Prostaglandins Contribute to Activation of Baroreceptors in Rabbits
Possible Paracrine Influence of Endothelium

Hsing I. Chen, Mark W. Chapleau, Thomas S. McDowell, and Francois M. Abboud

The purpose of this study was to test the hypothesis that prostaglandins released from vascular endothelial cells contribute to activation of baroreceptors during increases in arterial pressure. Baroreceptor activity was recorded from the vascularly isolated carotid sinus in rabbits anesthetized with chloralose. Baroreceptor activity was measured during ramp or step increases in nonpulsatile carotid sinus pressure over a range of 0–175 mm Hg. Exposure of the isolated carotid sinus to inhibitors of prostaglandin formation (indomethacin [n=10] or aspirin [n=6]) decreased baroreceptor activity significantly (p<0.05). The slope of the pressure–activity relation averaged 0.80±0.07 %/mm Hg (mean±SEM) during control measurements and 0.72±0.06 and 0.63±0.05 %/mm Hg during exposure to 10 and 20 μM indomethacin, respectively. Exposure of the carotid sinus to exogenous prostacyclin (PGI2 [n=11]) increased baroreceptor activity significantly. The slope of the pressure–activity relation averaged 0.89±0.10, 1.09±0.09, and 1.26±0.16 %/mm Hg during control and during exposure to 10 and 20 μM PGI2, respectively. Activity returned to control after removal of PGI2 (0.89±0.12 %/mm Hg). Removal of endothelium with either a balloon catheter (n=4) or a jet of a 95% O2-5% CO2 gas mixture (n=6) decreased the slope of the pressure–activity relation from 0.92±0.09 to 0.56±0.08 %/mm Hg (p<0.05). Exposure of the denuded sinus to exogenous PGI2 (20 μM [n=4]) restored activity (slope=1.09±0.24 %/mm Hg). Neither indomethacin (n=5) nor PGI2 (n=5) nor denudation (n=5) significantly altered the pressure–diameter relation of the carotid sinus (sonomicrometers), suggesting that the effects on baroreceptor discharge are not caused by altered stretch of the carotid sinus at a given pressure. The results suggest that prostaglandins (e.g., PGI2) released from endothelium contribute in a paracrine manner to activation of baroreceptors during increases in arterial pressure. (Circulation Research 1990;67:1394–1404)

Stimulation of arterial baroreceptors during an increase in arterial pressure triggers reflex circulatory adjustments that buffer the rise in pressure.1 The baroreceptors do not sense changes in arterial pressure directly but are activated by vascular stretch and deformation of the nerve endings.1 Several studies have shown that the vascular endothelium can sense changes in shear stress2 and vascular stretch3 and that these mechanical stimuli release endothelial factors such as endothelium-derived relaxing factor (EDRF)4 and prostacyclin (PGI2).4–6 We have demonstrated recently that exposure of the isolated carotid sinuses in the rabbit to exogenous PGI2 or arachidonic acid enhances the reflex inhibition of lumbar sympathetic nerve activity during increases in carotid sinus pressure and that inhibition of prostaglandin production with indomethacin or aspirin impairs the baroreflex.7 These findings suggest that prostaglandins released during vascular stretch contribute in a paracrine manner to activation of baroreceptors.

The goal of the present study was to directly test this hypothesis by recording carotid sinus baroreceptor activity during exposure of the isolated sinus to PGI2, indomethacin, or aspirin. In addition, we sought to determine whether 1) the effect of prostaglandins on baroreceptor activity can be explained by vasodilatation and increased diameter or stretch of the carotid sinus at a given level of pressure and 2) removal of endothelial cells from the carotid sinus

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Figure 1. Schematic of the isolated carotid sinus preparation. The sinus was isolated as a “blind sac,” and pressure was altered by changing the pressure in a reservoir attached to a pressurized air source. Multifiber baroreceptor activity was recorded with standard techniques (see text). The diameter of the sinus was measured with sonomicrometer crystals, and the temperature of the physiological saline was maintained at 37–38°C with a heated water jacket and water pump.

Males and Methods

Male New Zealand White rabbits were anesthetized with α-chloralose (80–90 mg/kg) injected through the marginal ear vein. Supplemental doses (10 mg/kg) were given as needed. The trachea was intubated to provide artificial ventilation with a mixture of oxygen and room air. The femoral artery and vein were catheterized for measurement of arterial pressure and administration of anesthetic, respectively.

