Peptidergic Modulation of Mechanotransduction in Rat Arterial Baroreceptors

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Regularly discharging baroreceptors in a rat in vitro aortic arch preparation were exposed to increasing concentrations of one of four vasoactive peptides: angiotensin II, arginine vasopressin, atrial natriuretic factor, or substance P. Slow ramps of pressure evoked discharge responses in single-fiber baroreceptors. Instantaneous discharge frequency was measured simultaneously with aortic diameter and pressure. During constriction induced by angiotensin II or arginine vasopressin, baroreceptor diameter threshold (Dth) decreased and pressure threshold (Pth) tended to increase; these effects were reduced or eliminated by nitroprusside. Atrial natriuretic factor and substance P by themselves were without effect on vessel diameter or on baroreceptor discharge. In preparations preconstricted with a moderate concentration of phenylephrine (10^{-8} M), atrial natriuretic factor reduced the phenylephrine-induced constriction and increased Dth and decreased Pth. Substance P, even at high concentrations, was less effective than atrial natriuretic factor in reducing phenylephrine constriction and in altering baroreceptor discharge. Baroreceptor gain was unaffected by any of these peptides. Thus, changes in smooth muscle tone altered mechanotransduction by shifts in 1) the vessel pressure-diameter relation and 2) baroreceptor threshold requirements (Pth and Dth). Changes in the baroreceptor mechanical threshold (Dth) reduced the effects on Pth expected from changes in vessel wall mechanics. Pth reflects the net effects of vessel wall and Dth changes. Pth generally increased during constrictions and decreased during dilations. The changes in Dth and their selectivity (no changes in gain) during vasoactive peptide action closely resemble rapid resetting of baroreceptors. We propose that vascular smooth muscle lies in a parallel arrangement with aortic baroreceptors and that a common compensatory mechanism regulates Dth during sustained changes in vessel diameter. Activation of smooth muscle and reductions in transmural pressure would reduce loading of baroreceptors, and the proposed compensatory mechanism would tend to keep discharge constant by decreasing Dth. Our experiments, however, cannot distinguish between hypotheses for local micromechanical changes in coupling or for changes modulating excitability within the baroreceptor neuron itself as the basis for Dth adjustments. (Circulation Research 1990;66:804–813)

Peptides have been implicated as neurotransmitters or modulators in the peripheral and central portions of the nervous system concerned with cardiovascular regulation.\(^1\)\(^{-4}\) The evidence includes anatomic localization, accumulation/depletion/release, responses to exogenous peptide, and competitive inhibition of some of these actions by synthetic analogues. Although the physiological function of many peptides is uncertain, there is a growing consensus that peptides serve as hormonal and neurotransmitter modulators in various tissues.

Recently, investigators have suggested that several different peptides may act to modify the discharge characteristics of cardiovascular mechanoreceptors and, thereby, influence baroreflex control systems.\(^5\)\(^{-7}\) Intravenous infusions of arginine vasopressin (AVP) enhanced discharge during pressure elevation in single aortic baroreceptors of rabbits and in left ventricular mechanoreceptors of cats.\(^8\) Sensitization of afferents could contribute to baroreflex augmentation.\(^9\)\(^{,10}\) In the rat, infusion of atrial natriuretic factor (ANF) evoked smaller than expected reflex changes in renal sympathetic nerve activity, and the authors suggested that ANF sensitized vagal afferents.\(^5\)\(^{,11}\)

A third peptide, substance P (subP), has been considered a potential candidate for local modula-
tion of baroreceptors. Immunoreactive labeling indicates substantial concentrations of subP in the aortic arch and carotid sinus regions.\textsuperscript{12-14} Since other sensory nerves are known to release subP at their peripheral endings,\textsuperscript{12} some have speculated that subP might have some local regulatory actions on baroreceptors.\textsuperscript{13} It is unclear whether subP is released by peripheral baroreceptor endings\textsuperscript{12} or under which conditions release might occur.

