Reflex Heart Rate Control Via Specific Aortic Nerve Afferents in the Rabbit

By Merrill B. Kardon, D. Fred Peterson, and Vernon S. Bishop

ABSTRACT

Reflex bradycardia was elicited in rabbits via repetitive electrical stimulation of the central end of the sectioned left aortic nerve. Supramaximal stimulation produced a 16.9 ± 1.3% (±SE) increase in the R-R interval when vagal and sympathetic efferent pathways were intact. Reducing the stimulation voltage allowed selective stimulation of the myelinated (A) fibers, and polarizing electrodes placed central to the stimulus site permitted A fiber blockade and selective stimulation of the unmyelinated (C) fibers. When afferent A fibers were selectively stimulated, 64% of the maximum response was obtained; selective C fiber activation elicited 63% of the maximum observed response. Selective stimulation of A or C fibers after either vagotomy or stellectomy indicated that A fiber afferents elicit heart rate responses via both vagal and sympathetic efferents, whereas C fiber afferent information is mediated predominantly via vagal efferents. This afferent-efferent specificity of the aortic baroreceptor pathways suggests baroreceptor mechanisms normally used to modulate heart rate. Small increments in blood pressure would activate low-threshold A fibers and result in reciprocal changes in vagal and sympathetic efferent activity. More substantial increases in blood pressure would activate C fibers and produce additional heart rate effects via vagal efferents.

The influence of baroreceptors on heart rate has been shown to involve afferent myelinated (1) and unmyelinated (2) fibers. Central stimulation of the rabbit aortic nerve causes a reduction in both heart rate and arterial blood pressure (2-4). The mechanisms whereby each of the aortic nerve afferent fiber groups cause reflex changes in heart rate have not been well defined. Recent data indicate that unmyelinated afferent (C) fibers, which have been shown to arise in the wall of the aortic arch (5), may carry a significant portion of the overall baroreceptor information in the rabbit (2) as well as in the dog and the cat (6).

Previous investigators have shown that arterial blood pressure is elevated reflex peripheral vascular resistance changes occur at a lower pressure than do heart rate changes (7-9). This finding indicates the possibility that a functional separation of baroreceptor-induced vagal and sympathetic cardiovascular influences may be attributable to the differences in their respective afferent mechanisms. Although reflex heart rate effects have been shown to involve reciprocal changes in cardiac vagal and sympathetic efferent activity (10), the specific afferent fiber groups responsible for these reciprocal changes have not been identified. The present study was designed to evaluate the ability of the rabbit's myelinated (A) and unmyelinated (C) aortic nerve groups to vary heart rate by way of either vagal or sympathetic efferent pathways.

Methods

Twenty-three albino rabbits, 1.5-2.5 kg, were anesthetized with sodium pentobarbital (30 mg/kg, iv). Catheters were placed in the femoral vein so that anesthetic could be administered and the thoracic aorta arterial blood pressure could be monitored with a Statham P23db transducer. A midventral cervical incision was made from the point of the sternum to the angle of the jaw, and artificial respiration was begun using a Harvard Apparatus ventilator (model 665) at a volume of 30 ml and a rate of 100 /min. The low-volume, high-frequency air flow produced synchronization (with the respirator) of the rabbit's spontaneous respiratory movements; minimum reflex changes in arterial blood pressure were observed. This technique has been shown to cause little, if any, qualitative change in baroreceptor-mediated responses (11) or in blood gas levels (2, 12). A pouch was formed by suspending the free ends of the incised skin from a horizontal ring in place above the incision. Using a binocular dissecting microscope (Olympus model SZ), the left aortic nerve was dissected free in the neck and cut as close as possible to the sternal. Insofar as A fibers respond at lower electrical thresholds than do C fibers, it was possible to stimulate A fibers selectively by reducing the stimulus voltage from supramaximal levels to sub-C fiber threshold levels. The reverse, however, was not

From the Department of Pharmacology, The University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284.

This work was supported by the Texas Heart Association, U. S. Public Health Service Grant HL12415-07 from the National Heart and Lung Institute, and Air Force Contract AFOSR 73-2525.

