Chronic Localized Hypotension and Resetting of Carotid Sinus Baroreceptors
Electrophysiological and Histological Studies in the Dog

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SUMMARY Resetting of carotid sinus (CS) baroreceptors to chronically elevated systemic pressure in hypertension has been demonstrated. The effects of chronic systemic hypotension on CS baroreceptor afferents has not been elucidated, however. The purpose of the present study was to determine the electrophysiological and histological characteristics of CS baroreceptors exposed to chronic hypotension. Chronic unilateral hypotension was produced by anastomosis of the common carotid artery to the external jugular vein. CS nerve activity and histology of the normotensive sinus (mean CS pressure \( \pm \) SD = 96 \( \pm \) 10.9 torr) were compared to those of the hypotensive sinus (mean CS pressure 50 \( \pm \) 14.2 torr) 44-48 days after anastomosis in nine mongrel dogs. An isolated CS pouch preparation was used to produce standard pressure changes. Threshold pressure for hypotensive sinuses (15.5 \( \pm \) 5.19 torr) was significantly less \((P < 0.05)\) than for normotensive sinuses (40.6 \( \pm \) 9.73 torr). Saturation pressure was also significantly less in the hypotensive sinus \((P < 0.05)\). There were no significant differences between slopes of the stimulus-response curves or in architecture of intima or media of the two sides. Thus, the stimulus-response curve was shifted to the left in chronic CS hypotension, and this effect could not be related to a change in CS light microscopic histology. Circ Res 49: 649-654, 1981

RESETTING of carotid and aortic baroreceptors to elevated systemic pressure has been demonstrated in established renal hypertension (Mc-Cubbin, 1958; Salgado and Krieger, 1973), in the spontaneously hypertensive rat (Nosaku and Okamoto, 1970; Nosaku and Wang, 1972; Sapru and Wang, 1976), and in coarctation of the aorta (Igler et al., 1981). Few studies have considered the effects of hypotensive levels of systemic pressure on the characteristics of the baroreflex arc (i.e., afferent, central, effenter, or end organ effects). Recently Salgado and Krieger (1976, 1978) examined aortic baroreceptor adaptation to hypotension at 1, 6, and 48 hours after lowering systemic blood pressure by hemorrhage or sympathetic blockade. They found decreased threshold and saturation for nerve discharge in hypotensive animals. The effect of chronic hypotension on carotid sinus baroreceptors has not been studied, nor has the effect of chronic hypotension on the stimulus-response curve relating pressure and baroreceptor activity been characterized.

Pathological changes (i.e., to vessel wall and/or receptor ending) due to chronic elevation of pressure in established hypertension have been demonstrated and have been postulated as a mechanism for baroreceptor resetting (Hilgenberg, 1958; Aars, 1968; Angell-James, 1973; Sapru and Wang, 1976). Studies by Brown et al. (1976), however, indicated that in early stages of hypertension in the spontaneously hypertensive rat the receptors themselves were primarily responsible for resetting. In addition, a recent study by Sapru and Krieger (1979) has shown partial upward resetting of the aortic baroreceptor in the spontaneously hypertensive rat in the absence of architectural changes of the aortic wall detectable by light microscopy.

To our knowledge the effects of chronic hypotension on carotid sinus wall architecture have not been studied. If no significant changes in vessel wall or nerve endings occur, it would seem unlikely that gross histological changes per se are necessary for the changes in baroreceptor characteristics which occur as a result of altered systemic pressure.

The purpose of the present study was 2-fold: (1) to determine the extent of resetting and stimulus-response characteristics of carotid sinus baroreceptors when chronic localized hypotension was the only known pathophysiological alteration; and (2) to determine if changes in carotid sinus wall or nerve ending architecture detectable by light microscopy occur, and if so, what relation these changes have to baroreceptor resetting. To eliminate possible systemic metabolic, humoral (Kirschheim, 1976), or electrolyte (Brown, 1980) effects which may alter baroreceptor nerve discharge characteristics, a preparation consisting of unilateral carotid sinus hypotension was utilized in the present study. With this experimental preparation,
a normotensive and a hypotensive carotid sinus were available for comparison in each animal studied.

