Acute Resetting in Two Functionally Different Types of Carotid Baroreceptors

J.L. Seagard, L.A. Gallenberg, F.A. Hopp, and C. Dean

The presence of two types of carotid sinus baroreceptors, as characterized by two different stimulus–response curves in an earlier study, suggests that each type may play a different role in the regulation of blood pressure. The discontinuous hyperbolic curve of the type I baroreceptors, marked by higher firing rates and greater sensitivity than the sigmoidal curve of type II baroreceptors, suggests that these baroreceptors would contribute more to the buffering of arterial pressure changes than the "tonically" active type II baroreceptors, which fired over greater pressure ranges and generally had spontaneous subthreshold discharge. The firing characteristics of type II baroreceptors suggest that these receptors would contribute more to regulation of tonic, baseline levels of arterial pressure. If this functional differentiation exists, the acute resetting characteristics of the two types of baroreceptors could be different. Resetting is defined as a shift in the response curve of a baroreceptor, marked by shifts in pressure threshold, in the same direction as the change in pressure to which it is exposed. Type I baroreceptors would be more likely to reset in response to a sustained acute change in pressure, since their primary role would be to prevent the initial change in pressure. However, type II baroreceptors would not reset to the acute change in pressure, since their primary role would be to maintain consistent information on the level of existing pressure. Therefore, this study was performed to examine the acute resetting ability of both types of baroreceptors by using a vasa vasorum isolated carotid sinus preparation in the dog. Single-fiber type I and II baroreceptor recordings were obtained during slow ramp changes in sinus pressure after pulsatile conditioning at mean pressures of 50, 100, and 150 mm Hg for 15–25 minutes. Seven type I baroreceptors were also conditioned using nonpulsatile mean pressures of the same values. Firing characteristics, including pressure threshold, were determined using nonlinear regression and best-fit analysis. Type I, but not type II, baroreceptors were found to reset to acute changes in pressures. There was no significant difference in resetting of type I baroreceptors in response to pulsatile versus nonpulsatile conditioning pressures. These results indicate that the resetting abilities of the two types of baroreceptors are different and suggest the possibility of functionally different roles for each in the regulation of the cardiovascular system. (Circulation Research 1992;70:559–565)

KEY WORDS • arterial baroreceptors • baroreceptor reflex • acute resetting

The ability of the arterial baroreceptors to acutely reset to short-term changes in pressure has been documented by many investigators.1–8 Resetting has been defined as the parallel shift of the baroreceptor stimulus–response curve in the direction of the change in pressure to which the baroreceptor has been exposed (conditioning pressure [CP]).9 As currently accepted, acute resetting is generally defined as the response of baroreceptors 5–30 minutes after a change in CP. The mechanism(s) responsible for this rapid response is not known but may be different from those responsible for the chronic resetting seen in animals made hypotensive or hypertensive for days to weeks. Although the mechanism(s) of acute resetting has not been established, some evidence indicates roles for both baroreceptor properties5,6,9–12 and mechanical effects of the sinus wall.1,4 The majority of previous studies have examined the resetting ability of baroreceptors with fast conducting, myelinated (A-fiber) axons, without determining the resetting ability of baroreceptors with slower conducting unmyelinated (C-fiber) axons. The evidence that does exist for the resetting ability of C-fiber baroreceptors is conflicting, with one study suggesting that C-fiber receptors acutely reset more than A-fiber baroreceptors,13 while two other reports indicate that C-fiber baroreceptors do not reset at all to acute pressure changes.14,15 The importance of acute baroreceptor resetting has not been clearly defined, because there is conflicting evidence as to whether the baroreflex as a whole will acutely reset. Some studies have shown that reflex control of sympathetic efferent nerve activity will reset within the same time frame as receptor resetting16–20; however, other studies have not found acute resetting of the reflex within the short time allotted to acute resetting.21