Dr. Kardon's present address is Department of Investigative Medicine, Mt. Sinai Hospital, 1800 E. 105th St., Cleveland, Ohio 44106.

Received December 20, 1974. Accepted for publication April 11, 1975.

Circulation Research, Vol. 37, July 1975

41
possible. To elicit the preferential activation of C fibers, the conduction of A fibers was first blocked using a pair of polarizing electrodes (13). Three sites were isolated along the length of the left aortic nerve. The free central end of the cut nerve was placed on a pair of platinum-iridium stimulating electrodes connected by way of a Grass SIU5 stimulus isolation unit to one channel of a Grass model S-88 stimulator. At the second more rostral site midway along the length of the left aortic nerve in the neck, a second pair of electrodes was positioned. These electrodes were covered with saline-soaked cotton wicks and connected via a second stimulus isolation unit to the 100,000-ohm d-c output of the second channel of the S-88 stimulator. As previously described, they served as A fiber blocking electrodes (13). Bipolar recording electrodes were placed at the third most rostral site. These electrodes were connected to a pair of cascaded Grass model P15 preamplifiers, and they served to monitor the evoked potential. The evoked potential was recorded on Polaroid film from the face of a Tektronix D12 storage oscilloscope. Bilateral vagotomies were performed in the cervical region. Stellectomies were performed by removing a section of the sympathetic chain formed in the cervical region. Stellectomies were performed between the first and the third thoracic interspace. Heart interval was monitored by way of needle electrodes placed along the sternum. These electrodes were connected to a Beckman type 9857B cardiotachometer coupler. Both heart rate and arterial blood pressure were recorded continuously using a Beckman type R-M (411) oscillograph.

On-line monitoring of the R-R interval and control of the nerve stimulus parameters were accomplished using a DEC PDP 8/E digital computer. Bursts of stimulus impulses were applied to the left aortic nerve during each R-R interval in fixed synchrony with each R wave. The duration of each impulse was 0.3 msec as established by the stimulator. The run duration and the stimulation sequence, which consisted of impulse frequency, impulse number, burst duration, and timing of the stimulus burst within each R-R interval, were established by the computer in accordance with the experimental protocol. The computer stored each R-R interval in a given run and averaged it with the equivalent interval in previous runs made under identical stimulus conditions. The sequence of average R-R interval values for each group of stimulus-response runs was then printed out on command. Three key parameters, latency to onset, latency to peak, and the value of the peak response were printed out along with their respective standard deviations. The latency to onset represents the number of heart intervals from the beginning of the stimulus to the end of the interval which first exceeded the control. The latency to peak represents the number of heart intervals from the beginning of the stimulus to the end of the longest interval during the response. Peak response represents the maximum percent change from the control R-R interval.

Comparisons were made between the magnitude of the reflex bradycardia elicited by either A or C fiber stimulation before and after selective deafferentation (vagotomy or stellectomy). To activate aortic afferent A fibers alone, the stimulus intensity was adjusted while the evoked potential was monitored. When the stimulus voltage was reduced enough to eliminate activation of C fiber afferents, while causing little or no change in A fiber activation (Fig. 1A), a stimulus-response run was begun. In a separate group of rabbits, the C fiber afferents were activated to the exclusion of the A fibers by using the technique of Manfredi (13). While the left aortic nerve was stimulated at supramaximal intensity (sufficient to activate all fibers), a small blocking current (5-15 μA) was applied to the nerve via the interposed blocking electrodes. By adjusting the blocking current while the evoked potential was monitored, it was possible to eliminate A fiber conduction and leave C fiber conduction little affected (Fig. 1B). A stimulus-response run could then be made while only C fibers were being

**FIGURE 1**

**A:** Single stimulus compound-evoked potential recorded from the left aortic nerve. Top: At a supramaximal stimulus intensity, A fibers (fast component at left) and C fibers (slow wave at center) are activated. Bottom: At a reduced stimulus voltage, C fibers are no longer activated, but the A wave is unchanged. Interelectrode distance = 30 mm, sweep speed = 5 msec/cm. **B:** Overlay of three single stimulus compound-evoked potentials which were recorded in rapid succession illustrating blockage of A fiber conduction. Top: At a supramaximal stimulus intensity all fibers are activated. Bottom: When the blocking current (5-15 μA), is on, the A wave is blocked; the C wave is reduced slightly. Interelectrode distance = 35 mm, sweep speed = 5 msec/cm. The horizontal bar represents 1 cm of the oscilloscope sweep or 5 msec in time.