Little is known about the precise mechanism of action of infusions of AVP or ANF on baroreceptor or baroreflex responses. Many peptides are vasoactive. AVP is a potent vasoconstrictor. ANF and subP are vasodilatory. Contraction or relaxation of vascular smooth muscle near arterial baroreceptors is known to alter discharge of arterial baroreceptors.\textsuperscript{15,16} In this report, we examine the response of aortic baroreceptors to the peptides angiotensin II (Ang II), AVP, ANF, and subP using an in vitro preparation in which conditioning and stimulating pressures can be rigorously controlled. We monitored discharge, pressure, and aortic diameter simultaneously to assess effects on baroreceptor pressure and mechanical responsiveness.

**Materials and Methods**

Single-fiber baroreceptors were studied in an in vitro preparation of the aortic arch using male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Indiana). Methods for testing baroreceptors have been described in detail previously.\textsuperscript{17} Briefly, under sodium pentobarbital anesthesia (30–50 mg/kg), the aortic arch and aortic nerve were exposed. Metal cannulas were placed in the innominate artery and the descending aorta. Ligatures were placed on the ascending aorta and the left common carotid and left subclavian arteries. The aortic arch and nerve were removed and transferred to a temperature-regulated perfusion bath in which the vessel was fixed to approximate its in situ length and shape. The aortic lumen was perfused with Krebs-Henseleit solution equilibrated with 95% O\textsubscript{2}-5% CO\textsubscript{2} gas mixture, and the preparation was covered with warm mineral oil.\textsuperscript{17}

To control for rapid baroreceptor resetting,\textsuperscript{18} the mean arterial pressure during perfusion was held constant at 80 mm Hg throughout the experiments except when testing baroreceptor discharge characteristics. Only regularly discharging receptors were tested, and these were presumed to have myelinated axons.\textsuperscript{19} When a single active baroreceptor was isolated, perfusion was halted and mean arterial pressure was reduced to 20 mm Hg. After 30 seconds at 20 mm Hg, pressure was increased in a slow ramp (<2 mm Hg/sec) by use of a shaker-driver-bellow system (model 411, Ling Dynamic Systems, Yaleville, Connecticut). These ramp rates were sufficient slow to evoke quasadiapated baroreceptor responses.\textsuperscript{17} The diameter of the descending aorta adjacent to the arch was measured simultaneously with discharge using a custom-made (Iwazumi, Canada) high-resolution (0.02% or 1 μm) photoelectronic caliper.\textsuperscript{18,20} At 20 mm Hg, aortic diameter was generally at or near its minimum.

Experiments were recorded on analog FM magnetic tape and later played back for digitization by a microcomputer (PDP 11/23). Diameter and pressure were sampled each 100 msec. Spike sorting\textsuperscript{21} was used in one animal to separate a pair of baroreceptors recorded simultaneously. Discharge rate was measured by the computer as spike-triggered interrupts and expressed as the instantaneous frequency (reciprocal of the interspike interval). Pressure-discharge and diameter-discharge relations were constructed for each ramp response. Typically, these relations have a distinct minimum pressure and diameter at which discharge begins and a suprathermal region in which discharge increases linearly in response to increases in pressure.\textsuperscript{17,18} In these regularly discharging baroreceptors, discharge at threshold generally begins by a jump from zero to a rate of between 15 and 30 spikes/sec as pressure exceeds the threshold level. Pressure and diameter values corresponding to the first 10 action potentials were averaged to represent the pressure threshold (Pth) and diameter threshold (Dth), respectively. The range of pressures for this averaging was generally less than 1 mm Hg. This averaging weighted these critical measures on a short interval of values rather than on the occurrence of a single action potential (the first one). The solutions tested did not consistently alter the rate of discharge at threshold or the slope of the linear suprathreshold region (an index of receptor gain or sensitivity to pressure or diameter). Therefore, Pth and Dth were used as the basic parameters for comparison.