In a previous study, we have identified two functionally different types of carotid baroreceptors, based on their responses to slow ramp increases in carotid sinus pressure (CSP).22 As seen by the neurograph in Figure 1, type I baroreceptors are characterized by a sudden onset of
Figure 1. Responses of a type I and type II baroreceptor to slow ramp increases in carotid sinus pressure. Single-fiber baroreceptor activity is presented as raw activity (spikes) and time-averaged nerve activity (averaged). Only the raw activity was used in data analysis. For the type I baroreceptor (upper tracings), note the abrupt onset in baroreceptor discharge at threshold pressure followed by a more gradual increase to a saturation frequency. For the type II baroreceptor (lower tracings), note the spontaneous discharge at pressures below threshold pressure followed by a linear increase in firing above threshold until activity plateaus above saturation pressure.

discharge at a relatively high frequency at pressure threshold. As shown in the same figure, type II baroreceptors typically demonstrate a spontaneous, subthreshold discharge that changes to a pressure-sensitive discharge in response to increases in CSP, but the rates of firing are usually lower than those of type I baroreceptors. Stimulus (CSP)–response (baroreceptor activity) curves of a type I and type II baroreceptor are shown in Figure 2. Type I response curves are characterized by discontinuous hyperbolic curves, marked by high rates of discharge, relatively high sensitivities, and narrow operating ranges. Type II baroreceptor response curves are characterized by continuous, sigmoidal discharge curves with low rates of discharge, lower sensitivities, and wider operating ranges. Based on conduction velocities, type I baroreceptors in the previous study were found to generally have large-diameter myelinated A-afferent axons, with only 10 of 49 reported as having unmyelinated C-fiber axons.22 In contrast, type II baroreceptors were found to have small-diameter A-fiber or C-fiber axons, with an average conduction velocity of 3.8 m/sec in contrast to 6.3 m/sec for type I baroreceptors. Therefore, although the distinction is not absolute, most baroreceptors with smaller afferent axons have type II discharge curves, while baroreceptors with larger myelinated axons usually have type I discharge patterns.

The presence of two different response curves led us to suggest that the roles played by each type of baroreceptor in the control of blood pressure may be different.22 The firing characteristics of type I baroreceptors suggest that these receptors may play a greater role in preventing changes in pressure (buffering ability), while the firing characteristics and spontaneous subthreshold discharges of the type II baroreceptors indicate that these receptors may be more suited to maintaining the baseline, ongoing level of pressure. If these assumptions are true, type I baroreceptors would acutely reset in response to changes in the level of conditioning pressure, because their role in buffering pressure changes would be somewhat independent of ongoing pressure. However, type II baroreceptors would not reset to acute changes in pressure, because their role in maintaining baseline pressure would not be effective if their input did not remain proportional to absolute levels in baseline pressure. Therefore, in an attempt to provide evidence as to the possible functional roles of each baroreceptor type, this study was performed to compare the acute resetting ability of type I versus type II baroreceptors. Our results indicate that type I baroreceptors will acutely reset to both increases and decreases in conditioning pressures, while type II baroreceptors do not alter their response curves in response to acute pressure changes.

Materials and Methods

Acute resetting of the baroreceptors has been defined as a shift in the baroreceptor stimulus–response curve
in the direction of the change in CP, resulting in a change in threshold \( (P_{th}) \) and saturation \( (P_{sat}) \) pressures. This ability of carotid baroreceptors to reset was studied using an isolated carotid sinus preparation in anesthetized (25 mg/kg sodium thiopental bolus and 10 mg/kg/hr infusion) mongrel dogs as previously described.

Briefly, the left carotid sinus was vascularly isolated to permit either a flow-through pulsatile perfusion of the sinus region at constant mean CPs of 50, 100, or 150 mm Hg or a slow ramp increase in CSP (1–2 mm Hg/sec). Buffered lactated Ringer’s solution was used as the perfusate, oxygenated with 100% O\(_2\) to chemically denervate any chemoreceptors not physically eliminated by the isolation technique.

CSP was measured via a catheter in the lingual artery and recorded along with arterial pressure via Statham pressure transducers on a Model 7D polygraph (Grass Instrument Co., Quincy, Mass.). These and all other parameters were also recorded on a Model D FM tape recorder (A.R. Vetter Co., Rebersburg, Pa.) for later data analysis. The left cervical vagosympathetic trunk was sectioned to eliminate any sympathetic efferent activity to the isolated sinus.

