Dual Effects of Norepinephrine and Mechanisms of Baroreceptor Stimulation

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The aim of this study was to determine the mechanism of action of norepinephrine (NE) on arterial baroreceptors (BRs), with the focus on regularly discharging, presumably myelinated fibers. With the use of an in vitro aortic arch/aortic nerve preparation from rats, BR single-fiber discharge was recorded simultaneously with aortic pressure and diameter. At constant suprathreshold pressure, NE had two dose-dependent effects. Inhibition was produced at low concentrations (10^{-10}-10^{-7} M), whereas excitation was produced at high concentrations (10^{-4}-10^{-3} M). Inhibition was attributed to BR unloading since the response was consistent with the fall in diameter, was mimicked by angiotensin II (10^{-8}-10^{-6} M), and was prevented by pretreatment with the smooth muscle relaxant sodium nitroprusside (10^{-4} M) or the selective α₁-adrenergic antagonist, prazosin (10^{-6} M). Excitation was attributed to direct activation of the BR endings since this response was independent of changes in diameter, was not mimicked by angiotensin II, and was not prevented by sodium nitroprusside but was blocked by prazosin. These results indicate that NE has two modes of action, one mediated by contraction of local vascular smooth muscle and the other due to direct excitation of the nerve endings. It was also found that BR discharge at given diameters decreased more when pressure was lowered (smooth muscle passive) than when the aorta constricted (smooth muscle active). Furthermore, if diameter was held constant during smooth muscle contraction, discharge increased, as opposed to decreasing at constant pressure. These later results suggest that BR responses to vasoactive agents reflect not only changes in wall dimension but perhaps changes in wall tension and/or the coupling relation between BR and smooth muscle structures. (Circulation Research 1987;61:409-419)
In Vitro Preparation

The in vitro aortic arch/aortic nerve preparation has been described previously in detail. The in situ configuration, and the preparation was covered with a gas mixture of 95% O₂-5% CO₂, was pumped through the arch via cannulas placed in the descending aorta and innominate arteries; all remaining branches were ligated. The bath and perfusate were maintained at 37°C. Perfusion pressure, referred to as mean arterial pressure (MAP), was controlled with a modified Starling outflow resistor that also was used to produce slow descending pressure ramps without interrupting flow. A shaker-driven bellows apparatus was used to produce identical ascending pressure ramps.

Recording of Baroreceptor Single-Fiber Activity

The aortic nerve was teased apart until a small filament contained one or a few active fibers that discharged with pressure. In multifiber recordings, individual BRs were functionally separated, using a time-dependent dual-window voltage discriminator (Bak Electronics, Inc., Rockville, Md.). Successful separation was possible if the action potential waveforms of each unit were sufficiently dissimilar; if they were not, the filament was split further. Once an acceptable recording was obtained, a slow pressure ramp (2 mm Hg/sec) from subthreshold to about 160 mm Hg was produced to determine the BR’s overall pressure-frequency (P-F) characteristics. These characteristics were used as a basis for selecting a homogeneous population of regularly discharging units, and they were typical of myelinated fibers. Conduction velocities were not measured because of an insufficient working length of nerve. Recordings lasted as long as the discharge characteristics and signal-to-noise ratio did not deteriorate, which was anywhere from one-half to several hours.

Measurement of Aortic Arch Diameter

The external diameter of the arch was measured with a newly developed photoelectric device that projects a shadow image of the vessel onto a pair of linear photodiode arrays. The arrays are used to track the location of the shadow’s edges, thus enabling diameter to be calculated electronically. Diameter was initially measured between the left carotid and left subclavian arteries since this region contains the endings of most aortic BRs. To produce a well-defined shadow, however, it was necessary to remove loose connective tissue and fat, which usually destroyed the aortic nerve and required separate preparations for recording diameter and discharge. To make simultaneous recordings, diameter was subsequently measured just downstream from the left subclavian artery where the nerve was not disrupted and where BR endings were found by gently probing the wall.