Pressure-response curves were measured each 5 minutes throughout the experiments. In all experiments, there was an initial control period of testing lasting 20–30 minutes during perfusion with the normal Krebs-Henseleit solution. After this, the aortic arch was perfused with a peptide-containing test solution, and ramps were repeated each 5 minutes. For most experiments, the test perfusion at a given concentration was continued for a total of 15 minutes with three ramp tests. The peptide concentration was then increased 10-fold, and the perfusion period was repeated. The concentration ranges for each peptide were similar; testing was begun below the concentration required for minimum vasoactive effect and increased through the clearly unphysiologically high concentration of 10\textsuperscript{−6} M. Because we found no evidence of a sensitization of aortic baroreceptors, the AVP range was extended to 10\textsuperscript{−5} M. The plasma concentrations of these peptides in humans and in experimental animals are normally between 10\textsuperscript{−12} and 10\textsuperscript{−11} M, and various physiological and pathophysiological challenges can increase plasma levels 10–100-fold.\textsuperscript{5,12,22,23} The concentration ranges tested for each peptide were (M) Ang II 10\textsuperscript{−10}–10\textsuperscript{−6}, AVP 10\textsuperscript{−9}–10\textsuperscript{−5}, ANF 10\textsuperscript{−11}–10\textsuperscript{−6}, and subP 10\textsuperscript{−10}–10\textsuperscript{−6}. Since these peptides have well-known vasoactive proper-
during constrictions, aortic diameter, and pressure were measured simultaneously during a slow ramp increase in pressure. Three panels display three representative aortic arch pressure-diameter (top panel), baroreceptor diameter-discharge (middle panel), and baroreceptor pressure-discharge (bottom panel) curves during control (C) and during constriction with 10^{-8} M Ang II (-8) and 10^{-6} M Ang II (-6). Insets plot the average diameter at 100 mm Hg (top inset), diameter threshold (middle inset), and pressure threshold (bottom inset) for three ramp tests at each concentration of Ang II tested from 10^{-16}-10^{-6} M Ang II. Ang II was increased stepwise in order of greater concentrations. At NP, nitroprusside was added to the maximal Ang II concentration. Note that during aortic constriction, the baroreceptor diameter-discharge curve is shifted to lower diameters while the pressure-discharge is shifted to higher pressures.

FIGURE 1. Curves and graphs showing typical response of a single baroreceptor to angiotensin II (Ang II). Discharge frequency, aortic diameter, and pressure were measured simultaneously during a slow ramp increase in pressure. Three panels display three representative aortic arch pressure-diameter (top panel), baroreceptor diameter-discharge (middle panel), and baroreceptor pressure-discharge (bottom panel) curves during control (C) and during constriction with 10^{-8} M Ang II (-8) and 10^{-6} M Ang II (-6). Insets plot the average diameter at 100 mm Hg (top inset), diameter threshold (middle inset), and pressure threshold (bottom inset) for three ramp tests at each concentration of Ang II tested from 10^{-16}-10^{-6} M Ang II. Ang II was increased stepwise in order of greater concentrations. At NP, nitroprusside was added to the maximal Ang II concentration. Note that during aortic constriction, the baroreceptor diameter-discharge curve is shifted to lower diameters while the pressure-discharge is shifted to higher pressures.

ties, we also tested the vasoconstrictors, Ang II and AVP, in the presence of high concentrations of nitroprusside (NP, 10^{-6}-10^{-5} M) to relax the smooth muscle. For the vasodilating peptides, ANF and subP, we also did experiments in the presence of vasoconstriction using phenylephrine (PE, 10^{-8} M, a dose that avoided the excitatory α1-adrenergic effect).

All drugs were dissolved in cold (1°-3° C) Krebs-Henseleit solution just minutes before use in each experiment. The solutions were warmed to 37° C by the temperature regulation system (servocontrolled Peltier device, Midland-Ross, Cambion, Cambridge, Massachusetts) just before entering the aorta. Peptide suppliers were Sigma Chemical, St. Louis, Missouri (AVP, Ang II, and subP), and Calbiochem, LaJolla, California (ANF). NP and PE were both from Sigma Chemical.

