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DISCHARGE CHARACTERISTICS AND RAPID RESETTING OF AUTOACTIVE AORTIC BARORECEPTORS IN RATS

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SUMMARY

1. An *in vitro* aortic arch–aortic nerve preparation was used to characterize the pressure–discharge properties of aortic 'autoactive' baroreceptors (aBRs), a functionally unique group of baroreceptors that discharge continuously below pressure threshold (P_{th}). These units contrast to more familiar 'quiescent' BRs (qBRs) that are silent below P_{th}. This study also examined whether aBRs rapidly reset to sustained changes in mean arterial pressure (MAP), and whether they respond to local vasoconstriction, as found in qBRs.

2. Pressure–discharge curves were constructed using slow pressure ramps (2 mmHg s^{-1}) in a total of fifty-four aBRs and sixty-four qBRs from fifty-three Wistar-Kyoto rats. Rapid resetting was tested by comparing the curves before and 15 min after adjusting MAP to selected levels between 40 and 180 mmHg. Response curves were also compared before and after constricting the arch with 10^{-8} M angiotensin II.

3. Compared to qBRs, aBRs had significantly lower P_{th} values (83 ± 2 *versus* 91 ± 2 mmHg, mean $\pm$ S.E.M, $P < 0{\cdot}05$) but similar threshold frequencies (13 ± 1 *versus* 16 ± 2 Hz), higher saturation pressures (138 ± 4 *versus* 123 ± 2 mmHg) but similar saturation frequencies (55 ± 3 *versus* 55 ± 3 Hz), and lower suprathreshold sensitivities (slope of linear region: $1{\cdot}1 \pm 0{\cdot}1$ *versus* $1{\cdot}4 \pm 0{\cdot}1$ Hz $mmHg^{-1}$) but wider operating ranges (57 ± 4 *versus* 35 ± 3 mmHg).

4. The aBRs rapidly reset to changes in MAP ($n = 16$), and the extent of resetting ($\Delta P_{th}/\Delta MAP = 0{\cdot}26$) was similar to that in qBRs (0·23).

5. Vasoconstriction had no effect on aBR subthreshold discharge ($n = 5$), but inhibited suprathreshold responses to pressure much like that in qBRs.

6. These results suggest that aortic aBRs may extend the range of the baroreflex, but probably do not improve its sensitivity to transient fluctuations in pressure or its ability to correct changes in mean pressure over extended periods.

7. Subthreshold discharge in aBRs appears to be an intrinsic property of these units, which was not affected by resetting or changes in vascular tone. At suprathreshold pressures, contraction of local smooth muscle modulates the aBRs and qBRs in a similar fashion by mechanically unloading the sensory endings.

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INTRODUCTION

The relationship between pressure and discharge in arterial baroreceptors (BRs) is fundamentally important with regard to neural control of the circulation because BR afferents provide the primary signals that inform the brain of rapid changes in blood pressure. In individual BRs, steady-state pressure–discharge curves have been commonly characterized using isolated preparations in which the barosensory regions can be stimulated with controlled pressure inputs. In such preparations, a gradual rise in pressure extending over a wide range produces BR response curves that are typically non-linear. The fibres remain quiescent until pressure reaches a threshold level (P_{th}), at which point discharge is initiated. The impulse activity then increases with pressure up to a maximal (saturation) frequency (Kircheim, 1976; Brown, 1980). However, not all BRs respond in this manner. There are reports of anomalous fibres that are continuously active even at very low pressures. These fibres discharge at a constant frequency until pressure reaches what can be considered their threshold level, at which point their frequency begins to follow pressure. Such fibres have been referred to as spontaneously or paradoxically active BRs (Brown, Saum & Tuley, 1976; Coleridge, Coleridge & Schultz, 1987), or arbitrarily as type II or type III fibres (Angell-James, 1971; Seagard, van Brederode, Dean, Hopp, Gallenberg & Kampine, 1990). Here they will be referred to simply as 'autoactive' BRs (aBRs), to distinguish them functionally from the more familiar and common 'quiescent' BRs (qBRs) that are silent below P_{th}.

Although previous investigators have casually mentioned BRs that were active at subthreshold pressures, only one study has examined these units systematically and only in the carotid sinus (Seagard *et al.* 1990). This earlier study found that, collectively, the carotid aBRs were less sensitive to changes in pressure than the qBRs, but that the aBRs operated over wider ranges of pressure. These differences prompted the suggestion that the aBRs may represent a unique type of BR that signals primarily the absolute mean pressure, whereas the qBRs signal mainly dynamic fluctuations. If this indeed is the case, one would expect that the pressure–discharge relationship of the aBRs would remain constant regardless of the prevailing mean arterial pressure, otherwise their afferent signal would be in error. Pressure–discharge curves of qBRs, however, have been shown to rapidly shift (reset) along the pressure axis shortly after a change in mean pressure (Coleridge, Coleridge, Kaufman & Dangel, 1981; Munch, Andresen & Brown, 1983). This shift attenuates the ability of the baroreflex to restore the original pressure (Kunze, 1985). The existence of BRs that do not reset, therefore, would be an important finding with regard to factors that determine the operating set-point of the reflex. In a recent study, Seagard and colleagues found that aBRs in the dog carotid sinus indeed did not rapidly reset (Seagard, Gallenberg, Dean & Hopp, 1991), which would represent a functional departure from the rapid resetting found in the carotid qBRs (Heesch, Thames & Abboud, 1984; Seagard *et al.* 1991). Such findings raise the question of whether a similar population of aBRs exists in the aortic arch and if they too are incapable of rapid resetting.

To answer this question, the present study had three objectives. The first was to identify and characterize aBRs in the aortic arch and to compare and contrast their

pressure–discharge curves with aortic qBRs. The second was to determine if the aortic aBRs rapidly reset to changes in mean arterial pressure, and if so, did they reset to the same extent as the aortic qBRs. And finally, because changes in vasoactive tone have been shown to affect the pressure–discharge curves of aortic qBRs (Munch & Brown, 1985; Munch, Thoren & Brown, 1987), the third objective was to examine the effect of drug-induced vasconstriction on the aBR response curves.