Application of Drugs

Drugs were dissolved and serially diluted in cold Krebs-Henseleit solution, and small aliquots were added to the perfusate reservoir to a final specific molar concentration. A two-reservoir delivery system was used so that solutions could be altered without affecting MAP or interrupting flow. The following drugs were used: L-norepinephrine hydrochloride, angiotensin II amide, sodium nitroprusside, phenolamine methanesulfonate, DL-propranolol hydrochloride, and prazosin hydrochloride (initially dissolved 1:1,000 in polyethylene glycol).

Data Acquisition and Analysis

The effect of drug-evoked smooth muscle contraction and relaxation on aortic arch diameter was first determined over a wide range of pressures since in subsequent experiments drugs were applied at various pressures set relative to the pressure threshold (Pš) of each BR. Drugs were tested by comparing pressure-diameter (P-D) curves before and after treatment as follows: A series of control curves was first constructed by subjecting the arch to identical slow pressure ramps at 5-minute intervals. All ramps went from near zero to 160 mm Hg, with a constant rate of rise of 2 mm Hg/sec. Between ramps, the arch was perfused at 100 mm Hg. Ramps were repeated until the curves were reproducible. Drugs were then applied during the interramp interval and a new set of curves obtained. Once the new curves were stable, a second drug was sometimes applied to test for drug interaction.

The effect of NE on BR discharge was next examined at constant MAP set between 80 and 120 mm Hg at a level 10–15 mm Hg above BR Pš. After adjusting MAP and before adding NE (or any drug), 15 minutes were allowed for discharge to stabilize so that the response was not affected by pressure adaptation or rapid resetting. NE concentrations differing by a factor of 10 were then applied, either as single doses with each followed by washout to full recovery or as an ascending cumulative series. For comparison, these experiments were repeated using similar concentrations of angiotensin II (AII). BR responses to NE were also determined when smooth muscle contraction was prevented with either the smooth muscle relaxant sodium nitroprusside (NP) or the selective α₁-antagonist prazosin.

In the preceding experiments at constant MAP, BR activity was altered when smooth muscle contraction constricted the arch (see “Results”). It was also of interest, with regard to the mechanical mechanism of BR activation, to determine what effect contraction would have if vasoconstriction was prevented. To examine this response in the presence of NE, diameter was held constant by increasing the perfusion outflow resistance so that MAP rose just enough to offset the contraction. Under these conditions, wall tension increased, as opposed to decreasing at constant MAP.
Results

Effect of NE, All, and Selected Vasoactive Antagonists on the Aortic Arch Pressure-Diameter (P-D) Curve

Smooth muscle contraction with NE (10^{-7} M) or All (10^{-7} M) shifted (reversibly) the P-D curves to higher pressures, with the largest and nearly parallel shift occurring at pressures from 80 to 120 mm Hg (Figure 1). Smooth muscle relaxation with NP (10^{-6} M) had the opposite effect, although the shift was not usually pronounced. When NE or All was reapplied in the presence of NP, contractions were prevented. Treatment with the nonselective α-antagonist phentolamine (10^{-6} M) or the selective α_1-antagonist prazosin (10^{-6} M) blocked the response to NE (up to 10^{-7} M), whereas the β-antagonist propranolol (10^{-6} M) was ineffective.

Dose-Dependent Response to NE at Constant MAP

Perfusing at constant MAP 10–15 mm Hg above P_a provided a means of continuously monitoring BR activity. By setting MAP between 80 and 120 mm Hg, smooth muscle effects on diameter were maximum and fairly uniform and within the steepest and most linear region of the arch P-D and BR P-F curves (Figure 1).^{26,35}

Aortic Arch Response. NE constricted the arch as shown in Figure 2A. The time course of the response displayed typical fast and slow components, as described for thoracic aortic strips.^{36} The fast component developed rapidly over the initial 20–30 seconds, whereas the slow component developed over 2–5 minutes and was maintained until NE was washed out. Recovery periods were often quite long, 5–90 minutes depending on concentration, which limited the number of single doses that could be tested on a given BR. Therefore, NE was usually applied cumulatively until a maximum response was obtained.

