Clonidine Attenuation of a Cardiogenic Hypertensive Chemoreflex

GILBERT R. HAGEMAN, PH.D., FERDINAND URTHALER, M.D., THOMAS N. JAMES, M.D. AND ROY H. SWATZELL, JR., M.S.

SUMMARY Clonidine is an antihypertensive agent with a primary action mediated by alpha adrenergic stimulation in the central nervous system, thus inhibiting sympathetic efferent activity. Serotonin activates a cardiogenic hypertensive chemoreflex which induces discharges of sympathetic efferent neurons. The purpose of this study was to determine the effects of intravenous clonidine upon the thoracic sympathetic efferent discharges in chloralose-anesthetized dogs, their peripheral autonomic receptors being blocked with atropine, propranolol, and phentolamine. Efferent nerve traffic was quantified using a Schmitt trigger and Digital PDP8/e computer. Control spontaneous activity (tone) following autonomic blockade was normalized at 100%. Serotonin (100 µg/ml, 2 ml, left atrium) caused an increase in the reflex efferent sympathetic activity to 192% ± 16% of control (p < 0.001). Following clonidine, the tone was decreased to 63% ± 6% of control, and the reflex sympathetic discharge elicited by serotonin was significantly (p < 0.001) reduced from 192% to 116% ± 9% of control tone (before clonidine). The attenuation of the reflexly elicited discharge was significantly (p < 0.05) greater than the attenuation of the tone. In four dogs that did not receive atropine, the vago-vagal reflex sinus bradycardia induced by serotonin was not affected by clonidine. (Hypertension 3: 240–244, 1981)

KEY WORDS • serotonin • sympathetic nervous system • chemoreceptor • computer analysis

Activation of a coronary chemoreceptor with 5-hydroxytryptamine (serotonin) elicits a cardiogenic hypertensive chemoreflex. Afferent traffic from the coronary chemoreceptor travels via the thoracic cardiac nerves (the left and right recurrent cardiac nerves serving as the preferential routes) to the vagus nerves and then into the central nervous system (CNS). Integration of this vagal chemoreceptor traffic from the heart induces a simultaneous reflex activation of the parasympathetic and sympathetic efferent limbs of the autonomic nervous system. The hemodynamic effects of this reflex are changes in heart rate and rhythm, in atrioventricular (AV) conduction, in atrial and ventricular contractile force, and in distribution of blood flow to various organs, with the net effect being a brisk hypertension. Clonidine is a powerful antihypertensive agent with a primary action thought to be mediated by an alpha adrenergic stimulation in the CNS, thus inhibiting sympathetic efferent traffic from the bulbar cardioaccelerator and vasoconstrictor centers. Furthermore, Krier et al. have reported that selected spinal sympathetic (lumbar) pathways may be depressed to various degrees by clonidine in anesthetized cats. Bernthal and Koss have reported that clonidine may inhibit reflexly evoked electrodermal responses in a sympathetic-cholinergic system of the spinal cat. In addition, clonidine has an effect upon peripheral autonomic nerves and modulates the release of neurotransmitters. The cardiogenic hypertensive chemoreflex can be prevented by intravenous administration of the serotonin antagonist, cyproheptadine, and the site of interdiction is at the coronary chemoreceptor.

The purpose of the present experiments was to determine whether the reflex sympathetic effects of the cardiogenic hypertensive chemoreflex were significantly attenuated in the CNS by clonidine. For this purpose, a quantitative evaluation was made of the sympathetic efferent discharge in the thoracic cardiac nerves before and after intravenous administration of clonidine. The peripheral alpha-stimulating actions of clonidine and the stimulating effect of the reflex hypertension upon the peripheral baroreceptors were attenuated by pretreatment with both atropine and with the adrenergic antagonists, propranolol and phentolamine.
Methods

Nine adult male and female mongrel dogs, weighing 14 to 23 kg, were pre-anesthetized with ketamine hydrochloride (5 mg/kg i.m.) and anesthetized with sodium pentobarbital (80–100 mg/kg i.v.). Ventilation was maintained through auffed endotracheal tube with intermittent positive pressure (Harvard respirator) and a bilateral thoracotomy performed. The animals were paralyzed with succinyl choline (60 mg i.m., supplemented as needed). Catheters were placed in the left atrium for administration of serotonin (5-hydroxytryptamine HCl) and into a femoral or carotid artery for the monitoring of systemic blood pressure. A surface lead II or III electrocardiogram was also recorded. Efferent sympathetic neural traffic was recorded with stainless steel (0.01 inch diameter) contiguous bipolar electrodes. Nerve signals were amplified, filtered (200–1000 Hz bandwidth), and recorded on a Hewlett-Packard storage oscilloscope and tape recorder. Pictures of oscilloscope tracings were made with a HP 197A camera and Polaroid film. The neurograms were later analyzed by a Digital Equipment Corporation Lab 8/e computer with a Schmitt trigger. The sympathetic discharges were recorded from the distally severed, split, and desheathed left anterior ansa (5 dogs), the RT-3 input to the stellate ganglion (1 dog), the RT-4 input to the stellate ganglion (1 dog), and the right craniovalgal cardiac nerve (2 dogs).

