Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 7399–7403, October 1988
Physiological Sciences

Rheoreceptors in the carotid sinus of dog

(arterial baroreceptors/cardiac output sensor/endothelium/mechanoreceptors/shear stress)

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Communicated by Eugene Braunwald, June 13, 1988 (received for review January 7, 1988)

ABSTRACT **The arterial baroreceptors are known to be sensitive to changes in pressure but there are no known sensors in the cardiovascular system for changes in flow. We tested the hypothesis that changes in flow at constant pressure alter carotid sinus multi-unit nerve activity. In anesthetized dogs with vascularly isolated carotid sinuses, increases in flow at constant pressure resulted in increases in carotid sinus nerve activity in relation to the increase in flow. The increased activity during flow was not caused by an increase in strain of the sinus wall but was directly related to the increase in shear stress (36.6 ± 11.7% increase in activity per dyne/cm^2; 1 dyne = 0.1 MN). The pressure threshold of single baroreceptor units was determined during a slow pressure ramp with and without flow. Flow caused a significant decrease in pressure threshold from 81.1 ± 6.1 mmHg (1 mmHg = 1.333 × 10^2 Pa) in the absence of flow to 69.3 ± 5.7 mmHg with flow. We conclude that there are arterial "rheoreceptors" in the carotid sinus that respond to flow at constant pressure and strain. The results with single baroreceptor units indicate also that baroreceptors may be sensitized by increases in flow. Thus, changes in flow *per se* in addition to changes in arterial pressure may be important determinants of reflex circulatory adjustments.**

Changes in arterial pressure cause changes in baroreceptor activity, which elicit neurally mediated changes in heart rate, cardiac output, and peripheral vascular resistance (1, 2). Similarly, changes in blood gases and pH change the activity of chemoreceptors and cause reflex changes in ventilation and the distribution of blood flow to maintain oxygen delivery to vital organs. However, to date there has been no evidence that changes in blood flow *per se* in the absence of a change in pressure or blood gases and pH can be sensed directly by arterial receptors. The location of such arterial receptors in the aortic arch and carotid sinus would be ideal for sensing changes in cardiac output or cerebral blood flow.

Flow may modulate the release of a number of vasoactive substances from the endothelium (3–6). It is possible that a change in flow through an effect of shear stress on the endothelium may mechanically or chemically activate nerve endings in the arterial wall. Accordingly, the purpose of the present investigation was to determine the effects of flow on carotid sinus nerve activity at a constant distending pressure.

METHODS

Mongrel dogs (17–25 kg) were anesthetized with thiopental sodium (30 mg/kg, i.v.) and α-chloralose (80 mg/mg, i.v.). Supplemental doses of α-chloralose were administered hourly. The dogs were mechanically ventilated with room air supplemented with oxygen. Femoral arterial and venous catheters were inserted for pressure measurements and α-chloralose administration, respectively. The animal temperature was maintained within normal limits (37–39°C).

Isolated Carotid Sinus Preparation. The left carotid sinus region was exposed through a midline incision in the neck (7). Catheters were placed in the common, external, and internal carotid arteries (Fig. 1). Carotid sinus pressure (CSP) was measured from the external carotid arterial catheter (model P23AA transducer; Statham, Hato Rey, PR) with its tip located at the bifurcation of the external and internal carotid arteries. The tip of this catheter was positioned such that a lateral pressure was measured in the sinus region. The isolated sinus was flushed and filled from a pressurized reservoir with a physiological salt solution (PSS) of the following composition: 2.5 mM $CaCl_2$/4.7 mM KCl/1.2 mM $MgSO_4$/1.1 mM KH_2PO_4/20.0 mM CH_3COONa/90.0 mM NaCl/24.0 mM $NaHCO_3$/10.0 mM glucose equilibrated with a 95% O_2/5% CO_2 mixture and warmed to 38°C. A peristaltic pump (model 1210, Harvard Apparatus, South Natick, MA) was placed upstream from the reservoir to regulate flow into the sinus and a capacitance chamber containing 5 ml of PSS and 15 ml of air at the outflow end of the sinus served to dampen any residual pulsations from the pump. Thus, flow was nonpulsatile in this study. A thermistor was placed near the carotid sinus ostium and also into the reservoir.