Single-fiber baroreceptor activity was recorded from the left carotid sinus nerve. The sinus nerve was identified, desheathed, and dissected into smaller bundles, which were then covered with mineral oil; surrounding tissue was used to create a recording chamber. To record single-fiber activity, tungsten carbide recording needles were connected to a high impedance differential preamplifier (gain, 1,000; passband, 0.1–10 kHz), followed by a filter/amplifier, which provided additional gain (up to 400) and high and low pass filtering (fourth order Butterworth, 10 Hz–3 kHz). Amplifier output was recorded on the FM tape recorder, averaged, and displayed on the Grass recorder. Small nerve bundles were dissected to obtain a single active fiber preparation, which remained viable for the entire experiment.

To determine the ability of the baroreceptors to reset to acute changes in conditioning pressures, the isolated carotid sinus was perfused at 50, 100, or 150 mm Hg for 25 minutes. Constant pressure was maintained using a servocontroller developed in this laboratory. After a 25-minute perfusion of the carotid sinus at one of the CPs, pump perfusion of the carotid sinus was abruptly halted and the outflow cannula was clamped, temporarily making the sinus a closed pouch. A syringe pump (Harvard Apparatus, South Natick, Mass.), in-line with the inflow cannula, was used to infuse lactated Ringer’s solution into the sinus pouch at a constant rate producing a slow, linear increase in CSP at a rate of 1–2 mm Hg/sec from 0 to 250 mm Hg. The pressure ramp was repeated before the perfusion circuit was reopened and constant CSP reestablished. The response to the second pressure ramp was used to construct baroreceptor stimulus–response curves and determine the firing characteristics of each baroreceptor. The sinus was then exposed to one of the remaining CPs for 25 minutes, and the process was repeated. For several fibers, resetting was also tested 15 and 20 minutes after the pressure change. A slight difference in \( P_{th} \) was observed in these fibers after 15 and 20 minutes of conditioning versus the values obtained at 25 minutes. Therefore, 25 minutes was used as the time interval for resetting in this study.

Responses measured with slow ramp pressure inputs are similar to responses measured after 30 seconds of adaptation to previous levels of step inputs and were therefore considered to represent the static firing characteristics of carotid sinus baroreceptors to a pressure stimulus. Thus, this study did not investigate the dynamic firing characteristics examined in some other studies. In addition, some previous studies have shown that type I-like baroreceptors will not reset in response to a pulsatile CP, while other studies have shown that pulsatile CPs do not prevent acute resetting in these receptors. Therefore, the effects of using pulsatile versus nonpulsatile CPs were examined for seven type I baroreceptors in this study. Following the acute resetting protocol and using pulsatile conditioning as described above, we added a Windkessel to the perfusion circuit to eliminate pulsations from the CP. The protocol was repeated for all three mean CPs, with the response curves determined using the slow ramp pressure changes as described above. To ensure that order was not an important factor, for three of the seven baroreceptors, static CPs were used before pulsatile pressures.

Individual action potentials were displayed on a storage oscilloscope, and only recordings with an identical action potential at all pressures were considered single-fiber recordings and analyzed for this study. In our experience, careful dissection produces single A- and C-fiber recordings with signal-to-noise ratios that allow discrimination of an individual action potential. For analysis of the single-fiber baroreceptor afferent nerve activity, tape-recorded raw activity was processed using a window discriminator that generated a voltage pulse for each spike. The pulse was fed into a digital counter/timer whose analog output was proportional to the number of spikes per unit time (1 second). The
Table 1. Effects of Changes in Conditioning Pressure on Firing Characteristics of Type I and Type II Baroreceptors

