A Direct Excitatory Action of Catecholamines on Rat Aortic Baroreceptors in Vitro

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SUMMARY. A persistent question with regard to the effects of catecholamines on baroreceptor activity has been whether the adrenergic action is indirectly mediated through an alteration in vessel wall tone or whether it is a direct action on the excitability of baroreceptor nerve endings. The effects of norepinephrine on the pressure-impulse frequency relationship of rat single aortic baroreceptor fibers were studied in vitro. Perfusion of the aortic arch with norepinephrine produced an excitatory response which consisted of: a drug-evoked stimulation of previously quiescent fibers at 0 mm Hg, and a sensitization of the response to pressure. The norepinephrine-induced facilitation was blocked by prazosin but not by yohimbine. Phenylephrine mimicked the effects of norepinephrine, whereas clonidine had no excitatory effects on baroreceptor activity. Baroreceptor activity was unaffected by β-adrenergic agonists or antagonists. Norepinephrine had no effect on the pressure-volume relationship of the aortic arch, but produced a contraction of helical aortic strips. However, there was no correlation between the concentration-response relationship of norepinephrine on aortic smooth muscle and its excitatory action on baroreceptors. 5-Hydroxytryptamine and angiotensin II produced a contraction of helical strips, but had no effect on baroreceptor activity. Sodium nitrate reduced the norepinephrine-induced contractile response by 75%, but did not antagonize the baroreceptor facilitation. It is proposed that the excitatory action of norepinephrine is independent of smooth muscle activity, and is mediated by activation of α₁-adrenergic receptors located on the aortic baroreceptor nerve endings. (Circ Res 55: 18-30, 1984)

PALME (1936) showed that local application of epinephrine to the walls of the carotid sinus of the dog and rabbit produced a fall in systemic blood pressure and a blockade of the bilateral carotid occlusion reflex. Since that time, these results have been confirmed and extended to show that the catecholamine-induced hypotension was reflex in nature and mediated by an increase in carotid sinus nerve activity (Kirchheim, 1976). Furthermore, electrical stimulation of the sympathetic innervation to the carotid sinus mimicked the effects of exogenously administered norepinephrine (Kezdi, 1954; Sampson and Mills, 1970; Bolter and Ledsome, 1976). These findings suggested a possible role for the sympathetic nervous system in modulating baroreceptor activity. It was generally believed that endogenous release of norepinephrine activated (α-adrenergic receptors in the smooth muscle of the carotid sinus wall. This was thought to result in a change in intramural tension or diameter which might then increase baroreceptor activity by mechanical stimulation (Landgren et al., 1952; Bergel et al., 1980).

On the other hand, Koizumi and Sato (1969) demonstrated, in the opossum, that sinus nerve afferent discharge was augmented at a very short latency (16-18 msec) following stimulation of the cervical sympathetic trunk. They proposed a direct synaptic connection between the sympathetic nerve fibers and the carotid baroreceptors. This hypothesis has been supported by a report which showed that sympathetic nerve stimulation (2-5 Hz) or low concentrations (10⁻⁹ M) of norepinephrine increased baroreceptor activity, but had no effect on carotid sinus smooth muscle (Tomomatsu and Nishi, 1981).

Thus, it seems clear that either exogenously administered or endogenously released catecholamines will enhance baroreceptor activity. What is equivocal is the site and mechanism of action of the catecholamines on arterial baroreceptors. That is, do catecholamines act indirectly, modulating baroreceptor activity through an action on vascular smooth muscle, or do they act directly on the afferent nerve terminal altering its excitability?

In the present investigation, we have addressed this question by using an in vitro rat aortic arch-aortic nerve preparation. The effects of norepinephrine on the pressure-impulse frequency relationship of single aortic baroreceptor fibers has been characterized. It is concluded that norepinephrine increased baroreceptor activity by a direct action on the baroreceptor ending which was independent of smooth muscle tone.

Methods

Isolation and Perfusion of the Aortic Arch in Vitro

Experiments were performed on 10- to 14-week-old (300-400 g) male Wistar-Kyoto rats. The animals were

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'anesthetized with sodium pentobarbital (30-40 mg/kg) administered intraperitoneally, after initial induction with ether. The procedure for isolation and perfusion of the aortic arch was similar to that described by Brown et al. (1976). The surgically isolated aortic arch-aortic depressor nerve preparation excised and pinned to the bottom of a water-jacketed Plexiglas chamber to approximate its in vivo configuration. The aortic arch was perfused with Krebs-Henseleit solution through the descending aorta by means of a Holter infusion pump at 7 ml/min. The effluent was drained via the brachiocephalic trunk. The control perfusate had the following composition: NaCl, 120 mM; KCl, 4.8 mM; MgSO4, 1.2 mM; CaCl2, 1.1 mM; H2PO4, 1.2 mM; NaHCO3, 25 mM and dextrose, 5.5 mM. The solution was equilibrated with 95% O2 and 5% CO2 to give a final pH of 7.35 to 7.45. The aortic arch and aortic nerve were covered with warm mineral oil, and the temperature of the perfusate and the Plexiglas chamber was maintained at 35°-37°C.

Application of Pressure Inputs

Slow ramp inputs, as well as pressure steps of variable amplitude and duration, were delivered to the aortic arch with a Ling 411 shaker (Saum et al., 1977). The shaker was connected to the inflow cannula of the aortic arch via electrically activated solenoid valves (General Valves) which isolated the preparation from the perfusion system. The aortic pressure was measured from a side arm of the outflow cannula with a strain gauge pressure transducer (Statham P23Db).