METHODS

Isolation of aortic arch and BR single fibres

Stimulus–response characteristics of single-fibre BRs were studied using an *in vitro* aortic arch–aortic nerve preparation from adult male Wistar–Kyoto rats. The *in vitro* preparation has been described previously in detail (Munch *et al.* 1983, 1987; Munch & Brown, 1985). Briefly, the animals were anaesthetized with sodium pentobarbitone (30–50 mg kg^{-1} I.P.) and placed on positive-pressure ventilation. The chest was opened through a mid-line sternotomy and the arch and nerve were separated from surrounding tissue. The nerve was cut as high in the neck as possible, and then the ascending aorta was ligated and stainless steel cannulas were placed retrogradely in the descending aorta and innominate arteries; all remaining arterial branches were ligated. The arch and nerve then were removed to a temperature-controlled plexiglass dish, where the arch was positioned in its approximate *in situ* configuration and covered with warm mineral oil (37 °C). Using a roller pump, the arch was perfused continuously with Krebs–Henseleit solution, which was prewarmed to 37 °C and equilibrated with a gas mixture of 95% O_2–5% CO_2. The perfusate was pumped through the lumen and then discarded. To apply drugs, concentrated solutions were added directly to the perfusate, which was contained in a large calibrated reservoir. Two reservoirs were used so that solutions could be alternated without affecting temperature, flow, or pressure. The perfusion pressure was adjusted and controlled with a modified Starling outflow resistor, which consisted of an inflatable balloon placed inside the outflow tubing. The pressure was monitored with a strain-gauge transducer (Gould Stratham Instruments, Hato Rey, Puerto Rico) connected to the perfusion circuit and was normally set at 80 mmHg. A shaker/bellows system driven by a function generator also was connected to the perfusion circuit to produce ramp increases in pressure that were used to test the BR response characteristics (Brown *et al.* 1976).

Single-fibre BR recordings were obtained by repeatedly teasing apart the aortic nerve until a small filament contained one or a few (2–3) active fibres that responded to pressure. Each filament was tested for pressure-related activity by briefly raising pressure to about 200 mmHg. If a filament contained several active fibres, action potentials from individual units were separated electronically using a dual voltage- and time-dependent window discriminator (BAK Electronics, Inc., Rockville, MD, USA). When separation was not possible (due to insufficient differences in spike waveforms), the filament was divided further. Extracellular recordings of nerve activity were obtained by draping the filaments over bipolar platinum-irridium wire electrodes. The signals were recorded with a high-gain (25000×) capacitance-coupled amplifier and were filtered to best accentuate each fibre's signal-to-noise ratio (band pass generally 0·1–10 kHz). The amplifier output was fed to an oscilloscope and audio monitor and was recorded along with aortic arch pressure on FM analog tape. The data were later digitized off-line and plotted with a PDP 11/70 computer. Two-dimensional plots were constructed in which pressure and frequency were plotted *versus* time, or in which pressure was plotted *versus* frequency. The pressure–frequency plots were used to characterize and compare discharge patterns of individual BRs in the following protocols.

Protocols

Ramp-testing BR pressure–discharge curves. The pressure–discharge relationship of each BR was determined by inflating the arch with a slow ramp increase in pressure. Slow pressure ramps are commonly used to characterize BR steady-state response characteristics because they provide a continuous measure of activity over a wide range of pressures. They also are completed within a relatively short period of time (1·5 min) and they approximate steady-state curves produced with staircase inputs. The ramps were produced by first reducing the pressure to zero (from the normal level of 80 mmHg) and then gradually increasing the pressure at a fixed rate (2 mmHg s^{-1}) up to

approximately 180 mmHg (Fig. 1). The same ramp rate was used for all fibres so that any differences in the response curves would not be complicated by individual differences in rate sensitivity (Wilson, Munch, Andresen & Brown, 1980). The rate that was applied was also used in previous related studies, which allowed direct comparisons to be made with the current data. After

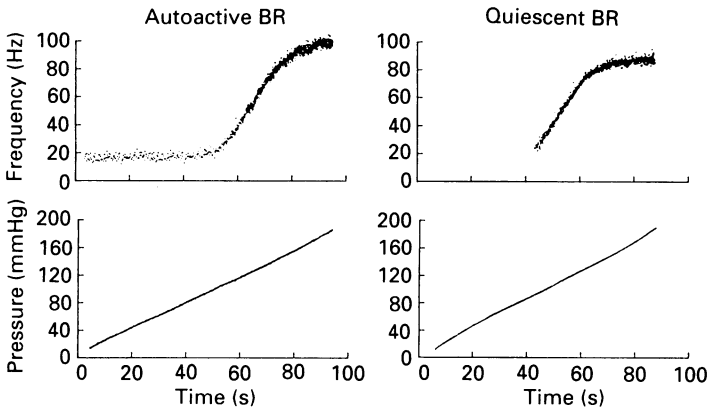

Fig. 1. Responses of a single autoactive and quiescent BR to a slow ramp increase in pressure (2 mmHg s^{-1}). These are computer-generated digital plots in which aortic pressure was sampled at 5 ms intervals and BR instantaneous frequency was calculated as the reciprocal of the interspike interval. To reduce the number of plotted points in rapidly discharging BRs such as these, the average frequency for each consecutive five spikes was plotted. Data from these ramps were used to construct individual BR pressure–frequency plots, as shown in Fig. 2.

completion of the ramp, pressure was returned to the usual perfusion level and was held there until the next ramp was delivered. To establish a reproducible response curve, at least three ramps were applied to each BR at regular 5 min intervals. From the ramp data, pressure–discharge curves were constructed by plotting the unit's instantaneous spike frequency (taken as the reciprocal of the interspike interval) against the ramp pressure.