*Circulation Research, Vol. 37, July 1975*
AORTIC AFFERENT HEART RATE CONTROL

stimulated. Without left aortic nerve stimulation, the blocking current never elicited a persistent change in either arterial blood pressure or heart rate. Subsequent to the control runs, selective deafferentations were performed (bilateral vagotomy or bilateral stellectomy). Repeat runs were carried out to indicate the relative importance of vagal and sympathetic pathways in the control of heart rate via either myelinated or unmyelinated aortic afferents.

Standard errors were calculated for the onset and peak latencies as well as for the peak responses. The significance of the difference was determined by the t-test. P values < 0.05 were considered significant.

CRITIQUE OF METHODS

The polarizing blocking technique used in this series of experiments has been shown to cause selective blockade of myelinated fiber conduction by producing a failure of conduction between the blocking anode and cathode (13). The A beta group is most sensitive to the d-c blockade. However, at current levels sufficient to block some of the beta group, asynchronous firing of some less sensitive nerve fibers occurs. This phenomenon has been shown to result from cathodal excitation. With complete blockade of the beta fibers (and subsequent blockade of the delta group as well), the asynchronous firing no longer is seen at the recording site due to conduction failure at the anode. The conduction of unmyelinated C fibers is only slightly affected by this level of current.

In the present experiments, the direct current was adjusted to block myelinated fiber conduction while causing as little asynchronous firing as possible. However, as d-c levels that completely suppressed synchronous firing also caused significant C wave depression, the level was adjusted to yield A wave blockade with as little C wave depression as possible. The amount of asynchronous firing passing the blocking anode was judged to have little qualitative effect on the observed response. During the course of the electrical nerve block, the blocking current often fell somewhat during the first 15–30 seconds, no doubt as a result of electrode polarization during the blocking phase. This fall sometimes led to a partial loss of block selectivity. However, a run was not made until the block was reestablished (by raising the blocking current slightly) and its stability verified for at least 30 seconds. Subsequently, during the blocking period, no detectable blocking current creep was seen nor was there a significant tendency for the block to degrade. Selective nerve block was not normally maintained for periods longer than 2 minutes. The current level necessary to effect a selective aortic nerve block was essentially unchanged for all trials in each rabbit. Barbiturates are well known for their ability to depress the vagal centers. However, in our experiments, the rabbits were maintained at a light surgical anesthetic level with little apparent depression of the vagal component as indicated by the slope of the heart rate response.

Results

In 23 rabbits, repetitive synchronous stimulus bursts were applied to the left aortic nerve at frequencies of 50 and 100 Hz. In 13 rabbits, a total of 48 trial runs was made while stimulating at supramaximal intensity (all aortic nerve fibers activated) (Fig. 2). The average R-R interval increased 16.9% from the control level of 209 ± 3 msec. The latency to onset and the latency to peak were increased to 8.4 and 32.6 intervals, respectively. Bilateral vagotomy had no significant effect on resting heart rate, as previously reported (2). It did result in a reduction of the peak response to 11.0%. The latency to onset and the latency to peak were increased to 8.4 and 32.6 intervals,
respectively. Thus, vagotomy reduced the peak response by 35% \( (P < 0.001) \), while the latencies to onset and to peak were significantly delayed in time. In the remaining 10 rabbits, 33 trial runs produced an average bradycardia of 14.9%. The onset and the peak bradycardia occurred at 3.8 and 17.9 intervals, respectively, from the stimulus onset. Stellectomy slightly decreased the resting heart rate and reduced the reflex bradycardia by 41% \( (P < 0.01) \). The latency to onset was not significantly affected, and the peak bradycardia occurred 4.5 intervals sooner \( (P < 0.05) \). Thus, when the entire aortic nerve was activated, loss of the sympathetic efferent pathways reduced the magnitude of the reflex bradycardia, and the latency to peak response but had no effect on the latency to onset. In contrast, loss of the parasympathetic pathways extended both the onset and the peak latency while it, too, reduced the magnitude of bradycardia.

