Characteristics of Rat Aortic Baroreceptors with Nonmedullated Afferent Nerve Fibers

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SUMMARY  Nervous activity was recorded from nonmedullated afferent baroreceptor fibers in the left aortic nerve of normotensive rats. The aortic arch was perfused in vitro with oxygenated Krebs-Henseleit solution. Two different patterns of aortic C fiber discharge were found. One type (5 of 29 fibers) had a regular discharge at constant pressure whereas the other type had an irregular discharge. The differences between the two types were independent of the mean steady state rates of discharge. The average threshold pressure for the receptors with the regular discharge was 87 mm Hg (range, 65-125); it was 152 mm Hg (range, 85-260) for the irregularly discharging receptors. During a pressure step the receptors in either group normally showed an initial high frequency burst of activity and a sustained discharge which had a lower frequency. Furthermore, the peak discharge and the sustained discharge were lower on the average in the receptors with the irregular type of firing. We also found that addition of norepinephrine to the perfusate lowered threshold. Since unmyelinated axons are the predominant fiber type in the rat aortic nerve and since some receptors are activated at physiological pressures they may contribute to the tonic control of blood pressure. Their effects should be particularly important when the blood pressure is increased.

THE EXISTENCE of aortic baroreceptors with medullated afferent nerve fibers has been demonstrated in many species and their functional characteristics have been examined extensively.1,2 The aortic nerve also contains a large number of nonmedullated fibers, some of which are definitely baroreceptors;3 in the rat such fibers predominate histologically.2 When unmyelinated baroreceptor fibers are stimulated electrically, powerful depressor reflexes are evoked.1 Recordings from nonmedullated baroreceptor afferents identified by measurements of conduction velocity were obtained in 1969 by Fidone and Sato3 using the carotid sinus nerve. Landgren4 had earlier described baroreceptor afferents in the same nerve which had a low signal to noise ratio and presumably were also nonmedullated or C fibers. In thoracic vessels, vascular endings connected to C fibers have been reported by Coleridge et al.5 These receptors either have very high thresholds, or can be excited only by nonphysiological stimuli. Their discharge characteristics are peculiar and they are not aortic arch baroreceptors. Thus, a systematic analysis of the functional characteristics of nonmedullated aortic baroreceptors has not yet been made. Therefore, the present study of threshold, pressure-response relationships, and discharge patterns of these unmyelinated fibers was undertaken.

The rat was used as the experimental animal because we have recently developed an in vitro aortic arch-aortic nerve preparation6 which permits better experimental control and allows the use of different pressure waveforms to test the receptors. This is a great advantage when the characteristics of the receptors are to be studied.

We found that, in comparison with the medullated baro-

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Receptors, most of the aortic baroreceptors with C fibers have higher thresholds, lower discharge frequencies, and an irregular type of discharge. We suggest that the baroreceptor C fibers might be important in control of blood pressure by exerting an inhibitory effect on the vasomotor center. Their importance will be enhanced when arterial blood pressure is raised, due to the marked increase in activity which will occur in these receptors at higher pressures.

Methods

Experiments were performed on normotensive rats (Wistar-Lewis strain, 250-400 g) of either sex and of ages 3-6 months. The rats were anesthetized with sodium pentobarbital (Nembutal, 30-40 mg/kg) administered intraperitoneally after initial induction with ether. The trachea was cannulated and the rats were artificially ventilated with a positive-pressure respirator. A midline thoracotomy was performed and the ascending aorta, the right and left subclavian arteries, and the right and left common carotid arteries were prepared for subsequent ligation. The left aortic nerve was freed from connective tissue on the surface of the aortic arch, dissected proximally for 1-2 cm, and cut. The brachiocephalic trunk and the descending aorta at the level of the right atrium were cannulated in a retrograde manner using stainless steel cannulas with an outer diameter of 1.42 mm, and the isolated segment was excised and pinned out in a plexiglass chamber. The aortic arch was perfused with a Krebs-Henseleit solution through the descending aorta and the effluent drained out by the brachiocephalic trunk. The perfusate had the following composition: NaCl, 120 mm; KCl, 4.8 mm; MgSO4, 1.2 mm; CaCl2, 1.1 mm; KH2PO4, 1.2 mm; NaHCO3, 25 mm; and dextrose, 5.5 mm. The solution was saturated with 95% O2 and 5% CO2 to give a final pH of 7.35-7.45. The flow through the arch could be varied by adjusting the flow rate of a Holter infusion pump. The aortic arch and the aortic nerve were covered with warm mineral oil (temperature, 37-37.5°C).
Pressure steps of varying amplitude from zero to 260 mm Hg and durations of 5-60 seconds were applied using a mercury manometer. The manometer was connected to the inflow cannula by an electrically activated solenoid valve. The aortic arch pressure was measured from a side arm of the inflow cannula with a strain gauge pressure transducer (Statham P23Db). The pressure signal was displayed on an oscilloscope and recorded on an FM tape recorder. Pressure ramps and pressure sine waves were generated by a DC servomotor (Electrocraft) and cam coupled to a syringe system having an adjustable air reservoir.