The level of CSP was controlled by adjusting a regulator valve connected to a pressurized air source. Flow was typically set at 18 ml/min but was also varied from 0 to 24 ml/min. In this closed system, when flow was increased, the pressure at the sinus could be maintained constant by small adjustments of the pressure reservoir.

Carotid Sinus Diameter Measurements. The external diameter of the carotid sinus was measured with sonomicrometers (8). Briefly, two 7-MHz piezoelectric crystals, which were mounted onto a low-resistance stainless steel clip, were aligned across the sinus and secured by suturing one side of the clip to the tissue around the carotid sinus (9). Relative circumferential carotid sinus wall strain was calculated by the following equation:

$$\text{Strain} = (d - d_o)/d_o,$$

where d_o represents the initial diameter at CSP = 25 mmHg (1 mmHg = 1.333 × 10^2 Pa) and d is the diameter at any given CSP.

Shear stresses at the wall (S_w) were calculated using the steady-laminar flow-cylindrical tube model (10):

$$S_w = \frac{4\,n\,Q}{\pi\,R^3},$$

where n = viscosity of saline, Q = flow, π = 3.1416, and R = carotid sinus internal radius. Because we did not measure internal radius, the external radius was substituted introducing only a small error into the calculation because the ratio of wall thickness to radius was assumed to be <0.1 (10).

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Abbreviations: CSP, carotid sinus pressure; PSS, physiological salt solution; P_{th}, pressure threshold; S_w, wall shear stress.

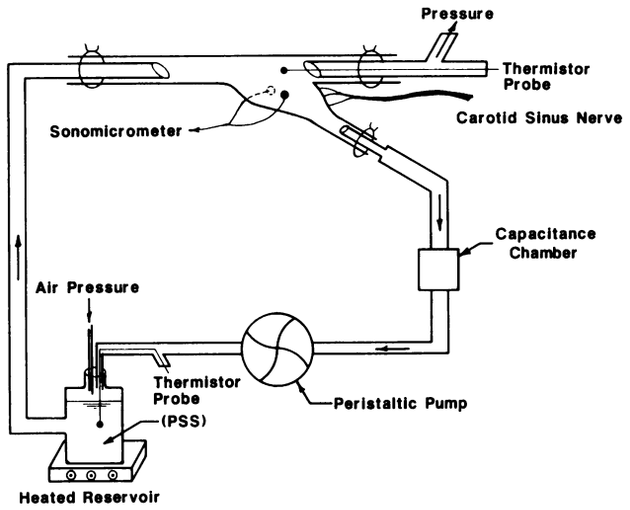

FIG. 1. Schematic representation of the experimental preparation.

Carotid Sinus Nerve Recordings. The carotid sinus nerve was cut at its junction with the glossopharyngeal nerve, covered with paraffin oil, and desheathed; multi-unit activity was recorded as described (7, 9). Through repeated splitting of the sinus nerve, a fine strand that exhibited single-unit characteristics could also be isolated (7, 9) for recording of nerve activity. The vagosympathetic trunk was sectioned. A nerve traffic analyzer that counts spikes above a preset voltage was used to quantify nerve activity. Carotid sinus pressure, systemic arterial pressure, diameter, mean nerve activity per sec, and integrated activity were continuously monitored on a Beckman dynograph recorder (model R411). Undamped pressures and diameters were recorded with a 30-Hz cutoff frequency amplifier. Prior to the nerve activity recording, decamethonium bromide, 0.3 mg/kg, was administered to each dog to prevent muscular movement.

Protocol. In multi-unit preparations, the carotid sinuses were exposed to step increases in pressure beginning at 25 mmHg and increasing to 200 mmHg in increments of 25 mmHg. Activity was allowed to stabilize after each step increase at zero flow for 30–60 sec; flow was then initiated at 18 ml/min and then returned to 0 ml/min (recovery). In several experiments, we studied the effect of progressive increases in flow on a multi-unit activity at a constant CSP of 50 mmHg.

