Effects of Changes in Extracellular Ionic Concentrations on Aortic Baroreceptors with Nonmyelinated Afferent Fibers

Peter Thoren, Michael C. Andresen, and Arthur M. Brown
From the Department of Physiology and Biophysics, University of Texas Medical Branch Galveston, Texas

SUMMARY. The effects of ionic changes upon one of the two classes of aortic baroreceptors, those having unmyelinated axons or C-fibers, have not been examined heretofore. Differences from results in aortic baroreceptors with myelinated axons might be expected because of differences in accessible surface area-volume relationships. Recordings were obtained using an in vitro aortic arch-aortic nerve preparation from 21 aortic C-fibers in 15 normotensive Wistar-Kyoto rats (WKY) 13–17 weeks old. During a slow pressure ramp, the endings responded with an irregular discharge. However, if the discharge was averaged over 10–20 seconds, the average discharge correlated well with the pressure level in the arch. The maximal discharge increased with faster ramp rates but the threshold was not shifted over the range of ramp rates employed. The relationship between pressure and discharge was tested during alterations in the extracellular concentrations of Na, K, and Ca ([Na]o, [K]o, and [Ca]o). A 12% decrease in [Na]o clearly decreased the firing in the aortic C-fibers during a ramp stimulus. During a pressure step, the initial discharge was not decreased but the steady state discharge was clearly depressed. When the aortic arch was perfused continuously at constant pressure, a decrease in Na of only 6% depressed the activity. Doubling [K]o increased firing for a few minutes and was followed by a reduction of discharge. Previous reports have shown no effect on myelinated baroreceptor discharge by a 12% decrease in [Na]o, a change which significantly depressed firing in all C-fiber baroreceptors in this study. Thus, C-fiber baroreceptors are more sensitive to changes in extracellular ionic composition than are baroreceptors with myelinated axons. (Circ Res 50: 413–418, 1982)
Results

Recordings were obtained from 21 irregularly firing baroreceptors identified in 15 rats. Conduction velocities were measured in 10 afferent fibers and were between 0.6 and 1.7 m/sec confirming earlier observations (Thoren et al., 1977) that these irregularly discharging fibers are all unmyelinated C-fibers.

Effects of Different Ramp Rates on Discharge

Figure 1 shows the typical response of an irregularly discharging UB to a 2 mm Hg/sec ramp from 50 to 240 mm Hg. In A, the instantaneous frequency is plotted against time after onset of the ramp. In B, the discharge rate is plotted against pressure. The scatter in the data makes interpretation difficult. Figure 1C shows the discharge rate averaged over 10 mm Hg pressure rises and plotted against pressure. The scatter is greatly reduced and, at suprathreshold pressures (<120 mm Hg), there is a good correlation between average discharge and aortic pressure.

Threshold in UB's does not appear to be strongly dependent on ramp rate over the range used in the present experiments. Figure 2 shows the pressure-discharge relationship for one receptor using ramps with different rates of rise. There is little change in threshold for ramps of 2, 8, or 20 mm Hg/sec. Maximal discharge, however, is higher with faster ramps which might be due to rate sensitivity (Brown et al., 1978), but could also be related to differences in the effects of electrogenic Na pumping (Saum et al., 1976).

Effects of Changes in [Na]o on Discharge Produced by Slow Pressure Ramps

The relationship between pressure and discharge rate for five irregular baroreceptors was studied in control solution and after perfusion with 3/4 and 7/8 [Na]o using ramp pressure stimulation (Fig. 3). To better satisfy the assumptions of normality and homogeneity of variance required by the analysis of variance technique, discharge frequencies (F) were transformed to ln (F+1). The analysis indicates that the increase in average discharge rate with increasing pressure was highly significant (P < 0.0001). Although the analysis indicates that the discharge frequencies in the two experimental conditions of reduced [Na]o are not significantly different (comparison C1 of Table 2, P = 0.9511), the discharge during these lowered [Na]o conditions are significantly less than control (comparison C2 of Table 2, P = 0.0328). In addition, the analysis shows a significant interaction between pressure and Na-condition (P = 0.0015). Further ex-

---

### Table 1

<table>
<thead>
<tr>
<th>Composition of Control and Experimental Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na (mM)</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>15/16 Na</td>
</tr>
<tr>
<td>7/8 Na</td>
</tr>
<tr>
<td>3/4 Na</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>Ca</td>
</tr>
</tbody>
</table>

In addition, all solutions contained Mg²⁺, 1.2 mM; HCO₃⁻, 25 mM; H₂PO₄⁻, 1.2 mM; SO₄²⁻, 1.2 mM; Cl, 127 mM; dextrose, 5.5 mM. pH 7.4.