For a given experiment to be accepted for analysis, test solutions at a minimum of four log concentrations (10,000-fold) had to be successfully completed. Across all protocols, dose-response curves in a total of 27 single-fiber baroreceptors from 26 animals met these criteria. In one animal, a pair of baroreceptors were recorded simultaneously and separated by spike sorting. Only one drug protocol was tested on each baroreceptor. Effective test solutions generally produced dose-dependent parallel shifts of the baroreceptor pressure-discharge or baroreceptor diameter-discharge curves along the x axis, that is, changes in threshold values. Dose-response curves were constructed for each test solution by averaging the magnitude of these curve shifts from the several ramp tests at each concentration and plotting these values against the log of the peptide concentration. Since we had multiple measurements of baroreceptor properties at each concentration within each experiment, difference testing was carried out at two levels: effects within experiments and summary normalized results averaged across experiments. Relations for individual baroreceptors were tested by analysis of variance or multiple linear regression analysis. The pattern of these individual within-experiment results are noted in each section of “Results.” To display average effects and to assess the general response across all baroreceptors tested with the same protocol, the data in individual experiments were normalized within experiments as the mean value of the multiple measurements relative to control. Thus, each value of the test parameter (Pth, Dth, or diameter at a fixed pressure) was divided by the mean of measurements during control (drug-free) conditions. For comparison of pressure-diameter relations, a pressure of 120 mm Hg was used in all experiments except Ang II. In Ang II experiments, control Pth values were somewhat lower, and a pressure (100 mm Hg) that was closer to the linear discharge-response range of this group of baroreceptors was used. Results were pooled by averaging one set of mean values for each drug level for each experiment (i.e., baroreceptor), and these normalized pooled data were subjected to another analysis of variance. The summary results are expressed as mean±SEM of
these normalized values and are discussed below as the “average” results in percent changes from control.

Results

Angiotensin

Concentrations of Ang II above $10^{-10}$ M (Figure 1, top panel) produced progressive constrictions of the aortic arch as previously reported.16 Two effects on baroreceptor discharge properties invariably accompanied these vessel wall constrictions. First, with each increase in Ang II concentration, the diameter-discharge curves progressively shifted in a parallel manner to lower diameter ranges (Figure 1, middle panel). There was no general change in the slope (gain) of the diameter-discharge curves during these shifts. Dth, the minimum diameter required for baroreceptor excitation, decreased in a dose-dependent manner (inset to middle panel of Figure 1). Second, as Ang II was increased, the pressure-discharge curves shifted in a parallel manner to higher pressures (Figure 1, bottom panel). Pth requirements were substantially higher during Ang II constriction (inset to bottom panel of Figure 1), but the suprathreshold pressure gain (slope) was unchanged. Addition of NP greatly reduced or eliminated the aortic constriction and the effects on baroreceptor discharge curves (Dth and Pth), even in the continuing presence of the maximal Ang II concentration (NP points in insets of Figure 1). Individual results in all four baroreceptors tested with Ang II alone shared this same basic pattern of significantly ($p<0.01$) increased Pth and decreased Dth. On average, compared with drug-free controls ($n=4$ from four rats), maximal Ang II constrictions reduced aortic diameter and Dth to $91.2\pm3.2\%$ and $90.9\pm2.5\%$, respectively, and increased Pth to $111.7\pm4.0\%$ (Figure 2). In separate experiments ($n=4$ from three rats) in which NP was present throughout, concentrations of Ang II up to $10^{-6}$ M did not affect diameter, Pth, or Dth ($p>0.54$).

Vasopressin

The effects of AVP on aortic baroreceptors were more variable than Ang II, and this appears to be related to the more variable response of aortic smooth muscle to AVP. AVP ($>10^{-10}$ M) constricted the aortic arch (Figure 3, top panel). The AVP constrictions, however, tended to relax somewhat (inset to top panel of Figure 3) after several minutes of perfusion at high concentrations ($>10^{-8}$ M). With increasing AVP, baroreceptor diameter-discharge curves shifted to lower diameter ranges in a parallel manner (Figure 3, middle panel). The changes in Dth followed the general response pattern for aortic diameter constriction as found with Ang II. Dth decreased substantially with the decreases in diameter from control to $10^{-8}$ M AVP, but at variable concentrations greater than $10^{-8}$ M, aortic diameter and Dth increased with further increases in AVP (inset to middle panel of Figure 3). The shifts in the pressure-discharge curves of individual baroreceptors (Figure 3, bottom panel) were parallel, but the magnitude of the shifts was generally small. Addition of NP for this baroreceptor eliminated most of the change in diameter and Dth.

