
<tr>
<td>Ve, L/min</td>
<td>7.0±0.7</td>
<td>21.2±1.2*</td>
<td>7.7±0.5</td>
<td>24.9±1.9*</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td>99±0.4</td>
<td>100±0.0*</td>
<td>99±0.6</td>
<td>100±0.2*</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td>41±0.5</td>
<td>54±0.3*</td>
<td>41±0.6</td>
<td>54±0.5*</td>
</tr>
<tr>
<td>SNA, U/min</td>
<td>167±26</td>
<td>222±35*</td>
<td>187±32</td>
<td>260±31*</td>
</tr>
</tbody>
</table>

MAP indicates mean arterial pressure; CVP, central venous pressure; HR, heart rate; bpm, beats per minute; Ve, minute ventilation; Pco2, end-tidal carbon dioxide; and SNA, sympathetic nerve activity. n=9 except where noted.

*P<.05, control vs hypercapnia.
before digitalis compared with 144±47% after digitalis (n=8, P=NS) (Fig 3). In accordance with our earlier findings, the elimination of ventilation by apnea increased the SNA response to hypoxia more than it did the SNA response to hypercapnia. Placebo did not alter the effects of apnea on SNA responses to hypoxia and hypercapnia.

Effects of Digitalis on Responses to the Cold Pressor Test

To assess for specificity of effects of digitalis on chemoreflex-mediated mechanisms, we examined responses to the non-chemoreflex-mediated CPT before and after administration of digitalis and placebo in nine subjects (Table 3). All subjects were able to tolerate the CPT for 2 minutes without undue distress. The CPT resulted in significant increases in mean arterial pressure, heart rate, and SNA. Neither digitalis nor placebo had any significant effect on these responses.

Discussion

The novel finding in this study is that digitalis selectively potentiates the ventilatory response to hypoxia but not hypercapnia in healthy humans. Sympathetic nerve responses to hypoxia after digitalis are not increased significantly. These studies are the first in humans examining the effects of digitalis on chemoreflex stimulation and confirm earlier animal studies suggesting that digitalis sensitizes peripheral chemoreceptors.6-8 Differences in stimulus intensity during hypoxia with and without digitalis cannot explain our findings, because if anything, oxygen saturation during hypoxia averaged 85% after digitalis compared with 83% without digitalis. Our data further indicate that this sensitization selectively affects peripheral but not central chemoreceptors and predominantly potentiates the ventilatory responses. Placebo had little effect on responses to hypoxia or hypercapnia, indicating that these responses are highly reproducible in the short term.

In addition, these data confirm our earlier findings that the inhibitory effect of ventilation on SNA more potently influences the response to hypoxia than the response to hypercapnia.14 Elimination of the ventilatory response (by apnea) is associated with a greater sympathetic response during hypoxia (increase of 329%) than during hypercapnia (increase of 184%), despite the sympathetic response to hypoxia and free breathing (increase of 25%) being less than the sympathetic response to hypercapnia and free breathing (increase of 38%).

Hypoxia primarily activates peripheral chemoreceptors in the carotid bodies, whereas hypercapnia acts via central chemoreceptors located on the ventral surface of the medulla. Changes in CO2 levels can influence the effects of hypoxia on chemoreflex responses. Approximately 12% of the ventilatory response to hypercapnia is mediated by peripheral chemoreceptors. We sought to minimize these factors first by maintaining isocapnia during hypoxia, and second by maintaining hyperoxia during hypercapnia.

Both hypoxia and hypercapnia elicit increases in sympathetic activity and ventilation in humans. The ventilatory response is predominant and inhibits the sympathetic response; this inhibition affects the sympathetic response to hypoxia more than the response to hypercapnia. Baroreceptor reflex activation induced by increases in blood pressure also inhibits sympathetic responses to hypoxia more than it does the responses to hypercapnia. These differential interactions may be because thoracic, baroreceptor, and peripheral chemoreceptor afferents relay in close proximity in the region of the nucleus tractus solitarius.

These interactions may also help explain the lack of significant potentiation of the sympathetic responses to hypoxia after digitalis, despite the increased ventilatory response. First, the ventilatory response to hypoxia is predominant and inhibits the sympathetic response. Second, digitalis has a sympathoinhibitory baroreceptor-sensitizing effect and baroreceptor reflex activation also inhibits the sympathetic response to hypoxia. We speculate that both the higher ventilatory response and the increased baroreceptor reflex sensitivity after digitalis would oppose any increased sympathetic activation resulting from digitalis-induced peripheral
chemoreflex potentiation. Nevertheless, despite the sympathoinhibitory influences of increased ventilation and possible increased baroreceptor reflex sensitivity, the peripheral chemoreceptors might be more easily influenced by blood-borne agents. The central chemoreceptors, located in the brainstem, may not be that easily accessible to digitalis in the bloodstream. It is conceivable that chronic digitalis administration may allow selective potentiation of peripheral chemoreflex responses by endogenous digitalis-like substances. We speculate that the potentiation of peripheral chemoreflex responses by endogenous digitalis-like substances may help explain the increased chemoreflex responses to hypoxia in patients with essential hypertension.