Methods

Unilateral side-to-side anastomosis (Fig. 1) of the common carotid artery and external jugular vein was performed on nine mongrel dogs under sodium pentobarbital anesthesia (30 mg/kg, iv) to produce local, sustained, carotid sinus hypotension (Table 1). The anastomosis was made distant from the carotid sinus to avoid effects of local scarring. The common carotid artery proximal to and external jugular vein distal from the anastomosis were ligated. The anastomosis was made on the right side in four animals and on the left side in the remainder. The carotid sinus on the unoperated side was exposed to normal, systemic blood pressure (Table 1). At no time after the operation did animals show clinical signs of high output heart failure or cerebrovascular insufficiency. Forty-four to 49 days after anastomosis the animals' trachea was intubated with a cuffed endotracheal tube after sodium thiopental administration; animals were then anesthetized with 0.5–1.0% halothane in 50% oxygen and ventilated using a Bird respirator. Baseline carotid sinus pressures for hypotensive and normotensive sides were then simultaneously recorded for a minimum of 2 minutes through a lingual arterial cannula utilizing Statham P23Db pressure transducers before further surgical procedures. A carotid sinus pouch preparation was used to systematically manipulate pressure as described previously (Igler et al., 1981). Carotid sinus baroreceptor function was studied by recording multifiber nerve activity from the peripheral end of the carotid sinus nerve which was divided at its junction with the glossopharyngeal nerve, desheathed, placed across tungsten carbide bipolar electrodes, and immersed in a chamber filled with warm mineral oil. The pressure-response run took 7–10 minutes for the normotensive side and 6–8 minutes for the hypotensive side. During pressure manipulations the pressure in the opposite sinus remained near that initially recorded.

Data Analysis and Statistics

Sufficient neural data were obtained from eight of the nine animals studied. The sinus to be studied first was selected randomly. Threshold pressure (i.e., the pressure at which distinct neural discharge became clearly increased) was determined by slowly increasing carotid sinus pressure from subthreshold levels (Fig. 2). This procedure was repeated several times for each nerve preparation. Saturation pressure (i.e., the pressure at which no further increase in nerve activity occurred with increases in pressure) was determined during systematic manipulations of carotid sinus pressure (see below). To generate stimulus-response curves of carotid sinus pressure versus nerve activity, pressure was increased in 10-mm Hg increments from threshold to saturation pressures. It was thought that chemoreceptor afferents did not significantly influence results because of the high PaO₂ and low flow differences between steps in pressure. Nerve activity was filtered by an active low pass filter to obtain electrically time-averaged activity (Irisawa and Ninomya, 1967; Kirchheim, 1976). To obtain adapted, steady state, nerve activity (Franz et al., 1971) at each level of pressure, the activity occurring between 5 and 20 seconds after the change in carotid sinus pressure was averaged using a PdPll digital computer. Nerve data was normalized by dividing all data points by maximum nerve activity (activity occurring at saturation pressure) to obtain a plot of carotid sinus pressure versus percent maximum nerve activity. Normalization was done so comparisons could be made between different whole nerve preparations in which a different number of active fibers were at differing distances from the recording electrodes.

Sensitivity (slope) was determined and compared utilizing linear regression statistics on data points between 20 and 80% of maximum nerve activity. Comparisons between threshold and saturation pressures were made using Student's t-test and the paired t-test. Mean values were considered significantly different when P < 0.05 in two-tailed tests.

Histology

The carotid sinus were removed from each dog and fixed in mixed glutaraldehyde and formalin solution. After fixation they were divided into three equal sections from the tip to the base and labeled accordingly. These sections were stained with hematoxylin-eosin and Morat stains. The thickness of the media and the intima of the carotid sinuses was determined microscopically with a micrometer disc attached to the eyepiece. The ratio between

**Figure 1** Schematic diagram depicting unilateral side-to-side anastomosis of the common carotid artery to the external jugular vein. The jugular vein distal to and common carotid artery proximal to the anastomosis were ligated. The procedure was done distant from the carotid sinus to avoid the effects of local scarring. The arrow shows the direction of blood flow.
Results