Testing rapid resetting in autoactive BRs. Rapid resetting was tested in the aBRs using a protocol similar to that used previously to examine resetting in qBRs (Munch *et al.* 1983). The arch was perfused at different mean arterial pressure (MAP) levels, during which time BR response curves were repeatedly ramp tested at regular intervals. The initial MAP was set arbitrarily at 80 or 100 mmHg, and in one case at 40 mmHg. When the control curve was stable, MAP was increased in a step-like manner to a selected new level. The steps ranged in magnitude from 20 to 80 mmHg and each was held for 15–60 min. After any changes in the response curve were completed and a new stable curve was obtained, MAP was returned to the initial control level to test for reversibility. In some experiments, depending on the recording life of each fibre, the response curve was then tested at several additional MAPs. These steps involved both upward and downward adjustments of varying magnitudes, presented in random order. To be included in the analysis, each BR had to survive at least three ramp tests at each of two MAPs.

Testing responses of autoactive BRs to local vasoconstriction. The effect of vasoconstriction on the aBR pressure–discharge curves was tested by constructing the curves under control conditions and after contracting the vascular smooth muscle with 10^{-8} M angiotensin II (Ang II). The Ang II concentration employed was chosen because it was found previously to produce roughly 50% maximal vasoconstriction in the rat arch (Munch *et al.* 1987). The peptide was initially dissolved in Krebs–Henseleit solution and then a concentrated aliquot was added to a known volume of perfusate to achieve the specific molar concentration. Throughout the control period and application of the peptide, the perfusion pressure was held constant at 80 mmHg. This was possible because the arch was perfused with a flow-through system and the pressure was controlled by means of the outflow resistor. After 5–10 min were allowed for full development of the vascular

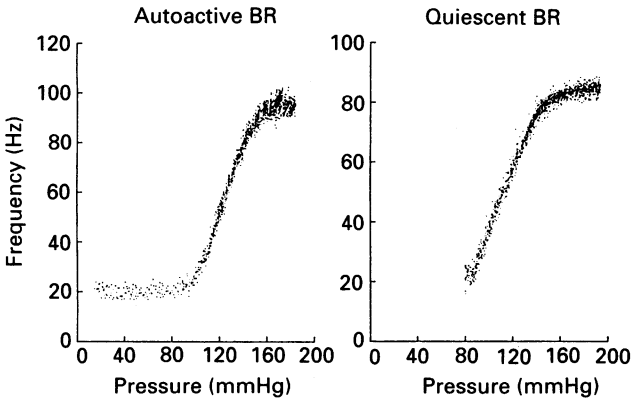

Fig. 2. Examples of ramp-evoked pressure–frequency plots of a single autoactive and a single quiescent BR (same units as in Fig. 1).

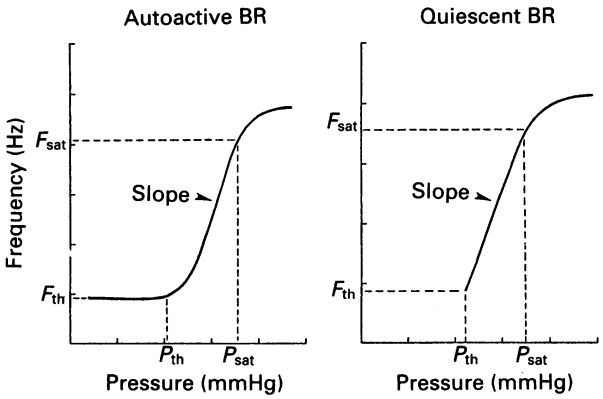

Fig. 3. Specific regions of individual BR pressure–frequency relationships that were used to characterize and compare autoactive and quiescent fibres. Curved lines represent scatter plots in Fig. 2 and were drawn for illustrative reference. Measurements were taken from the actual plots at the following regions, which are defined in text: P_{th}, threshold pressure; F_{th}, threshold frequency; P_{sat}, saturation pressure; F_{sat}, saturation frequency. Frequency range was calculated as F_{sat}–F_{th} and pressure range was calculated as P_{sat}–P_{th}. BR sensitivity was taken as the slope of the suprathreshold linear region of the curve.

response, ramp-evoked pressure–discharge curves were tested again and compared to the control curves.

Analysis

Each fibre was identified as either autoactive or quiescent based on its ramp response. As shown in Fig. 1, aBRs discharged continuously at a constant frequency until pressure reached a threshold level, at which point discharge began to rise gradually. In contrast, qBRs typically were silent until pressure reached threshold, at which point discharge began abruptly. From the ramp data, pressure–frequency plots were constructed for each fibre, as shown in Fig. 2. Generally, individual BRs exhibit unique response patterns under given controlled conditions. However, most BR curves have several features in common, which allow fibres to be compared quantitatively. Therefore, to characterize and compare the aBR and qBR response curves, measurements were taken at several specific locations. These are illustrated in Fig. 3 and are defined as follows.

The threshold pressure (P_{th}) was the pressure that first evoked an increase in activity, and the threshold frequency (F_{th}) was the frequency at P_{th}. Since the qBRs were initially silent, P_{th} was

simply the point at which they became active. However, since the aBRs were already active, but at a frequency unrelated to pressure, their P_{th} was the point at which discharge first began to rise. This was not always obvious because the increase in frequency occurred gradually and because all BR plots have some inherent degree of scatter. The measurement was taken, therefore, when the frequency values of all the plotted points first exceeded the average autoactive frequency. This was determined graphically by drawing a horizontal line through the subthreshold data points, which were horizontally distributed because the autoactive discharge was constant. This line was fit by eye and was closely described by the narrow point scatter. P_{th} then was taken where the plotted points first fell entirely above the line. F_{th} was considered the autoactive frequency (y-intercept of horizontal line), since their discharge rose from this level. With regard to these defined threshold measurements, there were some exceptions to the typical response patterns. Occasionally, discharge increased in some aBRs or was initiated in some qBRs at near-zero ramp pressures. This has been reported previously and was attributed to distortion of the BR endings due to collapse of the vessel wall (Landgren, 1952). Such activity was not considered to be autoactive discharge because it ceased with a rise in pressure that distended the vessel, but was still below P_{th}. In addition, there was a momentary decrease in the frequency of some qBRs just after they became active at P_{th}, and then their discharge rose with pressure. This response pattern also has been encountered by others (e.g. Fig. 1 in Andresen, Kuraoka & Brown, 1979) but the reason for it is unknown. In these units, P_{th} was measured when discharge first increased with pressure.

