Aggregating Human Platelets in Carotid Sinus of Rabbits Decrease Sensitivity of Baroreceptors

Zhi Li, Francois M. Abboud, and Mark W. Chapleau

Aggregating platelets release factors that act in a local paracrine manner to alter vascular tone. The purpose of the present study was to explore the possibility that factors released from aggregating platelets may alter the sensitivity of arterial baroreceptors. Baroreceptor activity was recorded from the vascularily isolated carotid sinus of rabbits anesthetized with sodium pentobarbital. The carotid sinus was filled with oxygenated Krebs-Henseleit buffer and distended with slow ramp increases in nonpulsatile pressure. Sensitivity of baroreceptors to increased pressure was determined before and during intraluminal exposure of the sinus to washed human platelets suspended in Krebs' buffer. Platelets activated with thrombin (0.4 units/ml) decreased baroreceptor activity and the slope of the pressure–activity curve significantly (n = 6). The platelet-induced decrease in baroreceptor sensitivity was related to the duration of exposure to platelets with no change in baroreceptor activity after 4 minutes and a progressive decrease in activity over the next 12 minutes. The slope of the pressure–nerve activity relation averaged 1.26 ± 0.08 %/mm Hg during control and decreased to 0.97 ± 0.22, 0.80 ± 0.19, and 0.53 ± 0.15 %/mm Hg after 12-16 minutes of exposure to 10^6, 10^7, and 3–6 × 10^8 activated platelets/ml, respectively (p < 0.05). Baroreceptor sensitivity was restored after removal of platelets from the carotid sinus. Thrombin alone had no effect on baroreceptor sensitivity. Activated platelets did not alter the carotid pressure–diameter relation, suggesting a direct inhibitory effect on baroreceptors. The slope of the pressure–activity curve and maximum baroreceptor activity were not decreased by the stable thromboxane analogue U46619, serotonin, or ADP. We conclude that an as-yet-unidentified factor released from aggregating platelets acts in a paracrine manner to decrease baroreceptor sensitivity. We speculate that in pathological states such as atherosclerosis platelets aggregating in carotid sinuses may contribute to decreased baroreceptor sensitivity and trigger reflex sympathetic activation with potential deleterious effects (vasospasm and hypertension). (Circulation Research 1992;70:644–650)

**Key Words** • pressoreceptors • carotid sinus • platelet aggregation • atherosclerosis • thrombin

Arterial baroreceptors are activated by vascular stretch during increases in arterial pressure. Increased baroreceptor activity triggers reflex inhibition of sympathetic nerve activity and an increase in parasympathetic nerve activity that buffer the rise in pressure.

The sensitivity of baroreceptors is modulated by various neurohumoral factors. Recent studies in our laboratory have demonstrated that prostacyclin (PGI2) released from vascular endothelium increases baroreceptor sensitivity and therefore contributes in a paracrine manner to activation of baroreceptors. We have also shown that impaired formation of PGI2 in pathological states associated with endothelial cell damage such as chronic hypertension and atherosclerosis is responsible in part for decreased baroreceptor sensitivity in these diseases.

Endothelial cell damage and decreased levels of PGI2 also promote aggregation of platelets and release of mediators, with significant effects on vascular tone. Local vasoconstriction in response to aggregating platelets is considered an important mechanism that contributes to vasospasm in pathological states. The goal of the present study was to explore the hypothesis that factors released from aggregating platelets in carotid sinus exert a paracrine inhibitory influence on the sensitivity of arterial baroreceptors.