Isolation of the Carotid Sinus

One carotid sinus was vascu larly isolated by ligating the thyroid, internal carotid, occipital and lingual arteries, and other small branches from the sinus region (Figure 1). The vagus and aortic nerves on the same side as the isolated sinus were severed. After identification of the carotid sinus nerve, all other nerves were ligated and sectioned to eliminate the possible influence of the sympathetic innervation to the sinus. A catheter (PE-90) was inserted into the common carotid artery until its tip reached a level approximately 1.5 mm below the sinus. Carotid sinus pressure was measured through a catheter (PE-50) in the external carotid artery connected to a Statham transducer (model P23ID, Hato Rey, Puerto Rico). The common carotid catheter was connected to a pressure reservoir filled with a physiological saline solution of the following composition (mM): NaCl 98.0, KCl 4.7, NaHCO3 24.0, KH2PO4 1.1, MgSO4 1.2, CH3COONa 20.0, CaCl2 2.5, and glucose 10.0. Before the experiment, the saline solution was warmed to 37°C and equilibrated with 95% O2-5% CO2 (PO2 >200 mm Hg, Pco2 =25–40 mm Hg, pH 7.3–7.4). The solution was kept near 37°C throughout the experiment by periodically advancing new solution into the sinus through a temperature-controlled water jacket, where temperature was measured and regulated (Figure 1), and by maintaining the rabbit’s body temperature at 37–38°C with external heating. The rabbit’s temperature tended to maintain the temperature of the saline solution in and around the sinus relatively constant until it was refilled from the water jacket. The “blind-sac” isolated sinus was also periodically refilled with freshly oxygenated solution. Nonpulsatile carotid sinus pressure was controlled by the pressure reservoir connected to a gas regulator valve and a pressurized air source. Thus, the sinus and its nerve were essentially a totally isolated preparation (Figure 1).

Recording of Carotid Sinus Nerve Activity

The carotid sinus nerve was isolated and sectioned at its junction with the glossopharyngeal nerve. The nerve was placed on a platinum unipolar electrode and encased in silicone gel (Wacker Silicones Corp., Adrian, Mich.). At least 30 minutes was allowed for the silicone gel to harden. Care was taken not to cover the internal carotid artery and sinus region with the gel. The sinus region was bathed externally with physiological saline solution throughout the experiment to avoid drying.

Each rabbit was paralyzed with decamethonium bromide (2 mg/kg i.v.) before recording nerve activity. Activity was recorded with a Grass probe (model HIP511E, Grass Instrument Co., Quincy, Mass.) and a Grass band-pass amplifier (model P511). The high-frequency cutoff was set at 1,000 or 3,000 Hz, and the low-frequency cutoff was set at 30 Hz. The amplifier output was led to a loudspeaker and a dual-beam storage oscilloscope (model D13, Tektronix, Beaver-
A nerve traffic analyzer (model 706C, University of Iowa Bioengineering, Iowa City) that counts spikes exceeding a selected voltage was used to quantitate baroreceptor activity. Since the absolute amount of whole nerve activity recorded depends on the number of active fibers in contact with the recording electrode and may vary between preparations, activity is expressed as a percentage of the maximum activity recorded during the initial control increase in carotid sinus pressure. Integrated and mean baroreceptor activity (electronically damped) and systemic arterial and carotid sinus pressure were continuously displayed on a Dynograph recorder (model R411, Beckman, Schiller Park, Ill.).

**Carotid Sinus Diameter**

The diameter of the carotid sinus was measured with sonomicrometer crystals. In brief, two miniature piezoelectric crystals (5 MHz) attached to a low resistance stainless steel clip were placed on either side of the carotid sinus. The clip was secured by suturing it to the adventitia. The pressure–diameter relation of the carotid sinus was determined with ramp or step increases in pressure over a range of 50–175 mm Hg. Capacitively induced electrical noise from the transmitter crystal prevented accurate measurement of diameter at very low pressures (<30–50 mm Hg). Each diameter value is expressed as a percent change from the initial diameter measured in the control condition at 50 mm Hg.

**Drugs**

PGI$_2$ (Sigma) was dissolved in cold sodium bicarbonate solution to obtain a 1-mM stock solution (pH 9–10). Aliquots (50–100 µl) were prepared in plastic tubes and lyophilized with a freeze dryer (20 µg PGI$_2$/tube). Indomethacin (Sigma) was dissolved in physiological saline solution along with sodium carbonate (3:1 ratio) to increase solubility. Aspirin (acetylsalicylic acid) was dissolved in a small volume of ethanol (<0.5% vol/vol). The stock solutions of each drug were diluted with physiological saline solution immediately before use to obtain the required concentration. Vehicles for each drug were prepared for control injections.

