Activated Endothelial Cells in Culture Suppress Baroreceptors in the Carotid Sinus of Dog

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SUMMARY  Vascular stretch increases the activity of arterial baroreceptors along with the production and release of substances from the endothelium. We hypothesized that endothelial factors modulate the sensitivity of baroreceptors during increases in arterial pressure. Baroreceptor activity was recorded from single fibers innervating the isolated carotid sinus of dogs anesthetized with chloralose after removal of the endothelium (balloon denudation) and after replacing into the denuded sinus bovine aortic endothelial cells cultured on microcarrier beads. The endothelial cells were activated with either the calcium ionophore A23187 (2 μM) or bradykinin (10 μM). The threshold pressure (n = 7) determined with a slow ramp increase in static pressure averaged 73 ± 7 (SEM) mm Hg during exposure to naked beads and was increased significantly (96 ± 18 mm Hg; p < 0.05) during exposure to endothelial cell cultures. During stepwise increases in pressure, activity (n = 6) averaged 14 ± 5, 40 ± 8, and 54 ± 8 spikes/sec at 75, 125, and 175 mm Hg during exposure to naked beads and decreased significantly to 2 ± 2, 30 ± 11, and 35 ± 12 spikes/sec at equivalent pressures during exposure to the cell cultures. The activity was restored after replacement of the cell cultures with naked beads. The suppressed activity was not caused by changes in carotid sinus diameter or strain (sonomicrometers) or by the chemical activators that were also added to the naked beads. The results indicate that chemically activated endothelial cells release an inhibitory factor that suppresses baroreceptor activity. (Hypertension 11: 586-590, 1988)

KEY WORDS • endothelium • baroreceptor reflex • microcarrier beads • calcium ionophore • bradykinin • dogs

ARTERIAL baroreceptors are stimulated by mechanical deformation during increases in arterial pressure. Increased vascular stretch increases the production of endothelial factors including prostaglandins, which may modulate the increase in baroreceptor activity. We have observed recently that prostacyclin (PGI2) increases and indomethacin decreases baroreceptor activity in the rabbit, suggesting an excitatory role of prostaglandins in baroreceptor activation.

To further explore the contribution of the endothelium to baroreceptor activation, we measured carotid sinus baroreceptor activity in dogs after removal of the endothelium and again after reinstating, into the denuded sinus, endothelium grown in culture on microcarrier beads. The endothelium was stimulated with calcium ionophore (A23187) or bradykinin. The results indicate that chemical activation of the endothelium in this preparation suppresses baroreceptor activity.

Materials and Methods

Mongrel dogs (weight, 16–24 kg) were anesthetized with thiopental sodium (30 mg/kg i.v.) and α-chloralose (80 mg/kg i.v.). Additional doses of chloralose were administered as needed. The dogs were intubated and mechanically ventilated with room air supplemented with oxygen. Arterial pH and CO2 tension were maintained within normal limits by adjusting the ventilation. Catheters were placed in a femoral artery and vein for measurements of arterial pressure and administration of chloralose, respectively.

Isolated Carotid Sinus Preparation

The isolated carotid sinus and baroreceptor nerve recording techniques have been described elsewhere.
and will be explained briefly. The left carotid sinus was exposed, and all arteries in the vicinity of the sinus were ligated. Catheters were placed in the common, external, and internal carotid arteries. The isolated sinus was flushed and filled with a physiological saline solution equilibrated with 95% O₂, 5% CO₂, and warmed to 37°C. The sinus was connected to a pressure reservoir, and carotid sinus pressure was measured through the external carotid catheter with a Statham (Model P23AA, Hato Rey, Puerto Rico) transducer. Adjustment of a regulator valve connected to a pressurized air source enabled control of nonpulsatile carotid sinus pressure (Figure 1).

**Recordings of Baroreceptor Activity**

The carotid sinus nerve was cut, placed on a dissection stage, covered with paraffin oil, and desheathed. The vagosympathetic trunk and other nerves innervating the sinus region were sectioned. Baroreceptor activity was recorded with a bipolar platinum electrode connected to a Grass high-impedance probe (Model HIP 511E, Quincy, MA, USA) and amplified by a Grass (Model P511) bandpass amplifier (30 Hz to 3-10 kHz). Nerve traffic was visualized on a Tektronix (Model 5113, Beaverton, OR, USA) dual-beam storage oscilloscope and heard on a loudspeaker. A nerve traffic analyzer that counted spikes that exceeded a selected voltage was used to quantify nerve activity.

Baroreceptor activity was recorded from seven single fibers in seven dogs. Single units were identified by splitting the sinus nerve until a fine strand was obtained that exhibited increased activity of uniform spike height in response to increases in pressure, relative constant interspike interval during maintained static pressure, and lack of activity from other units above the preselected voltage. Decamethonium bromide, 0.3 mg/kg, was administered to each dog to prevent muscular movement while nerve activity was recorded.