**Materials and Methods**

New Zealand White rabbits of either sex were anesthetized with sodium pentobarbital (30–35 mg/kg) injected through an ear vein. The trachea was cannulated to provide artificial ventilation with room air supplemented with oxygen. The pH of arterial blood was maintained between 7.30 and 7.45, arterial PCO2 was maintained between 30 and 45 mm Hg, and PO2 was maintained >100 mm Hg by adjusting the frequency of the ventilator. The femoral artery and vein were catheterized for measurement of arterial pressure and administration of anesthetic, respectively. Body temperature was maintained between 36°C and 38°C by external warming.
**Isolation of the Carotid Sinus**

In each experiment, one carotid sinus was vascularity isolated as described previously. All visible branches of the common and external carotid arteries were ligated in the region of the carotid sinus. Catheters were placed in the common, external, and internal carotid arteries. The carotid sinus was filled with a physiological saline solution (Krebs-Henseleit) of the following composition (mM): NaCl 118.0, KCl 4.7, NaHCO₃ 24.0, MgSO₄ 1.2, CaCl₂ 2.5, KH₂PO₄ 1.1, and glucose 10.0. The Krebs’ solution was bubbled beforehand with a 95% O₂-5% CO₂ gas mixture; pH was 7.3-7.4, Po₂ was >200 mm Hg, and PCO₂ was 30-40 mm Hg. The carotid sinus was refilled periodically with fresh, warm (37°C) Krebs’ solution.

The common carotid catheter was connected to a pressure bottle filled with Krebs’ solution, and nonpulsatile pressure in the carotid sinus was controlled by regulating the air inflow to the bottle from a pressurized air source. In this way, the carotid sinus was pressurized as a blind sac in the absence of flow through the sinus. Pressure in the carotid sinus was measured with a transducer (Statham model P23XL) connected to the external carotid catheter. The criteria for successful isolation of the carotid sinus was absence of leak of blood into the sinus when pressure was lowered to 0 mm Hg and the absence of leak of Krebs’ solution out of the sinus when pressure was held at 60 mm Hg. Thus, the carotid sinus and its nerve were essentially a totally isolated preparation. The vagal, aortic, and cervical sympathetic nerves were cut below the carotid sinus to avoid a possible influence of sympathetic activity on baroreceptor discharge. Decamethonium bromide (0.3 mg/kg i.v.) was administered before recording nerve activity to eliminate skeletal muscle contraction. Supplemental doses of anesthetic and decamethonium were given as needed.

**Measurement of Baroreceptor Nerve Activity**

The carotid sinus nerve was carefully isolated and sectioned at its junction with the glossopharyngeal nerve. The nerve was desheathed, placed on a unipolar platinum electrode, and encased in silicone gel. Care was taken to not cover the internal carotid artery and sinus region with the gel. The preparation was bathed in warm (37°C) paraaffin oil. Nerve activity was recorded with a high impedance probe (model HIP511J, Grass Instrument Co., Quincy, Mass.) and a Grass band-pass amplifier (model PS11J; band width from 100–300 Hz to 3–10 kHz). The electroneurogram was displayed on a dual-beam storage oscilloscope (model 5113, Tektronix, Beaverton, Ore.) and heard through a loudspeaker. A nerve traffic analyzer (model 706C, Department of Bioengineering, University of Iowa, Iowa City) was used to count the frequency of action potentials that exceeded a selected threshold voltage level set just above the electrical noise. The output of the counter, the raw neurogram, and carotid sinus pressure were recorded continuously on an electrostatic recorder (model ES1000, Gould Inc., Cleveland, Ohio).

**Measurement of Carotid Pressure–Diameter Relation**

A video technique was used to measure the diameter of the carotid artery at the origin of the carotid sinus. The carotid artery and sinus were viewed through a stereomicroscope (model M3C, Wild Corp., Heerbrugg, Switzerland) and recorded on videotape using a camera (model JE2362, Javelin Electronics, Inc., Torrance, Calif.), videocassette recorder (model SLV-585HF, SONY Corp., Tokyo), and video monitor (model BWM15, Javelin Electronics). A digital readout of the carotid sinus pressure on the Gould recorder was filmed simultaneously (camera model JE7542B, Javelin Electronics) and projected on the same monitor as the carotid sinus with the use of a beam splitter (model MPS-50, Image Laboratories Corp., Pearl River, N.Y.). Measurements of carotid diameter were obtained with a videomicroimeter (model VIA-100, Boeckeler Instrument, Tucson, Ariz.) and controller (model KS-30, Boeckeler Instrument).