In single-unit preparations, the sinuses were exposed to a slow ramp increase in CSP (<2 mmHg/sec) from 25 to 200 mmHg under zero flow or flow conditions. Carotid sinus pressure was maintained at 25 mmHg prior to the start of each ramp for 5–10 min with or without flow. Single-fiber activity was recorded during the pressure ramp, and the pressure at which activity was initiated (pressure threshold, P_{th}) was determined.

Data Analysis. A one-way analysis of variance was used to determine significant differences between the no flow, flow, and recovery (no flow) conditions at each pressure (11). The Neuman–Kuel test for multiple comparisons was used to determine differences between individual pairs of means when the F-test was significant (12). P_{th} values were compared using the paired t test. A value of $P < 0.05$ was considered to be statistically significant.

RESULTS

Effect of Flow on Multi-Unit Baroreceptor Activity. Compared to the response without flow, when flow at a rate of 18 ml/min was initiated, multi-unit nerve activity increased at carotid sinus pressure below 125 mmHg (Figs. 2 and 3) but did not change at higher pressures (Fig. 3). The increase in activity was promptly reversed upon cessation of flow (Fig. 2). Flow did not alter the pressure–strain relationship of the carotid sinus (Fig. 3 *Right*). At constant flow, shear stress decreased with increased pressure because of increased diameter.

Effect of Graded Increases in Flow. When carotid sinus pressure was held at 50 mmHg, increases in flow over the range of 0 to 24 ml/min resulted in proportional increases in multi-unit activity with no significant changes in diameter; the activity returned to control levels upon cessation of flow (Fig. 4).

Relation Between Shear Stress and Activity. There was a linear relationship between S_w and multi-unit nerve activity (36.6 ± 11.7% increase in activity per dyne/cm^2; 1 dyne = 1 × 10^6 N) at shear stresses >0.8 dynes/cm^2 whether the change in S_w was accomplished by increased flow at a constant pressure (50 mmHg) or increased pressure at a constant flow (18 ml/min) (Fig. 5).

Effect of Flow on Single Unit Baroreceptor Activity. The P_{th} determined with a slow pressure ramp (<2 mmHg/sec) decreased in the presence of flow (18 ml/min) from an average value of 80.1 ± 6.1 mmHg to 69.3 ± 5.7 mmHg (Fig. 6). Maximal activity was not significantly altered by flow

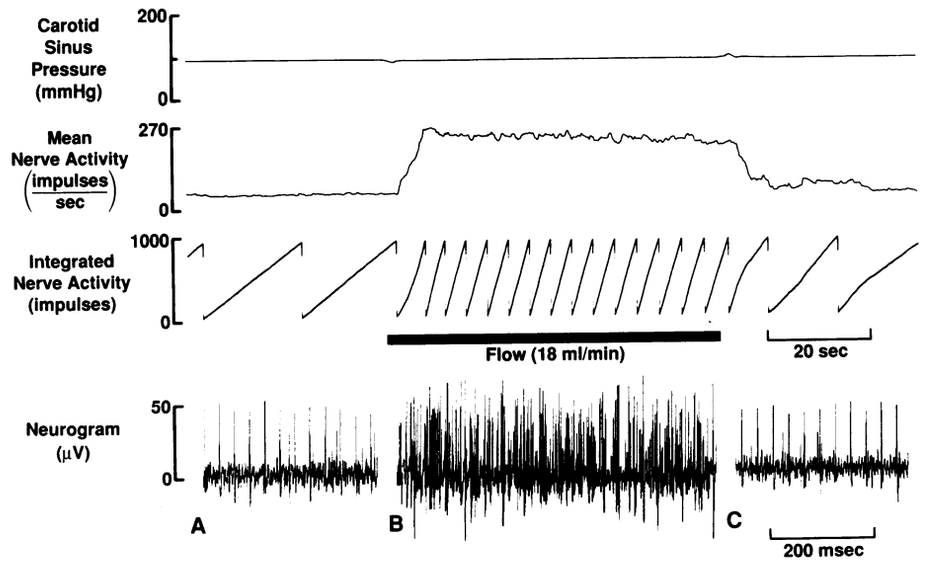

FIG. 2. Experimental record showing that exposure of the isolated carotid sinus to a step increase in flow (shaded bar) increases multi-unit nerve activity per unit time. Carotid sinus pressure was maintained at 90 mmHg. Bottom trace represents a neurogram recorded at a fast paper speed during no flow (control) (*A*), flow (18 ml/min, 38°C) (*B*), and recovery (*C*).