**ELECTROPHYSIOLOGICAL MEASUREMENTS**

Slips of baroreceptor fibers were teased from the left aortic nerve and mounted on platinum-iridium electrodes. Dissection was continued until single or few fiber preparations with satisfactory signal to noise ratios were obtained. The action potentials were recorded with a capacitance-coupled amplifier (Princeton Applied Research, model 113) and displayed on an oscilloscope. The output from the amplifier was also fed to an audiomonitor and to an FM tape recorder. The spikes could also be displayed together with the pressure on a Honeywell Visicorder (model 1508). The output from the amplifier was also connected to a rate meter equipped with a discriminator, which could count either spikes exceeding a certain value or spikes with amplitudes between two predetermined levels. The output of the rate meter was recorded on a Grass (model 5) recorder together with the pressure signal.

The conduction time of afferent fibers was determined by applying an electrical stimulus (Grass stimulator S4 with SIU) to the area of the arch where the receptor could be maximally activated by mechanical means. The evoked potential was then recorded and the amplitude and the morphology of the evoked potential were compared with the potentials recorded during the pressure steps. In several filaments the electrical stimulation also activated silent fibers, but then it was possible to identify the active fiber from the spike configuration and amplitude. The distance between the stimulating and the recording electrodes was measured under the microscope with a transparent plastic ruler, and since the conduction time was known, the conduction velocity could be calculated.

The major error in the measurement of conduction velocity is the estimation of the length of the nerve between the stimulating and recording electrodes because it is difficult to clearly establish the pathway of the afferent nerve along the aortic arch. However, this error is probably relatively small. Another error is the setting up time of the inputs at the stimulating electrode. However, this time cannot be longer than the duration of the electrical stimulus (0.3-0.5 msec) and since the total conduction time in the fiber was between 5 and 40 msec, this error could never exceed 10% and is in most cases much less.

**DATA ANALYSIS**

The methods of digitizing the pressure and action potential inputs and the calculation of instantaneous impulse frequency and isochronal pressure-frequency curves were previously described. The computer processes spike trains by measuring directly the interspike intervals with an accuracy of 0.1 msec. The computer converts each interval to an "instantaneous frequency" point. It is helpful to regard these points as a continuous function of the generator potential which initiates axonal discharge.

**EXPERIMENTAL PROCEDURES**

Because aortic baroreceptor C fibers normally have a low or absent discharge, the activity in all filaments dissected from the aortic nerve was observed during a brief (3-6 seconds) pressure ramp applied from zero to about 260 mm Hg. Every filament that responded with increased activity to this maneuver was dissected further until a filament with only one or two to three fibers was obtained. Recordings were made from few fiber preparations only when the amplitude of the individual spikes was clearly different so that it was possible to calculate the activity in at least one single filament. Recordings in which the conduction velocity of units could not be established or was higher than 2 m/sec were excluded. The anatomical location of the receptors was obtained by exploring the aortic arch with a fine probe.

Receptor activity was observed during alternations in aortic pressure in a stepwise (10- or 60-second) or ramp fashion.

The receptor response to a ramp stimulus was repeated after adding norepinephrine (1 µg/ml) to the perfusate.

The significance of changes was determined by Student's t-test for paired observations.

**Results**

Recordings were made from 29 aortic baroreceptor C fibers in 19 rats. Twenty-one recordings were made with only one active fiber and the others with two or three active fibers. The conduction velocity varied from 0.3 to 1.8 m/sec (mean ± se, 0.9 ± 0.1 m/sec). Thus, all of these fibers belong to the C fiber category.