Hemodynamic characteristics of normotensive and hypotensive carotid sinuses are presented in Table 1. Systolic, diastolic, mean, and pulse pressures were significantly less ($P < 0.05$) in the hypotensive sinus. Paired analysis revealed an average decrease of 46 ± 7.04 mm Hg for mean pressure in the hypotensive sinus. Figure 2 shows an example of baseline carotid sinus pressures (pressure after anesthesia, recorded simultaneously for both sinuses) and determination of threshold for a typical experimental animal. Carotid sinus pressure was increased until the first distinct increase in averaged nerve activity was detected. In this animal threshold pressure was 40 mm Hg for the normotensive sinus and 16 mm Hg for the hypotensive sinus. Saturation pressure was determined during systematic manipulations of pressure as shown in Figure 3. Figure 3 depicts manipulations of pressure in the carotid pouch preparations comparing a normotensive sinus to a hypotensive sinus. The continuous firing pattern at steady state pressure occurring between 5 and 20 seconds after an increase in pressure is shown. The typical overshoot of nerve activity with change in pressure which occurs before a steady state discharge pattern is not shown. For purposes of illustration, only 5 seconds of carotid sinus pressure, averaged nerve activity, and raw nerve activity at each level are depicted. The first increase in nerve activity (represented by the solid dot on the pressure axis) occurred between 40 and

<table>
<thead>
<tr>
<th>Baseline Pressure (mm Hg)</th>
<th>Normotensive</th>
<th>Hypotensive</th>
<th>Paired Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic</td>
<td>115</td>
<td>55†</td>
<td>60†</td>
</tr>
<tr>
<td></td>
<td>(14.27)</td>
<td>(17.23)</td>
<td>(7.56)</td>
</tr>
<tr>
<td>Diastolic</td>
<td>89</td>
<td>46†</td>
<td>43†</td>
</tr>
<tr>
<td></td>
<td>(12.75)</td>
<td>(13.07)</td>
<td>(7.05)</td>
</tr>
<tr>
<td>Mean</td>
<td>96</td>
<td>50†</td>
<td>46†</td>
</tr>
<tr>
<td></td>
<td>(10.53)</td>
<td>(14.02)</td>
<td>(7.04)</td>
</tr>
<tr>
<td>Pulse</td>
<td>27.5</td>
<td>8.9†</td>
<td>18.5‡</td>
</tr>
<tr>
<td></td>
<td>(0.56)</td>
<td>(4.90)</td>
<td>(3.14)</td>
</tr>
<tr>
<td>Threshold Pressure (mm Hg)</td>
<td>40.6</td>
<td>15.5‡</td>
<td>23.1‡</td>
</tr>
<tr>
<td></td>
<td>(9.73)</td>
<td>(5.16)</td>
<td>(3.62)</td>
</tr>
<tr>
<td>Saturation Pressure (mm Hg)</td>
<td>158.1</td>
<td>121.3‡</td>
<td>36.9‡</td>
</tr>
<tr>
<td></td>
<td>(9.23)</td>
<td>(18.7)</td>
<td>(5.51)</td>
</tr>
<tr>
<td>Slope §</td>
<td>1.15</td>
<td>1.32</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>(0.21)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* All values expressed as means (SD).
† Values expressed as $t$ (df).
‡ Significant difference $P$ ≤ 0.05
§ Data compared using linear regression statistics.
FIGURE 3 A typical example of systematic manipulation of carotid sinus pressure (CSP) in normotensive (top traces) and hypotensive (bottom traces) sinus. Note that threshold (T, represented as a solid dot on the pressure axis) and saturation (S, represented by an asterisk) are substantially lower for the hypotensive sinus.

50 mm Hg for the normotensive sinus but between 10 and 20 mm Hg for the hypotensive sinus. It should be noted that the greatest increase in nerve activity with a single 10-mm Hg step in pressure occurred at 70 mm Hg for the normotensive sinus but at 50 mm Hg for the hypotensive sinus. Saturation of nerve activity (represented by the asterisk on the pressure axis) occurred at 150 mm Hg for the normotensive compared to 110 mm Hg for the hypotensive sinus.

Figure 4 depicts stimulus-response curves characterizing baroreceptor activity. There was a shift of the curve to the left with a slight increase in slope. The greatest increase in nerve activity with a 10-mm Hg increase in pressure occurred between 50 and 60 mm Hg for hypotensive sinus and between 60 and 70 mm Hg for normotensive sinuses. Table 1 shows results from both unpaired and paired analysis of data. Threshold and saturation pressures were both significantly lower (P ≤ 0.05) for the hypotensive sinuses with an average decrease (d ± se) of 23.1 ± 3.62 for threshold and 36.9 ± 5.31 for saturation pressure. Slope determined from data between 20 and 80% of maximum nerve activity was slightly higher for the hypotensive sinuses (1.32) compared to normotensive sinuses (1.15) but not significantly different.