A histogram of the discharges of the multifiber preparations was plotted from 12 seconds of data following an injection signal. A poststimulus histogram was constructed, composed of 120 bins of 100 msec each. An integration of the histogram data was computed and total discharge activity determined. Twelve seconds of control resting tone was normalized at 100% in each dog to compare discharges between dogs and preparations with a differing number of active fibers. The Schmitt trigger of the Digital Equipment Corporation Lab 8/e computer provided a voltage threshold level that was used to count the positive-going firings of the nerve fibers. The nerve fiber data had been alternating current (AC) coupled to eliminate voltage shifts between recordings. The Schmitt trigger setting was adjusted using the control sympathetic efferent tone (the normal degree of spontaneous activity at resting blood pressure) so that no bin contained less than 1 count nor more than 20 counts. In this way little or no neural traffic remained undetected while only a small fraction of the noise of the system was counted. Control resting tones and reflex discharges were obtained following the intravenous administration of atropine (0.1–0.5 mg/kg), propranolol (0.5 mg/kg), and phenolamine (1–3 mg/kg). The cardiogenic hypertensive chemoreflex was elicited in a standardized fashion with 2 ml of serotonin 100 μg/ml, injected via the left atrial catheter. Clonidine was administered intravenously (100 μg/kg) in all nine dogs. The dose was increased to 300 μg/kg in four of the dogs in which the serotonin discharge failed to abolish the sympathetic discharge.

In four additional dogs, blockade of the cholinergic muscarinic receptor with atropine was omitted from the above protocol. In these experiments the reflexly induced parasympathetic efferent sinus bradycardia was evaluated before and after intravenous clonidine.

The numerical data are expressed as the arithmetic mean ± standard error of the mean. Statistical analysis was performed using the Student's t test. Differences between means were considered significant when p < 0.05.

Results

Injection of serotonin into the left atrium of nine dogs regularly produced the cardiogenic hypertensive chemoreflex. Control systolic blood pressure was 133 ± 5 mm Hg and diastolic pressure was 92 ± 6 mm Hg. Activation of the cardiac chemoreceptor with serotonin induced a significant (p < 0.001) and rapid (within 4 to 6 seconds) peak blood pressure increase to 246 ± 8 over 171 ± 9 mm Hg. This hypertension is similar to previously reported data.

The reflex efferent discharge in the thoracic sympathetic nerves has been quantitated by computer analysis of the multifiber traffic. The reflex discharge has a duration of 4.9 ± 0.8 seconds and the intensity for a 12-second time period is 199% ± 16% of the control resting tone. Following peripheral autonomic blockade, the resting blood pressure was significantly (p < 0.05) reduced to 155 ± 5 over 75 ± 3 mm Hg. The peak blood pressure response to serotonin administration was significantly (p < 0.001) reduced to 139 ± 7 over 101 ± 5 mm Hg. Attenuation of the baroreceptor feedback by pharmacological blockade of the peripheral autonomic receptors intensifies the sympathetic discharge to 268% ± 37% of control tone. For the evaluation of the actions of clonidine during this cardiogenic chemoreflex, the control sympathetic resting tone (spontaneous activity) and the serotonin elicited discharge were determined following peripheral autonomic blockade with atropine, propranolol, and phenolamine. By 15 to 30 minutes following clonidine administration, the resting blood pressure was 129 ± 8 over 92 ± 10 mm Hg, which is not significantly different from pre-clonidine levels. The peak blood pressure response to serotonin administration was 148 ± 11 over 108 ± 11 mm Hg, which is not significantly different from the pre-clonidine response. These blood pressure responses after peripheral autonomic blockade were similar to those reported by Hageman et al.

Figure 1 illustrates the control resting electrocardiogram, blood pressure, and sympathetic efferent traffic along with the computer-generated histogram of the nerve traffic from a typical experiment. The heart is in a sinus rhythm, the blood pressure is stable, and there is spontaneous sympathetic efferent activity. Administration of serotonin via the left atrial catheter
induces a brief but intense sympathetic discharge (fig. 2). The concomitant change in heart rate, peripheral resistance, pulse pressure, and severe hypertension have been attenuated by the pharmacological blockade of the peripheral autonomic receptors. The slowly developing small increase in blood pressure was not eliminated by vagotomy. Those relatively late pressure changes are most likely due to the combination of the direct action of serotonin upon peripheral vessels and the release of catecholamines from the adrenal glands.6,9

Administration of clonidine attenuated the resting spontaneous neural tone in each of the nine sympathetic efferent preparations investigated. Figures 3 and 4 illustrate a recording from the same dog represented in figures 1 and 2. Within 15 minutes after clonidine administration, the resting tone has diminished. In this dog, injection of serotonin after clonidine induced a diminished sympathetic discharge (fig. 4). This reflex discharge following clonidine was the maximal discharge recorded in the nine preparations studied. In the eight other neural preparations, the serotonin discharge was either totally eliminated (five dogs) or less intense than the one illustrated in figure 4. Increasing the clonidine from 100 to 300 µg/kg did not further depress either the sympathetic tone or the serotonin discharge in the four dogs so studied.