When the activity in the individual fibers was recorded in response to pressure steps the aortic C fiber afferents showed two different patterns of response. Five baroreceptors showed a pattern similar to that described earlier for medullated afferents from the aortic arch, i.e., a transient discharge which adapted over a few seconds to a steady state discharge. The adapted discharge frequency was quite regular during the pressure step, as shown in Figure 1A. After the initial 3 seconds the variation in discharge in this fiber was less than ±15% from the mean value. The other 24 receptors had an irregular pattern of discharge during the pressure step with a marked variation in the instantaneous frequency as shown in Figure 1B. When similar average values of interspike intervals were compared between these two groups, the standard deviations of the interspike intervals were 6 times greater in the irregular group. These striking differences in discharge patterns are analyzed in more detail in a subsequent section. However, the conduction velocities for the receptors with the regular and irregular type of firing were not significantly different (1.06 ± 0.20 and 0.89 ± 0.10 m/sec, respectively). The discharge patterns of individual fibers in both groups were reproducible over periods of...
many hours, and when deterioration appeared the fiber was not studied further.

SPONTANEOUS DISCHARGE

Nine receptors (three receptors, regular type of firing) showed a sustained spontaneous discharge with frequencies of 1.5–10 impulses/sec, thought to arise from distortion of the in vitro preparation while it is partially collapsed. The mean spontaneous discharge for the regular firing receptors was 4.3 ± 1.9 impulses/sec, and for the irregular, 1.0 ± 0.34 impulses/sec. Six other receptors showed only occasional spontaneous spikes and the other 14 receptors showed no activity at all.

THRESHOLD FOR ACTIVATION DURING A RAMP STIMULUS

The threshold for activation during a ramp stimulus (Fig. 2A) having a rate of rise of about 40 mm Hg/sec was examined in 27 receptors, as shown in Figure 3. Five receptors with a regular type of firing had a lower threshold than 22 receptors with an irregular type of firing (Fig. 3). Some of the receptors with an irregular type of firing had thresholds in excess of 200 mm Hg. The mean threshold values for these two groups of receptors were 87 ± 11 mm Hg (regular) and 152 ± 11 mm Hg (irregular).

THE RECEPTOR RESPONSE TO PRESSURE STEPS

The receptor response to 10-second pressure steps ranging from 80 to 260 mm Hg was examined in 26 receptors. Examples are shown in Figure 2B and C. At equivalent pressures the regularly discharging receptors had higher frequencies during the early and later portions of the pressure step. This is shown in Figure 4. Three irregularly discharging receptors with high thresholds (more than 180 mm Hg) showed only an initial burst of activity during the pressure step and can be classified as rapidly adapting receptors. Two other receptors had a sustained discharge throughout the pressure step without any initial higher frequency burst.

DIFFERENCE IN REGULARITY OF DISCHARGE

Since the regularly discharging receptors had higher discharge frequencies than the irregularly discharging group, regularity might be attributed to differences in frequency alone. Therefore, we examined the variability of the interspike intervals at comparable mean frequencies during the 6th to 9th seconds of the pressure steps. We had to select the lower end of the frequency-pressure curve of regularly discharging receptors and the higher end of irregularly discharging receptors to obtain comparable mean interspike intervals (Fig. 4). Table 1 shows the number of interspike intervals counted during the 6th to
9th seconds, the average interspike interval values that were compared, and the standard deviation of the interspike intervals at each mean interspike interval. There were no significant differences between the average interspike intervals that were compared. However, the standard deviations of the interspike intervals were about 6 times greater for the irregularly discharging groups (Table 1, column 3), and these differences are highly significant statistically.

THE RESPONSE TO A LONG-LASTING PRESSURE STEP

The receptor response to a 40-second pressure step of 220 mm Hg was tested in eight receptors (one receptor with a regular type of firing). The discharge frequency was recorded by the spike-counting device (time constant = 0.4 second), as shown in Figure 5. Notice that the sustained discharge at the end of the 40-second pressure step is much lower than the initial discharge. This is especially prominent in the seven receptors with an irregular type of firing (panels 1-7).