Measurements of the Mechanical Properties of the Aortic Arch in Vitro

Pressure-volume measurements of the aortic arch were performed in the same perfusion chamber and with the same vessel configuration as used for the experiments in which baroreceptor nerve activity was measured. Krebs-Henseleit solution (at 37°C) was injected into the aortic arch in 10-μl increments, with a 100-μl Hamilton micro-syringe. Twenty to 30 seconds after injection, the resulting steady state pressures were measured, and these measurements were repeated 2–3 times, always starting at 0 mm Hg. The volume of the solution in the aortic arch at 0 mm Hg was not determined; however, the total volume of the system was 1.5 ml.

Helical aortic strips, identical to those used to record baroreceptor activity, were obtained from the thoracic aortas and aortic arches of Wistar-Kyoto rats. The aortas were cut into helical strips (1.5–2.0 mm wide and 20–25 mm long) and placed in a muscle chamber under a resting tension of 1.5 g. Both thoracic aorta and aortic arch strips were equilibrated for 2 hours in Krebs-Henseleit solution in a manner described previously (Altura and Altura, 1970). The drug-evoked contractile responses of the aortic arch strips were 50% to 60% smaller than those elicited from thoracic aortic strips. This difference was presumably due to the unevenness and irregularity of the aortic arch strips, which was the result of cutting the tissue from the curvature of the aortic arch. However, all of the drugs tested evoked responses from both groups of helical strips that were qualitatively identical. Therefore, in most experiments, helical thoracic aortic strips were used. In addition, this allowed the responses of aortic baroreceptors and aortic smooth muscle to be compared in the same experiment. Drugs were added directly into the muscle bath to achieve the desired concentration. Standard isometric recordings were obtained with a Grass FT03C force-displacement transducer.

Experimental Design

To examine the effects of catecholamines on aortic baroreceptor activity, primarily two types of experiments were performed: First, the baroreceptor response to a pressure ramp of from 0 to 200 mm Hg was studied. Typically, we applied three or four pressure ramps (8 mm Hg/sec) to the aortic arch under control conditions to determine the baroreceptor discharge characteristics. The duration of each ramp was 50 seconds, and they were applied in a series, with one ramp immediately following the last. The perfusion system was closed during this period, and there was no perfusion between ramps. Using this procedure, little variability (<5%) was observed in the response between pressure ramps with regard to baroreceptor threshold, slope of the linear portion of the pressure-frequency curve, or maximum impulse frequency. Since the pressure ramps were sufficiently slow, the pressure-frequency curve was considered to reflect steady state responses much the same as those obtained in the steady state for pressures below those producing a plateau in impulse frequency (Saum et al., 1977; Andresen et al., 1979). This equivalency was verified by using pressure staircases. In 10 experiments, pressure was increased from 0 to 200 mm Hg in a series of 20 or 40 mm Hg increments. Each step or increment had a duration of 10–30 seconds, i.e., until the elicited impulse frequency had reached a steady state.

Once the baroreceptor response had been characterized, norepinephrine (NE) was perfused for 3 minutes and the pressure ramps were repeated. In those experiments in which the interaction of another compound with NE was tested, the aortic arch was perfused with the test compound for 3 minutes and the baroreceptor response to three to four pressure ramps was elicited. This was immediately followed by a 3-minute perfusion of the test compound plus NE, and the ramps were repeated. The results of the various drug treatments were compared by constructing pressure-impulse frequency curves. In the second type of experiment, we studied the ability of a drug to evoke a baroreceptor discharge at 0 mm Hg. Initially, the response to NE was elicited, and all other drug responses were subsequently compared to the NE response as follows. In some experiments, the aortic arch was perfused for 3 minutes with the test compound, and this was followed immediately by perfusion of the test compound plus NE. In other experiments, the response to NE was elicited first, and the test compound then was added to the NE perfusion.

Data Analysis

The methods for recording single unit baroreceptor fibers as the aortic nerve have been described previously (Brown et al., 1976). The impulse frequency/sec of single baroreceptor units was measured by a rate meter after selection with a window discriminator. Threshold was defined as the pressure at which the impulse frequency began to increase as a function of pressure. An index of sensitivity was calculated from the slope of the pressure-frequency curve during the linear portion of the curve, using a linear least-mean squares fit. Statistical analysis was performed using Student’s paired t-test (two-tail).
Drugs

The desired drug concentrations were achieved by dissolving 0.1–0.5 ml of concentrated stock solutions in 100 ml of prewarmed (37°C) and oxygenated Krebs-Henseleit solution immediately prior to perfusion through the aortic arch. All concentrations were expressed as the salt. The following drugs were used: dopamine hydrochloride, l-epinephrine hydrochloride, dl-isoproterenol hydrochloride, l-norepinephrine hydrochloride, clonidine hydrochloride, phenylephrine hydrochloride, phentolamine methanesulfonate, prazosin hydrochloride, dl-propranolol hydrochloride, yohimbine hydrochloride, angiotensin amide, 5-hydroxytryptamine creatinine sulfate, and sodium nitrate.

Results

The effects of a series of pressure ramps and steps (0–200 mm Hg) on the discharge characteristics of 104 single aortic baroreceptor fibers in vitro were examined. These stimuli elicited patterns of baroreceptor activity which could be divided into three distinct types: Slowly adapting fibers which increased their activity during norepinephrine infusion, and rapidly adapting and irregularly discharging units which were unresponsive to norepinephrine. The former group of fibers constitute the subject of this report. Although each type of response could be observed in the same preparation, a transformation from one type of discharge pattern to another in the same nerve fiber was never observed. Fibers were generally studied for 3–6 hours without any dramatic changes in their response to pressure.