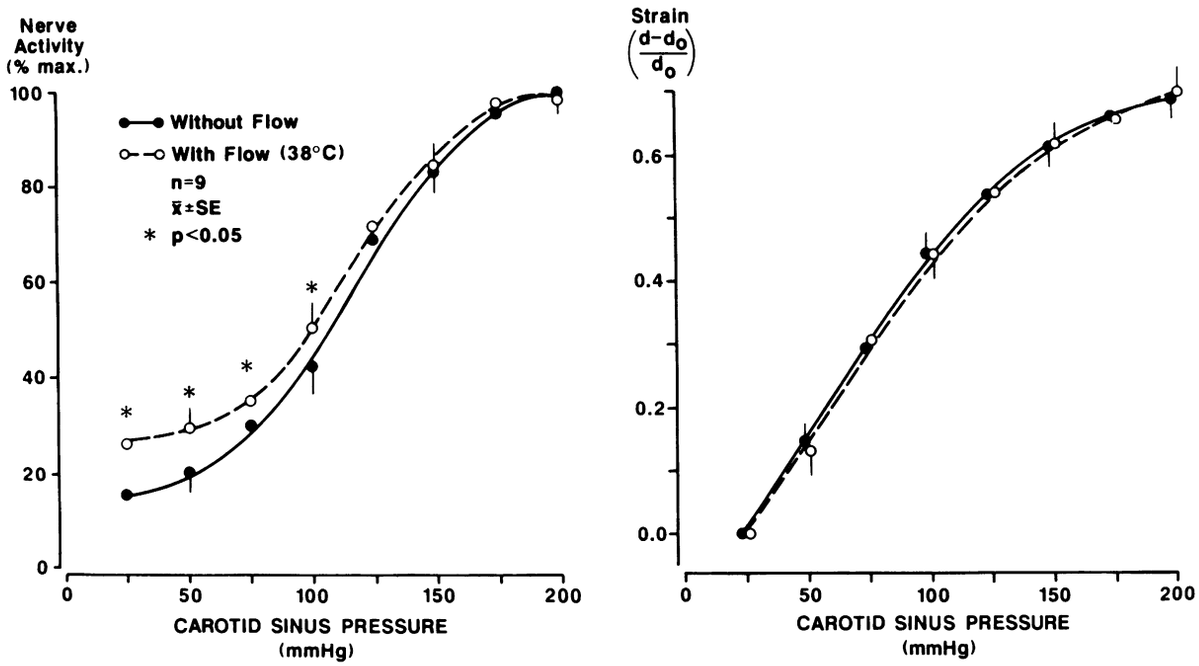

FIG. 3. Effect of flow (18 ml/min) with perfusate maintained at 38°C on carotid sinus nerve activity (*Left*) and strain (*Right*).

(Fig. 6). Fig. 7 represents data from four separate single units with various responses to flow. Decreased P_{th} was noted in 11 of 12 fibers, increased slope in 3 of 12 with no change in 6, and increased maximal activity in 7 of 12.

DISCUSSION

The possibility that there are receptors in the circulation that are capable of responding to changes in cardiac output or blood flow has not been studied. It has been suggested that because atrial type B mechanoreceptors are sensitive to the rate of change in pressure (13–15), they may signal the rate of venous return and, therefore, cardiac output (16). Charlton and Baertschi (17) have shown that aortic baroreceptors are *indirectly* sensitive to changes in aortic flow as a consequence of their sensitivity to the rate of pressure change. The effect of changes in flow in the absence of any alterations in pressure cannot be determined from their study.

Our results indicate that at a constant pressure and diameter, increases in flow elicit increases in activity in the carotid sinus nerve of the dog and increase the sensitivity of single baroreceptor units as defined by a consistent reduction of the pressure threshold. The flows through the internal carotid artery used in the present study were within the physiologic range reported to be 8–20 ml/min for both conscious and anesthetized dogs (18, 19). The discussion will cover the possible mechanisms of activation of these receptors and their physiologic significance.