EFFECT OF HYPOXIA

The effect of hypoxia on the receptor response to a pressure step was examined further in two irregular receptors. Hypoxia was induced by perfusion of the aortic arch for 15 minutes with a solution gassed with 95% N₂ and 5% CO₂. One receptor showed depressed activity and one receptor was unaffected by this hypoxia.

Stopping the perfusion pump for 2 minutes did not affect the receptor response to a subsequent 10-second pressure step in four receptors. The adaptation during the 40-second pressure step can thus not be ascribed to hypoxia during the prolonged pressure step.

EFFECT OF NOREPINEPHRINE ON THE RECEPTOR ACTIVITY

The effect of norepinephrine added to the perfusate in a dose of 1 µg/ml was tested on seven irregularly firing receptors. The control threshold for activation during a ramp stimulus of 30-40 mm Hg/sec was 156.4 ± 15.7 mm Hg before and 142.1 ± 14.9 mm Hg after norepinephrine. This difference is statistically significant (P < 0.05).

RESPONSE TO A PULSATILE STIMULUS

The activity of seven receptors (two regularly discharging receptors) was recorded during sine wave modulated

FIGURE 2  The activity in a single aortic C fiber of the irregular type and the aortic arch pressure during a ramp stimulus (A), constant step stimuli of 120 mm Hg (B) and 160 mm Hg (C), and a pulsatile step of 160 mm Hg (D). Notice that during the step stimuli B and C, the activity shows a marked burst during the 1st second and that the sustained discharge is very irregular. Likewise, the early pulses in D were associated with increased discharge.

FIGURE 3  Threshold during ramp stimuli for five receptors of the regular type and 22 receptors of the irregular type. The first evidence of increased activity during the ramp stimuli (rate of rise = 40 mm Hg/sec) was considered as the threshold.
pressure steps ranging from 80 to 240 mm Hg. This is illustrated in Figure 2D. The amplitudes of the pressure step and the sine wave in Figure 2D were 160 and 45 mm Hg, respectively, and the frequency of the sine wave was 4 Hz. Six of the receptors showed a discharge modulated by the sine wave pressure. At pressures higher than 180 mm Hg, three of the receptors showed a continuous discharge.

POSTEXCITATORY DEPRESSION (PED) IN BARORECEPTOR C FIBERS

Since PED has been described in myelinated fibers,7 we examined C fibers for its presence. Three of four fibers with irregular discharge and all three regular types showed PED (Fig. 6). When ouabain (5 x 10^-4 M) was added to the perfusate the period of (PED) was abolished as tested in one receptor with a regular type of firing.

Discussion

RECEPTOR CHARACTERISTICS AND MECHANISM OF ACTIVATION

The 29 aortic baroreceptors with C fiber afferents could be divided into two groups. Five receptors showed a regular steady state firing pattern with small variations in the interspike intervals, and 24 receptors showed a marked variation in the interspike intervals during the pressure step. The receptors with an irregular type of discharge had, on the average, higher thresholds for activation and lower initial and sustained discharge rates during the pres-
Figure 6  *Top:* the activity of single aortic C fibers of the regular type (A) and the irregular type (B) during constant pressure steps of 180 mm Hg. Both receptors had a spontaneous discharge and showed a clear postexcitatory depression (PED) on cessation of the pressure step. Receptor A was firing regularly and receptor B irregularly during pressure steps. The spontaneous discharge of A was less regular than the pressure-evoked discharge. *Bottom:* the duration of PED is plotted against the amplitude of the preceding pressure steps (regular, A; irregular, B).

The difference in regularity between the two types appears to be largely independent of discharge rate, since it did not occur in regularly discharging fibers which had steady state discharge rates equal to or lower than those of irregularly discharging fibers.

Stretch of the aortic arch is the prime determinant of activity in aortic baroreceptors with C fiber afferents. The receptors with a regular type of firing must be classified as slowly adapting stretch receptors. The receptors with an irregular type of firing are more rapidly adapting even if most of the receptors showed an irregular, sustained discharge during the pressure steps. Three receptors are classified as rapidly adapting because they lacked sustained discharge entirely during the pressure step even at the highest pressures used. Slowly adapting receptors never developed a rapidly adapting pattern, therefore the effect is not the result of deterioration of these fibers.