Response of Slowly Adapting Fibers

Eighty-eight of the 104 fibers studied were considered to be slowly adapting. These fibers exhibited a regular discharge pattern in response to a pressure ramp over the range of 0 to 200 mm Hg. This is characteristic of slowly adapting baroreceptors with myelinated axons (Fig. 1; see also, Brown et al., 1976); however, conduction velocities were not measured, due to the short length of isolated nerve (5–15 mm). Fifty-one of the fibers studied were quiescent at 0 mm Hg, and responded to a pressure ramp when a threshold pressure level had been attained (Figs. 1A and 2A). The remaining 37 fibers were spontaneously active at 0 mm Hg (Fig. 2B). These fibers exhibited a spontaneous firing rate (3–25 impulses/sec) which remained fairly constant throughout the experiment. Spontaneous activity in an in vitro baroreceptor preparation has been described previously (Brown et al., 1976), and was attributed to distortion of the baroreceptor endings as a result of excision and realignment of the aortic arch in the perfusion chamber. In that regard, it was observed that, at the onset of the pressure ramp in 40% of the preparations exhibiting spontaneous activity, the impulse frequency would initially decrease and then increase. In these preparations, it was noticed that the aortic arch was distorted or collapsed at 0 mm Hg. Once the aortic pressure had reached 10–20 mm Hg, the arch would inflate and assume a more natural configuration. In addition, in some of the fibers, the spontaneous firing rate was cyclic in nature, and was associated with the cyclic nature of the perfusion pump. This was shown by the fact that, when the pump was turned off, the fibers either became quiescent or fired continuously at a reduced rate.

The mean threshold pressure for the slowly adapting fibers was 97 ± 4 mm Hg. As pressure was increased above threshold, the fibers responded with an increase in impulse frequency which was relatively linear between 90 and 120 mm Hg at the lower end, and between 150 and 160 mm Hg at the upper end of the linear range. At higher pressures, the firing rate reached a plateau. Maximum impulse frequencies ranged from 48 to 159 impulses/second and were usually attained at 160 to 180 mm Hg.

Response to Norepinephrine

Perfusion of norepinephrine (10⁻⁷ M to 10⁻⁵ M) through the aortic arch produced a concentration-dependent increase in baroreceptor activity in 89% of the fibers (Figs. 1 and 2). The pressure-frequency curve was shifted to the left of the control curve,
FIGURE 2. Effects of NE on pressure-frequency curves of a quiescent (panel A) and a spontaneously active (panel B) baroreceptor. Panel A: filled circles, control response to pressure ramp (0–200 mm Hg; 8 mm Hg/sec; open circles, response 3 minutes after perfusion with 3 × 10⁻⁶ M NE. In both curves, symbols represent mean response to three ramps; bars on the symbols indicate SEM for symbols lacking bars are smaller than symbol. In all later figures, bars omitted for clarity. Panel B: filled circles, control response to pressure ramp (0–200 mm Hg; 8 mm Hg/sec) of aortic baroreceptor having spontaneous discharge; open circles, response obtained 3 minutes after perfusion with 3 × 10⁻⁶ M NE.

but not in a parallel manner, i.e., there was a greater effect of NE at the low and high ends of the pressure curve. For example, in previously quiescent fibers, NE usually (see below) initiated a regular discharge (10–50 impulses/sec) at 0 mm Hg (Figs. 1B and 2A) or increased the impulse frequency of spontaneously active fibers by a similar amount (Fig. 2B). The degree of excitation was dependent on the drug concentration and was independent of the sponta-
neous firing rate. The mean threshold pressure observed in the presence of NE was not significantly different from control (95 ± 3 mm Hg). However, in two quiescent fibers, NE failed to initiate a discharge at 0 mm Hg, although the activity at suprathreshold pressures was enhanced by the drug. In both fibers, the threshold pressure was reduced (from 124 mm Hg to 116 mm Hg and from 142 mm Hg to 122 mm Hg). This suggests that the failure to detect a NE-induced change in threshold pressure may be due to the difficulty of measuring the precise threshold of fibers that are exhibiting a background discharge.

As shown in Figure 2, the magnitude of the NE-induced increase in baroreceptor impulse frequency diminished in the linear portion of the pressure-frequency curve. At pressures between 120 and 150 mm Hg, the discharge frequency increased by only 10–20%. Coinciding with the increase in frequency was a small but statistically significant increase in the slope of the linear portion of the pressure-frequency curve as measured in 30 baroreceptor fibers (1.04 ± 0.06 vs. 1.12 ± 0.06 impulses/sec per mm Hg, P < 0.05). At higher pressures (160–200 mm Hg), the impulse frequency began to approach a plateau, and, at these pressures, the NE-induced enhancement again became more striking. The impulse frequency was increased by 20–40%. There was no apparent difference in the effects of NE on quiescent or spontaneously active fibers.

The NE-induced discharge was produced within 5–10 seconds after the start of perfusion, and a maximal response was attained within 1 to 2 minutes (Fig. 3A). The enhanced activity was maintained for the duration of the norepinephrine perfusion (usually 3 minutes), and there was no apparent desensitization up to 30 minutes. Baroreceptor activity diminished gradually after return to the control solution, and complete recovery was attained within 15–30 minutes, depending upon the drug concentration.

The mean threshold concentration of norepinephrine which produced a change in impulse frequency was 10⁻⁷ M (five experiments; Fig. 4). Maximal excitation was, in general, produced by 5 × 10⁻⁶ M to 10⁻⁵ M. Higher concentrations of norepinephrine produced irreversible effects characterized by high frequency firing followed by an irregular discharge. Eventually, the amplitude of the action potential decreased, and baroreceptors became silent and unresponsive to pressure stimuli.

In several preparations in which more than one fiber was isolated, it was found that one of the fibers would respond to NE, whereas the other fiber was unresponsive even to large concentrations of the agent (10⁻³ M). Thus, 11% of the slowly adapting fibers isolated did not respond to NE. In these instances, there was no apparent relationship between the location of the receptive field of the baroreceptor endings, determined by probing the surface of the aortic arch with a blunt glass pipette.
FIGURE 3. Time course of NE-induced effects on a single aortic baroreceptor unit (panel A) and on aortic smooth muscle (panel B). Panel A: effect of $3 \times 10^{-4}$ M NE on baroreceptor impulse frequency at 0 mm Hg. Panel B: effect of $3 \times 10^{-6}$ M NE on development of contractile tension in helical aortic strip. NE applied at onset of arrow and lasted for duration of tracing.