**Measurement of Carotid Sinus Diameter**

The diameter of the carotid sinus was measured with sonomicrometers. Two 7-MHz piezoelectric crystals were mounted on the opposite tips of a low resistance stainless steel clip that was placed around the carotid sinus (see Figure 1). The crystals were aligned across the sinus and secured by suturing one side of the clip to the tissue around the carotid sinus. The clip enabled the placement of the crystals with minimal trauma to the wall of the sinus. Carotid sinus diameter, carotid sinus pressure, integrated baroreceptor activity, mean baroreceptor activity, and systemic arterial pressure were displayed on a Beckman recorder (Model R411, Schiller Park, IL, USA).

**Endothelial Cell Cultures**

Bovine aortic endothelial cell cultures were initiated from vessels obtained from freshly slaughtered animals. The cells were grown on Cytodex 3 microcarrier beads (Pharmacia Fine Chemicals, Uppsala, Sweden) and used 3 to 5 days after seeding. The cells released PGI₂ in response to chemical stimulation and contained angiotensin converting enzyme and plasminogen activator. The cells had a uniform appearance, demonstrated an epithelioid growth pattern, and were positive for Factor VIII antigen when grown on tissue culture plates. The cultures were maintained at 37°C in modified medium 199 with Earle's salts and 20% heat-inactivated fetal bovine serum in an atmosphere containing 5% CO₂. The cultures were studied between Passages 7 and 13.

On the day of the experiment several sets of naked microcarrier beads were suspended in media (1.6 x 10⁶ beads/10 ml) without fetal bovine serum as a set of beads with attached endothelial cells (1-2 x 10⁶ cells/10 ml). The naked or endothelial beads were flushed into the carotid sinus through the common carotid artery and withdrawn through the internal and external carotid arteries.

**Protocol**

The threshold pressure (Pₒ) of single units was determined with slow ramp increases in nonpulsatile pressure (<3 mm Hg/sec), and baroreceptor activity and carotid sinus diameter were recorded over a pressure range of 25 to 200 mm Hg during stepwise changes of 25 mm Hg. Pressure was always held at 25 mm Hg for at least 10 minutes before the pressure stimulus was applied.

The carotid sinus was denuded of endothelium 2 to 3 hours before recording baroreceptor activity. Denudation was accomplished by inserting an embolectomy balloon catheter (2-4F, Edwards Laboratories, Santa Ana, CA, USA) into the isolated carotid sinus, inflating the balloon until the vessel was visibly distended by 25 to 50%, and slowly withdrawing the catheter. This procedure was repeated three to four times. The
efficacy of this method to remove endothelium has been confirmed in our laboratory by scanning electron microscopy and by elimination of acetylcholine-induced vasodilatation of the carotid sinus.18

First, the naked bead-media mixture was removed from the incubator, mixed with either the calcium ionophore A23187 (2 μM; n = 5; Sigma Chemical, St. Louis, MO, USA) or bradykinin (10 μM; n = 2; Vega Biotechnologies, Tucson, AZ, USA), stirred gently, and injected into the carotid sinus. Following placement of the beads, carotid sinus diameter and single unit activity were recorded during the ramp and stepwise increases in pressure. Measurements were repeated once or twice at 10-minute intervals in three of the seven experiments.

Endothelial beads were then stimulated with the same chemicals and injected into the sinus in an identical manner. As the endothelial beads entered the sinus, the naked beads were passively flushed out. Measurements were obtained every 10 minutes after exposure of the sinus to the cells for periods up to 50 minutes in three experiments, 20 minutes in three experiments, and 10 minutes in one experiment.

Recovery measurements were obtained after re-injection of naked beads mixed with the chemicals.

Data Analysis

All data are expressed as the mean ± SEM. Circumferential wall strain (%) was calculated as \((D - Do) \times 100/Do\), where \(D\) is the diameter at a given pressure and \(Do\) is the diameter at 25 mm Hg.

The pressure-activity and pressure-strain relations were analyzed by two-way analysis of variance. When a significant effect was shown, Tukey’s test was used to determine which groups were different. \(P_a\) values were compared by the Wilcoxon paired-sample test. 19 A p level of 0.05 or less was considered significant.

Results

In preliminary experiments the placement of unstimulated endothelial cell cultures in the denuded sinus caused only slight and inconsistent increases in \(P_a\) and decreases in baroreceptor activity. Therefore, we elected to activate the endothelial cells chemically in the remaining experiments.

Exposure of the denuded carotid sinus to chemically stimulated endothelial beads caused significant increases in \(P_a\) and decreases in activity as compared with values obtained with naked beads plus chemicals (Figures 2 and 3). The \(P_a\) averaged 73 ± 7 mm Hg during exposure to naked beads and was increased significantly (96 ± 18 mm Hg) during exposure to endothelial cell cultures. During stepwise increases in pressure, activity (n = 6) averaged 14 ± 5, 40 ± 8, and 54 ± 8 spikes/sec at 75, 125, and 175 mm Hg during exposure to naked beads and decreased significantly to 2 ± 2, 30 ± 11, and 35 ± 12 spikes/sec at equivalent pressures during exposure to the cell cultures. The decrease in sensitivity was related to the duration of exposure to endothelium in some of the experiments (see Figure 2) and was reversed after replacement of the endothelial beads with naked beads (see Figures 2 and 3). The stimulated endothelial beads caused small and variable constriction of the sinus that was not statistically significant (see Figure 3).