**Preparation of Platelets**

Platelets were isolated from venous blood drawn from human donors as described previously. Blood was anticoagulated with acid citrate dextrose, and platelets were isolated and washed by a modification of the method described by Mustard et al. A stock solution of platelets was routinely prepared in buffered Tyrode’s albumin (3.5 mg/ml) solution at a concentration of 5-6 x 10⁸ platelets/ml and maintained at 37°C in a water bath. Platelets were diluted into Krebs-Henseleit buffer before testing responses in the isolated carotid sinus. The number of platelets was determined with a Coulter counter (Coulter Corp., Hialeah, Fla.). The ability of platelets to aggregate was verified by measuring the change in light transmission in a dual-chamber aggregometer in response to the addition of bovine thrombin (0.1 units/ml). Thrombin induced 80–100% aggregation of platelets within ~3 minutes.

**Protocols**

Baroreceptor activity was recorded during slow ramp increases (2–4 mm Hg/sec) in nonpulsatile carotid sinus pressure (from 0 to 150 mm Hg). The rate of rise in pressure (dP/dt) was equivalent during all pressure ramps within an experiment. Pressure was held constant at 60 mm Hg whenever ramps were not being applied. Three to six consecutive pressure ramps with reproducible responses were obtained every 4 minutes to ensure a stable preparation before the injection of platelets into the carotid sinus.

**Influence of platelets on baroreceptor activity (n=6)**

After obtaining control responses, platelets were injected into the isolated carotid sinus at concentrations of 1 x 10⁸, 1 x 10⁷, and 3 x 10⁶ or 6 x 10⁶ platelets/ml (6 x 10⁴/ml in one experiment). The desired concentration of platelets was obtained by dilution of the concentrated platelet solution into Krebs’ buffer. During exposure of the sinus to each concentration of platelets, four pressure ramps were applied at 4-minute intervals over a total period of 16 minutes. Platelets were then flushed out of the carotid sinus and replaced with fresh Krebs’ solution. Baroreceptor responses to three to four additional ramps at 4-minute intervals were obtained to demonstrate recovery from the effects of platelets before raising the platelet concentration.

After determining responses to platelets alone, the protocol was repeated using aggregating platelets. Bo-
Vine thrombin was added to the platelet suspension (0.4 units/ml) to cause aggregation immediately before injecting the platelets into the carotid sinus. As described above, pressure ramps were applied at 4-minute intervals for each platelet concentration. Platelets were removed from the carotid sinus after exposure to each concentration and replaced with fresh Krebs’ solution, and the response of baroreceptors to increases in pressure was obtained to demonstrate reversibility.

In two of these experiments and in two additional ones (n=4), baroreceptor activity was measured during pressure ramps before and in the presence of thrombin alone (0.4 units/ml).

**Influence of activated platelets on the carotid pressure-diameter relation (n=4).** The effect of thrombin-activated platelets on the carotid pressure-diameter relation was examined in four experiments. Pressure ramps were applied, and platelets were injected into the isolated carotid sinus as described above for the other protocols.

**Influence of thromboxane (U46619), serotonin, and ADP on baroreceptor activity (n=15).** The effects of several factors known to be released from aggregating platelets

![Graph showing baroreceptor activity and gain](image_url)

**Figure 1.** Original recordings from an individual experiment that demonstrate the effect of platelets on baroreceptor activity. Mean baroreceptor discharge frequency increased in response to ramp increases in carotid sinus pressure (CSP). Intraluminal exposure of the sinus to 3×10⁸ platelets/ml alone for 16 minutes slightly decreased baroreceptor activity. Removal of platelets from the carotid sinus restored baroreceptor activity (not shown). Subsequently, exposure to the same concentration of thrombin-activated (0.4 units/ml) platelets for 16 minutes decreased baroreceptor activity markedly. Baroreceptor activity was restored after removal of platelets from the carotid sinus (recovery). The pressure ramp is shown only once for clarity and was superimposable for the four conditions shown.