Type of Receptor. These flow-sensitive receptors or rheoreceptors may be activated by chemicals released from the endothelium by an increase in shear forces during flow or by subtle mechanical deformation of the endings through vibration of the carotid sinus wall without actual changes in strain. They may be baroreceptors, chemoreceptors, or "chemically sensitive" receptors. First, the results from our single-unit preparations suggest that the receptors that were activated during flow are baroreceptors. Second, chemoreceptors are typically activated by decreases in O_2 and pH and increases in CO_2. The preparation was such that the gases of the physiologic salt solution would preclude any possible change in the metabolic environment of the receptors (Po_2 > 150 mmHg, Pco_2 = 25–35 mmHg, pH = 7.35–7.45). Furthermore, the glomus of the carotid body, which contains the chemoreceptors, was excluded in this preparation by ligation of the occipital artery close to its origin. Finally, flow may activate chemically sensitive endings that are neither baronor chemoreceptors. These receptors would be sensitive to chemicals released from the endothelium or smooth muscle under conditions of changes in flow.

We identified single-unit baroreceptors by an increase in

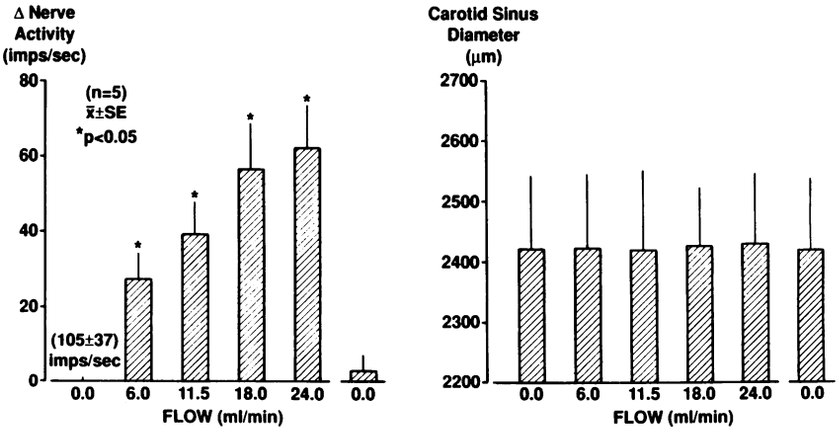

FIG. 4. Influence of varying levels of flow (38°C) on carotid sinus multi-unit nerve activity (*Left*) and diameter (*Right*). With CSP held constant at 50 mmHg, increments in flow resulted in proportional increases in activity without altering diameter.

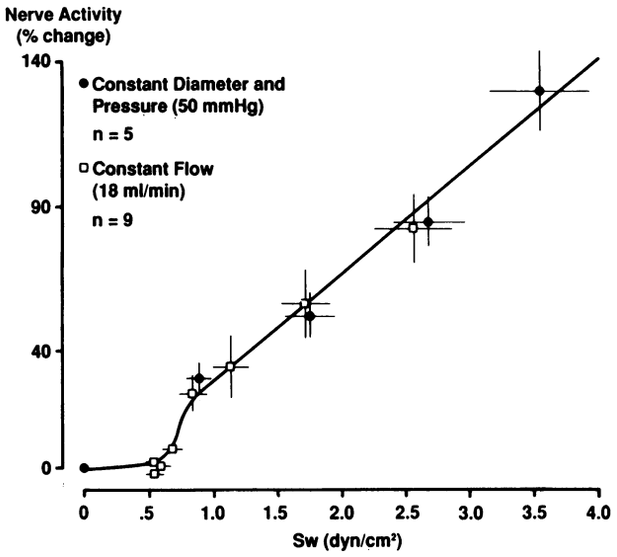

FIG. 5. Influence of wall shear stress (S_w) on carotid sinus multi-unit nerve activity. Data expressed as mean ± SE. ●, Data from Fig. 4 obtained under constant diameters and pressures with varying flow conditions. □, Data from Fig. 3 under constant flow conditions with varying pressures and diameters.

discharge rate in response to an increase in carotid sinus pressure. Although sensitization was observed by a consistent decrease in P_{th} during flow, the individual single units did exhibit variable responses (Fig. 7). This may indicate that different baroreceptors may respond differently to the flow stimulus. Whether this is due to different fiber types cannot be determined from this study. While we show that baroreceptors are flow sensitive, one could also propose that they are rheoreceptors sensitive to pressure. We cannot rule out the possibility that chemically sensitive endings as those shown by others (20, 21) are sensitive to flow.