The dose-response relation was determined by measuring diameter after a given period of drug exposure. This period was sufficiently long to allow completion of the fast component at all concentrations. Generally, subsequent diameter changes were relatively small and did not produce further changes in BR discharge. Measurements were normalized as a percent of the maximum change in diameter and were plotted versus the negative log of the NE concentration (see Figure 4).

Baroreceptor Response. NE produced two distinctly different dose-related effects on BR activity. At concentrations from 10^{-10} to 10^{-7} M, referred to as “low” concentrations, discharge fell relative to control (Figure 2B). The response followed a roughly similar time course as vasoconstriction, and when recorded simultaneously, discharge and diameter clearly decreased concomitantly (Figure 2C). Changes in frequency varied across units but were always dose-dependent within a given unit. Discharge remained depressed for as long as NE was applied, which at times was up to 20 minutes. During washout, discharge gradually returned to control levels as the arch relaxed. At concentrations of 10^{-6} – 10^{-5} M, referred to as “high” concentrations, discharge increased relative to control (Figure 2B and 2C). This excitatory response was also dose-dependent but did not correspond to changes in...
FIGURE 2. Dose-dependent effect of NE on aortic arch diameter and BR discharge at constant MAP. A: Decrease in diameter with increasing concentration (top to bottom). Each concentration was applied with arch at initial reference diameter (indicated by zero). B: Change in BR discharge produced by same concentrations as in panel A. Typical response at each concentration is shown by these single-fiber recordings from several different fibers from different preparations (fourth and fifth plot from top are from same fiber). Gain of ordinate axis was made to show each unit's response optimally. Note that discharge decreased at concentrations up to 10^{-7} M but increased at 10^{-6} M and 10^{-5} M. Nature of response was not due to different types of BRs, since both effects were always seen when high and low concentrations were tested on a given fiber (panel C). Wide point scatter in some units resulted from frequency oscillations in response to small undamped perfusion pump pressures (1–3 mm Hg). These oscillations appear band-like when compressed in time. C: Simultaneous record of discharge, diameter, and MAP during cumulative application of concentrations that decreased and increased activity in a single fiber. Note that frequency and diameter decreased together with 10^{-7} M but changed oppositely with 10^{-6} M. Excitation was immediately reversed by NE washout, whereas relaxation was delayed.

diameter. Based on the estimated time when NE first reached the arch, onset times for inhibition and excitation appeared roughly the same. High concentrations sometimes initiated firing in previously inactive BRs, making isolation of single fibers more difficult. Low concentrations, on the other hand, did not activate silent units.

The dose-response relation was constructed by measuring the change in spike frequency from control at the same point in time that diameter was measured. The average change for all units was then plotted against the negative log of the NE concentration (see Figure 4, left panels). Two units were not included in the pooled data since they shut off completely at 10^{-9} and 10^{-8} M; they later resumed firing when NE was removed. Note that the most striking feature of the curve is that at low concentrations, discharge decreased with diameter, whereas at high concentrations, where the arch was fully constricted, discharge increased with concentration. Although the maximum fall in discharge generally occurred at 10^{-7} M, in 4 of 17 units the change was less than at 10^{-8} M.

Effect of Angiotensin II

The effect of ATII on arch diameter and BR discharge was examined in a similar set of experiments. ATII produced dose-dependent constriction that followed a typical monophasic time course (Figure 3A). As with NE, constriction reduced BR discharge, the response again resembling the change in diameter (Figure 3B). When recorded simultaneously during cumulative applications, discharge and diameter clearly decreased together (Figure 3C). ATII inhibition was completely reversed by vasoconstriction during washout. In some preparations, constriction began to fade at high concentrations that were applied cumulatively. Similar response fade is typically seen in aortic strips exposed to high or successive doses.37,38 In these instances, discharge rose slightly with diameter, but neither approached control levels. Concentrations higher than 10^{-6} M were not employed in order to avoid fading.