We always used ramps with rates of rise of 30–40 mm Hg/sec, which are at the lower level of values that have been employed for baroreceptors (see Figure 15 of Landgren). Baroreceptor threshold as a function of ramp rate is unchanged over the range of 40–470 mm Hg/sec and is only slightly higher than values obtained with much higher rates. Thus differences between the receptors are unlikely to be due to differences in accommodation.

The baroreceptors with C fiber afferents had lower thresholds when norepinephrine was added to the perfusate. This is in agreement with the findings of Landgren et al. that baroreceptors with low amplitude spikes were sensitized by norepinephrine. This has been interpreted as indicating that baroreceptor C fiber receptors are coupled "in series" with the muscular elements in the vessel wall. The effects of norepinephrine and sympathetic stimulation on the activity in arterial baroreceptors with medullated vagal afferents is less clear. Some authors claim that these receptors are unaffected or sensitized by sympathetic stimulation. However, it has also been reported that these baroreceptors are desensitized by norepinephrine or sympathetic stimulation.

The basis for the highly irregular type of firing in the majority of baroreceptors with unmyelinated afferent fibers is not understood. As noted earlier it is not attributable to differences in mean discharge frequency. Irregular discharge is an unusual occurrence in mechanore-
The C fibers seem to affect mainly the vagal outflow to the heart and have only a small effect on the sympathetic outflow to the heart. The reflex effects of the C fibers on the different vascular beds have not been studied in detail.

**ARE REGULARLY DISCHARGING C FIBERS AN EXPERIMENTAL ARTIFACT?**

The existence of C fibers with firing characteristics similar to medullated fibers was surprising in light of the fact that mechanoreceptors with C fibers or unmyelinated fibers generally have discharge characteristics that are quite different. Is it possible that the measurement of the conduction velocity is incorrect in these experiments? Undoubtedly several errors might be involved in the measurement of the conduction velocity, e.g., the setting up time of the impulses and the measurement of the conduction pathway. However, the error with the setting up time is small, as discussed in the Methods, and the error involved in the measurement of the conduction pathway was also probably less than 10%. It is unlikely that these errors could be systematized for only one category of fiber. It could also be argued that even if the electrically evoked potentials are of amplitude and morphology similar to those of mechanically evoked potentials, they represent two different fibers. However, great care was taken to avoid this, and in one instance when the conduction velocity was measured by applying the stimulus on the nerve close to the aortic arch, occlusion occurred between the electrical and mechanically evoked potential, indicating that the two potentials were from the same fiber. Finally, it can be argued that these high frequency C fibers with a regular type of discharge represent medullated afferents where the conduction, for some reason, had been slowed. However, it is difficult to understand how such a markedly disturbed medullated fiber can conduct up to 150 impulses/sec with a regular type of discharge. For all of these reasons we believe that part of the baroreceptor population with affenter C fibers in the rat may show a behavior similar to that of baroreceptors with medullated afferents.

**REFLEX EFFECTS OF AORTIC BARORECEPTOR C FIBERS**

Reflex effects of aortic baroreceptor C fibers have been studied during differential electrical stimulation of the aortic nerve in the cat and the rabbit. Douglas et al. reported in 1956 that electrical activation of aortic baroreceptor C fibers induced powerful depressor reflexes at low stimulation frequencies. The reflex pattern from the aortic baroreceptor C fibers also seems to be different from the aortic baroreceptors with medullated fibers. The C fibers seem to affect mainly the vagal outflow to the heart and have only a small effect on the sympathetic outflow to the heart. The reflex effects of the C fibers on the different vascular beds have not been studied in detail.

**PHYSIOLOGICAL ROLE OF THE AORTIC BARORECEPTOR C FIBERS**

These normotensive rats have mean systolic arterial blood pressures of about 110-115 mm Hg measured with the indirect tail occlusion technique. This means that the discharge in individual baroreceptor C fibers is normally rather sparse. This is especially so in the irregular firing C fibers. Thus, these C fibers may not be of major importance for cardiovascular control during decreases in blood pressure resulting from hemorrhage because the firing in the C fibers is so sparse during normal conditions. However, when the blood pressure is increased for some reason, e.g., during anger or fear reactions, the C fibers might contribute significantly to the inhibitory traffic reaching the vasomotor centers.

**References**