<table>
<thead>
<tr>
<th></th>
<th>Type I (n=33) with CP:</th>
<th>Type II (n=18) with CP:</th>
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<tbody>
<tr>
<td></td>
<td>50 mm Hg</td>
<td>100 mm Hg</td>
</tr>
<tr>
<td>( P_{th} ) (mm Hg)</td>
<td>69.7±5.1</td>
<td>77.3±4.8*</td>
</tr>
<tr>
<td>( P_{sat} ) (mm Hg)</td>
<td>175.6±6.7</td>
<td>178.9±6.3*</td>
</tr>
<tr>
<td>( F_{in} ) (spikes/sec)</td>
<td>24.6±1.6</td>
<td>24.7±1.5</td>
</tr>
<tr>
<td>( F_{sat} ) (spikes/sec)</td>
<td>64.5±6.2</td>
<td>64.7±6.1</td>
</tr>
<tr>
<td>Slope (spikes/sec/mm Hg)</td>
<td>0.78±0.11</td>
<td>0.92±0.13*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CP, conditioning pressure; \( P_{th} \), threshold pressure; \( P_{sat} \), saturation pressure; \( F_{in} \), threshold frequency; \( F_{sat} \), saturation frequency.

*Significantly different \((p<0.05)\) from values at CP=50 mm Hg.
†Significantly different \((p<0.05)\) from values at CP=50 and 100 mm Hg.

counter output and ramp CSP were sampled (1 Hz) using a Hewlett-Packard 310 computer and stored on computer disk for later analysis, as follows.

Single-fiber baroreceptor activity (response) versus ramp changes in CSP (stimulus) was plotted for each baroreceptor with the HP 310 computer. Steady-state stimulus–response curves for the CSP–baroreceptor frequency relation belonged to one of two previously described categories22: baroreceptors showing 1) a discontinuous, hyperbolic stimulus–response curve (type I); or 2) a continuous, sigmoidal stimulus–response curve (type II). Standard nonlinear least-squares regression techniques described previously22 were used to obtain optimum fit of the experimental data and thereby obtain the best-fit estimates of the following parameters: threshold \( (F_{th}) \) and saturation \( (F_{sat}) \) firing rates, \( P_{th} \) and \( P_{sat} \), and maximum gain (slope). Type I stimulus–response curves were analyzed using a hyperbolic curve-fitting regression, whereas type II curves were analyzed with a sigmoidal curve-fitting routine. For both types of receptors, the maximum gain (slope) of each curve was determined to quantitate the sensitivity of each receptor. The maximum gain for type I baroreceptors was the initial slope of the hyperbolic stimulus–response curve, whereas the maximum gain of each sigmoidal type II stimulus–response curve was the slope at the midpoint of the linear portion of the curve. Because of the presence of spontaneous activity with type II baroreceptors, \( P_{th} \) was calculated as \( P_{th} = P_{1/2(T)} - (2.634/k) \) and \( P_{sat} = P_{1/2(T)} + (2.634/k) \), based on the analysis of Sagawa.31 \( P_{th} \) was identified as the inflection point of the sigmoidal baroreceptor activity–CSP curve at which firing frequency starts to increase from the spontaneous firing rate and \( P_{sat} \) as the inflection point at which firing frequency starts to plateau. Although the low rate of discharge at \( P_{th} \) is not much different from the spontaneous rate of discharge at subthreshold pressures, this threshold frequency does not change in response to changes in CP. Therefore, the curve-fitting technique was applied to a consistent pattern of discharge for a given fiber at all levels of CP. A similar analysis for type I receptors calculated \( P_{sat} \) as \( P_{sat} = P_{th} + (2.534/k) \). \( P_{th} \) for these receptors was easily determined as the pressure at which discharge abruptly began.

The values for \( P_{th}, P_{sat}, F_{in}, F_{sat}, \) and slope for type I or type II baroreceptors for each CP were compared within groups (type I versus type II) by using a one-way analysis of variance with repeated measures. Significantly different means were located using the Newman-Keuls test. All levels of significance were set at \( p<0.05 \) a priori. To determine the effects of pulsatile versus nonpulsatile pressure on resetting ability, firing characteristics of the seven type I baroreceptors tested with both types of CPs were compared across groups by using a paired \( t \) test, with significance set at \( p<0.05 \).
baroreceptors at all pressures. Analysis indicated that there was a 20% possibility of a type II error for differences in $P_a$ at 100 mm Hg versus either 50 or 150 mm Hg. This percentage increased to 30% for the difference in $P_a$ between 50 versus 150 mm Hg. These levels of $\beta$ are considered acceptable for a significance level set at $p<0.05$.\textsuperscript{34}