In individual experiments with complete dose-response curves ($n=5$ from five rats), most (four of five) baroreceptors had small increases in Pth with AVP up to a $+7$ mm Hg shift (not shown), a direction of change similar to Ang II baroreceptor responses. One baroreceptor had small, but significant ($p<0.05$), decreases in Pth (Figure 3). On average, compared with drug-free controls ($n=5$ from five rats), AVP maximally reduced aortic diameter and Dth to $84.2\pm1.2\%$ and $90.7\pm1.2\%$, respectively, but Pth increased insignificantly to only $103.4\pm1.3\%$ during AVP (Figure 2). The presentation and group statistics of the AVP data (Figure 2) is complicated by variation from preparation to preparation in the concentration at which the diameter relaxation began and the severity of this relaxation. In separate experiments ($n=4$ from four rats) with NP continuously
present, AVP was without effect on wall or baroreceptor properties (p>0.45). In one additional baroreceptor, AVP concentrations of up to 10^{-5} M were tested in the continuous presence of Ang II (10^{-6} M). The Ang II caused a large near-maximal vasoconstriction and increases in Dth and Pth similar to Figure 1, but addition of AVP was without further effect.

**Atrial Natriuretic Factor**

ANF alone, even at very high concentrations (10^{-6} M), was without effect on diameter, Dth, Pth, or the slopes of the baroreceptor discharge-response curves (p>0.5). Low to moderate concentrations of the α₁-agonist PE constricted the aortic arch and reduced baroreceptor discharge at any given pressure (Figure 4, bottom panel). Addition of ANF to perfusate containing PE dilated the aortic arch from the PE-alone value (Figure 4, top panel). PE constriction decreased Dth substantially (insert of middle panel of Figure 4), and addition of ANF to the PE perfusate increased Dth values. Pth was substantially increased during PE alone, and ANF reduced the PE effect (Figure 4, bottom panel). High concentrations of ANF (10^{-6} M) returned diameter, Dth, and Pth to values close to those of the initial drug-free state. This pattern was found in all baroreceptors tested. On average, compared with drug-free controls (n=3 from three rats), PE constriction reduced aortic diameter and Dth to 81.6±1.9% and 83.7±1.4%, respectively, and increased Pth (107.6±1.2%; Figure 5). On average, high concentrations of ANF restored diameter, Dth, and Pth values to near their drug-free control values (Figure 5; p>0.5).

**Substance P**

SubP alone failed to affect diameter, Dth, Pth, or the slopes of the baroreceptor discharge-response curve (p>0.5). In one of five cases, subP, like ANF, relaxed PE-constricted aortas (Figure 6, top panel). In this baroreceptor, subP-induced relaxation was accompanied by a return of Dth and Pth to near drug-free values despite the presence of PE (Figure 6, middle and bottom panels). Of the five baroreceptors in which dose-response curves were completed, high concentrations of subP failed to normalize the vessel wall and baroreceptor discharge curves in four cases. Thus, on average, compared with drug-free controls (n=5 from five rats), subP only partially dilated aortic diameter from 84.4±2.1% during PE to 90.2±3.9% during subP plus PE (Figure 5). Across the five-unit averages, addition of subP failed to affect PE-induced changes in Dth or Pth (Figure 5, p>0.5). However, when subP produced aortic relaxation, the pattern of baroreceptor response was similar to that of ANF (Figure 6).