Arch and BR dose-response curves were constructed as described for NE (Figure 4, right panels). Note that the discharge was below control at all concentrations, which was consistent with the change in diameter. For
FIGURE 3. Dose-dependent effect of All on aortic arch diameter and BR discharge at constant MAP. Data are presented as in Figure 2. Note that in contrast to NE results, discharge was below control at all concentrations.

FIGURE 4. Comparison of aortic arch and BR dose-response curves for NE (left) and All (right) at constant MAP set between 80 and 120 mm Hg. Data points represent average responses at equimolar concentrations (numbers = sample size, vertical bars = ± SEM). Arch response is plotted as percent of maximum decrease in diameter from control. BR response is plotted as change in frequency relative to control discharge (control indicated by dashed line at zero). Curved lines were fit by eye. Relatively large error bars at 10^{-4} M and 10^{-3} M NE (lower left) were due to substantial increases in discharge in a few units (maximum change observed was +32 Hz); none of the BR had discharge below control at these concentrations.
NE, however, this observation was true only up to $10^{-7}$ M. At higher NE concentrations, discharge increased considerably while diameter remained constant. These results suggest that at constant MAP, smooth muscle contraction mechanically unloads the nerve endings. Moreover, if NE concentrations are relatively high, the endings may be excited directly, an effect that appears to predominate above $10^{-6}$ M.

**Effect of Smooth Muscle Relaxation**

If NE has a direct excitatory action on BRs, then relaxing the smooth muscle without blocking the adrenergic receptors should prevent mechanical unloading but not direct excitation. To test this possibility, NP was used to relax the smooth muscle since it does not exhibit any inhibitory or excitatory effects on either α- or β-adrenergic receptors, nor does it affect BRs other than through changes in smooth muscle tone.

In 5 experiments in the presence of NP ($10^{-6}$ M), constrictions were reduced at all NE concentrations, with about a hundredfold increase in the threshold dose (Figure 5A). BR unloading was also reduced (up through $10^{-4}$ M), although excitation was not affected; that is, discharge again increased at the same NE concentration ($10^{-6}$ M) as in untreated arches.

With the initial application of NP, one might have expected smooth muscle relaxation to increase BR activity since contraction had the opposite effect. However, the isolated arch has little resting tone (Figure 1), and thus, there was generally little if any initial change in diameter or discharge. On the other hand, if the arch was first constricted so that discharge was reduced, then subsequent relaxation with NP increased diameter and discharge back to original control levels, the BR effect presumably being due to reloading of the sensory endings.

**Effect of Adrenergic Antagonism**

Whether competitive adrenergic antagonism could prevent BR excitation at high NE concentrations, in addition to preventing unloading associated with vasoconstriction, was also tested. In 6 experiments, NE was applied cumulatively in the presence of prazosin ($10^{-6}$ M). Vasoconstriction and BR unloading were reduced (up to $10^{-5}$ M NE), and excitation normally seen at $10^{-8}$ to $10^{-3}$ M was absent (Figure 5B). Thus, the fact that excitation was not prevented by NP, a noncompetitive adrenergic antagonist, but was blocked by prazosin, a competitive adrenergic antagonist, suggests that high NE concentrations act directly on adrenergic receptors on the nerve endings.

The experiments so far were all done at constant MAP so that direct comparisons of drug actions could be made. The ensuing experiments examined the mechanical stimulus that activates the endings.