Above P_{th}, aBR and qBR response curves both exhibited steep linear regions, which represented the range over which they were most sensitive to pressure. Their sensitivity, therefore, was quantified by taking the slope of the best-fit line drawn through the points in this region. This line also was well defined by the narrow point scatter, and the slope was determined directly by measuring the change in frequency for a given change in pressure between two selected points on the line.

Above the linear region, the increases in frequency with pressure became progressively less and often reached a maximal (saturation) level. However, individual response curves were variable, so it was difficult to define a saturation region that could be applied to all units. In some fibres, discharge never completely saturated, but continued to rise gradually; in other fibres, discharge saturated and then either fell or continued to rise. Therefore, to compare a region common to the vast majority of fibres, saturation pressure (P_{sat}) was defined as the pressure at which the curves first bent downward. Since the bend was often gradual, the measurement was taken when all the plotted points first fell entirely below the line through the linear region, which was extended upward. The saturation frequency (F_{sat}), then, was the frequency at P_{sat}.

Lastly, the operating range of pressure (P_{range}) was considered to be the difference between the pressures at threshold and saturation (i.e. P_{sat}–P_{th}), and the operating range of frequencies (F_{range}) was the difference between the threshold and saturation frequencies (i.e. $F_{sat}-F_{th}$). Because some additional change in frequency does occur above P_{sat}, as mentioned above, these definitions somewhat underestimate the complete response range. However, P_{sat} was the most common landmark that could be quantified, and a similar definition has been used previously by others (Seagard *et al.* 1990). Furthermore, the changes in discharge that occur above P_{sat} are relatively small compared to the changes that occur below P_{sat} where the curve is much steeper.

The pressure and frequency values at the defined locations were all measured directly from the individual BR plots. To compare grouped data, differences between mean values were tested using Student's t test for unpaired observations, when comparing means of different BR populations (i.e. aBRs and qBRs), or Student's t test for paired observations, when each BR served as its own control (i.e. resetting protocol). Differences were considered statistically significant at $P < 0{\cdot}05$.

RESULTS

Characteristic pressure–discharge curves of autoactive and quiescent BRs

Pressure–discharge curves were examined in a total of 118 BRs from fifty-three rats. Based on their ramp responses, these BRs were separated into fifty-four aBRs and sixty-four qBRs. Both types of fibre were often found in the same preparation, and in some cases were electronically isolated from the same multifibre recording. The aBRs were easily distinguished by their continuous activity at subthreshold

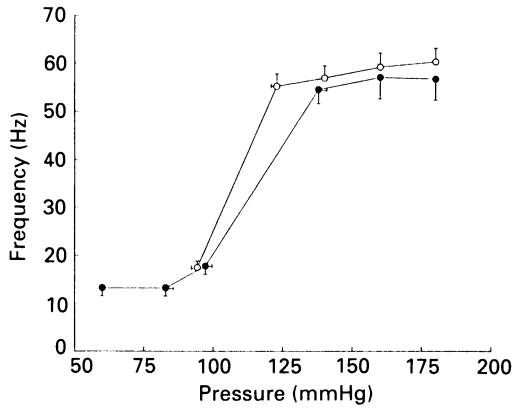

Fig. 4. Average pressure–frequency relationship of fifty-four autoactive BRs (●) and sixty-four quiescent BRs (○). Data points and error bars represent means ± S.E.M. Points with vertical and horizontal bars represent average values at threshold, start of linear region, and saturation (end of linear region). Single point below threshold on aBR curve represents the average autoactive frequency, which was taken at 60 mmHg pressure. Points on asymptotic portions of curves are average frequencies taken at 20 mmHg intervals of pressure.

TABLE 1. Comparisons between specific regions of autoactive and quiescent BR pressure–frequency curves

	Autoactive BRs ($n = 54$)	Quiescent BRs ($n = 64$)
P_{th} (mmHg)	83 ± 2	91 ± 2*
F_{th} (Hz)	13 ± 2	16 ± 1
P_{sat} (mmHg)	138 ± 4	123 ± 2*
F_{sat} (Hz)	55 ± 3	55 ± 3
P_{range} (mmHg)	57 ± 4	35 ± 3*
F_{range} (Hz)	41 ± 2	37 ± 2
Slope (Hz mmHg^{-1})	1·1 ± 0·1	1·4 ± 0·1*

Values are means ± S.E.M. * Values significantly different using unpaired t test ($P < 0{\cdot}05$). P_{th}, threshold pressure; F_{th}, threshold frequency; P_{sat}, saturation pressure; F_{sat}, saturation frequency; P_{range}, range of pressure response; F_{range}, range of frequency response; Slope, BR sensitivity to pressure over linear region (Δ frequency/Δ pressure).

pressures, whereas the qBRs were quiescent unless pressure exceeded P_{th}. Other than these obvious features, the aBRs and qBRs did not appear remarkably different when initially recorded. However, when the fibres in each group were combined, there were significant differences in their overall response patterns. Table 1 shows the average pressure and frequency values at the specific defined regions on the response curves, and Fig. 4 shows the average pressure–frequency relationship for each group. In general, the aBRs responded to pressure at significantly lower P_{th} values and significantly higher P_{sat} values than the qBRs. Thus, the response range of the aBRs was significantly wider than that of the qBRs. The discharge frequencies at threshold and saturation were not significantly different, however, so both groups were capable

of firing over similar ranges of frequency. Lastly, the slopes of the aBR curves were significantly less than the slopes of the qBR curves, so within their linear regions, the aBRs were less sensitive to pressure than the qBRs.