Histology

The results of the histological examination of the sinuses are summarized in Table 2. The thickness of the media and intima and the media/intima ratio did not differ when the sinuses exposed to long term hypotension were compared to those exposed to normotension. In no animal were microscopically visible differences noted between the vessel walls of the two sides. Examination of the nerve fibers in the area of the sinuses revealed no pathological findings. Although there were some fibers in which axonal swelling or an increase in Schwann cells were present, the changes were slight and were seen both in normal and hypotensive sinuses.

Discussion

The effect of chronic hypotension on carotid sinus baroreceptor function has not been reported. The present study shows that a significant shift in the stimulus-response curve to the left occurs. These results are consistent with studies of Salgado and Krieger (1976, 1978) which showed a decrease in threshold and saturation pressure and the pressure necessary to obtain a "normal" pattern of firing of aortic baroreceptors after acute hemorrhagic hypotension and sympathetic blockade. The present study has also demonstrated that in the carotid sinus a local decrease in pressure apart from systemic metabolic, humoral (Kirchheim, 1976), electrolyte, or acid-base (Brown, 1980) effects decreases baroreceptor firing range. The only other report of the effect of local pressure changes and baroreceptor function was by Kezdi et al. (1973). They showed that in renal hypertension if one carotid sinus was protected from the elevation in pressure no resetting to the elevated systemic pressure occurred. The present study supports the hypothesis that a local change in vascular pressure is sufficient for resetting of baroreceptor afferents. Intimal thickening (Angell-James, 1973), medial necrosis and
chronic hypertension and baroreceptor resetting/Igler et al.

Figure 4  Stimulus-response curves of carotid sinus pressure versus percent maximum nerve activity for normotensive and hypotensive sinuses. Note the significant shift to the left of the curve for the hypotensive sinus. All values are means ± SE.

calcification (Angell-James, 1973), and nerve fiber damage (Hilgenberg, 1958; Angell-James, 1973) have been demonstrated in chronic hypertension and have been thought to play an important role in baroreceptor resetting (Hilgenberg, 1948; Aars, 1968; Angell-James, 1973; Sapru and Wang, 1976). In the present study no histological change could be demonstrated using similar techniques of fixation and staining. However, a significant shift of the stimulus-response curve to the left in chronic hypotension was demonstrated. As previously suggested by Aars (1968), pathological changes in the baroreceptor nerve fibers or vessel wall may have the effect of changing the slope of the stimulus-response curve relating whole nerve activity and pressure rather than simply shifting the stimulus-response curve. The pathological changes found in hypertension may serve to modify and/or intensify baroreceptor resetting which may be due simply to changes such as vessel wall and receptor ionic content (Brown, 1980), occurring with alteration of local vessel wall pressure dynamics.

In conclusion, the present study has demonstrated that chronic, localized carotid sinus hypotension results in a significant decrease in the operating range of pressure of carotid sinus baroreceptors with no significant change in slope of the stimulus-response curve relating pressure and afferent discharge. No significant differences in vessel wall or nerve fiber architecture between normotensive and hypotensive sinuses could be demonstrated. It appears, therefore, that the local mechanical factor play the predominant role in the resetting of baroreceptors and pathological and histological changes previously demonstrated serve only to further modify this initial resetting.

Table 2  Comparison of the Normotensive and Hypotensive Carotid Sinus (n = 9)

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
<th>Hypotensive</th>
<th>Paired analysis</th>
</tr>
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<tbody>
<tr>
<td>Media (mm)</td>
<td>0.152</td>
<td>0.173</td>
<td>0.020†</td>
</tr>
<tr>
<td>Intima (mm)</td>
<td>0.014</td>
<td>0.016</td>
<td>0.020</td>
</tr>
<tr>
<td>Intima:media ratio</td>
<td>0.002</td>
<td>0.020</td>
<td>0.033†</td>
</tr>
<tr>
<td>Paired analysis</td>
<td>0.032</td>
<td>0.009</td>
<td>0.034</td>
</tr>
</tbody>
</table>

* All values expressed as means (SE).
† Values expressed as d (SE).
‡ Not significant.

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