**Endothelial Denudation**

The endothelium was removed from the isolated carotid sinus with either a Fogarty embolectomy balloon-tipped catheter (2F, Edwards Laboratories, Santa Ana, Calif.) or by intraluminal exposure to a jet of a 95% O$_2$–5% CO$_2$ gas mixture. Two established methods of denudation were used so as to minimize the chance that nonspecific effects of the denudation procedure, for example, vascular and baroreceptor stretch with balloon dilatation, might be responsible for changes in baroreceptor activity. Since both methods were performed for the same purpose, that of removing the endothelium, and since both methods had a similar effect on baroreceptor activity, we have chosen to report the combined results from these two groups.

Endothelial denudation with the gas mixture (n=11) was achieved by attaching the gas line to the common carotid catheter and by placing a small hole in the internal carotid artery to provide an outlet for the gas mixture. The carotid sinus was exposed to the jet of the O$_2$–CO$_2$ gas mixture for periods ranging from 18 to 30 minutes. The flow was adjusted to maintain the carotid sinus pressure at approximately 50 mm Hg. After this exposure, the isolated sinus was flushed and filled with physiological saline solution, and the internal carotid artery was ligated proximal to the perforation.

For balloon denudation (n=4), the common carotid catheter was carefully withdrawn, and the embolectomy catheter was inserted and advanced into the internal carotid artery. The balloon was distended with air to a degree that did not overstretch the carotid sinus as viewed under the dissecting microscope. The balloon was gently pulled back from the sinus region into the common carotid artery and deflated. After the procedure was repeated three times, the common carotid catheter was replaced, and the sinus region was flushed and filled with physiological saline solution.

Endothelial denudation was verified histologically with scanning electron microscopy. At the end of each experiment, the carotid sinus was perfused with 50% Karnovsky’s solution for 5 minutes at a pressure of approximately 50 mm Hg. The preserved carotid sinus was opened longitudinally, and the intraluminal surface (approximately 4–8 mm$^2$) was scanned. Nondenuded isolated carotid sinuses were also scanned for comparison.

**Protocols**

Baroreceptor activity and carotid sinus diameter were measured over a wide range of carotid sinus pressure to generate pressure–activity and pressure–diameter relations. In most of the experiments, pressure was increased in a continuous ramp from 0 to 175 mm Hg. The rate of rise in pressure (dP/dt) was equivalent during all pressure ramps applied within an experiment and usually was less than 5 mm Hg/sec. In other experiments, pressure was varied over a range of pressure in incremental steps: 25, 50, 70, 90, 110, 130, and 140 mm Hg. The step change in pressure was achieved within 1–3 seconds, and pressure was held constant for 10–30 seconds before raising pressure to the next level. Measurements were taken within the last 2–10 seconds of each pressure step after baroreceptor activity had adapted to a relatively stable level. Baroreceptor responses measured in this way are essentially the same as measured during a slow pressure ramp. The same method of increasing pressure (ramp or step) was used throughout a given experiment. Pressure was held at or below 70 mm Hg whenever measurements were not taken.

Three to six consecutive trials of increased pressure with reproducible responses were obtained in
the beginning of most of the experiments to assure a stable preparation before drug or denudation procedures were performed.

Effects of indomethacin and aspirin. Baroreceptor activity was measured during increases in carotid sinus pressure before \((n=10)\) and during exposure of the isolated sinus to sequentially increasing doses of indomethacin \((10 \ [n=9], \ 20 \ [n=10], \ \text{and} \ 40 \ [n=9] \ \mu M; \ \text{and} \ 80 \ \text{or} \ 100 \ \mu M \ [n=5])\) or aspirin \((0.5 \ \text{and} \ 2.0 \ \text{mM} \ [n=6])\). These doses of indomethacin and aspirin have been used routinely in numerous preparations, including the carotid artery of the rabbit, \(^{14}\) to inhibit the formation of prostaglandins. Recent studies in our laboratory, \(^{15}\) using the same preparation as in the present study, have demonstrated that indomethacin \((50 \ \mu M)\) completely prevents the increase in PGI₂ production \(\text{(measured as the stable metabolite 6-ke-}
\text{toprostaglandin F₁₉)}\) that occurs in response to arachidonic acid \((10 \ \mu M)\). The purpose of exposing the carotid sinus to indomethacin and aspirin was to inhibit the formation of prostaglandins in endothelial cells. Therefore, the drugs were placed inside the carotid sinus to allow direct contact with endothelium. The responses to increases in pressure were measured several times after each dose of indomethacin with intervals of 2–3 minutes between tests. The values reported are those obtained after 5–10 minutes of exposure to indomethacin when the response was stable. Responses were measured after 10 minutes of exposure to aspirin.