Rapid resetting of autoactive BRs

Rapid resetting was examined in a total of sixteen aBRs. In each case, a sustained change in MAP shifted the suprathreshold portion of the pressure–frequency curves

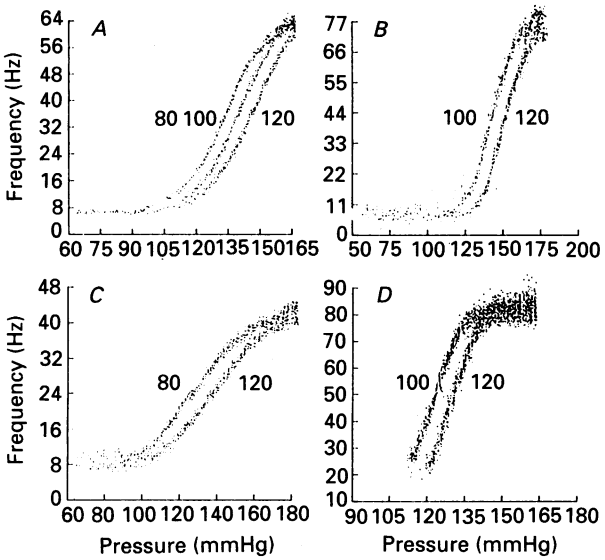

Fig. 5. Rapid resetting of pressure–frequency relationship in three autoactive BRs (panels *A–C*) and one quiescent BR (panel *D*) following a sustained change in MAP. For each BR, the different ramp-evoked plots shown were produced after perfusing the arch for 15 min at a different MAP (indicated adjacent to each plot). In panel *A*, the plots left to right correspond to MAPs of 80, 100 and 120 mmHg; these MAPs were tested in the order of 100, 120 and 80 mmHg. Each plot shown represents a reproducible pattern that was confirmed by repeated ramp testing at the specified MAP. The duplicate plots were not included, to avoid confusion of points. Fibres in panels *B* and *D* are from the same arch; fibres in panels *A* and *C* are from different arches.

along the pressure axis in the direction of the change in MAP. This is shown by examples of three individual fibres in Fig. 5*A–C*. Note the roughly parallel shifts in the curves without a change in the threshold or saturation frequencies. This was quite similar to the rapid resetting found in qBRs (example in Fig. 5*D*), as reported previously (Munch *et al.* 1983). The shifts in the curve were clearly evident upon visual inspection, but to quantify the relative position of the curves on the pressure axis, measurements were taken at P_{th} and at the midpoint of the linear region. P_{th} has been conventionally used to examine resetting in single-unit afferents, but can be somewhat less precise in aBRs because their discharge increases gradually. The linear mid-point (P_{mp}) is more precise because the curve is rising sharply and the point scatter is often less than at the threshold region. P_{mp} also represents the steepest (most sensitive) region of the response curve. Regardless of which method was

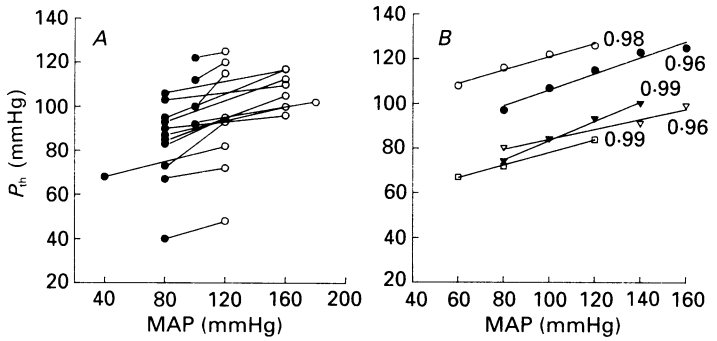

Fig. 6. Relationship between pressure threshold (P_{th}) and mean arterial pressure (MAP) in individual aBRs tested for resetting. Panel *A* shows P_{th} values for each of sixteen aBRs measured at the initial control MAP (●) and 15 min after a step increase in MAP (○). Resetting is indicated by an increase in P_{th} with an increase in MAP, and the extent of resetting is given by the slope of this relationship ($\Delta P_{th}/\Delta MAP$). For these combined aBRs, their average slope was 0·26 ± 0·04 (mean ± S.E.M.). In eleven of these fibres, when the MAP was subsequently returned to the control level, the changes in P_{th} were reversed. The recovery points are not shown, however, since they would fall essentially on top of the control points (●). Panel *B* is similar to panel *A*, but shows data from five individual aBRs that were tested at multiple MAPs. Each fibre is represented by a different symbol. Lines through the data are best-fit linear regressions determined using least-squares analysis; correlation coefficients (r) are given next to each line.

TABLE 2. Changes in defined regions of autoactive BR pressure–discharge curves following a step increase in mean arterial pressure from an initial control level (MAP_1) to an elevated level (MAP_2)

	MAP_1	MAP_2
P_{th} (mmHg)	86 ± 5	97 ± 6*
P_{mp} (mmHg)	129 ± 6	139 ± 7*
F_{th} (Hz)	13 ± 4	14 ± 4
P_{sat} (mmHg)	153 ± 4	159 ± 8*
F_{sat} (Hz)	55 ± 8	53 ± 5
P_{range} (mmHg)	66 ± 7	65 ± 8
F_{range} (Hz)	41 ± 4	42 ± 5
Slope (Hz $mmHg^{-1}$)	0·89 ± 0·13	0·97 ± 0·13

Values are means ± S.E.M. for all sixteen aBRs tested. * Values significantly different using paired t test ($P < 0·05$). P_{mp}, pressure at midpoint of linear region; other abbreviations same as in Table 1. Magnitude of step changes in MAP ranged from 20 to 80 mmHg. All measurements were taken when the response curves were stable or after holding the MAP at least 15 min.

employed, however, both indicated a significant shift in the curve to higher pressures following an increase in MAP. This was assessed using the data taken at the first two MAPs, which included all sixteen aBRs. As shown in Table 2, there was a significant increase in both P_{th} and P_{mp}, as well as P_{sat}, without a significant change in the corresponding frequencies at these locations. There also were no significant differences in the slopes of the curves or the range of operation, which was consistent with a parallel shift. In eleven of the sixteen aBRs, MAP was subsequently returned to the initial control level to test for reversibility. In each case, the curves shifted

back to their original location. In the five remaining aBRs, the fibres survived long enough so that the response curves could be tested at several additional MAPs; one fibre was tested at six different MAPs, one at five MAPs, and three at three MAPs. In each of these units, the shifts in the response curves again were consistent with the changes in MAP (Fig. 5*A*).