---

lated baroreceptors (Thoren et al., 1977). It was mainly these "irregular" receptors that were included in the present study. Measurement of conduction velocities confirmed that these irregular receptors were C-fibers. A large number of units with regular discharge was also found, but as these endings have been the object of extensive studies earlier, they were not examined further (with one exception, see last paragraph in Results).

The receptors were tested in control solution during a slow ramp from 50 to 200 mm Hg at a rate of 2 to 2.5 mm Hg/sec. The ramps were repeated after perfusion of the arch with 3/4 and 7/8 [Na]o. The new solutions were perfused for 2-10 minutes to achieve equilibration (Table 1). The ramp responses were then tested again after control perfusion for 15-20 minutes.

In five receptors the response to a step of 200-225 mm Hg for 30-40 seconds was tested in the control solution and in solutions of 3/4 and 7/8 [Na]o.

In some experiments, discharge was recorded continually while solution changes were made. A modified Starling resistor (Noresson et al., 1979) was added to the outflow tubing from the perfused aortic arch in order to keep the pressure constant while flow was continuous. Alterations in steady state discharge then were studied during the following changes in ionic composition of the Krebs-Henseleit perfusate: 7/8 [Na]o, 3/4 [Na]o, 2 [K]o, 2 [Ca]o.

Data Analysis

Nerve activity and aortic pressure were recorded on an FM tape-recorder and analyzed off line on a PDP 11/70 computer (Brown et al., 1978). The computer calculated the instantaneous frequency as the reciprocal of the interspike interval. The instantaneous frequency and pressure could then be plotted against time, or frequency could be plotted against pressure. In addition, the computer was used to calculate average discharge (average number of impulses/sec) over intervals of pressure or time. This greatly facilitated analysis because of the irregular nature of the receptor discharge. The significance of changes in threshold pressures was determined by Student’s t-test for paired observations. For comparison of pressure-response relationships of baroreceptors during changes in [Na]o, analysis of variance for a single-factor experiment (receptor discharge) with repeated measures on two factors ([Na]o and pressure) was used (Winer, 1971).

It is often difficult in irregular fibers to define the pressure threshold exactly, due to the irregular nature of their discharge. Often these receptors discharge spontaneously at a low rate at subthreshold pressures. The discharge-pressure curve produced by ramp inputs could be divided into two components discernible by eye; a flat component at the lowest pressures with no change in firing rate with increasing pressure—the subthreshold region—and a roughly linear rising phase in which discharge increased with increasing pressure. The pressure-discharge relationship often reached generally asymptotic discharge rate at high pressures, but this nonlinearity was not used for the analysis. Pressure threshold was determined as the intersection of two lines fit to these two components by eye and thus represented the pressure level at which discharge began to increase with increasing pressure.
amination indicates that the pattern of the pressure-response relationship (slope) is significantly reduced from control during Na reduction ($P = 0.0006$), whereas no difference is found between the slopes of the two low sodium pressure-response relationships ($P = 0.9986$).

For five endings, the estimated pressure threshold (see Methods) was higher ($121 \pm 11$ mm Hg) at $\frac{3}{4}$ [Na], than in control solution ($104 \pm 5$ mm Hg), but this difference was not significant due to the large variance. However, at $\frac{3}{4}$ [Na], the threshold ($131 \pm 9.0$ mm Hg) was significantly higher than control ($P < 0.05$).

Effects of Alterations in [Na]$_o$ on Discharge Produced by Pressure Steps

The responses of five irregularly discharging receptors were examined during 30- to 40-second pressure steps from zero to 200 or 225 mm Hg. "Peak" discharge was calculated as the average mean instantaneous frequency for the first five impulses, and steady state discharge was calculated as the average discharge during the period 20-30 seconds after the onset of the pressure step. The data are summarized in Figure 4. Both $\frac{3}{4}$ and $\frac{3}{4}$ [Na], depressed steady state discharge, but did not alter "peak" discharge significantly.

Effects of Changes in [Na]$_o$ during Constant Perfusion

The effects of changing [Na]$_o$ during constant perfusion at 200 mm Hg pressure were tested in five endings (Fig. 5). A decrease in [Na]$_o$ to $94\%$ of control clearly depressed firing and the depression was further augmented at $\frac{7}{8}$ and $\frac{3}{4}$ [Na]$_o$. The depression was reversed when perfusion with control solution was restored.

![Figure 1](image1.png)

**Figure 1.** A: Instantaneous frequency (upper record) and aortic pressure (lower record) plotted against time after onset of a slow ramp of 2 mm Hg/sec from 60 to 240 mm Hg. B: Instantaneous frequency plotted against aortic pressure. (Same data as in A). C: Average discharge recorded over a 10 mm Hg pressure change plotted against aortic pressure (same data as B).