(1 mm outside diameter), and the ability of the fiber to respond to NE.

Response to Epinephrine, Dopamine, and Isoproterenol

Qualitatively similar effects could be produced by epinephrine ($3 \times 10^{-7}$ M to $5 \times 10^{-6}$ M; 10 experiments) and dopamine ($10^{-6}$ M to $10^{-4}$ M; three experiments). The threshold concentration to initiate baroreceptor activity varied in different experiments; however, when tested on the same baroreceptor fiber, norepinephrine was approximately equipotent to epinephrine but 100–200 times more potent than dopamine. With regard to time course and maximal facilitation, the three catecholamines were identical. On the other hand, the $\beta$-adrenergic receptor agonist, isoproterenol ($10^{-7}$ M to $10^{-5}$ M), had no effect on baroreceptor activity (eight experiments). No depression of baroreceptor activity was ever observed with any of the catecholamines.

Effects of $\alpha$-Adrenergic Receptor Agonists and Antagonists on Aortic Baroreceptors

The above finding that isoproterenol did not affect baroreceptor activity suggested that the effects of the other catecholamines were mediated by activation of $\alpha$-adrenergic receptors. At least two types of $\alpha$-adrenergic receptors have been identified in the peripheral nervous system; therefore, we attempted to characterize the type of $\alpha$-receptor mediating the increase in baroreceptor activity (Berthelsen and Pettenger, 1977; Hoffman and Lefkowitz, 1980). The relatively selective $\alpha_1$-adrenergic receptor agonist, phenylephrine ($10^{-7}$ M to $5 \times 10^{-6}$ M; three experiments), mimicked the effects of NE and was approximately equipotent to the catecholamine (Fig. 5A).

On the other hand, clonidine ($10^{-7}$ M to $10^{-5}$ M), an $\alpha_2$-adrenergic receptor agonist, had no excitatory effects on baroreceptor activity (Fig. 5B; six experiments). Instead, higher concentrations of clonidine ($10^{-6}$ M to $10^{-5}$ M) produced a partial blockade of the NE-induced enhancement. The maximal amount of antagonism produced by clonidine ranged from 30 to 90%, and was more prominent at the lower portion of the pressure-frequency curve. Recovery occurred in 45–60 minutes. This effect was seen whether clonidine was administered for 3 minutes prior to the test concentration of NE, or whether it was perfused after NE had already produced an increase in baroreceptor activity.

The $\alpha$-adrenergic receptor antagonist, phenolamine ($10^{-7}$ M to $10^{-6}$ M; six experiments), completely blocked the increase in baroreceptor activity produced by NE, but had no effect on the control response. This effect was observed after a 6-minute perfusion with phenolamine, and the antagonism persisted for the remainder of the experiment. Prazosin ($10^{-7}$ M to $5 \times 10^{-7}$ M; six experiments), which is reported to be a selective $\alpha_1$-adrenergic receptor antagonist (Borowski et al., 1977; Doxey et al., 1977), reversibly blocked norepinephrine’s ability to increase baroreceptor firing (Fig. 6A). This antagonism could be produced by perfusing the aortic arch with prazosin for 3 minutes prior to NE administration, or by administering the antagonist once NE had initiated a baroreceptor discharge. Under the latter conditions, the time for onset of antagonism by prazosin and phenolamine was prolonged by 5–10
FIGURE 5. Effect of α1- and α2-adrenergic receptor agonists on pressure-frequency curve of aortic baroreceptors. Panel A: filled circles represent the control response to pressure ramps (0–200 mm Hg; 8 mm Hg/sec; open circles, effect of α1-agonist, phenylephrine (3 × 10⁻⁶ M), on the pressure-frequency curve of a slowly adapting aortic baroreceptor fiber. Panel B: filled circles, control response to pressure ramp (0–200 mm Hg; 8 mm Hg/sec); open circles, response obtained after 3 minutes of perfusion with 3 × 10⁻⁶ M clonidine.

FIGURE 6. Effect of α1- and α2-adrenergic receptor antagonists on pressure-frequency curves of aortic baroreceptor fibers in the presence of NE. Panel A: effect of α1-antagonist, prazosin, on NE-induced enhancement of a baroreceptor's response to a pressure ramp (0–200 mm Hg; 8 mm Hg/sec). Filled circles, control response to pressure ramp; open circles, response obtained 3 minutes after perfusion with 3 × 10⁻⁶ M NE; open squares, response obtained 3 minutes after perfusion with 3 × 10⁻⁶ M NE in the presence of prazosin (10⁻⁷ M). Panel B: effect of α2-adrenergic receptor antagonist, yohimbine, on NE-induced enhancement of a different, spontaneously active baroreceptor fiber. Filled circles, control response to pressure ramp (0–200 mm Hg; 8 mm Hg/sec); open circles, response obtained after 3 minutes perfusion with 3 × 10⁻⁶ M NE; open squares, response obtained after 3 minutes of perfusion of NE in the presence of yohimbine (10⁻⁷ M).

minutes, or, in some experiments, larger concentrations of the antagonist (10⁻⁸ M) were required to produce blockade. Complete recovery occurred in approximately 1 hour.