**BR Response to Active vs. Passive Changes in Aortic Arch Diameter**

BR discharge appears closely associated with diameter during vasoconstriction (these results) and during changes in aortic pressure (see diameter-frequency plots in Goldman and Saum27 and Munch and Brown26). An interesting question is whether the diameter-frequency (D-F) relation is the same under these two conditions, i.e., when the smooth muscle is active versus when it is passive. To make a comparison, the
D-F relation was examined when the arch was constricted with cumulative concentrations of NE or All and when deflated by gradually lowering pressure, as shown in Figure 6A. In the case of NE, because it had dual effects, D-F curves over all concentrations were distinctly nonlinear (Figure 6B). Therefore, NE curves were compared only at diameters where discharge was reduced, which corresponded to NE concentrations that were below threshold for direct BR excitation based on the NP results. As shown in Figure 6C, both the active and passive D-F plots were approximately linear and intersected at diameters corresponding to the initial perfusion pressure. There was a distinct difference, however, in the slope of the curves. At given diameters, there was a greater fall in discharge with pressure (smooth muscle passive) than with vasoconstriction (smooth muscle active). Results with All were similar (Figure 6D).

Response to NE at Constant Diameter

The observation that BRs were unloaded by vasoconstriction at constant MAP raised the question of whether discharge would be affected if diameter did not change. To address this question, the smooth muscle was contracted with NE (up to $10^{-8}$ M) while diameter was held constant. For smooth muscle oriented circumferentially, contractions were essentially isometric. Under these conditions, wall tension increased with pressure (Laplacean relation, tension = pressure × radius), whereas in experiments at constant MAP, wall tension decreased with diameter.

In 5 experiments, it was found that discharge increased with each successive contraction, the response closely paralleling the change in offsetting pressure (Figure 7). Increases in frequency were directly proportional to the increases in pressure and, on the average, rose 0.60 ± 0.09 Hz/mm Hg (mean ± SEM). Since only NE concentrations were used that in all cases unloaded BRs at constant MAP, the increase could not be attributed to direct BR activation. BR responses were thus possible without changes in diameter and were directionally consistent with the change in wall tension. Furthermore, the responses to isometric versus isotonic contraction were opposite.

**Figure 6.** Comparison between BR passive and active diameter-frequency (D-F) relation. Passive relation, shown by lower plots in panels C and D, was determined by gradually decreasing perfusion pressure from initial suprathreshold level, as illustrated in panel A. Active relation was determined by applying cumulative concentrations of NE (upper plot in panel C) or All (upper plot in panel D) at constant MAP. Both types of plots were produced right to left in time and intersect at diameters corresponding to initial suprathreshold perfusion pressure. Passive plots have fewer points than active plots because deflation occurred somewhat faster than constriction. In NE experiments, only data at low concentrations were plotted for comparison since excitation was unrelated to diameter at high concentrations. Over entire range of NE concentrations, the D-F plots were dramatically nonlinear, as shown in panel C (data reduced by spike and time-bin averaging).
Discussion

The findings in the present study provide evidence that NE has both inhibitory and excitatory effects on regularly discharging aortic BRs with presumably myelinated fibers. At constant pressure, inhibition is mediated indirectly by contraction of local vascular smooth muscle, whereas excitation is independent of smooth muscle contraction and probably results from direct activation of adrenoceptors on the sensory endings. The dual effects of NE, which can occur in one and the same fiber, were separated functionally by differences in their respective dose-response relations and by selective pharmacologic modification of the aortic smooth muscle. In addition, our results show that BR responses to vasoactive drugs are strongly dependent on the experimental conditions.

Indirect Effects of NE on BR Discharge

BR endings at constant MAP appear to be unloaded by NE-induced smooth muscle contraction for the following reasons: 1) Decreases in discharge and diameter were similar between 10^{-10} and 10^{-7} M NE. The diameter measurements suggested that unloading also occurred at higher concentrations, but as will be discussed below, direct excitation of the endings predominated. 2) Unloading was mimicked by smooth muscle contraction with AII. This effect was not due to induced liberation of NE from sympathetic efferents because liberation requires higher AII concentrations than were used and ongoing sympathetic activity. Moreover, sympathetic efferents are either absent or very scarce in the rat arch and if present, were eliminated in the in vitro preparation. 3) Preventing smooth muscle contraction by pretreatment with either NP or prazosin prevented unloading. In the case of NP, if BRs were first unloaded by NE-evoked contraction, subsequent NP-evoked relaxation increased discharge by reloading the endings. 4) BR inhibition was not a result of desensitization since the response depended on vessel wall behavior, as shown by experiments at constant diameter, and because, after inhibition, BRs could be excited by higher NE concentrations.