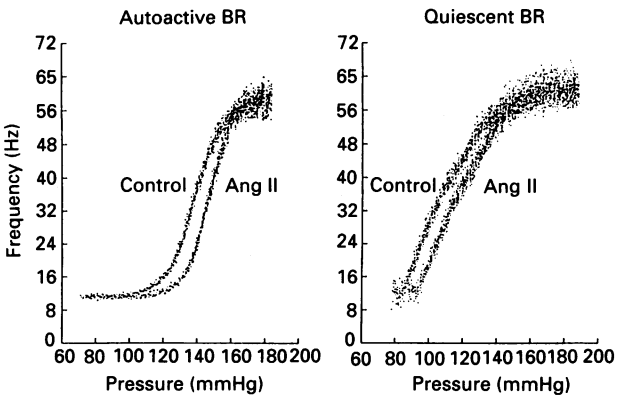

Fig. 7. Effect of vasoconstriction on pressure–frequency relationship of a single autoactive and quiescent BR. In each case, response curves were constructed under control conditions and 5–10 min after perfusing the arch with 10^{-8} M angiotensin II (Ang II).

To quantify the extent of resetting, P_{th} was plotted against MAP using the data for all sixteen aBRs at the initial two MAPs (Fig. 6*A*). The slope of the relationship ($\Delta P_{th}/\Delta$MAP) indicates the relative shift in the curve for a given change in MAP. When this quotient equals 1·0, the BRs are said to be fully reset. For each aBR tested, the extent of resetting was always less than 1·0; and for all sixteen units combined, the average was $0{\cdot}26 \pm 0{\cdot}04$ (mean ± S.E.M.). This value was not significantly different from that reported previously for the qBRs (0·23) (Munch *et al.* 1983). In addition, a similar plot of P_{th} *versus* MAP was constructed for the individual aBRs that were tested at multiple MAPs (Fig. 6*B*). For each of these fibres, the data were closely fit with a linear regression (least-squares analysis method), as shown by the high correlation coefficients. This indicated that the process of resetting was linear over a wide range of MAPs, and also that the extent of resetting was not dependent on the magnitude or direction of the change in MAP.

Responses of autoactive BRs to vasoconstriction

The effect of Ang II-evoked vasoconstriction on the aBR pressure–discharge curves was tested in five fibres. In each case, the suprathreshold portion of the curves was shifted to the right, resulting in lower discharge frequencies at given ramp pressures (Fig. 7). These changes were not the result of BR resetting, since the perfusion pressure was held constant during the application of Ang II. The shifts in the response curves were largest over the linear mid-region and became progressively smaller at lower and higher pressures. The autoactive discharge below P_{th} was entirely unaffected by vasoconstriction, so the subthreshold portion of the curves

remained constant. With regard to the suprathreshold portion, the effect of vasoconstriction on the aBRs was similar to that on the qBRs. This is shown in Fig. 7 and was reported previously by Munch *et al.* (1987).

DISCUSSION

The results of this study demonstrate for the first time the existence of a functionally unique group of BRs in the aortic arch that discharge continuously below P_{th}. These units contrast to the more familiar quiescent BRs that are silent below P_{th}. The most distinguishing feature of the 'autoactive' units was their subthreshold activity, which was independent of pressure. This produced pressure–discharge curves that were typically sigmoidal in shape, unlike the hyperbolic patterns characteristic of qBRs. There also were significant quantitative differences between the suprathreshold regions of the response curves when the two types of fibres were considered collectively. The aBRs generally were less sensitive to pressure, but responded over a wider range than the qBRs. These findings agree with recent results of Seagard *et al.* (1990), who reported similar differences between aBR and qBR fibres in the dog carotid sinus (which they termed type II and type I fibres, respectively). However, when comparing the aBR populations in the two studies, the aortic aBRs generally fired at faster threshold and saturation frequencies and were more sensitive to pressure than the carotid aBRs (slope = 1·1 and 0·3 Hz $mmHg^{-1}$, respectively; Table 1 and Seagard *et al.* 1990). These differences may be due to the proportion of myelinated and unmyelinated fibres in each sample. Both types of fibres have been shown to be autoactive (Table 3), but unmyelinated fibres typically discharge slower and are less sensitive to pressure than myelinated fibres (Thoren, Saum & Brown, 1977; Coleridge *et al.* 1987). Most of the carotid aBRs were unmyelinated fibres (ten out of seventeen), whereas most of the aortic aBRs were presumably myelinated fibres, based on discharge characteristics typical of myelinated units (Thoren *et al.* 1977; Brown, 1980). Conduction velocities could not be measured in the aortic group, though, due to an insufficient working length of nerve. A second possible explanation is that the aortic aBRs were studied *in vitro* whereas the carotid aBRs were studied *in situ*. Previous investigators have found that BR curves are steeper in excised compared to intact preparations (Angell-James, 1971). Third, the vascular wall in which the aBR endings are embedded is structurally different in the carotid sinus and aortic arch regions. This could contribute to differences in the process of mechano-electrical transduction in the aBRs from these two regions. And finally, there may be inherent differences between the aBRs from the two regions, or between the aBRs from the two species studied (rat and dog).

Perhaps the most interesting feature of the aBRs is their subthreshold activity, which appears to be an intrinsic property of these units rather than the result of mechanical stimulation. This activity was not attributed to collapse of the vessel wall at very low pressures because it persisted at pressures that clearly distended the wall, but were still below threshold. The endings did not seem to be mechanically loaded when placing the arch in the dish, since attempts to unload the endings by manipulating the arch geometry were unsuccessful. It also seems unlikely that the

aBR endings were pinched under sutures used to ligate nearby arteries or tie in the perfusion cannulas, since their receptive fields would have been unresponsive to pressure. Furthermore, aBRs have been reported in *in situ* preparations in which the natural orientation and tethering of the vessel were intact (Coleridge *et al.* 1987; Seagard *et al.* 1990), and roughly equivalent numbers of aBRs have been found in both intact and excised arches (Angell-James, 1971).