**Figure 2.** Bar graphs showing the influence of the concentration of platelets alone (no thrombin) on maximum baroreceptor activity (panel A) and baroreceptor gain (panel B). Control (C) and recovery (R) data are shown by the hatched bars. Duration of exposure to platelets was 12 minutes in two experiments and 16 minutes in four experiments (C, n=6; 10⁷ platelets/ml, n=3; 10⁸ platelets/ml, n=5; 3–6×10⁸ platelets/ml, n=5; and R, n=6). *p<0.05 compared with control by one-factor analysis of variance and Fisher’s protected least significant difference test.
on baroreceptor sensitivity were studied. Carotid sinus pressure–nerve activity curves were generated before and during intraluminal exposure of the isolated sinus to 1) the stable endoperoxide thromboxane A2 analogue U46619 (from $10^{-8}$ to $10^{-4}$ M, n=5), 2) serotonin (5-hydroxytryptamine creatinine sulfate [5-HT], $10^{-9}$ M, n=5), and 3) ADP (from $10^{-9}$ to $10^{-4}$ M, n=5). The concentrations provided are final concentrations of the drugs dissolved in Krebs-Henseleit buffer placed into the isolated carotid sinus. 5-HT and ADP were obtained from Sigma Chemical Co., St. Louis, Mo., and U46619 was from Cayman Chemical Co., Ann Arbor, Mich.

Data Analysis

Since the absolute amount of nerve activity recorded depends on the number of active fibers in contact with the recording electrode and varies between preparations, baroreceptor activity was expressed as a percentage of the maximum activity recorded during the initial increase in carotid sinus pressure (control). The relation between carotid sinus pressure and baroreceptor activity was calculated for each pressure ramp. The slope of the linear portion of the pressure–nerve activity curve (40–80 mm Hg) was determined by linear regression analysis. The influence of platelets and platelet factors on baroreceptor slope (gain) and maximum baroreceptor activity were analyzed by one-factor analysis of variance (ANOVA). The pressure–diameter curves were analyzed by two-factor ANOVA. When an overall effect was determined to be significant by ANOVA, Fisher’s protected least significant difference test was used to compare mean values. All data are presented as mean±SEM. Differences were considered significant at $p<0.05$.

Results

Influence of Platelets on Baroreceptor Activity

Platelets alone in the carotid sinus (no thrombin) caused a small but significant decrease in maximum baroreceptor activity but did not significantly influence baroreceptor gain (Figures 1 and 2).

In contrast, thrombin-activated platelets decreased baroreceptor activity markedly (Figure 1). The inhibitory effect of aggregating platelets on baroreceptor activity was related to the duration of exposure to platelets and the platelet concentration. The inhibitory effect developed slowly with no change in baroreceptor activity for up to 4 minutes after placement of activated platelets in the isolated carotid sinus. Baroreceptor activity was progressively inhibited as the duration of exposure was increased (Figure 3). Baroreceptor activity was restored after removal of platelets from the isolated carotid sinus (Figures 1 and 3).

Platelet-induced suppression of baroreceptor activity was also dependent on the concentration of platelets with inhibition of activity after 12–16 minutes of exposure to concentrations as low as $10^7$ platelets/ml (n=3) and 50% inhibition of baroreceptor activity during exposure to the physiological concentration of $3\times10^8$.
platelets/ml (Figures 1, 4, and 5). Both maximum discharge frequency of baroreceptors and the slope or gain of the pressure–activity curve were decreased significantly by activated platelets (Figure 5). Exposure of the isolated carotid sinus to thrombin alone did not influence maximum baroreceptor activity or the slope of the pressure–activity curve (Figure 5).

Influence of Platelets on the Carotid Pressure–Diameter Relation

Exposure of the isolated carotid sinus to thrombin-activated platelets did not influence the carotid pressure–diameter relation (n=4, Figure 6).