In other experiments \((n=5)\), carotid sinus diameter was measured during changes in pressure before and during exposure of the sinus to indomethacin \((10, 20, \ \text{and} \ 40 \ \mu M)\).

Effect of PGI₂. Baroreceptor activity was measured during increases in pressure both before \((n=11)\) and during exposure of the carotid sinus to increasing doses of PGI₂ \((5 \ [n=7], \ 10 \ [n=11], \ 20 \ [n=10], \ \text{and} \ 40 \ [n=5] \ \mu M)\) and after removal of PGI₂ from the sinus \((n=5)\). The responses to increased pressure were determined within 1–2 minutes after the PGI₂ was added to the sinus. We hypothesized that PGI₂ would increase baroreceptor activity through a direct action on the nerve endings. We were concerned that the endothelium might provide a diffusion barrier and prevent exogenous PGI₂ placed inside the carotid sinus from reaching the nerve endings. It has been suggested that the diffusion of intraluminal PGI₂ across the endothelial barrier may be restricted, whereas endogenous PGI₂ is released from endothelial cells toward smooth muscle. \(^{16}\) Therefore, we placed PGI₂ both inside and outside the carotid sinus to ensure that it reached the nerve endings.

In separate experiments \((n=5)\), the pressure–diameter relation of the carotid sinus was determined before and during exposure of the sinus to PGI₂ \((20 \ \mu M)\).

Effect of endothelial denudation. The baroreceptor response to increases in pressure was obtained before and approximately every 5–10 minutes for up to an hour after removal of the endothelium \(\text{(gas mixture} \ [n=6], \ \text{balloon} \ [n=4])\). Responses became stable within 35 minutes. In four of these experiments \(\text{(air} \ [n=2], \ \text{balloon} \ [n=2])\), responses were then obtained after placement of PGI₂ \((20 \ \mu M)\) into and around the denuded carotid sinus and again after removal of PGI₂.

The pressure–diameter relation of the carotid sinus was determined in separate experiments before and approximately every 5–10 minutes for up to an hour after denudation with the gas mixture \((n=5)\). In these experiments, the carotid sinuses were exposed to the gas mixture for 29±1 minutes.

The endothelium-denuded carotid sinuses used in nerve recording experiments were examined with electron microscopy for the presence of endothelial cells and were compared with micrographs obtained from noneneduded isolated carotid sinuses. Results obtained from denuded sinuses were included only after histological confirmation that a majority of the surface area examined was either totally denuded of endothelium or exhibited clear disruption and damage of endothelial cells.

Data Analysis

Baroreceptor activity was expressed as a percent of the maximum activity obtained during the initial control increase in pressure. Changes in carotid sinus diameter were expressed as a percent change from the initial value at 50 mm Hg in the control condition. The slope of the linear portion of the pressure–nerve activity relation over the pressure range of 50–125 mm Hg was calculated with linear regression analysis. \(^{17}\) Slopes and maximum baroreceptor activities were compared with analysis of variance \(\text{(repeated measures)}\) and Fisher’s least significant difference test. \(^{17}\) The effect of the interventions on carotid sinus diameter at the same level of pressure was tested with the paired \(t\) test. \(^{17}\) All values represent mean±SEM. Differences were considered significant at \(p<0.05\).

Results

Effects of Indomethacin and Aspirin

Exposure of the carotid sinus to indomethacin caused dose-related decreases in maximum baroreceptor activity and a shift of the pressure–activity relation to the right \((\text{Figures} \ 2 \ \text{and} \ 3, \ \text{Table} \ 1)\). The decreased baroreceptor activity in the presence of indomethacin was not reversible within the first 30–45 minutes after removing the indomethacin. Indomethacin did not alter the pressure–diameter relation of the carotid sinus \((\text{Figure} \ 3)\).

Exposure of the isolated carotid sinus to aspirin \((2 \ \text{mM})\) also decreased maximum baroreceptor activity to 69±7\% \((p<0.05)\) and the slope of the pressure–activity curve from 1.38±0.07 to 0.95±0.08 \%/mm Hg \((p<0.05)\). The smaller dose of aspirin \((0.5 \ \text{mM})\) had no effect.