On the other hand, the effects of the α2-adrenergic receptor antagonist, yohimbine (Starke et al., 1975; Doxey et al., 1977), were more complex. At low concentrations (5 × 10⁻⁸ M to 10⁻⁷ M; six experiments), yohimbine had no effect on the augmented baroreceptor activity produced by NE (Fig. 6B), whereas perfusion with higher concentrations of the drug (5 × 10⁻⁷ M; three experiments) partially antagonized the effects of NE. The selectivity of the α-adrenergic antagonists was compared in two experiments in which equal concentrations (10⁻⁷ M) of prazosin and yohimbine were tested against NE in the same baroreceptor fiber. The increased baroreceptor activity was antagonized only by prazosin;
yohimbine had no effect. Interestingly, in two experiments, low concentrations ($5 \times 10^{-8} \text{ M}$) of yohimbine actually mimicked the effects of NE. That is, yohimbine elicited baroreceptor activity at subthreshold pressures and shifted the pressure-frequency curve to the left in a manner identical to that seen with NE. In one experiment, prazosin ($10^{-7} \text{ M}$) completely blocked the increased baroreceptor activity. At higher concentrations of yohimbine ($10^{-7} \text{ M}$ to $5 \times 10^{-7} \text{ M}$), this enhancement was diminished or completely absent. Although this response to yohimbine occurred in only two of six experiments, it was replicated several times in each preparation.

**Effects of NE on Smooth Muscle in the Aortic Arch**

Since it was possible that catecholamines might be modifying baroreceptor function by an action on the aortic smooth muscle, the ability of NE to alter smooth muscle tone, and thus influence baroreceptor activity, was examined. One possible manifestation of an alteration in vascular tone is a change in the compliance of the vessel wall (Dobrin, 1978). To determine whether a change in compliance of the aortic arch mediated all or part of the NE-induced augmentation of baroreceptor activity, pressure-volume curves were generated in the absence and presence of NE. The pressure-volume curves of the isolated aortic arch were unaffected by perfusion for 6 minutes with NE concentrations of $10^{-7} \text{ M}$ to $10^{-5} \text{ M}$ (Fig. 7) which enhanced baroreceptor activity. Similar findings have been reported previously (Andresen et al., 1979), and, based on these observations, it was suggested that alterations in smooth muscle have very little effect on aortic arch distensibility in the rat.

However, to determine whether NE was acting directly on the baroreceptor nerve endings or indirectly via the vascular smooth muscle, it was important to have some measure of smooth muscle activity. Therefore, the effects of NE on helical aortic arch strips were examined. The smooth muscle response to NE consisted of an initial rapid contraction which attained a peak in 12–25 seconds, followed by a much slower increase in contractile tension over the next 10 minutes (which is the longest time the drug was tested) (Fig. 3B). As shown in Figure 8, the increase in contractile force produced by NE was dose-dependent. The threshold concentration for the response was $10^{-9} \text{ M}$, whereas a maximal response was attained with $10^{-6} \text{ M}$. The mean half-maximal response was obtained at approximately $10^{-8} \text{ M}$, which was well below the threshold concentration necessary to elicit a baroreceptor discharge (compare Figs. 4 and 8).

**Effects of Propranolol on Aortic Baroreceptors**

In the carotid sinus of cats and dogs (Tuttle and Mc Cleary, 1978; Schultz and Zehr, 1981), propranolol enhances carotid sinus nerve activity, presumably by acting on the smooth muscle of this structure. However, as shown in Figure 9, propranolol ($10^{-7} \text{ M}$), which enhanced the contractile response of the aortic arch to NE, had no effect on aortic baroreceptor activity (eight experiments). In addition, propranolol did not affect the increase in baroreceptor activity elicited by NE (five experiments). At higher concentrations ($10^{-6} \text{ M}$ to $10^{-5} \text{ M}$), a 3-minute perfusion with propranolol depressed both the response to control pressure ramps, and the NE-induced enhancement of baroreceptor activity. The reduced impulse frequency was accompanied by a reduction in action potential amplitude, suggesting that depression of baroreceptor activity was due to the local anesthetic properties of propranolol (Morales-Aguilera and Vaughan Williams, 1965; Tarr et al., 1973).

**Effect of Vasoactive Agents on Aortic Baroreceptors**

If the effects of NE on baroreceptor activity were mediated by an action on aortic smooth muscle, it might be possible to mimic these effects with other vasoactive agents (Landgren et al., 1952). Therefore,
the effects of these drugs on the helical aortic strip preparation and their ability to influence baroreceptor activity were compared.

5-Hydroxytryptamine (5-HT, $10^{-8}$ M to $10^{-4}$ M) produced a contraction equal to that elicited by norepinephrine, although the concentration-response curve was shifted to the right (Fig. 8). However, 5-HT had no effect on the baroreceptor response to a pressure ramp stimulus (13 of 13 experiments; Fig. 10A). In addition, angiotensin II ($10^{-9}$ M to $10^{-6}$ M) which elicited a maximal contractile response which was 30% that of the NE contraction, also had no effect on baroreceptor activity in seven single fibers (Fig. 10B).

Since we could not increase baroreceptor activity with agents producing a contractile response in the
helical aortic strip, we attempted to influence the NE-induced response by agents which were known to relax vascular smooth muscle. Administration of the vasodilator, sodium nitrite (10⁻³ M), reduced by 60-90% the contractile response elicited by concentrations of NE which facilitated baroreceptor activity (Fig. 8). On the other hand, it did not antagonize the ability of NE to increase baroreceptor activity (Figure 11, four experiments). The inability of sodium nitrite to affect the facilitation was observed whether the vasodilator was perfused through the aortic arch for 3-5 minutes prior to the NE, and then in combination with it, or if it was administered during the NE-induced increase in impulse frequency. Sodium nitrite also had no effect on the baroreceptor response under control conditions (Fig. 11). Similarly, isoproterenol (10⁻⁷ M to 10⁻⁵ M) reduced the contractile response to NE by 40-60%, but had no effect on baroreceptor facilitation (six experiments).