Previous studies involving the effects of NE, NP, or AII are consistent with our results. Landgren reported that BRs with large spike amplitudes, presumably myelinated, had smaller responses to pressure steps after NE treatment. Likewise, others found that at pressures similar to ours, vasoconstriction reduced BR activity. In some cases, the diameter or strain-discharge relation remained constant, consistent with unloading, whereas in other cases, the discharge was enhanced at given diameters, suggesting either a direct NE effect or smooth muscle acting in series. At the time, neither possibility could be excluded. Our NP results are consistent with our earlier study in which BR pressure-frequency curves were shifted to lower pressures and with the Aars study in which relaxation increased BR activity in anesthetized rabbits. Goldman and Saum, on the other hand, found that relaxation was ineffective in the rat arch. This finding is not surprising given that the rat arch has little resting tone in vitro. Muscle relaxants produce minimal dilation and thus negligible changes in BR activity unless preceded by vasoconstriction. Lastly, unloading produced by AII may explain why McCubbin et al found that injecting angiotonin into the isolated carotid sinus produced a moderate rise in systemic pressure. Others found that very high AII concentrations (10^{-7} M) increased BR activity, but because the response was absent some hours after postganglionic sympathectomy or following reserpine application, excitation was attributed to NE liberation from sympathetic terminals.

Mechanical Stimulus for Impulse Initiation in Regularly Discharging BRs

The actual mechanical stimulus for BR activation is not fully understood. Earlier studies by Brown and coworkers found that discharge was closely related to wall strain during both maturation and development of spontaneous hypertension. The strain expression takes into account changes in unstrained vessel diameter. In our experiments, changes in diameter were essentially equivalent to strain since smooth muscle contraction had little or no effect on diameter at zero pressure. However, two new observations indicate that the strain hypothesis needs revision. One finding was that when diameter rather than MAP was held constant,
discharge increased rather than decreased. This increase was not due to direct NE activation because the increments in frequency were directly related to the offsetting pressure and because, when MAP was held constant, the same NE concentrations decreased discharge. The second observation was that at given diameters, discharge was greater on the active compared with the passive D-F plots. This result also was not due to direct NE activation since similar differences between these plots also occurred with All. Furthermore, for both observations, the NE concentrations were below threshold for direct BR activation. Given, then, that these responses were mechanically induced, two explanations seem plausible.

One explanation is that BRs respond not only to changes in whole-wall dimension but also to changes in tension or stress, as proposed by Coleridge et al.\(^\text{44}\) For instance, tension increased with NE at constant diameter because of the increase in offsetting pressure (Laplacean relation), and in this case, discharge likewise increased. Conversely, tension decreased with NE at constant MAP because of the decrease in diameter, and in this case, discharge likewise decreased. Thus, smooth muscle contraction produced responses that were directionally opposite but were consistent with the change in tension. This explanation might also account for the difference between the active and passive D-F plots. Tension decreased with vasoconstriction at constant pressure because of the fall in diameter alone, whereas tension decreased more with deflation as a result of the fall in pressure and diameter. Against the tension explanation is the fact that the majority of BRs are outside the media and that when BRs are subjected to increasingly higher pressures, they typically approach maximum discharge rates, although tension continues to rise. However, whatever independent variable is used, a maximum value might be anticipated.

A second explanation is that BR responses depend on how the nerve endings are coupled with respect to local smooth muscle. It seems likely that they are both in series and in parallel, given their complex threedimensional structure.\(^\text{44}\) Conceivably, discharge will reflect which arrangement predominates. A preferred circumferential distribution has been described for sinus BRs.\(^\text{7}\) Since constriction unloaded the endings, regularly discharging BRs are probably coupled predominantly in parallel. Given that they are primarily sensitive to circumferential stretch, the parallel elements are probably oriented in the hoop direction, which suggests that at constant diameter, they are "clamped" and thus have no effect on discharge. Meanwhile, series elements oriented away from circumferential might distort and thereby activate the endings. A series contribution might also explain why discharge fell less with vasoconstriction than with deflation when the smooth muscle was passive. The major difficulty with this explanation is that although we have some idea of the endings' structure, their functional relation with respect to local and distal wall components is unknown.