Effect of PGI₂

Exposure of the isolated carotid sinus to PGI₂ caused dose-related increases in maximum barore-
Effect damaged the carotid sinus during a pressure experiment. Carotid sinus receptor activity returned to control within 1–2 minutes after removal of PGI2. The equivalent baroreceptor response to increased pressure before the addition of PGI2 and after removal of PGI2 (Table 2) indicates that the increased nerve activity during PGI2 was caused by the drug and not by time alone and that the preparation was stable over the duration of the experiment.

PGI2 did not significantly alter the pressure–diameter relation of the carotid sinus (Figure 4).

Effect of Endothelial Denudation

Both balloon and “air” denudation removed or damaged the endothelium over a majority of the surface area scanned. Scanning electron micrographs of an intact and a balloon-denuded carotid sinus are shown in Figure 5. The balloon and air methods of denudation decreased baroreceptor activity to a similar degree, and the decrease in activity was significant (p<0.05) in both groups when each group was analyzed separately or when analyzed together. Both the slope of the pressure–activity curve and maximum baroreceptor activity were decreased significantly after removal of endothelium (Figures 6 and 7). Removal of endothelium with the O2-CO2 gas mixture did not influence the pressure–diameter relation of the carotid sinus significantly (Table 3).

Table 1. Effect of Indomethacin on Baroreceptor Pressure–Activity Relation and on Maximum Nerve Activity

<table>
<thead>
<tr>
<th>Normalized baroreceptor activity</th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Slope</td>
<td>Maximum</td>
</tr>
<tr>
<td>%/mmHg</td>
<td>(% of max)</td>
</tr>
<tr>
<td>n</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.80±0.07</td>
</tr>
<tr>
<td>Indomethacin</td>
<td></td>
</tr>
<tr>
<td>10 µM</td>
<td>0.72±0.06</td>
</tr>
<tr>
<td>20 µM</td>
<td>0.63±0.05</td>
</tr>
<tr>
<td>40 µM</td>
<td>0.63±0.04</td>
</tr>
<tr>
<td>80–100 µM</td>
<td>0.60±0.03</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The slope of the baroreceptor pressure–activity relation was calculated over the linear range between 50 and 125 mm Hg (r=0.96±0.01). Activity was normalized with reference to the maximum activity (max) recorded during the control condition in each experiment. Maximum activity decreased in a dose-dependent manner; there was a significant shift of the curve to the right, and the decline in slope was not statistically significant (see also Figures 2 and 3).

*Significant difference from control at p<0.05.
slope of the pressure–activity curve to a level not different than control (Figures 6 and 7). Baroreceptor activity was again suppressed within 1–2 minutes after removal of PGI2 (Figures 6 and 7).

Discussion

Four main results were obtained in the present study: 1) Exposure of the isolated carotid sinus of rabbits to inhibitors of prostaglandin formation (indomethacin or aspirin) decreased baroreceptor activity. 2) Exposure of the sinus to exogenous PGI2 increased baroreceptor activity. 3) Removal of the endothelium from the carotid sinus reduced baroreceptor activity, and addition of exogenous PG12 to the denuded sinus restored activity back to control levels. 4) The effects of indomethacin, PG12, and denudation on baroreceptor activity were not caused by changes in carotid sinus diameter and vascular stretch.

The results extend our previous findings7 that intrasinus administration of indomethacin or aspirin attenuates the baroreflex inhibition of sympathetic nerve activity during increases in carotid sinus pressure and that PG12 or arachidonic acid enhances the baroreflex.

Arterial baroreceptors are activated by stretch of the vascular wall and deformation of the nerve endings during increases in arterial pressure.1 Our results suggest an additional mechanism of activation of baroreceptors—that of chemical sensitization of the endings by PG12 or other prostanoids released from the endothelium during vascular stretch. The “Discussion” section will focus on three areas: 1) studies from other laboratories related to prostaglandins and baroreflex function, 2) the mechanisms involved in the release of prostaglandins and their excitatory influence on baroreceptors, and 3) the physiological and pathophysiological implications of the findings.

Studies by Others

Previous studies18–24 that have examined the influence of prostaglandins on arterial baroreceptor and baroreflex function have provided variable results. Kaplan et al18 observed a decrease in systemic arterial pressure in response to intracarotid administration of PGE1 in vivo that did not occur after sectioning of the carotid sinus nerve, suggesting that activation of baroreceptors caused the hypotension. McQueen and Belmonte19 recorded baroreceptor and chemoreceptor activity from the carotid sinus nerve in anesthetized cats during intravenous and intracarotid injections of prostaglandin E2, prostaglandin A2, and prostaglandin F20. The authors attributed the changes in baroreceptor activity to the changes in arterial pressure induced by the drugs and concluded that prostaglandins in the concentrations used do not directly affect baroreceptors or chemoreceptors.