**Discussion**

Several investigators have reported changes in baroreceptor and baroreflex sensitivity during exposure to exogenous Ang II, AVP, or ANF.5-7 Interpretation of infusion studies, however, is often diffi-
cult. Plasma concentrations were generally not measured, but the range of concentrations of many studies probably reaches or exceeds the maximum plasma levels likely to be found even under extreme circumstances. These peptides often alter systemic hemodynamics and make it difficult to control the stimulus to the baroreceptor afferents. The effects on baroreceptor afferents of changes in smooth muscle tone produced by these peptides have not been assessed previously. Using an in vitro preparation, we have studied the discharge properties of aortic baroreceptors under controlled conditions in which peptide concentrations and the baroreceptor stimulus were regulated and pressure, discharge, and vessel diameter were measured simultaneously. Our experiments clearly indicate that changes in vascular smooth muscle are required for these peptides to alter baroreceptor discharge properties and that only baroreceptor threshold and not baroreceptor sensitivity (gain) is affected.

Smooth Muscle Modulates Baroreceptor Threshold

Vasoactive peptides affect arterial baroreceptor discharge by at least two mechanisms. First, peptides might act indirectly by changing vessel wall mechanics through their actions on vascular smooth muscle as shown for Ang II in previous\(^1^6\) and the present studies. Second, peptides might act directly by binding to specific sites on baroreceptor neurons, as recently suggested for norepinephrine,\(^1^6\) and affect excitability through some intracellular mechanism. Our results indicate that Ang II, AVP, ANF, and subP act indirectly on aortic baroreceptors via vascular smooth muscle, although the concentrations required are generally very high. For example, substantial hemorrhage (30 ml/kg) in the conscious dog raises AVP levels to \(10^{-10}\) M,\(^2^2\) a concentration that in our preparation produced very modest effects. Ang II and AVP substantially reduced aortic arch diameter at the lowest concentrations \((10^{-10}-10^{-8}\) M). Pth generally increased during this vasoconstriction so that less discharge was found at any given pressure. Although there appear to be differences between Ang II and AVP in the net effect on Pth, the general response pattern was similar, NP prevented all effects of Ang II and AVP on baroreceptors, and maximal preconstriction with Ang II prevented baroreceptor changes induced by AVP. We conclude that Ang II and AVP have similar actions on baroreceptors. Reductions in baroreceptor discharge by vasoconstriction are consistent with a mechanical restraint of vessel wall distention often referred to as baroreceptor “unloading.” At a given pressure, the baroreceptor ending in the adventitial layers of the vessel should be subjected to less stretch and, therefore, less stimulation in the contracted aorta. With the vasodilating peptides, ANF and subP, or with AVP at high concentrations, increases in diameter (dilations) generally resulted in greater discharge; that is, vasodilation activated baroreceptors.

Baroreceptor discharge was unaffected by any of the peptides when changes in diameter were prevented or did not occur: 1) during NP with both Ang II and AVP,
2) during control conditions for ANF and subP, since this in vitro preparation has little smooth muscle tone under control conditions, 3) during apparent desensitization with long exposures or high doses such as found with AVP and subP, and 4) during AVP after maximal preconstriction with Ang II. This suggests that, in the rat, these peptides lack any direct actions that might be mediated by peptide binding to sites on the baroreceptor membrane and emphasizes that diameter changes are an absolute requirement for the Dth and Pth changes observed. Limited experiments in the dog carotid sinus and, recently, in the rabbit aortic arch in vitro also found that AVP had no excitatory effect on arterial baroreceptors. Since AVP is a potent vasoconstrictor and should, therefore, mechanically unload baroreceptors, the reported sensitization is unlikely to be the result of any local actions via smooth muscle. In the rat, AVP alters baroreflex control of heart rate but not of lumbar sympathetic activity. It seems more likely that the changes in baroreflex responses and the increase in baroreceptor discharge reported during AVP and ANF are related to the significant hemodynamic changes or central nervous system effects rather than to actions on baroreceptor transduction. The interplay of hemodynamic and local vasoactive changes was evident in recent experiments with ANF in anesthetized rabbits. Although pressure decreased during ANF infusions, aortic baroreceptor discharge did not change. The level of baroreceptor stimulation appears to have been maintained constant by the increased diameter of the aorta near the baroreceptors.