Response of Rapidly Adapting and Irregularly Discharging Fibers

Eight of the baroreceptors which were split from the aortic nerve could be characterized as rapidly adapting. In response to a pressure step (80-120 mm Hg), these fibers demonstrated a transient response during the dynamic phase of the stimulus, but ceased firing, or discharged only infrequently, during the static phase, even at very high pressures (180-200 mm Hg). This group did not respond to a slow ramp stimulus. A third type of baroreceptor response which was observed approximately as often as the rapidly adapting discharge was characterized by a high threshold for onset of activity (140-180 mm Hg) and an irregular firing pattern. Irregularly discharging fibers have been previously described in the aortic arch and are characteristic of unmyelinated C-fibers (Thoren et al., 1977); however, in our experiments, the conduction velocity of these units was not determined.

Response to Norepinephrine

In contrast to its effects on slowly adapting fibers, NE (3 × 10⁻⁸ M to 10⁻⁵ M) did not enhance the activity of rapidly adapting fibers (eight of eight experiments); neither did it alter the fiber rate of adaptation to a pressure step. In addition, the activity of those fibers which exhibited a high threshold for initiating a response and an irregular discharge pattern were unaffected by NE (eight of eight experiments).

Discussion

Effects of Norepinephrine on Slowly Adapting Baroreceptor Discharge

A persistent question with regard to the effects of NE or sympathetic nerve stimulation on baroreceptor activity has been whether the adrenergic action is indirectly mediated through an alteration in vessel wall tone, or whether it is a direct effect of catecholamines on baroreceptor nerve endings. One of the major conclusions of this study was that NE produced both stimulation and augmentation of slowly adapting aortic baroreceptor activity which was mediated by activation of α₁-adrenergic receptors. These effects were independent of smooth muscle activity, and it is proposed that these α₁-adrenergic receptors are located directly on the aortic baroreceptor nerve endings.

This hypothesis is supported by the following observations: First, NE always elicited an increase in baroreceptor activity, regardless of intra-aortic pressure. This excitatory response had two components. The first was a direct drug-evoked stimulation of baroreceptor activity which was independent of pressure. NE-induced activity in the absence of an adequate mechanical stimulus has been described in rapidly adapting frog cutaneous mechanoreceptors (Loewenstein, 1956); however, NE-induced activity has not been reported previously in arterial baroreceptors. The ability of NE to evoke a baroreceptor discharge in the absence of pressure might explain why applications of either NE or epinephrine to the carotid sinus (Palme, 1943; Heymans and Heuvel-Heymans, 1950, 1951; Heymans et al., 1953; Landgren et al., 1952), as well as electrical stimulation of the cervical sympathetic nerve (Kezdi, 1954; Wurster and Trobiani, 1973), blocked the rise in systemic pressure elicited by bilateral carotid occlusion. Thus, in the presence of NE, baroreceptor activity would be maintained, despite the low intra-
sinus pressure, and there would be reflex inhibition of sympathetic tone preventing the rise in blood pressure.

The second component of the catecholamine-induced enhancement of baroreceptor activity was a sensitization of the response to pressure. This increase in sensitivity was reflected by a significant increase in the slope of the linear portion of the pressure-frequency curve. On the other hand, no significant change in threshold pressure was observed. In the only other investigation in which the effects of NE on baroreceptor threshold has been studied (Tomomatsu and Nishi, 1981), the results were similar to those reported here. The threshold pressure was reduced in five of the nine carotid baroreceptor fibers studied, but, as a group, there was no significant change in threshold. In the present study, the NE-induced discharge might have masked the magnitude of any change in threshold elicited by the drug. A decrease in threshold pressure for baroreceptor activity might be expected to have a significant reflex effect on blood pressure or heart rate, since there would be an increase in the activity of those fibers already activated by pressure, as well as a recruitment of baroreceptors with higher thresholds.

At suprathereshold pressures, NE produced a non-parallel shift in the pressure-frequency curve. Although there have been numerous studies on the effects of drugs on cardiovascular baroreceptors (for review, see Paintal, 1977), there are only a few studies in which the effects of drugs on the pressure-impulse frequency curves have been examined in any detail. For example, ether, chloroform, trichlorethylene (Robertson et al., 1956), and halothane (Biscoe and Millar, 1964) produced a nonparallel shift to the left in the pressure-frequency relationship of aortic and carotid baroreceptors of the cat. There was also a small reduction in the threshold pressure. It was concluded that these excitatory effects were due to a direct sensitization of the baroreceptors and were independent of any change in the properties of the arterial wall.

Our findings are in contrast to other reports on the effects of NE on the response of baroreceptors to pressure, in that increases in activity were always observed, regardless of intra-aortic pressure. For example, Aars (1971a, 1971b) noted that, in the presence of NE, baroreceptor activity in the aortic arch of the rabbit was unchanged or slightly reduced at any given pressure. On the other hand, since NE decreased the aortic diameter, when baroreceptor activity was compared at equal diameters, the discharge rate was actually increased. However, Aars (1971a) could not determine whether the observed enhancement was due to an increased intramural tension, or a direct effect on the baroreceptors. Furthermore, it was observed that NE depressed carotid sinus baroreceptor activity at low intrasinus pressures, but increased activity at higher pressure (Landgren, 1952; Landgren et al., 1952; Bergel et al., 1980). Thus, Landgren (1952) reported that at pressures below 100 mm Hg, catecholamines reduced the activity of baroreceptors possessing large amplitude action potentials, whereas at pressures greater than 100 mm Hg, the activity of these receptors was increased. Similarly, Bergel et al. (1980) reported that, typically, NE decreased carotid baroreceptor activity in the dog at pressures below 117 mm Hg and increased activity above that pressure. Landgren et al. (1952) interpreted their findings to suggest that the large myelinated, slowly adapting baroreceptor fibers were functionally "in parallel" with the smooth muscle, whereas Bergel et al. (1978, 1980) concluded that the receptor element was "in series" with the smooth muscle and that baroreceptor activity was correlated with changes in sinus wall tension.