Table 2. Effect of Prostacyclin on Baroreceptor Pressure–Activity Relation and on Maximum Nerve Activity

<table>
<thead>
<tr>
<th>Normalized baroreceptor activity</th>
<th>Slope (%/mm Hg)</th>
<th>Maximum (% of max)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.89±0.10</td>
<td>100±0</td>
<td>11</td>
</tr>
<tr>
<td>PGI2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 μM</td>
<td>0.79±0.11</td>
<td>102±3</td>
<td>7</td>
</tr>
<tr>
<td>10 μM</td>
<td>1.09±0.09</td>
<td>118±4*</td>
<td>11</td>
</tr>
<tr>
<td>20 μM</td>
<td>1.26±0.16*</td>
<td>148±6*</td>
<td>10</td>
</tr>
<tr>
<td>40 μM</td>
<td>1.29±0.24*</td>
<td>161±8*</td>
<td>5</td>
</tr>
<tr>
<td>Recovery</td>
<td>0.89±0.12</td>
<td>94±4</td>
<td>5</td>
</tr>
</tbody>
</table>

Values are mean±SEM. PGI2, prostacyclin. The slope of the baroreceptor pressure–activity relation was calculated over the linear range between 50 and 125 mm Hg (r=0.94±0.02). Activity was normalized with reference to the maximum activity (max) recorded during the control condition in each experiment.

*Significant difference from control at p<0.05.
Figure 5. Panel A: Scanning electron micrograph of the intraluminal surface of a control, non-denuded carotid sinus showing intact endothelium. Panel B: Scanning electron micrograph of the intraluminal surface of a carotid sinus after balloon denudation. Magnification, ×1,000.

Figure 6. Original recordings from one experiment showing the magnitude of increased baroreceptor activity during control (panel A), after balloon denudation (panel B), after addition of 20 μM exogenous prostacyclin (PGI₂) to the denuded sinus (panel C), and after removal of PGI₂ (recovery) (panel D). Nerve activity was reduced after removal of endothelium and restored temporarily by PGI₂.
D’Souza and Biggs\textsuperscript{20} recorded carotid sinus nerve activity in anesthetized guinea pigs during intravenous administration of aspirin and indomethacin. The authors concluded that aspirin and indomethacin increase baroreceptor activity but suggested that the increased activity was not the result of cyclooxygenase inhibition. Arterial pressure was increased by aspirin and indomethacin, thus complicating the interpretation of the data. Hirooka et al in a preliminary report\textsuperscript{21} showed that the reduction in baroreceptor activity recorded from the aortic nerve of the rabbit was similar during nitroglycerin- and PGI\textsubscript{2}-induced decreases in arterial pressure, suggesting that PGI\textsubscript{2} does not influence the sensitivity of baroreceptors.

PGI\textsubscript{2} or other prostaglandins may attenuate the arterial baroreflex\textsuperscript{22-24} by 1) sensitizing cardiopulmonary receptors connected to vagal afferents\textsuperscript{25-27} whose activation interacts centrally with the baroreflex, 2) inhibiting the release of norepinephrine from sympathetic nerve terminals,\textsuperscript{28} and 3) blunting the vascular response to norepinephrine.\textsuperscript{28}

Thus, the variable results observed in previous studies may reflect the different prostaglandins studied, the range of doses that were used, the confounding influence of variable changes in arterial pressure that occurred during systemic administration of drugs, and the effect of prostaglandins on other reflexes or neuroeffector responsiveness.

The present study was designed to circumvent many of these complicating factors. The sympathetic-denervated, vascularly isolated carotid sinus preparation allowed us to measure directly baroreceptor responses to controlled levels of pressure without the confounding influence of activating other reflexes. In addition, the in situ preparation allowed us to test wide ranges of concentration of the pharmacological agents, to measure the diameter of the carotid sinus as an index of the mechanical stimulus to the receptors, and to test responses before and after removal of the endothelium.