Following clonidine the resting spontaneous tone was reduced to 63% ± 6% of the control value (fig. 5). The serotonin-induced sympathetic discharge was also significantly reduced from a control value of 192% ± 16% before clonidine to 116% ± 19% following clonidine (p < 0.001). This attenuation of the reflexly elicited discharge was significantly (p < 0.05) greater than the attenuation of the tone (fig. 5). When

---

**Figure 1.** Spontaneous activity recorded in a thoracic multifiber sympathetic efferent preparation. The dog has been pretreated with intravenous atropine, propranolol, and phentolamine. The computer histogram, composed of 100 msec bins, depicts 9 seconds of the sympathetic activity.

**Figure 2.** The sympathetic efferent discharge elicited with serotonin (STN) 2 ml, 100 µg/ml, via the left atrium (same dog as in fig 1).
the serotonin-induced discharge after clonidine was compared to the reduced tone after clonidine (rather than the control tone), the percent increase in traffic with serotonin was 184%.

In four other dogs that were not pretreated with atropine, the reflex parasympathetic depression in heart rate, which is characteristic of the serotonin-induced cardiogenic hypertensive chemoreflex was noted as a sinus pause of 1.2 ± 0.3 seconds. Following clonidine administration, this sinus pause was 1.3 ± 0.5 seconds (not significant).

Discussion

In this study we determined that clonidine effectively inhibits the resting spontaneous tonic activity in the thoracic sympathetic efferent nerves of the anesthetized dog and that clonidine significantly prevents the sympathetic discharge(s) specifically elicited by activation with serotonin of a cardiac chemoreceptor. Although clonidine's mode of action includes stimulation of CNS alpha receptors, these receptors were not blocked by the prior intravenous administration of phentolamine in our experiments. These results are similar to those reported by Robson et al. who found that clonidine-sensitive CNS presynaptic alpha receptors were not blocked by phentolamine in pithed spontaneously hypertensive rats. The attenuated sympathetic activity that was elicited with serotonin after intravenous clonidine in some dogs suggests the existence of clonidine-insensitive cells or pathways in this reflex. Our methods did not permit us to determine if the reduction of the resting tone from normalized 100% to 63% following clonidine was equivalent to full suppression of physiological tone.
Clonidine also failed to affect the characteristic, parasympathetically mediated sinus bradycardia, which is elicited during the cardiogenic hypertensive chemoreflex. This suggests that clonidine does not act as a peripheral serotonin antagonist, such as cyproheptadine, since the afferent neural signal is required for the reflex parasympathetic effect. Clonidine apparently failed to interrupt the central connections between the afferent signal and the parasympathetic nerves. In addition, clonidine did not apparently affect the preganglionic-to-postganglionic parasympathetic autonomic synapses, since the vago-vagal depression of the sinus node was unaffected by clonidine; the sympathetic ganglionic transmission was not quantified in our experiments.

Seven of the nine sympathetic neural preparations were from preganglionic nerves, and the two postganglionic nerves (craniovascals) were similarly affected by clonidine. Furthermore, clonidine did not appear to affect significantly the muscarinic receptors of the sinus node. Primm et al. reported an atropine-insensitive sinus bradycardia in response to clonidine administered via the canine sinus node artery and found inconsistent effects of clonidine upon the chronotropic response to electrical stimulation of the cervical vagus nerve. The chronotropic effects of stellate stimulation were attenuated following sinus node artery administration of clonidine; this result is consistent with the $\alpha_2$ antagonism of clonidine.

We conclude that the action of clonidine in our experiments was limited to the CNS modulation of serotonin-induced afferent signals and/or the central sympathetic efferent neurons.

**Figure 5.** Histograms illustrate the resting spontaneous control sympathetic efferent traffic (normalized at 100% for these multifiber preparations), the serotonin elicited response (shaded), and the activity and responses following clonidine in nine dogs.

<table>
<thead>
<tr>
<th>Control</th>
<th>After Clonidine 100-300 µg/kg IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Neural discharge</td>
<td>% Neural discharge</td>
</tr>
<tr>
<td>Control</td>
<td>STN</td>
</tr>
<tr>
<td>200</td>
<td>150</td>
</tr>
<tr>
<td>(9)</td>
<td>(9)</td>
</tr>
</tbody>
</table>

**References**

17. Ashe JH, Cooper CL. Multifiber efferent activity in postganglionic sympathetic and parasympathetic nerves related to the latency of spontaneous and evoked papillary dilation Exp Neurol 59: 413, 1978
Clonidine attenuation of a cardiogenic hypertensive chemoreflex.
G R Hageman, F Urthaler, T N James and R H Swatzell, Jr

Hypertension. 1981;3:240-244
doi: 10.1161/01.HYP.3.2.240

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/3/2/240

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/