![Figure 2](image2.png)

**Figure 2.** Pressure-discharge relationships evoked by different ramp rates: 2.5 mm Hg/sec in A, 8 mm Hg/sec in B, and 20 mm Hg/sec in C. The cluster of spikes at 80 mm Hg in panel A was spontaneous and not interpreted as threshold discharge which occurred at 120 mm Hg.
Effect of 2X [K]o and 2X [Ca]o on UB Discharge

During constant perfusion at 200 mm Hg pressure, a change in perfusate composition to 2X [K]o caused marked activation of discharge during the first minute in three receptors. The discharge rate increased from 1.9 to 5 Hz. With prolonged perfusion, the discharge became depressed in two receptors but remained elevated in one. The mean discharge after 3 minutes was 1.2 Hz.

Increasing [Ca]o either 1.5- or 2-fold during constant pressure perfusion reduced the steady discharge from 3 Hz to 1.2 Hz in one receptor.

Effect of 15Ae and 7Ae [Na]o on Regularly Discharging Receptors

In earlier experiments on MB's (Saum et al., 1977; Andresen et al., 1979), the effects of ionic changes during constant perfusion were not examined. Since we wished to compare present experiments of UB's with MB's, we decided to extend this aspect of the present study to MB's. The effects of 15Ae and 7Ae [Na]o were tested on four regularly discharging units during constant pressure perfusion. Changing to 7Ae [Na]o in one ending evoked a marked but transient depression in the firing (Fig. 6). With continued perfusion, activity returned almost to normal. In three

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>S.S.</th>
<th>dF</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor</td>
<td>10.5836</td>
<td>4</td>
<td>2.646</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condition ([Na]o)</td>
<td>4.7812</td>
<td>2</td>
<td>2.391</td>
<td></td>
<td>0.0891</td>
</tr>
<tr>
<td>7/8 [Na]o vs. 3/4 [Na]o (C1)</td>
<td>0.0029</td>
<td>(1)</td>
<td>0.003</td>
<td>0.004</td>
<td>0.9511</td>
</tr>
<tr>
<td>Control vs. 7/8 and 3/4 [Na]o (C2)</td>
<td>4.7783</td>
<td>(1)</td>
<td>4.778</td>
<td>6.636</td>
<td>0.0328</td>
</tr>
<tr>
<td>Pressure</td>
<td>30.6520</td>
<td>6</td>
<td>5.109</td>
<td>43.600</td>
<td>0.0001</td>
</tr>
<tr>
<td>Condition × pressure (slope)</td>
<td>1.8025</td>
<td>12</td>
<td>0.150</td>
<td>3.330</td>
<td>0.0015</td>
</tr>
<tr>
<td>C1 × pressure</td>
<td>0.1835</td>
<td>(6)</td>
<td>0.003</td>
<td>0.066</td>
<td>0.9986</td>
</tr>
<tr>
<td>C2 × pressure</td>
<td>1.6190</td>
<td>(6)</td>
<td>0.270</td>
<td>6.000</td>
<td>0.0006</td>
</tr>
<tr>
<td>Receptor × condition</td>
<td>5.7577</td>
<td>8</td>
<td>0.720</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptor × pressure</td>
<td>2.8023</td>
<td>24</td>
<td>0.117</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Receptor × condition × pressure</td>
<td>2.1691</td>
<td>48</td>
<td>0.045</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thoren et al. / Ionic Sensitivity of C-Fiber Baroreceptors

AVERAGE DISCHARGE, (Hz)

FIGURE 5. The means of the average discharge (spikes/sec) in five endings during a steady perfusion at a pressure of 200 mm Hg with control solution, 15/16 [Na]o, 7/8 [Na]o, 3/4 [Na]o, and control solution. The discharge was averaged over 30-second periods and the values were obtained after 3-6 minutes perfusion with the test solution. The P values refer to changes from control discharge.

endings. 15/16 [Na]o, induced a biphasic response with a small transient increase in discharge (110% of control) during the first 20 seconds and a transient depression (75% of control) lasting 2-3 minutes.

Discussion

A Comparison of The Sodium Sensitivities between UB's and MB's

The most significant finding in this study is that irregularly discharging baroreceptors with unmyelinated axons, UB's, are more sensitive to changes in [Na]o than regularly discharging baroreceptors with myelinated axons, MB's (Saum et al., 1977; Andresen et al., 1979). Thus 7/8 [Na]o did not significantly alter threshold or suprathreshold sensitivity of regularly discharging fibers (Andresen et al., 1979) but clearly reduced the slope of the pressure-response curve of irregularly discharging fibers. This slope change is consistent with an increase in pressure threshold and a reduction in suprathreshold sensitivity.