### Mechanisms of Prostaglandin Release and Baroreceptor Activation

The vascular endothelium is the primary source of PGI\textsubscript{2} and other prostanoids produced in the vessel wall, although smooth muscle is also capable of producing prostaglandins.\textsuperscript{29} Our finding that endothelial denudation (Figures 6 and 7) as well as inhibition of prostaglandin formation (Figures 2 and 3) suppressed baroreceptor activity suggests that endothelium is an important source of prostanoids that activate baroreceptors. Recent studies\textsuperscript{2-6} have demonstrated that endothelial cells are capable of detecting changes in mechanical forces acting on the endothelial cell membrane. Increases in shear stress or mechanical stretch increase the probability of opening of ion channels on the endothelial cell membrane.\textsuperscript{2-3} The corresponding movement of ions

### Table 3. Pressure–Diameter Relation of the Isolated Carotid Sinus Before and After Endothelial Denudation With O\textsubscript{2}-CO\textsubscript{2} Gas Mixture

<table>
<thead>
<tr>
<th>Pressure</th>
<th>Control (% change from control value at 50 mm Hg)</th>
<th>After denudation</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mm Hg</td>
<td>0.0±0.0</td>
<td>2.6±2.6</td>
</tr>
<tr>
<td>75 mm Hg</td>
<td>7.5±1.3</td>
<td>9.8±2.9</td>
</tr>
<tr>
<td>100 mm Hg</td>
<td>15.7±2.3</td>
<td>17.7±3.4</td>
</tr>
<tr>
<td>125 mm Hg</td>
<td>22.9±2.8</td>
<td>24.6±4.1</td>
</tr>
<tr>
<td>140 mm Hg</td>
<td>26.8±3.2</td>
<td>28.1±4.8</td>
</tr>
</tbody>
</table>

Values represent mean±SEM; n=5. The diameter of the carotid sinus averaged 1.571±198 μm in the control condition at a pressure of 50 mm Hg. The values reported were obtained before denudation and again 29±1 minutes after refilling the sinus with physiological saline after denudation. Carotid sinus pressure was held at approximately 20 mm Hg whenever pressure ramps were not being applied.

### Figure 7. Graphs showing group data from denudation experiments. D, denudation; PGI\textsubscript{2}, prostacyclin. Left panel: Pressure–activity relations. Right panels: Slopes and maximum nerve activities. Maximum activity and slope were decreased significantly after denudation (D; n=10), restored by prostacyclin (D+PGI\textsubscript{2}; n=4), and reduced again after removal of PGI\textsubscript{2} (D−PGI\textsubscript{2}; n=4). *Statistical significance of these changes (p<0.05).
may provide the initial signal for release of PGI₂ from endothelium in response to vascular stretch.¹⁻⁶

We considered the possibility that in our experiments removal of endothelium with the balloon catheter might damage baroreceptors by causing excessive stretch of the carotid sinus. Therefore, we were careful to limit the degree of stretch during balloon inflation. In addition, in other experiments the endothelium was removed with air desiccation, which did not mechanically stretch the carotid sinus. Both methods of denudation reduced baroreceptor activity significantly and to a similar degree. The finding that exposure of the denuded carotid sinus to PGI₂ restored baroreceptor sensitivity (Figures 6 and 7) suggests that the denudation procedures did not cause irreversible damage to the receptors and that the decreased activity after denudation reflects reduced production of PGI₂.

Removal of endothelium might also be expected to decrease formation of other endothelial factors besides PGI₂.³⁰ Although this may occur, we believe that decreased prostaglandin formation is likely to be responsible for the decreased nerve activity after denudation. There is no evidence that other endothelial factors increase baroreceptor discharge; in fact, we have shown that cultured endothelial cells produce a factor that suppresses baroreceptor activity.¹¹ Clearly, decreased production of an "inhibitory" factor after removal of endothelium could not be responsible for decreased nerve activity. Furthermore, removal of endothelium did not significantly alter the pressure–diameter relation of the carotid sinus in our preparation. Thus, one cannot explain our findings by altered release of vasoactive factors influencing baroreceptor activity indirectly through changes in vascular tone. The evidence points to a mediator that acts directly on baroreceptor neurons to increase activity; prostaglandins are known to have such direct effects on neurons.²⁵⁻²⁸

Indomethacin and PGI₂ also did not alter the pressure–diameter relation of the isolated carotid sinus. The lack of a dilator response to PGI₂ may be a consequence of the relatively low vascular tone present in the isolated, sympathetic-denervated carotid sinus, or alternatively, the carotid sinus of the rabbit, like the rabbit aorta, may not dilate in response to PGI₂.³¹ Our results suggest that increased baroreceptor activity during PGI₂ most likely results from sensitization of the endings to mechanical deformation rather than greater deformation. We speculate that the mechanism of sensitization by PGI₂ may involve cyclic AMP, which has been shown to mediate the vasodilating and antiplatelet-aggregating actions of PGI₂.³²

The increase in carotid sinus nerve activity with PGI₂ is pressure dependent (Figure 4). The insignificant effect of PGI₂ at low pressures and the marked increase in activity at higher pressures suggests that PGI₂ may not stimulate the endings unless some mechanical deformation is also present; that is, PGI₂ sensitizes the nerve endings to the mechanical stim-

ulus. The lack of an effect of PGI₂ on nerve activity at low levels of pressure also indicates that activation of chemoreceptors was not the reason for the increase in carotid sinus nerve activity.