**Direct Effects of NE on BR Discharge**

BR excitation produced by high NE concentrations (\(10^{-6}-10^{-5}\) M) at constant MAP was attributed to direct activation of the sensory endings for the following reasons: 1) Excitation and arch constriction were dissociated. Excitation did not occur at low concentrations that produced substantial constriction, yet occurred in a dose-dependent manner at high concentrations, which were supramaximal for constriction. Thus, BR activity rose sharply where the arch diameter dose-response curve was constant. 2) Threshold concentration for excitation was apparently near \(10^{-7}\) M, or about a thousandfold higher than for aortic constriction. Threshold was based on the fact that discharge in 4 of 17 units was greater at \(10^{-7}\) M than at \(10^{-8}\) M, although still below control. At lower concentrations, excitation was not simply offset by unloading since discharge did not increase when unloading was prevented (using NP). Furthermore, NE D-F plots were similar to All D-F plots and were approximately linear, a result not consistent with direct NE excitation. 3) In the presence of NP, a smooth muscle relaxant without \(\alpha\)-adrenergolytic or \(\beta\)-sympathomimetic effects, excitation occurred as in untreated arches. 4) All, which presumably has no direct effect on BRs, did not produce excitation but simply unloaded the endings at all concentrations. 5) Competitive adrenergic antagonism with prazosin inhibited not only unloading due to smooth muscle contraction but excitation as well.

The fact that adrenergic blockade but not smooth muscle relaxation prevented BR excitation suggests that the nerves were stimulated independently of the smooth muscle, perhaps via adrenergic receptors on the sensory endings. This possibility is consistent with NE effects on other types of vertebrate mechanoreceptors, including Pacinian corpuscles,\(^\text{25}\) muscle spindles,\(^\text{26}\) and cutaneous mechanoreceptors.\(^\text{27}\) In Pacinian corpuscles, excitation results from an increase in generator potential amplitude.\(^\text{28}\) Effects on other sensory structures, including BR afferents, may be similar.

BR activation by catecholamines has been reported since the early 1950s, and since it occurred in the open sinus, a direct effect was postulated.\(^\text{8}\) Interestingly, nearly all earlier studies used high concentrations, presumably to assure smooth muscle contraction. Thus, direct activation probably occurred regardless of smooth muscle effects. Because smooth muscle contraction could also activate the endings, the question of a direct effect was unsettled. Recent studies have addressed this problem. Goldman and Saum,\(^\text{29}\) using a preparation similar to ours, reported that NE directly excited rat aortic BRs since they also found that excitation was 1) dose-dependent at concentrations where smooth muscle contraction was supramaximal, 2) prevented by \(\alpha\)-adrenergic blockade but not by smooth muscle relaxation, and 3) not mimicked by All. However, their conclusion was based on not being able to detect any smooth muscle effects, probably because they focused only on high NE concentrations, where excitation predominates, and because when they per-
fused at zero pressure, the endings were already unloaded. In addition, they compared averaged pressure-response curves, which have poorer resolution than spike-by-spike plots. In another study, Kunze et al found that with the rat arch cut open and the media removed, BRs in the adventitia were activated by NE at \(10^{-7} - 10^{-5}\) M. All studies in the rat, therefore, support a direct effect at high concentrations.