We have concluded that there is no functional relationship between the baroreceptors and smooth muscle of the aortic arch of the rat. However, it is impossible, based on this study, to draw any conclusion with regard to their physical relationship. The ultrastructure of the baroreceptor endings in the aortic arch has not been thoroughly studied, although two electron microscopic studies (Krauhs, 1979; Yamauchi, 1979) have revealed no direct relationship between baroreceptor terminals and smooth muscle cells. Our results are consistent with this circumstance.

The Effects of Vascular Smooth Muscle Activity on Baroreceptor Discharge

The second observation supporting our hypothesis was the lack of any correlation between the effects of NE on aortic smooth muscle and its ability to produce an excitatory effect on aortic baroreceptors. Although neither the aortic diameter nor aortic wall tension were measured in the present study, we were unable to observe any changes in the pressure-volume relationship of the aortic arch in the presence of NE. This was consistent with a report by Andresen et al. (1979) in which they were unable to show any significant effects of NE on the static stress-strain curves of aortic arches from 4- to 6-month-old Wistar-Kyoto rats. They suggested that changes in the diameter or volume of the aortic arch might be expected to be small, since the aorta possesses relatively small amounts of smooth muscle which is arranged as "spannmuskeln" (Dobrin, 1978). On the other hand, NE produced a dose-dependent increase in contractile tension in helical aortic strips. The contractile response was biphasic in nature, consisting of an initial rapid phase which reached a maximum in 30 seconds, followed by a slow phase in which tension progressively increased throughout the duration of the 10-minute observation period. As shown in Figure 3, after a 1-minute exposure to NE, the contractile tension was 33% of the maximal response, whereas the NE-evoked baroreceptor re-
response developed gradually over 30–60 seconds, by which time it was maximal. Thus, even though contractile tension continued to increase, the baroreceptor response was maximal.

There was a similar lack of parallelism between the concentration-response curves for NE-induced baroreceptor activity and the norepinephrine-induced contractile response in aortic strips (compare Figs. 4 and 8). The threshold concentration (10^{-7} \text{M}) necessary to initiate a baroreceptor discharge was 100 times greater than the concentration (10^{-9} \text{M}) which produced a contractile response in the aortic arch. Furthermore, the concentration of NE (10^{-8} \text{M}) necessary to produce a half-maximal contractile response was an order of magnitude less than the threshold concentration, and 250 times lower than the concentration (2 \times 10^{-6} \text{M}) which produced a half-maximal increase in baroreceptor impulse frequency. Thus, the entire concentration-response relationship between NE and baroreceptor activity lies at the extreme high end of the smooth muscle dose-response curve. These results are consistent with the findings of Kunze (1980) who demonstrated in an isolated aortic arch adventitia preparation, which was devoid of the smooth muscle layer, that baroreceptor activity was significantly increased when the arch was superfused with NE at concentrations of 10^{-6} \text{M} and 10^{-5} \text{M}, but was unaffected by a concentration of 10^{-7} \text{M}. Similarly, Bergel et al. (1980) observed that carotid baroreceptor excitability was altered in the presence of 1 \times 10^{-6} \text{M} to 5 \times 10^{-6} \text{M} NE. On the other hand, Tomomatsu and Nishi (1981) reported that perfusion of the carotid sinus with similar concentrations of NE (5 \times 10^{-6} \text{M}) had no effect on baroreceptor activity, yet much smaller concentrations (5 \times 10^{-8} \text{M}) significantly increased afferent activity. Furthermore, the increase in baroreceptor activity was not antagonized by the \alpha-adrenergic receptor antagonist, phenotolamine (3.6 \times 10^{-6} \text{M}). This is in contrast to several previous studies, as well as the present experiments in which \alpha-receptor-blocking agents inhibited the effects of catecholamines on baroreceptors (Heymans and Heuvel-Heymans, 1951; Heymans et al., 1951; Landgren et al., 1952; Delaunois and Martini, 1953; Martini and Rovati, 1954). It remains unclear why such a large discrepancy exists between the results of Tomomatsu and Nishi (1981) and those of other investigators.

According to the hypothesis proposed by Landgren (1952) and reiterated most recently by Bergel et al. (1980), catecholamines enhance baroreceptor activity as a result of an increase in intramural tension which is secondary to smooth muscle contraction. Agents which would be expected to produce a contraction of vascular smooth muscle, such as pitressin (Heymans and Heuvel-Heymans, 1950, 1951), 5-hydroxytryptamine (Heymans and Heuvel-Heymans, 1953), and angiotensin II (McCubbin et al., 1957; Edmonson and Joels, 1969), mimicked the effects of NE in the carotid sinus. Conversely, sodium nitrite was shown to reduce smooth muscle tone in the carotid sinus, and thereby elicited a reflex rise in systemic pressure (Heymans and Heuvel-Heymans, 1950, 1951; Landgren et al., 1952) and decreased carotid sinus nerve activity (Landgren, 1952; Landgren et al., 1952).

In contrast, in the present investigation, the increase in baroreceptor activity induced by the catecholamines appeared to be a highly specific response. That is, no other vasoactive agent had any effect on baroreceptor impulse frequency. For example, 5-HT produced a contractile response in helical aortic strips which was equal to that of NE, yet it had no effect on baroreceptor activity. The results with angiotensin II were similar; although the smooth muscle contractile response elicited by angiotensin II was smaller than that produced by either NE or 5-HT. Furthermore, if the effects of NE were, in fact, due to smooth muscle activation, then it should have been possible to reduce or block the effect with a vasodilator. However, the NE-induced excitation of aortic baroreceptors was unaffected by a concentration of sodium nitrite which reduced by as much as 75%, the contractile responses elicited by NE.