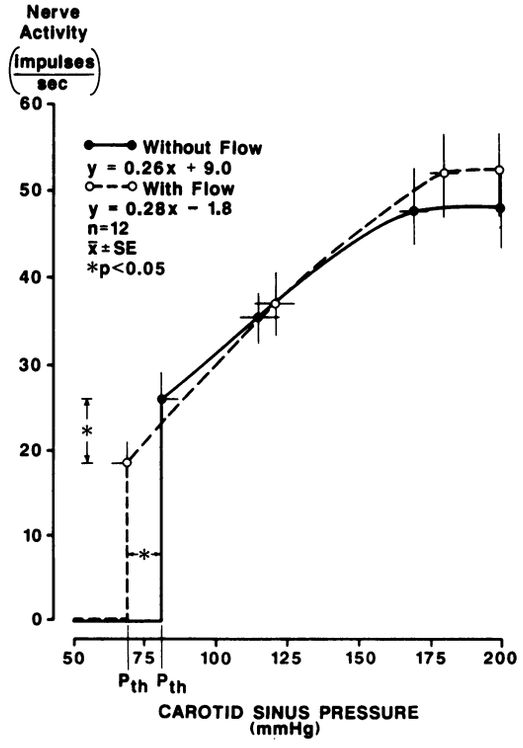

FIG. 6. Influence of flow on single-unit baroreceptor activity during a ramp increase in carotid sinus pressure in 12 experiments.

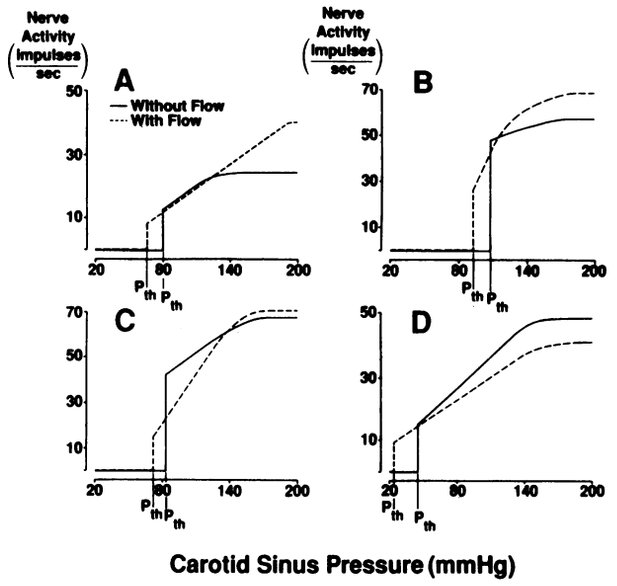

FIG. 7. Individual responses of four single units (*A–D*) to flow (18 ml/min) during a ramp increase in carotid sinus pressure.

Effect of Shear Stress. Increased shear forces during flow have been shown to have profound effects on the vascular endothelium resulting in the production and release of prostaglandin I_2 (prostacyclin) and endothelium-derived relaxing factors, increased endothelial cell turnover, and increased pinocytotic rates (3, 22–25). Although the role of the endothelium in regulating vascular smooth muscle tone has been of major interest for some time (26), the interaction of the endothelium with baroreceptor activity remains unknown. We have recently shown that exogenous administration of prostaglandin I_2 sensitizes and that the cyclooxygenase inhibitor indomethacin attenuates baroreceptor activity, indicating that arachidonic acid metabolites may tonically activate the baroreceptors (27, 28). Therefore, the mechanism of flow sensitization of the carotid sinus receptors may be related to a chemical activation of the nerve endings.