An exogenous PGI₂ concentration of 10⁻⁴⁻⁴⁰ µM was required to sensitize baroreceptors. This relatively high concentration raises the question as to whether PGI₂ released from endothelial cells in vivo achieves a high enough concentration at the baroreceptor endings to increase nerve activity. Several lines of evidence suggest that it does.

Exposure of the carotid sinus to inhibitors of prostaglandin formation (indomethacin or aspirin) decreased baroreceptor activity significantly, suggesting that a sufficient concentration of endogenous prostaglandins reached the nerve endings to enhance activity during vascular stretch. The significant inhibition of nerve activity with two different inhibitors of cyclooxygenase along with our finding that a comparable concentration of indomethacin (50 µM) abolishes arachidonic acid–induced PGI₂ formation in the same preparation¹⁵ suggests that the responses resulted from inhibition of prostaglandin formation rather than a nonspecific effect. In addition, removal of the primary source of PGI₂, the endothelium,²⁹ decreased baroreceptor activity.

Recent studies in our laboratory have demonstrated that intrasinus administration of arachidonic acid (10 µM) enhances the baroreflex control of sympathetic nerve activity⁷ and increases afferent baroreceptor activity (unpublished observation) to a similar degree as exogenous PGI₂. This dose of arachidonic acid, which is converted endogenously to PGI₂, increased the luminal concentration of PGI₂ to approximately 100 nM in our preparation,¹⁵ much less than the 10⁻⁴⁻⁴⁰ µM concentration of exogenous PGI₂ required to increase baroreceptor activity. This finding suggests that measurements of PGI₂ in the vessel lumen or circulating blood do not reflect the local concentration that can be achieved in the vascular wall. PGI₂ released into the lumen is diluted by blood or physiological saline. Therefore, the contribution of circulating PGI₂ to baroreceptor activation may be questioned. We propose that it is the local production of prostaglandins that is important. Prostaglandins may act in a local, paracrine manner to contribute to activation of baroreceptors. It has been shown that PGI₂ is released from the basolateral membrane of the endothelial cell into the vessel wall.¹⁶ Therefore, the local concentration of PGI₂ in media and at the baroreceptor endings, which are primarily located in the innermost region of the adventitia, may be very high, particularly since the carotid sinus media is very thin compared with other vessels of similar size.¹ It is also possible that endothelium of vasa vasorum in adventitia may provide a source of prostaglandins very close to the nerve endings.

A diffusion barrier may limit the efficacy of exogenous PGI₂ placed in the lumen of the carotid sinus to reach the nerve endings, particularly since the
half-life of PG1₂ is only approximately 3 minutes and it took us several minutes to prepare and administer
the PG₁₂ solution and generate the slow pressure ramp. This may explain in part the need to use
relatively high concentrations of exogenous PG₁₂ to obtain an effect.

In addition, it is possible that other prostanoids besides PG₁₂ may contribute to baroreceptor activation
during vascular stretch. The combination of lower concentrations of several prostanoids may cause a
similar effect as a higher concentration of PG₁₂ alone.

Pathophysiological Implications

Hypertension, atherosclerosis, and diabetes are associated with endothelial dysfunction and reduced
capacity for PG₁₂ production,33–36 which may partially account for the altered baroreceptor and
baroreflex function observed in these diseases.37–39 In considering the pathophysiological implications
of endothelial dysfunction, it is of interest that chemical activation of cultured endothelium releases an “inhibitory factor” that suppresses baroreceptor activity in anesthetized dogs.11 It is established that the endothelium produces various factors, some of which exert opposing actions.30 We speculate that the balance between the formation of the unidentified “inhibitory factor” and the production of prostaglandins may modulate baroreceptor sensitivity in a manner analogous to the modulation of smooth muscle tone by vasoconstrictor and dilator factors released from the endothelium.40 Because endothelial denudation decreased baroreceptor activity, it appears that the excitatory influence of endothelium on baroreceptors predominates in the normal rabbit. In pathophysiological states, the balance between PG₁₂ formation and production of the “inhibitory factor” may be altered. The physiological stimulus for release and the identity of the “inhibitory factor” are unknown, although preliminary evidence from our laboratory suggests that the recently discovered peptide endothelin⁴⁰ may decrease baroreceptor activity.⁴¹

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References


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