During perfusion with constant pressure, UB's responded to changes as small as 15/16 [Na]o, whereas 15/16 [Na]o appears to affect MB discharge transiently, 7/8 [Na]o, almost completely suppressing firing in one fiber for 1 minute. MB's may be transiently sensitive to small changes in [Na]o, but steady state changes appear to require larger changes in [Na]o.

Transient responses in UB's are difficult to evaluate because of the irregular nature of their discharge. However, such responses were not discernible in UB's. It is possible that a major difference in ionic sensitivity between UB and MB is on the time course so that the effect of small changes in [Na]o is prolonged in UB's but only transient in MB's.

Interestingly, the peak discharge of UB's during pressure steps is not affected by 7/8 and 3/4 [Na]o, but the steady state discharge was clearly depressed at these concentrations. This would indicate that rate effects may be as important as ionic driving forces in producing the receptor potential.

Sodium Sensitivity of Baroreceptors and Reflex Effects

Small reductions in Na concentrations of solutions perfusing an isolated carotid sinus produce reflex increases in arterial pressure and heart rate (Kunze and Brown, 1978). The response peaks transiently at 2 minutes and then subsides to a smaller steady value. Similar changes in [Na]o do not produce significant steady state changes (Saum et al., 1977; Andresen et al., 1979) in the discharge of MB's but may produce transient changes in discharge. It is possible that the differences between the results of the reflex experiments and earlier receptor experiments were due to the contribution of UB's to the reflex effect which were not known at that time. The peak response might then be due to the transient effects on MB's of the type shown in Figure 6.

Effects of Changes in [K]o

Doubling [K]o during constant pressure perfusion induced a biphasic discharge response with initial activation followed in a few minutes by depression. Aortic baroreceptors are unlikely to encounter such a marked, abrupt change in [K]o, and the response might appear to be an experimental curiosity. However, endings with unmyelinated afferent fibers are present in the left ventricle and are likely to be exposed to marked changes in [K]o during cardiac ischemia. Thus, extracellular [K]o rose to 3 times normal within 5 minutes following acute coronary occlusion in dogs (Guggi et al., 1978). The left ventricular C-fibers were also markedly activated immediately after onset of coronary occlusion (Thoren, 1972, 1976). Within 1-2 minutes, however, the firing
began to decline again (Thoren, 1976). It is possible that increased $[K]$ might cause this temporal pattern of discharge. The secondary depression of ventricular C-fibers might be of great clinical interest, for it could explain why bradyarrhythmia during myocardial infarction is so shortlasting (Thoren, 1979).

Possible Mechanisms for the Differences in Ionic Sensitivity of UB's and MB's

It is not surprising that alterations in extracellular ions affect UB discharge, because it was shown previously that MB's (Saum et al., 1978, Andresen et al., 1979) and other stretch receptors (Diamond et al., 1958; Edwards et al., 1963) are sensitive to low $[Na]_o$. The present study indicates that UB's are more sensitive than MB's. The following speculative explanation is offered for this difference. MB's should have a smaller area of membrane exposed to ionic changes than UB's, since the myelinated portions would be relatively impermeable to ion transport. MB's might be able to compensate for a small reduction in $[Na]_o$ by pumping out Na from the intracellular space (Deitmer and Ellis, 1980). In this way, $E_{Na}$ would return to normal and the response to a change in $[Na]_o$ would be transient. More pronounced changes in $[Na]_o$ might not be compensated for, however, and steady state changes in threshold and sensitivity will be observed. UB's might not have the same ability to compensate for small changes in $[Na]_o$ since intracellular Na concentration changes would be greater at a constant density of membrane pump sites. Restoration of $E_{Na}$ would therefore be incomplete. The foregoing account does not, however, explain the biphasic response seen in some fibers.

We would like to thank Dr. Harvey Bunce for his assistance with the analysis of variance.

This work was supported by National Institutes of Health Grant HL-16657.

Dr. Thoren's permanent address is: Department of Physiology, Fack S-70033 Göteborg, 33, Sweden.

Address for reprints: Dr. Arthur M. Brown, Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, Texas 77550.

Received November 3, 1980; accepted for publication: November 13, 1981.

References


Thoren, P (1976) Activation of left ventricular receptors with non-medullated vagal afferents during occlusion of a coronary artery in the cat. Am J Cardiol 37: 1046-1051


Effects of changes in extracellular ionic concentrations on aortic baroreceptors with nonmyelinated afferent fibers.
P Thoren, M C Andresen and A M Brown

Circ Res. 1982;50:413-418
doi: 10.1161/01.RES.50.3.413

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/50/3/413