**Table 1. Influence of U46619, Serotonin, and ADP on Carotid Sinus Nerve Activity Measured at 60 and 140 mm Hg and on the Slope of the Pressure–Nerve Activity Relation**

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Drug</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>U46619 (10⁻⁷ M, n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity at 60 mm Hg (%)</td>
<td>54±6</td>
<td>35±5*</td>
<td>52±7</td>
</tr>
<tr>
<td>Maximum activity at 140 mm Hg (%)</td>
<td>99±0</td>
<td>99±1</td>
<td>100±1</td>
</tr>
<tr>
<td>Slope (%/mm Hg)</td>
<td>1.41±0.10</td>
<td>1.37±0.10</td>
<td>1.35±0.10</td>
</tr>
<tr>
<td><strong>Serotonin (10⁻⁸ M, n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity at 60 mm Hg (%)</td>
<td>52±6</td>
<td>72±7*</td>
<td>54±4</td>
</tr>
<tr>
<td>Maximum activity at 140 mm Hg (%)</td>
<td>100±0</td>
<td>121±13*</td>
<td>101±1</td>
</tr>
<tr>
<td>Slope (%/mm Hg)</td>
<td>1.26±0.07</td>
<td>1.28±0.14</td>
<td>1.35±0.04</td>
</tr>
<tr>
<td><strong>ADP (10⁻⁴ M, n=5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activity at 60 mm Hg (%)</td>
<td>49±8</td>
<td>51±7</td>
<td>50±7</td>
</tr>
<tr>
<td>Maximum activity at 140 mm Hg (%)</td>
<td>99±1</td>
<td>100±0</td>
<td>99±1</td>
</tr>
<tr>
<td>Slope (%/mm Hg)</td>
<td>1.11±0.20</td>
<td>1.18±0.19</td>
<td>1.22±0.21</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Nerve activity is expressed as a percent of the maximum activity recorded at 140 mm Hg during three control pressure ramps. Baroreceptor slope or gain was determined by linear regression analysis of the relation between carotid sinus pressure and nerve activity over a pressure range of 40–80 mm Hg. *Significant change (p<0.05) during exposure to the drug compared with control.
ADP did not influence the pressure–nerve activity relation (Table 1).

**Discussion**

The main finding of the present study is that aggregating platelets in the carotid sinus decrease the sensitivity of baroreceptors. The decreased baroreceptor sensitivity is related to the platelet concentration and to the duration of exposure to platelets, is reversible, and cannot be explained by a change in vascular compliance, since there was no influence on the carotid pressure–diameter relation. Thrombin, the agonist used to activate platelets, has no effect on baroreceptor sensitivity. These results suggest that a factor or factors released from aggregating platelets act in a paracrine manner to suppress baroreceptor activity.

Numerous studies support the concept of paracrine modulation of baroreceptor sensitivity. In previous studies, we have demonstrated that PGH₂ released from endothelial cells sensitizes baroreceptors and contributes to increased baroreceptor activity during increased arterial pressure. We have also demonstrated that cultured endothelial cells, when activated chemically, release a factor that decreases sensitivity of baroreceptors. Norepinephrine released from sympathetic nerve terminals modulates the activity of arterial baroreceptors.

**Experimental Approach**

Several potential limitations in the methodology should be considered. First, there is the question as to whether any factor(s) besides platelets in our platelet suspensions, such as white blood cells, factors in plasma, or the diluted Tyrode’s solution, may have influenced baroreceptor sensitivity. To minimize this possibility, we used isolated and washed human platelets rather than platelet-rich plasma to avoid the influence of multiple factors present in plasma. There is essentially no contamination of the platelet suspensions with white blood cells when using this isolation procedure. The platelets were suspended in Tyrode’s buffer that was diluted into Krebs’ solution before placement in the isolated carotid sinus. Exposure of the isolated sinus to Tyrode’s solution had minimal effect on baroreceptor activity.