The effects of pulsatile versus nonpulsatile CP on type I baroreceptors are shown in Table 2. As indicated, there were no significant differences between the firing characteristics at each CP with pulsatile versus nonpulsatile CP. However, there were nonsignificant trends following nonpulsatile pressure for the $P_a$ and $P_{sat}$ to be higher and lower, respectively, than the $P_a$ and $P_{sat}$ following pulsatile conditioning at each CP. As seen in the above study, the percent resetting was greater for changes in CP between 50 and 100 mm Hg (27.8% pulsatile, 28.4% nonpulsatile) than for changes between 100 and 150 mm Hg (13.8% pulsatile, 14.0% nonpulsatile).

Discussion

Acute resetting was found to occur within 25 minutes of a pressure change for type I, but not type II, baroreceptors. No resetting changes in the response curves of type II baroreceptors were found in this study. These results complement the findings of Shultz et al.,\textsuperscript{14,15} who reported no resetting in C-fiber baroreceptors, most of which would be type II baroreceptors in the present study. However, these results disagree with the findings of Yao and Thoran,\textsuperscript{13} who found a greater degree of acute resetting in C-fiber versus A-fiber baroreceptors. The last study included data from only three C-fiber baroreceptors and may have been representative of the few C-fiber baroreceptors that have a type I discharge pattern. Accompanying the changes in $P_a$ of the type I baroreceptors was a decrease in slope at the lowest CP and an increase in $F_{sat}$ at the highest CP, responses not usually attributed to the resetting process. The reasons for these changes in firing characteristics are not known. However, the trend for a decrease in slope, or sensitivity, at the lower CP was also observed in an earlier study,\textsuperscript{7} although the response there was limited to a few fibers.

The percent of resetting seen for type I baroreceptors falls within the range reported by earlier studies for A-fiber baroreceptors,\textsuperscript{7} although it is somewhat less than that seen in most studies. The present study also found a trend for a greater degree of resetting for changes in CP between 50 and 100 mm Hg than for changes in CP between 100 and 150 mm Hg. Dorward et al.\textsuperscript{2} and Heesch et al.\textsuperscript{3} also found smaller percent changes in $P_a$ at higher versus lower CP. Part of this difference in degree of resetting may be due to the shape of the hyperbolic response curves for the type I baroreceptors but the lack of change in the sigmoidal curves of the type II baroreceptor.

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** Response curves for an “average” type I and type II carotid baroreceptor, demonstrating the acute resetting effects resulting from changes in conditioning pressure (CP) among 50, 100, or 150 mm Hg. Response curves were generated using best-fit equations and values obtained experimentally for threshold and saturation frequencies, threshold and saturation pressures, and slope shown in Table 1. Acute resetting occurred for type I but not type II baroreceptors, as seen by the right–left shifts in the hyperbolic response curves for the type I baroreceptors but the lack of change in the sigmoidal curves of the type II baroreceptor.

### Table 2. Effects of Pulsatile Versus Nonpulsatile Conditioning Pressures on Firing Characteristics of Type I Baroreceptors

<table>
<thead>
<tr>
<th></th>
<th>Pulsatile CP</th>
<th>Nonpulsatile CP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>50 mm Hg</td>
<td>100 mm Hg</td>
</tr>
<tr>
<td>$P_a$ (mm Hg)</td>
<td>78.3±8.9</td>
<td>92.2±9.5</td>
</tr>
<tr>
<td>$F_{sat}$ (mm Hg)</td>
<td>193.2±21.7</td>
<td>199.1±19.9</td>
</tr>
<tr>
<td>$F_a$ (spikes/sec)</td>
<td>23.3±4.1</td>
<td>23.0±4.0</td>
</tr>
<tr>
<td>$F_{sat}$ (spikes/sec)</td>
<td>57.6±5.5</td>
<td>57.5±5.0</td>
</tr>
<tr>
<td>Slope (spikes/sec/mm Hg)</td>
<td>0.51±0.09</td>
<td>0.55±0.10</td>
</tr>
</tbody>
</table>