TABLE 3. Numbers and types of autoactive and quiescent BRs reported in previous studies

	Autoactive BRs		Quiescent BRs	
References (preparation)	Number of fibres (types)	Percentage of total	Number of fibres (types)	Percentage of total
Angell-James, 1971* (rabbit *in situ* AA)	8 (NR)	22	28 (NR)	78
Brown *et al.* 1976† (rat *in vitro* AA)	10 (9A,1C)	32	21 (15A,6C)	68
Thoren *et al.* 1977 (rat *in vitro* AA)	9 (all C)	31	20 (all C)	69
Coleridge *et al.* 1981 (dog intact AA)	3 (all A)	9	32 (all A)	91
Coleridge *et al.* 1987 (dog *in situ* CS)	30 (3A, 27C)	31	67 (34A, 23C)	69
Seagard *et al.* 1990 (dog *in situ* CS)	17 (7A, 10C)	26	48 (38A, 10C)	74
Present paper (rat *in vitro* AA)	54 (NR)	46	64 (NR)	64

* Includes BRs from right and left aortic nerves. † Includes BRs from WKY and SHR rats. Numbers and types of BRs when given: A, myelinated (A fibres); C, unmyelinated (C fibres); NR, not reported. Preparations: AA, aortic arch; CS, carotid sinus.

A second consideration was that the axons of the aBRs may have been damaged during nerve dissection, an unavoidable possibility when recording single-unit afferents. However, the aBR spike waveforms and pressure–discharge patterns remained constant during the recording period, and the aBRs tended to survive as long as the qBRs. Subthreshold discharge also is evident in whole-nerve recordings that involve minimal nerve dissection. If axon damage is a factor, one would expect that the vast majority of aBRs would have been unmyelinated fibres, since their axons are thinner and lack a myelin sheath, and since more extensive dissection usually is needed to obtain an adequate signal-to-noise ratio. However, previous studies have shown that aBR fibres can be either myelinated or unmyelinated (Table 3). Whether there is an actual preferred distribution, though, is unclear because it is technically more difficult to record from unmyelinated units. Finally, if subthreshold impulses do originate from a damaged axon, their retrograde conduction would collide with pressure-evoked antegrade impulses from the sensory terminals. This would preclude the regular discharge pattern normally seen at suprathreshold pressures.

A third possibility, which may pertain to blood-perfused preparations, is that

subthreshold discharge could result from the decreases in tissue O_2, pH or temperature that are associated with reduced blood flow at low arterial pressures. In such preparations, though, experimental anoxia did not induce activity in previously quiescent fibres (Landgren, 1952), and these factors were controlled in the present *in vitro* study. The aBRs also were not mistaken as chemoreceptors, since their response to suprathreshold pressures was confirmed.

Finally, subthreshold discharge has been noted in several different species and preparations (Table 3), so aBRs appear to be fairly widespread. Here and in studies by others, aBRs were present together with qBRs in adult rats of various ages and in both Wistar–Kyoto, WKY and spontaneously hypertensive strains, SHR (Brown *et al.* 1976). Therefore, aBRs do not result from a compromised preparation or the degenerative processes associated with ageing or genetic hypertension. It seems likely, then, that they probably do occur naturally. What is not yet clear is whether they represent a unique type of fibre or simply a 'modified' qBR.

In recent studies using an isolated carotid sinus preparation, subthreshold discharge was reportedly induced in qBRs by blocking a transient K^+ current with 4-aminopyridine (4-AP) (van Brederode, Seagard, Dean, Hopp & Kampine, 1990). These results tend to suggest that the differences between aBRs and qBRs are due to the relative effectiveness of this conductance in controlling membrane excitability at the spike-initiating zone. However, the slope and saturation regions of the curves were not affected, whereas these regions were typically quite different in aBRs and qBRs. Therefore, this explanation is incomplete. In addition, 4-AP affects K^+ currents in other tissues, such as endothelial cells (Takeda, Schini & Stoeckel, 1987), which in turn could release substances that affect the BRs directly or through changes in vasoactive tone. Interestingly, other substances, such as noradrenaline, have been shown to initiate subthreshold discharge in qBRs, which resulted in the qBR curves becoming sigmoidal in shape (Goldman & Saum, 1984). This was attributed to a direct effect of the catecholamine on the BR endings. Therefore, it is not certain whether 4-AP actually affects a unique membrane process(es) that distinguishes aBRs from qBRs, or whether it simply activates qBRs in such a way that their curves resemble those of the aBRs.

Resetting in autoactive BRs

A number of studies have demonstrated that arterial BRs rapidly reset following changes in MAP, but until recently none have involved fibres that were autoactive. The possibility that some BRs might not reset has important implications because resetting of BR afferent nerves attenuates the ability of the baroreflex to buffer sustained changes in MAP (Kunze, 1985). This is due to the fact that the response curves shift along the pressure axis is the direction of the new MAP, such that the change in frequency produced by the change in pressure gradually diminishes. Recent studies by Seagard *et al.* (1991), however, have suggested that aBRs (type II fibres) in the dog carotid sinus do not reset, which would mean that these units would continue to signal the full change in MAP after the qBRs reset. This prompted the hypothesis that, by not resetting, aBRs may be important in maintaining the absolute mean pressure. The present results involving aortic aBRs, however, do not support this hypothesis. The parallel shifts in their response curves demonstrated

that these units did in fact rapidly reset, much like the aortic qBRs reported previously (Munch *et al.* 1983; Munch & Brown, 1985). Furthermore, the extent of resetting was not different between the aortic aBRs and qBRs (0·26 and 0·23, respectively), so neither group appears to be better suited for signalling the mean pressure over extended periods.