Compensatory Changes in Baroreceptor Dth

Shifts in the pressure-diameter curve of the vessel wall alone could account, in a qualitative fashion, for the loading and unloading of the baroreceptors during vasoactive peptide administration. If this were the case during vasoconstriction, Pth would increase simply because higher pressures would be required to reach the same diameter and, therefore, the same degree of baroreceptor stretch. Our simultaneous measurements of diameter, pressure, and discharge showed an additional, unexpected change in baroreceptors during changes in smooth muscle tone. The baroreceptor diameter-discharge relation always shifted along the diameter axis in the direction of the change in the vessel diameter. Since the slope (gain) of these baroreceptor curves was unaffected, this effect was a selective change in Dth, the baroreceptor set point. Vasoconstriction decreased both vessel diameter and Dth. The reduction in vessel diameter at a given pressure would be expected to reduce baroreceptor discharge, but the simultaneous decrease in Dth reduced the degree of stretch required for baroreceptor activation and, thus, reduced the effect or “compensated” for the change in vessel wall mechanics. Therefore, we propose that the changes in Dth represent a compensatory feedback relation between the degree of stretch and baroreceptor threshold.

In studies of norepinephrine and Ang II, Munch et al described aortic baroreceptor discharge responses during vasoconstriction under two conditions: 1) when aortic diameter was held constant by adjusting the transmural pressure (isometric cond-
tions) and 2) when pressure was held constant by a pressure regulator and diameter was free to constrict (isotonic conditions). Baroreceptor discharge decreased during isotonic vasoconstriction (Figure 6 of Reference 16), similar to our own results and conditions. Discharge increased, however, during isometric vasoconstriction in which transmural pressure was increased to offset the tendency for vessel diameter to decrease (Figure 7 of Reference 16). Munch et al reasoned, as we have, that discharge decreased during isotonic responses due to baroreceptor unloading. In light of the results of our study, we would add that baroreceptor unloading (increased Pth) during isotonic vasoconstriction resulted from the quantitative predominance of the change in vessel wall mechanics (the shift in the pressure-diameter curve to lower pressures) over the opposing compensatory reduction in Dth. The increases in discharge during isometric contractions, they suggested, might result from changes in wall tension or contraction of smooth muscle coupled in series to the baroreceptor endings. Munch et al produced an isotonic diameter-discharge curve by gradually increasing Ang II concentration and measuring diameter and discharge at a single fixed pressure (Figure 6D of Reference 16). This composite curve had a lower slope but higher discharge frequencies than the diameter-discharge curve evoked by changing pressure. Neither baroreceptor threshold nor the complete diameter-discharge relation was measured at each Ang II concentration in their studies. Our results suggest that the relative elevation in discharge rate during constriction at constant diameters (isometric) was due to the progressive decrease in baroreceptor setpoint, Dth. We observed no changes in sensitivity (slope) of the baroreceptor diameter-discharge curves between conditions of maximal vasoconstriction and complete relaxation (NP). Thus, we would attribute the apparent change in slope in the Munch et al study to a progressive shift in threshold with each change in vasoconstrictor dose and not to a change in baroreceptor gain.

Additional evidence of apparently compensatory changes in Dth comes from studies of arterial baroreceptors during both chronic and rapid resetting. In normotensive development and aging and in spontaneous and salt-induced hypertension, the expected effects of changes in distensibility on baroreceptor pressure-discharge properties were partially offset by changes in baroreceptor distortion-sensing properties including the strain threshold, a normalized form of Dth. In the present study, changes in smooth muscle tone shifted the baroreceptor diameter-discharge and pressure-discharge curves in opposite directions. The selectivity of the effects on threshold without changing gain, that is, parallel shifts of both of these curve types, is strikingly similar to the selective changes in Dth and Pth found during rapid resetting of arterial baroreceptors. In rapid resetting, however, gross vessel wall mechanics are not always present, so that