Values are mean±SEM. CP, conditioning pressure; $P_a$, threshold pressure; $P_{sat}$, saturation pressure; $F_a$, threshold frequency; $F_{sat}$, saturation frequency. No significant differences between variables for pulsatile versus nonpulsatile CPs were found.
in distension\textsuperscript{1,4} or changes in receptor electrogenic properties\textsuperscript{5,6,9–12} are involved. Whatever the actual mechanism involved, there must be differences in the way that type I versus type II baroreceptors respond to the change in CP, leading to the differences seen in acute resetting ability. An earlier study from this laboratory did find that a transient potassium current, the A-current, played a role in determining the firing rate of the type I, but not type II, baroreceptors.\textsuperscript{31} It is not known if this current plays a role in the acute resetting process, but it could be a possible source of difference in the resetting behavior of the two types of baroreceptors. A difference in the coupling of baroreceptor type within the sinus wall may also be involved. If C-fiber baroreceptors (mostly type II) are either more loosely coupled\textsuperscript{27} or coupled to less distensible wall components\textsuperscript{35} than are A-fiber baroreceptors (type I), the changes in CP leading to creep or stress relaxation may not produce the same degree of distension of both types of baroreceptors. Thus, whether acute resetting acts via wall or receptor mechanisms, the degree of distension may not be sufficient to initiate the appropriate mechanism in the C-fiber baroreceptor.

No difference was found in the acute resetting response to pulsatile versus nonpulsatile conditioning flow. This contrasts with prior studies that reported that pulsatile conditioning attenuated or prevented acute resetting\textsuperscript{28,29} but agrees with studies by other investigators that found pulsatility did not eliminate resetting\textsuperscript{1,2,8,30} Although there was a trend for the Pa, following pulsatile conditioning to be lower than Pa following nonpulsatile conditioning, this finding was not statistically significant in this study. However, other studies have reported a lowering in Pa\textsubscript{sh}\textsuperscript{36} and a greater baroreflex inhibition of blood pressure\textsuperscript{37–39} and sympathetic activity seen in response to conversion to a pulsatile baroreceptor stimulation from a nonpulsatile stimulus. These results suggest that some factor resulting from the pulsatile stimulus enhances baroreceptor control of pressure. The pattern of afferent baroreceptor activity that produces the most effective central inhibition of sympathetic outflow is not known but is obviously related to a pulsatile receptor stimulation.

The difference in resetting abilities of the two types of baroreceptors may have functional significance. As proposed earlier, if type I baroreceptors mainly serve to buffer changes in pressure, the occurrence of acute resetting is part of their role in control of pressure, serving as “floating” regulators that buffer large changes in mean pressure. However, if type II baroreceptors primarily regulate mean levels of pressure, the lack of resetting observed in the present study would result in “fixed” regulators of mean pressure within a given time frame. It is not known if the type II baroreceptors would reset over longer time periods in the face of chronic hypertension or hypotension. If so, it would be of interest to determine if distensibility of putative coupling elements in the sinus wall would undergo parallel changes, offering evidence of a role in differences in coupling mechanics as a possible reason for differences seen in the functional responses of the two types of baroreceptors. One study does suggest that C-fiber baroreceptors reset less to chronic changes in pressure,\textsuperscript{28} offering evidence that they could be more aptly suited to regulation of baseline levels of pressure than the resetting A-fiber baroreceptors. This led investigators to propose that C-fiber baroreceptors may play a primary role in the regulation of mean pressure,\textsuperscript{40} although the presence of high Pa implied that they may be most important as “antihypertensive” regulators.\textsuperscript{40–42} Again, the above studies used a different classification system to separate baroreceptors based on anatomic, and not functional, criteria. However, the fact that most C-fiber baroreceptors have type II response curves allows the extension of these findings to the present study.

Acknowledgment

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