Evidence regarding the carotid sinus is less clear. Yao and Thoren reported that in rabbits, NE had no general effect on myelinated fibers. However, when their units were considered individually, it was noted that discharge was reduced in over half, which suggests unloading. Since the concentration was high (10^(-6) g/ml), direct excitation might explain why discharge in the remainder was increased or unchanged. Tomomatsu and Nishi also concluded that NE has a direct action, but their results seem puzzling. At low concentrations (10^(-6) g/ml), BR responses to pressure steps were enhanced, yet wall properties were unaltered; at high concentrations (10^(-6) g/ml), BRs were unaffected, but wall stiffness increased. Furthermore, phentolamine inhibited smooth muscle contraction but not the BR activation resulting from sympathetic nerve stimulation. This last result could be attributed to the agonist properties of phentolamine on sympathetic endings.

Effects of NE Likely to Occur In Vivo

The dual effects of NE are significant in that they occur at physiologic concentrations and within the normal range of pressure. Since NE readily diffuses across the arterial wall, intramural NE in vivo will depend on levels in the blood and on release from local sympathetic efferents. Under control conditions, blood NE is \(4 \times 10^{-9}\) M in rats and \(2 \times 10^{-9}\) M in humans. Since \(\alpha\)-adrenergic threshold is generally between 10^(-9) and 10^(-8) M, most vessels at rest are scarcely influenced by circulating NE. However, during exhaustive exercise, NE may increase to 1.2 \(\times 10^{-8}\) M in rats and 7 \(\times 10^{-8}\) M in humans, and with hemorrhage in dogs, NE may reach 2.3 \(\times 10^{-7}\) M. Likewise, during continuous sympathetic stimulation of 1–10 Hz, estimated intrasynaptic and perisynaptic NE levels are 6 \(\times 10^{-4}\) to 5.5 \(\times 10^{-7}\) M. These concentrations are within the range where BRs were affected via smooth muscle contraction, and they approach or exceed levels necessary for direct activation.

The NE results suggest that local sympathetic nerves may have both excitatory and inhibitory actions on BRs. There is some evidence to support this possibility in the carotid sinus where reflex or electrical activation of the efferent nerves to the carotid sinus increased sinus afferent nerve activity. Depressed the baroreflex, and reduced mean arterial pressure. In single-fiber studies, some authors report an enhancement of BR activity, while others report a reduction (see Figure 1 of Bolter and Ledsome). Interestingly, two studies mentioned that some BRs were excited during sympathetic stimulation but afterward were inhibited. We recently made a similar observation using an in vitro preparation from rabbits.

Quite possibly, NE levels during stimulation are sufficiently high to excite some BRs directly but then fall rapidly so that after stimulation, unloading becomes evident because of persisting smooth muscle contraction. The fact that not all BRs respond alike may reflect their proximity to efferent nerves. Two studies suggested a direct synaptic relation. Koizumi and Sato found that sympathetic stimulation in the os- sum carotid sinus evoked BR action potentials after a latency of only 16–18 msec; and Brattstrom et al reported that the evoked reflex drop in MAP from sympathetic stimulation in rabbits was dependent on when the stimulus occurred during the cardiac cycle.

What role, if any, sympathetic modulation of BRs plays in blood pressure control is an interesting question. A general increase in sympathetic drive evoked by, say, a fall in MAP might contract local smooth muscle and further unload the endings. This increase would augment reflex adjustments operating to correct the pressure deficit, while also countering the effect of BRs rapidly resetting to lower pressures, which otherwise would attenuate the baroreflex. If BRs were activated directly, however, this effect would seem detrimental since it would tend to support rather than correct the hypotension.

Alternately, if there were an increase in sympathetic drive but with an elevation in MAP, such as during stress, BRs would not be unloaded if the arch were distended. Instead, smooth muscle contraction would increase wall tension, thereby stimulating the endings. This effect, as well as direct activation from high NE concentrations, would serve to maintain MAP within acceptable limits.

Obviously, sympathetic modulation of BRs is not straightforward and requires further investigation. The present study has examined only regularly discharging units exposed to exogenous NE at static pressures. Their behavior in vivo and the properties of irregularly discharging fibers may be different.

References


Key Words • baroreceptors • norepinephrine • aorta • angiotensin II • prazosin • sodium nitroprusside • vascular smooth muscle

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