**Baroreceptor reflex activation also inhibits ventilatory responses to hypoxia.** Although the possibility of this interaction attenuating the ventilatory response to hypoxia after digitalis, the potentiated ventilatory response was still apparent and thus may be underestimated. These data have several limitations. First, studies were acute in nature and do not necessarily predict long-term effects of digitalis glycosides in humans. Second, the present studies were performed in healthy humans and may not be readily extrapolated to specific disease states. Third, both hypercapnia and the CPT elicited stronger excitatory responses than did hypoxia. Thus, we cannot exclude the possibility that this stronger response may have masked a digitalis-induced potentiation of these responses. Any such potentiation may have been evident with a milder degree of hypercapnia than was used in these studies. However, even when the first minute of the response to hypercapnia was examined, digitalis did not exhibit any potentiation of responses to hypercapnia.

The mechanism of peripheral chemoreflex potentiation after digitalis is not known. The absence of potentiated responses to hypercapnia and the CPT suggests that digitalis does not induce a generalized reflex hyperresponsiveness. A possible mechanism may be the known membrane-sensitizing action of digitalis caused by blockade of sodium-potassium ATPase activity at the cellular membrane level. Selective potentiation of peripheral but not central chemoreflex responses may in part be explained by the anatomic location of the peripheral chemoreceptors in the carotid bodies, which makes them more easily influenced by blood-borne pharmacologic agents. The central chemoreceptors, located on the ventral surface of the medulla, may be less accessible to digitalis in the bloodstream. It is conceivable that chronic digitalis administration may allow potentiation of central chemoreceptor responses as well.

Our findings may have implications for both heart failure and hypertension. Digitalis-induced chemoreflex potentiation may explain the increased ventilatory response to exercise in patients with heart failure receiving digitalis therapy. Potentiated ventilatory responses to hypoxia may also be beneficial to patients with chronic obstructive lung disease and blunted hypoxic ventilatory drive, especially if these patients have coexisting heart failure. It is also possible that digitalis may favorably influence the Cheyne-Stokes breathing pattern noted in patients with severe heart failure; this consideration would be important in understanding the reasons for the beneficial effects of digitalis in heart failure patients and deserves further study.

Spontaneously hypertensive rats and hypertensive humans have potentiated chemoreflex responses to hypoxia. Hypertension is also associated with higher levels of endogenous digitalis-like substances. We speculate that the potentiation of peripheral chemoreflex responses by endogenous digitalis-like substances may help explain the increased chemoreflex responses to hypoxia in patients with essential hypertension.

**Acknowledgments**

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**References**


**Table 3. Effects of Digitalis and Placebo on Hemodynamic and Sympathetic Nerve Responses to Cold Pressor Test**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Control (Before)</th>
<th>CPT (After)</th>
<th>Control (Before)</th>
<th>CPT (After)</th>
<th>Control (Before)</th>
<th>CPT (After)</th>
<th>Control (Before)</th>
<th>CPT (After)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAP, mm Hg</td>
<td>80.1±0.6</td>
<td>69.4±3.2</td>
<td>82.2±2.5</td>
<td>104.3±4.2</td>
<td>79.6±2.1</td>
<td>98.6±3.0</td>
<td>81.6±2.7</td>
<td>99.4±3.2</td>
</tr>
<tr>
<td>CVP, mm Hg</td>
<td>5.7±0.6</td>
<td>6.1±0.8</td>
<td>4.1±0.5</td>
<td>4.0±0.6</td>
<td>5.2±0.5</td>
<td>5.4±0.7</td>
<td>5.0±0.9</td>
<td>4.8±0.9</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>65.2±4.6</td>
<td>73.7±5.9</td>
<td>62.6±5.3</td>
<td>71.6±6.3</td>
<td>85.3±3.0</td>
<td>71.8±4.3</td>
<td>83.9±3.2</td>
<td>69.2±4.6</td>
</tr>
<tr>
<td>SNA, U/mln</td>
<td>202±34</td>
<td>412±66</td>
<td>176±42</td>
<td>438±62</td>
<td>192±41</td>
<td>455±97</td>
<td>195±49</td>
<td>464±124</td>
</tr>
</tbody>
</table>

CPT indicates cold pressor test; MAP, mean arterial pressure; CVP, central venous pressure; HR, heart rate; bpm, beats per minute; and SNA, sympathetic nerve activity. *n=9.*

*P<.01, control vs CPT.

fp<.05, control before drug vs control after drug.