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Curves and graphs showing exceptional response of a single baroreceptor to substance P (Sub.P). In four of the five baroreceptors surveyed, Sub.P was without effect. This particular atypical baroreceptor responded to Sub.P with increases in diameter threshold and decreases in pressure threshold similar to vasodilation with ANF. Discharge frequency, aortic diameter, and pressure were measured simultaneously during a slow ramp increase in pressure. Three panels display four aortic pressure-diameter (top panel), baroreceptor diameter-discharge (middle panel), and baroreceptor pressure-discharge (bottom panel) curves from this baroreceptor during control (C), during phenylephrine (PE) alone, and during PE plus 10^{-9} M Sub.P. (–9) and 10^{-3} M Sub.P (–7). Inserts plot the average diameter at 120 mm Hg (top inset), diameter threshold (middle inset), and pressure threshold (bottom inset) for three ramp tests at each concentration tested from 10^{-9} to 10^{-5} M Sub.P. Sub.P was increased stepwise in order of greater concentrations. Note that during aortic constriction with PE, the baroreceptor diameter-discharge curve is shifted to lower diameters while the pressure-discharge is shifted to higher pressures. In this particular baroreceptor, addition of Sub.P returned the curves toward normal values in spite of the continuing presence of PE.
Pth, the composite of vessel wall and Dth effects, always changes in the direction of Dth.

Possible Mechanisms for Dth Changes

The nature or identity of the mechanism or structures responsible for changes in Dth is difficult to determine. It is tempting to speculate that the same mechanism is responsible for the adjustments in Dth both during changes in smooth muscle tone and during prolonged conditioning pressure changes (rapid resetting). We propose that at least two key elements in the mechanotransduction cascade could conceivably be responsible for Dth regulation: 1) a coupling element arranged in series with the baroreceptor ending or 2) excitability of the baroreceptor neuron itself. The experimental observations suggest a relation between sustained diameter and Dth that would, in effect, provide a negative feedback tendency to maintain baroreceptor discharge more constant.

In the mechanical coupling concept, a serial element is thought to link distortions of the vessel wall with distortions of the mechanosensitive baroreceptor endings.33 Viscous relaxation of this coupling element could give the time-dependent properties associated with rapid resetting and could unload baroreceptor endings at a microscopic level (i.e., undetectable by gross diameter measurements). To be consistent with our measurements of aortic diameter and baroreceptor activity, the major portion of the vessel wall (e.g., media and intimal layers remote from the baroreceptor endings) would have to function in parallel to the baroreceptor and the series-coupling element, which are probably confined to the adventitia.35 Local unloading of the baroreceptors would occur whenever diameter is increased for a prolonged time period of minutes to hours. Thus, vasodilation at constant pressure or increases in passive pressure distension (hypertensive rapid resetting) both lead to increases in diameter and Dth. The hypothesized serial coupling element would act micromechanically to reduce changes in baroreceptor distortion and, therefore, discharge output without affecting the gross vessel wall properties.

Alterations of membrane excitability within the baroreceptor are a second possible explanation of the changes in Dth.18,36,37 Mechanical transmission of vessel distortion to the baroreceptor ending could be unchanged during the challenge. Instead, the mechanotransduction process converting ending distortion to discharge could adapt to become less responsive. Precedence exists in vertebrate muscle spindle mechanoreceptors, in which direct measurements suggest that mechanical changes cannot account for adaptation.38

The present baroreceptor experiments cannot distinguish between hypotheses involving local micro-mechanical changes in coupling and changes modulating excitability within the baroreceptor neuron itself as the basis for Dth adjustments. Functionally, it makes little difference which mechanism is responsible. Our observations suggest that the change in Dth is quantitatively important. For instance, during AVP, there are only small changes in discharge at a given pressure despite larger changes in diameter, and this clearly results from the offsetting decrease in Dth. In rapid resetting, only minor changes in the vessel wall diameter have been observed, and change in Dth is clearly the predominant mechanism.18 The present studies suggest that changes in Dth may reflect a very generalized local-control mechanism of arterial baroreceptors and, therefore, of baroreflexes.

References

4. Haeseler G, Osterwalder R: Evidence suggesting a transmitter or neuromodulatory role for substance P at the first synapse of the baroreceptor reflex. Naunyn Schmiedebergs Arch Pharmacol 1980;314:111–121


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