The differences between the present results and those of Seagard *et al.* (1991) may be due to several methodological factors, even though both studies used similar protocols. Resetting was tested in the carotid aBRs by comparing P_{th} values derived by fitting ramp responses to a sigmoid function, whereas resetting was tested in the aortic aBRs by taking actual P_{th} and P_{mp} values directly from individual response curves. Computer-derived thresholds may be objective, but may not represent actual thresholds if the overall shape of the derived curve is determined mostly by regions away from threshold that contain far more data points (such as the asymptotic region, which can be variable, as noted earlier). Although aortic aBR P_{th} values were determined with the aid of lines fitted by eye, the shifts in the curves were clearly evident upon visual inspection (Fig. 5). Second, the carotid aBRs were tested using an unpaired comparison of average P_{th} values before and after changing MAP. Given the natural spread of P_{th} values in any BR population, significant changes can be missed without a large sample size. The aortic aBRs were tested using a paired comparison (since each BR served as its own control), in which case the changes in P_{th} and P_{mp} were significant for about the same number of units. Third, many of the carotid aBRs were unmyelinated fibres, which generally discharge irregularly compared to myelinated units (Thoren *et al.* 1977; Coleridge *et al.* 1987). The unmyelinated pressure–frequency plots are more scattered, therefore, which can limit the resolution of resetting if all units are averaged together. Because the aortic aBRs generally discharged regularly with pressure, their point scatter was relatively small. There also is disagreement as to whether unmyelinated fibres are capable of rapid resetting. Some investigators have reported that both A and C fibres reset in the rabbit carotid sinus (Yao & Thoren, 1983), whereas others have reported that in the dog carotid sinus, the A fibres reset but the C fibres did not (Schultz, Pisarri, Coleridge & Coleridge, 1985; Schultz, Coleridge & Coleridge, 1990). Finally, the process of resetting may be different between carotid and aortic aBRs, or between aBRs in rats and dogs, just as there are differences in their characteristic response curves. This may further distinguish the aBRs in these two regions. Such differences in resetting do not extend to the qBRs, though, which reset in both sino-aortic regions and in both species (Coleridge *et al.* 1981; Munch *et al.* 1983; Heesch *et al.* 1984).

Responses of autoactive BRs to vasoconstriction

The rightward shifts in the aBR response curves indicated that these fibres were inhibited by vasoconstriction. This is consistent with the idea that their sensory endings are 'coupled' primarily in parallel with respect to the vascular smooth muscle. Conceivably, when the muscle shortens, the endings are mechanically unloaded, which reduces their impulse activity. Greater pressures, then, are needed to evoke a given change in frequency. In earlier studies, similar responses were observed in the aortic qBRs (Munch & Brown, 1985; Munch *et al.* 1987). Thus, at suprathreshold pressures, the aBRs and qBRs seem to have a similar functional

relationship with respect to the vascular smooth muscle. The most prominent change in the response curves occurred within the linear mid-range. This corresponded to pressures over which the smooth muscle can constrict the arch most effectively (Munch & Brown, 1985; Munch *et al.* 1987). Progressively smaller changes occurred at higher pressures within the asymptotic portion of the curves, which reflected the inability of the smooth muscle to shorten against the greater force of distension. This was due most likely to the smooth muscle operating on the descending portion of its length–active tension curve (Murphy, 1980).

The changes in the aBR curves also became progressively smaller at pressures below the linear mid-range, with no change in the subthreshold autoactive frequency. This indicates that the process underlying autoactive discharge is independent of either passive (pressure) or active (smooth muscle) changes in the mechanical stimulus to the aBR endings. These results, then, further support the idea that autoactivity is intrinsic to the aBRs. These results also indicate that because subthreshold activity was not affected by vasoconstriction, BR responses to various vasoactive substances may not be evident in many experimental situations if afferent nerve recordings contain aBR fibres that are discharging below P_{th}.

Physiological role of autoactive BRs

The presence of aBRs in the carotid sinus and aortic arch raises the question of what role do these fibres play in neural control of the circulation? Although encountered less frequently than qBRs, aBRs have been noted by several other investigators using a variety of preparations. Table 3 lists studies in which the numbers of autoactive and quiescent fibres were given. If these studies are considered collectively, the aBRs accounted for approximately 25% of the fibres recorded, so they are not a rare anomaly. They also are evident in studies in which their numbers were not given (e.g. Landgren, 1952; Goldman & Saum, 1984). However, their percentage of the BR population may be only approximate, since sampling was not necessarily random. Some aBRs may have been discarded as damaged fibres or as chemoreceptors (if pressure was below P_{th}), and others may have been more noticeable because of their continuous activity. Nevertheless, despite their actual numbers, their rather frequent occurrence suggests that they may make a meaningful contribution to the baroreflex. The nature of that contribution, however, is spectulative at this time.

With regard to their constant subthreshold discharge, clearly this provides no information regarding changes in blood pressure. In fact, such activity would seem detrimental during a severe fall in arterial pressure, since it would tend to suppress reflex responses serving to correct the pressure deficit. In cases of severe and prolonged hypotension, autoactive discharge may actually initiate positive feedback responses that contribute to collapse of the circulation when compensatory reflex mechanisms fail (Downing, 1979). Some investigators, on the other hand, feel that autoactive discharge may play a tonic role in setting the baseline function of the baroreflex, since initiating subthreshold activity with 4-AP reportedly induced a reflex fall in renal and cardiac efferent nerve activity (Gallenberg, Seagard, Dean-Bernhoft, Hopp & Kampine, 1989). Any substance, though, that enhances BR afferent traffic might evoke a similar reflex response, even if the enhanced activity is not related to pressure.

With regard to their responses at suprathreshold pressures, the aBRs may buffer transient fluctuations much like the qBRs, but just less effectively because the slopes of their response curves were not as steep as the qBRs. However, the aBRs may be beneficial by expanding the range of the baroreflex, since their threshold pressures were lower and their saturation pressures were higher than the qBRs. Thus, the aBRs may continue to signal both progressive decreases in pressure after the qBRs have shut off, and progressive increases in pressure after the qBRs have saturated. This benefit most probably would be realized mainly at the high end of the response range, since the aBR curves continued to rise linearly after the qBR curves became asymptotic (Fig. 4). The benefit may be minimal at the low end, however, because even though their threshold pressures were lower than the qBRs, their discharge increased very little before the qBRs became active. The functional role of the aBRs, therefore, is not yet understood. Additional studies clearly are needed to define their contribution to the arterial baroreflex.

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