Discussion

The results indicate that placement of chemically activated endothelial cells in the carotid sinus denuded of endothelium in dogs suppresses baroreceptor activity. Removal of the endothelium with a balloon catheter has been widely used to eliminate endothelial function.20 The release of endothelium-derived relaxing factor (EDRF) and PGJ2 during chemical stimulation is essentially prevented following acute denuda-

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**Figure 2.** Exposure of the isolated carotid sinus to the beads with endothelium (ENDO beads) increased the threshold pressure \((P_{th})\) and decreased the activity recorded from single units. In this unit, baroreceptor sensitivity decreased progressively as the duration of exposure to the cells was increased. The activity was totally abolished up to a pressure of 199 mm Hg after 15 to 20 minutes of exposure to the cells. The decreased sensitivity was reversed after replacement of the endothelial beads with beads without endothelium (NAKED beads). A23187 (2 μM) was added to the beads with endothelium to stimulate the cells and to the naked beads, which provided the internal control and recovery values.
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We have confirmed these findings in our preparation and have verified the effective removal of endothelium by scanning electron microscopy. We have shown that the vasodilatation of the carotid sinus in response to acetylcholine (EDRF) is eliminated after denudation. In addition, we found in one experiment, as reported by others, that the concentration of PGI₂ in the carotid sinus effluent (6-keto prostaglandin F₁₀, radioimmunoassay) was greater in the denuded sinus but that the increase in PGI₂ in response to the addition of A23187 was essentially blocked after denudation (from 32 to 158 nM with endothelium intact vs 64 to 72 nM after denudation). Thus, our method of removing the endothelium prevented the chemically activated release of both EDRF and PGI₂.

The inhibitory factor responsible for the suppressed baroreceptor activity is unknown. We believe that it is endothelium-dependent and that neither A23187 nor bradykinin is directly responsible for the suppressed activity. The activity was restored after endothelial beads were replaced with naked beads despite continued presence of the chemicals in the sinus. Further studies are needed to determine what substance released from the endothelial cells is responsible for the decreased baroreceptor sensitivity. The primary arachidonic acid metabolites released in response to A23187 by the same line of cultured bovine aortic endothelial cells that we have used are PGI₂ and arachidonic acid. Lesser amounts of prostaglandin E₂, hydroxyeicosatetraenoic acid, and 12-hydroxyheptadecatrienoic acid are formed. PGI₂ is unlikely to be the factor responsible for the suppressed activity. PGI₂ can sensitize or stimulate cardiac receptors, and recently we have observed an excitatory influence of PGI₂ on carotid sinus baroreceptors in the rabbit. Other substances released from the endothelium that should be considered possible mediators of the suppressed activity include EDRF, metabolites of the lipoxygenase pathway, free radicals generated during arachidonic acid metabolism, and the endothelium-derived contracting factors.

The endothelium appears to be important in modulating baroreceptor activity. Our findings in rabbits that exogenous PGI₂ increases and indomethacin decreases baroreceptor activity during increases in carotid sinus pressure support this concept. Based on these earlier studies, we had expected the cultured endothelial cells to increase baroreceptor activity and were surprised by the suppression of activity. We believe, however, that the results do not necessarily conflict with our earlier studies. The different responses may reflect the nature of the stimulus applied to the endothelium. The excitatory role of PGI₂ was demonstrated during increases in carotid sinus pressure, whereas the suppression of baroreceptor activity reported in the present study was seen with chemical activation of the endothelial cells. The endothelium may produce several factors, some of which exert opposing actions. The balance between the production of excitatory and inhibitory endothelial factors may influence baroreceptor sensitivity in a manner analogous to their modulation of smooth muscle tone. For example, spontaneous hypertension causes a shift away from the production of EDRF to the production of an endothelium-derived contracting factor. Similarly, the production of PGI₂ by the endothelium...
lium may be attenuated in certain pathological states such as atherosclerosis,27 diabetes,28 or hypertension,29 and this attenuation may amplify the influence of the inhibitory factor(s). We do not know which substance may have the predominant physiological or pathological role in baroreceptor activation. Our earlier studies suggest a physiological role of PG12 or a cyclooxygenase metabolite since the stimulus applied to the carotid sinus was a change in arterial pressure. The role of the inhibitory substance released during chemical activation of cultured endothelial cells remains to be determined, but the demonstration of the potential for a physiological role of an inhibitory endothelial factor acting on baroreceptors is important.

Another possible explanation for the apparently conflicting findings in the two different experiments may reflect the fact that they were done in two different species; the earlier study was done in rabbits, and this study was done in dogs. The excitatory endothelial effect may predominate in rabbits, whereas the inhibitory effect may predominate in dogs. Endothelium from even the same animal but from different vascular beds is known to have different effects with a surprising degree of specificity.

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