It is noteworthy that the higher concentrations of platelets (3–6×10⁸/ml) decreased baroreceptor activity significantly in the absence of thrombin (Figure 2). One would not expect aggregation of platelets in an intact blood vessel. In our preparation, exposure of the platelets to the polyethylene catheters and to the damaged arterial wall at the site of catheter placement could contribute to an aggregation of platelets in the absence of thrombin. Our finding that exposure of platelets to a known aggregating agent, thrombin, markedly enhanced the inhibitory effect on baroreceptors suggests that the decrease in baroreceptor sensitivity was related to platelet aggregation.

In our experiments, baroreceptor activity was recorded from multiple fibers. Therefore, a possible influence of chemoreceptor activity should be considered. Our finding that a rapid decrease in carotid sinus pressure totally eliminated nerve activity (Figure 1) strongly suggests that the recorded nerve activity originated from baroreceptors with no contribution of activity from chemoreceptors. In addition, the preferential inhibition of carotid sinus nerve activity at high pressures by aggregating platelets indicates that activity of pressure–sensitive baroreceptors was decreased.

**Mechanism of Inhibitory Effect of Platelets on Baroreceptor Activity**

The mechanism of platelet-induced inhibition of baroreceptor activity is unclear. As discussed above, the results suggest that the aggregation of platelets is responsible for the inhibitory effect on baroreceptors. Thus, a factor or factors released from platelets is most likely responsible for the decreased baroreceptor sensitivity. During platelet aggregation and activation, numerous substances are released, including 5-HT, ADP, and thromboxane A₂, many of which exert significant effects on vascular tone. 5-HT and thromboxane A₂ act directly on vascular smooth muscle to cause vasoconstriction. In addition, 5-HT and ADP trigger release of endothelium-derived relaxing factor from endothelium, which can cause vasodilatation. The results of the present study do not support a role of these factors in causing the decrease in baroreceptor sensitivity by aggregating platelets. None of the factors decreased maximum baroreceptor activity or the slope of the pressure–baroreceptor activity curve (see Table 1), which contrasts with the response to aggregating platelets. Numerous additional mediators are released from platelets during aggregation and additional studies are needed to identify the one responsible for inhibition of baroreceptor activity.

An important question is whether the factor from aggregating platelets suppresses baroreceptor activity through a direct effect on neuronal excitability or an indirect effect on vascular tone. We found that intraluminal exposure of the isolated carotid sinus to activated platelets did not influence the carotid pressure–diameter relation, suggesting a direct effect on baroreceptors. The absence of a vasoactive effect of platelets in
our preparation may reflect the low vascular tone in the sympathetically denervated, isolated carotid sinus or, alternatively, the opposing influences of endothelial-dependent relaxation and direct contraction by 5-HT and thromboxane. Similar results were obtained in an independent study that examined the effects of human platelets on vascular responses of rabbit carotid arteries. Intraluminal perfusion of rabbit carotid arteries with aggregating platelets did not influence vessel diameter when basal tone was low, and it caused vasodilation only when tone was elevated beforehand with phenylephrine. Only extravascular application of aggregating platelets caused vasoconstriction. We speculate that a factor(s) released by aggregating platelets acts directly on baroreceptor endings to decrease their responsiveness to stretch.

**Pathophysiological Implications**

We believe that our results may be important in pathological states associated with endothelial cell dysfunction and platelet aggregation, such as atherosclerosis. Numerous studies have emphasized the important role of platelet aggregation and endothelial damage in triggering local vasoconstrictor responses and vasospasm. The results of the present study may suggest an additional mechanism by which platelets could promote vasoconstriction—that of decreased baroreceptor activity triggering a reflex increase in sympathetic discharge. This mechanism, along with impaired PGI₂ formation from endothelium, could conceivably contribute to vasospasm and/or neurogenic hypertension in atherosclerosis. The observation that atherosclerotic lesions in humans are particularly prominent in the carotid sinuses supports this concept. The potential pathophysiological significance of our findings is emphasized by the fact that baroreceptor activity was decreased by extremely low concentrations (10⁻⁹–10⁻⁸/ml) of human platelets. The normal circulating platelet concentration in humans is ~3x10⁹ platelets/ml. Platelet function differs between species. The use of human platelets suggests that our results may be relevant to human pathophysiology.

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**References**

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