The relative influence of afferent A and C fiber activation on heart rate response, when all efferent pathways are intact, was studied in 11 rabbits (Fig. 3). Activation of A fibers alone caused no significant change in the latency to onset. When C fibers were selectively activated, the latency to onset was increased from a control value of 4.6 intervals to 6.0 intervals, which was a significant 31% increase \( (P < 0.01) \). The latency to peak was greatly reduced when C fibers were not activated. The reduction observed was 19.7 intervals to 11.4 intervals \( (P < 0.01) \). When C fibers alone were activated, there was a reduction from control, 19.6 intervals to 15.3 intervals, which was not significant. The peak response was equally dependent on activation of both A and C fiber afferents. The reduction in peak response was from 19.0% to 12.2% \( (P < 0.02) \) when afferent A fibers were stimulated alone, whereas stimulation of C fibers alone reduced the response from 13.4% to 8.0% \( (P < 0.01) \).

In 11 rabbits, a comparison was made between the reflex heart rate response to A fiber activation before and after either vagotomy or stellectomy (Fig. 4). Selective A fiber stimulation produced an average fall (peak response) in heart rate of 10.5%. The latencies to onset and peak were 4.1 and 11.0 intervals, respectively. Vagotomy in 6 of these rabbits reduced the peak response by 63%, and the onset of the response was delayed by 3.0 intervals, \( (P < 0.05) \). In the remaining 5 rabbits, stellectomy reduced the peak response to 5.4% \( (P < 0.01) \) (a 61% reduction). The latencies to onset and peak were 4.8 and 10.2 intervals, respectively, but neither of these changes was statistically significant.

In ten rabbits, A fiber conduction was blocked, and a total of 30 trial runs was performed (Fig. 5). In trials performed in six of these rabbits, C fiber stimulation reduced heart rate by 8.5%. The latencies to onset and peak were 5.8 and 16.5 intervals, respectively. Vagotomy in this group reduced the peak response to 4.9% and increased the latencies to onset and peak to 8.8 and 22.8 intervals, respectively. Thus, vagotomy reduced the peak response by 42% \( (P < 0.01) \). The onset of the response was delayed by 3.0 intervals \( (P < 0.05) \). In a total of 11 trial runs performed in the remain-

---

**FIGURE 3**

Afferent aortic nerve fiber influences on reflex bradycardia. Each pair of bars compares either A fiber or C fiber stimulation with supramaximal stimulation carried out immediately prior to selective stimulation. Comparison of A fiber stimulation with control represents a mean of 31 averaged trials in 11 rabbits. C fiber comparisons with control are for 30 trials in 10 rabbits. Abbreviations are the same as they are in Figure 2.
AORTIC AFFERENT HEART RATE CONTROL

FIGURE 4
Influence of efferent neural pathways on the heart rate response during selective aortic A fiber stimulation. Each pair of bars compares responses after either vagotomy or cardiac sympathectomy with responses prior to nerve section. Definitions are the same as they are in Figure 2.

ing four rabbits, stellectomy did not cause a significant change in peak response. No significant change was observed in either latency to onset or the latency to peak. When aortic unmyelinated fibers were activated, loss of parasym pathetic efferent pathways caused a reduction in peak effect and a delay in both onset and peak bradycardia. Loss of sympathetic efferents, however, had no significant effect on the peak response, indicating that aortic nerve unmyelinated fibers mediate heart rate predominantly via vagal efferents.

Discussion
This study provides the first direct evidence that aortic nerve-mediated reflex bradycardia occurs in response to activation of either afferent A or C fibers and that its magnitude depends on the number and the type of fibers being activated.