Shear stress may account for the increased activity at lower pressures, and the lack of an influence of flow at high pressures may also be a consequence of the lower shear forces. Indeed, the wall shear stress decreased by 60% when pressure was raised from 25 to 100 mmHg. The wall shear stresses determined were based on a simple rigid tube model with laminar flow. The actual shear forces were slightly different because of the geometry and location of the carotid sinus and possible influences of turbulent flow. Nevertheless, our values for shear are in the range shown to affect endothelial processes (25). The relationship between shear stress and nerve activity was linear beyond values of shear stress of ≈ 0.8 dyne/cm^2 regardless of whether the stress was increased by increments in flow at constant pressure or decrements in pressure and diameter at constant flow.

The endothelium has recently been shown to possess mechanotransducing ion channels (29). It is possible that the shear forces exerted on the endothelium during flow increase the probability of a mechanosensitive Ca^{2+} (29) or K^+ channel (30) to exist in the open state, leading to the production and/or release of a chemical agent(s) that diffuses to the baroreceptor ending during flow. It is interesting to note that release of both prostaglandin I_2 and endothelium-derived relaxing factors are associated with increases in intracellular Ca^{2+} (31, 32). On the other hand, Yanagisawa *et al.* (6) recently isolated an endothelial vasoconstrictor peptide (endothelin), which behaves similarly to the neurotoxins that bind to tetrodotoxin-sensitive Na^+ channels and block

neural transmission. Endothelin production appears to be inhibited by chronic flow-mediated shear stress (6).

Direct Mechanical Activation. Once the endothelial factors are released, they may affect the nerve endings chemically or mechanically. Our data tend to rule out a mechanical factor. A flow-mediated vasodilation was not observed in this study, and no changes in carotid sinus wall strain were found. This is not surprising, as there is relatively little basal vasomotor tone in our vessel preparation. We have seen an endothelium-dependent relaxation of the carotid sinus in response to acetylcholine (33), under conditions of high tone.

It is possible that small vibrations or alterations in smooth muscle mechanics, undetectable by sonomicrometry, stimulated the baroreceptor endings. However, the increase in nerve activity during flow at 25 mmHg was comparable to that seen during a rise in distending pressure from 25 to 75 mmHg in the absence of flow (Fig. 3). The corresponding increase in carotid sinus diameter during a 50-mmHg increase in distending pressure over the same pressure range is $\approx$750 μm, which exceeds by almost 100-fold the detectable changes (5–10 μm) in diameter. Thus, the mechanism of flow sensitization does not seem to be a direct mechanical activation of the nerve endings.

Physiological Significance. The location of rheoreceptors in the circulation may be strategically important for two reasons. First, the shear stresses associated with flow are usually highest on the leading edges of flow dividers and around the circumference of entrance regions at branching vessels. Indeed, the measured shear stresses in these regions have been found to exceed those in the upstream parent vessel by approximately 2 to 4 times (34). This makes the endothelium in the carotid sinus region ideally suited for a flow sensor.

Second, the internal carotid artery is a major conduit vessel for brain blood flow. It would be advantageous for the organism to sense any change in blood flow to the brain that could potentially compromise homeostasis. The presence of a flow sensor would add another facet to the control of the circulation. A sudden decrease in pressure without any alteration in cardiac output would result in a decrease in baroreceptor activity and an increase in sympathetic output and decrease in parasympathetic output to the periphery. If, however, cardiac output were to also drop, which might occur during orthostasis or hemorrhage, afferent baroreceptor activity would decrease to a much greater extent for any given drop in pressure (Fig. 3). As a result, the system would be able to better compensate reflexly to maintain an adequate perfusion to vital organs.

The present findings may have important implications because certain pathologic states such as cardiogenic shock decrease cardiac output and arterial pressure, whereas septic shock lowers pressure and increases output (35, 36). If baroreceptor activity were directly related to flow at low pressure, the activity would be greater in septic shock than in cardiogenic shock at an equivalent pressure. Differences in baroreceptor activities may partially account for differences in neurohumoral control of the circulation in these two conditions.

We wish to thank Laurie Fankhauser and Scott Johnson for technical assistance, Carolyn Wagner for the preparation of figures, and Nancy Stamp for typing the manuscript. This research was supported by National Institutes of Health Grants HL14388, HL07121, and HL07762.

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