Interestingly, in large elastic arteries such as the aorta and pulmonary artery, classical synapses between the adrenergic nerve terminal and the smooth muscle do not appear to exist; instead, the distance between the two structures is as much as 100 nm, and the action of the transmitter is presumably rather diffuse (Bevan and Su, 1973). It was calculated that the mean extracellular concentration of norepinephrine released by repetitive stimulation of the postganglionic sympathetic nerve fibers (10 cycles/sec) to the pulmonary artery of the rabbit was approximately 5.5 \times 10^{-7} \text{M}. Furthermore, it has been demonstrated, in rabbit aorta, that the concentration of norepinephrine in the extracellular space at the medial-adventitial border was only 25–35% of that found in the intima following a 2-minute exposure of the intimal surface to 7.75 \times 10^{-7} \text{M} norepinephrine (Bevan and Torok, 1970). Thus, the effective perfused catecholamine concentrations in the present study were often several times greater than those produced through endogenous transmitter release. However, since the aortic baroreceptor areas are located at the medial-adventitial border (Krauhs, 1979; Yamauchi, 1979), the catecholamine concentration at the baroreceptor endings might be substantially less than that in the perfusion medium, and of the same order of magnitude as that released from sympathetic nerve terminals.

The idea that physiological concentrations of norepinephrine will increase the excitability of aortic baroreceptors is consistent with the in vivo findings of Aars (1971a). He observed that, regardless of smooth muscle tone, aortic baroreceptor discharge is reduced when the aortic diameter was reduced.
However, it was found that during norepinephrine-evoked vasoconstriction, baroreceptive activity and sensitivity were maintained and, in some instances, even exceeded normal levels. Thus, feedback control of sympathetic activity was maintained. Similarly, norepinephrine-evoked vasoconstriction, if it occurred in the present study, should have reduced baroreceptor activity, but it did not. Indeed, activity was increased.

**Effects of α1-Adrenergic Receptor Activation of Baroreceptor Discharge**

The enhancement of baroreceptor activity induced by NE was mediated by activation of α1-adrenergic receptors, since the drug’s effect would be mimicked or blocked by a selective α1-adrenergic receptor agonist and antagonist, respectively. These results are consistent with previous observations made at the carotid sinus in which intraconjunctival injections of the α1-adrenergic receptor agonist, phenylephrine (200 µg), elicited a fall in systemic pressure identical to that produced by NE (Heymans and Mazzella, 1952). Furthermore, phenotamine (10–20 µg) blocked the reflex fall in systemic blood pressure induced by intraconjunctival injection of epinephrine (Martini and Rovati, 1954), as well as cervical sympathetic nerve stimulation (Korner, 1954). However, the lack of an excitatory effect with α2-adrenergic receptor agonist is in contrast to the results of several in vivo studies in which it was shown that clonidine stimulated aortic baroreceptors in the rabbit (Aars, 1972; Korner et al., 1974; Sleight et al., 1975). The enhanced activity, which was similar to that produced by the α-antagonist, phenoxybenzamine (Aars, 1971a), was attributed to an increase in the diameter of the aortic arch (Aars, 1972). The dilation appeared to result from a clonidine-induced relaxation of the aortic smooth muscle; clonidine has been shown to have α-adrenergic-blocking effects in vascular smooth muscle (Constantine and McShane, 1968; Rees et al., 1979).

That yohimbine, an α2-adrenergic receptor antagonist, did not block the effects of NE is consistent with the response being mediated by α1-adrenergic receptors. However, in two experiments, low concentrations of yohimbine (5 × 10⁻⁸ M) consistently mimicked the effects of norepinephrine, and interestingly, the effects diminished as the concentration of perfused yohimbine was increased (from 10⁻⁸ M to 5 × 10⁻⁷ M). It is difficult to imagine that, in these instances, yohimbine was acting directly on an α1-receptor, since the results presented here indicate that it would be approximately 10–100 times more potent than norepinephrine in stimulating that receptor. This also fails to provide any explanation as to why the effect was intermittent, and why the effect lessened as the concentration of yohimbine was increased. Alternatively, yohimbine might be acting on presynaptic α2-receptors to cause release of norepinephrine from sympathetic nerve terminals located in the aortic arch. Yohimbine in concentrations of 3 × 10⁻⁸ M to 10⁻⁶ M has been demonstrated to enhance release from sympathetic nerve endings and subsequently increase stimulation-induced smooth muscle contractions, whereas higher concentrations of the drug reduced contractions, suggesting postsynaptic α1-receptor blockade (Borowski et al., 1977). The norepinephrine-like effect of yohimbine in the present investigation seems to be consistent with this report. However, in the present study, since the sympathetic innervation was not being activated, yohimbine would have to enhance the basal release of norepinephrine. In that regard, Rand et al. (1975) demonstrated that presynaptic α2-receptor blockade will also substantially increase resting release of norepinephrine.

**Effect of NE on Rapidly Adapting and High Threshold Fibers**

In contrast to its effects on slowly adapting baroreceptor fibers, norepinephrine did not enhance the activity of either the rapidly adapting fibers, or of those fibers which exhibited a high threshold for initiating a response and an irregular discharge pattern. Anatomically, these fibers innervate the same region of the adventitia as the slowly adapting fibers. The small unmyelinated fibers were observed to wind irregularly around the premyelinated axons of the larger fibers (Krauhs, 1979). It is conceivable that these fibers lack the α1-receptors which mediate norepinephrine’s action. On the other hand, Thoren et al. (1977) found that 5 × 10⁻⁶ M norepinephrine significantly decreased (from 156 mm Hg to 142 mm Hg) the threshold for activation of seven irregularly firing baroreceptors. More study is needed to resolve this discrepancy.

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