The effect of A and C fiber afferents as well as that of vagal and sympathetic efferents on the peak response indicates that A and C fiber afferents are approximately equipotent so long as both efferent pathways are intact. Vagal and sympathetic efferent pathways are equally important in eliciting a reflex bradycardia when all fibers or when only the A fibers are activated. However, removal of the sympathetic efferents has no effect on the heart rate response when only C fibers are activated. Since the bradycardia to selective C fiber stimulation is not abolished after vagal section, we cannot unequivocally disregard a sympathetic efferent involvement in the reflex response. Possibly, in the

FIGURE 5
Influence of efferent neural pathways on the heart rate response during selective aortic C fiber stimulation. Each pair of bars compares responses after either vagotomy or stellectomy with responses prior to nerve section. Definitions are the same as they are in Figure 2.
presence of intact vagal influences, sympathetic influence is minimal, whereas in the absence of vagal activity sympathetic influences are substantial during selective aortic C fiber stimulation. Similar responsiveness has been demonstrated in isolated atria exposed to acetylcholine and norepinephrine (14). In any case, insofar as baroreceptor effects on heart rate are concerned, the cardiac sympathetic influence appears to be primarily dependent on changes in afferent A fiber activity. In contrast, vagal influences are modulated by both afferent A and C fibers in the aortic nerve. These results are supported by similar findings during carotid sinus stimulation and neural physiological studies of the vasomotor areas of the brainstem (15, 16). In the brainstem of the cat and the rabbit, closely situated areas have been identified which receive information from either A or C fibers (15, 16). Furthermore, Kumada and Nakajima (15) have demonstrated that inputs from myelinated aortic nerve afferents innervate both the vagal and the sympathetic sensory areas of the rabbit brainstem and that unmyelinated afferents are absent from the sympathetic sensory areas.

Selective activation of either A or C fiber afferents demonstrates that the myelinated group is responsible for the earliest onset of bradycardia. This finding, of course, is predictable, since myelinated fibers can have many times the conduction velocity of unmyelinated fibers. Likewise, selective cardiac efferent nerve section demonstrates that intact vagi are essential to elicit the earliest onset of reflex bradycardia. Again, this finding is predictable, since the vagus contains many myelinated efferent fibers (17, 18) and the cardiac sympathetic nerves contain few myelinated fibers of any type (19, 20).

Our results illustrate the long time constant nature of the sympathetic influence on heart rate. The elimination of efferent cardiac sympathetic nerves causes a reduction in the number of intervals to peak effect with no significant effect on the latency to onset. This finding infers that rapidly conducting A fiber afferents which modify sympathetic efferent activity serve a function which is not dependent on their high conduction velocity. However, rapid modification of heart rate is vagally mediated (10). This study shows that myelinated aortic nerve fibers influence the vagal control of heart rate. Consequently, the fastest potential aortic baroreceptor influence on heart rate must occur by way of these afferent A fibers modulating efferent vagal activity. The efferent vagal fiber types which are modulated by these aortic afferents are not known at this time. However, working in the cat, Kunze (17) has shown that activation of the arterial baroreceptors causes demonstrable changes in the activity of vagal efferents which innervate the sinoatrial node. These vagal efferents are small-diameter myelinated fibers.

An indication of the order of activation of the A and C fiber aortic baroreceptor pool may be inferred from the known progression of peripheral vascular resistance and heart rate effects seen with increasing arterial blood pressure. Glick and Covell (8) and Allison et al. (7) have shown that with increasing arterial blood pressure in the dog, reflex reduction in peripheral vascular resistance (which is assumed to be largely under the influence of the sympathetic nervous system) occurs at a lower blood pressure threshold than does bradycardia. This finding indicates that during the course of an increase in arterial blood pressure the afferents which are activated at lower pressures are those which tend to suppress sympathetic efferent activity to the vasculature. Angell-James (21) has shown that, when they are activated physiologically, aortic nerve fibers fire at a frequency which is largely independent of the rate of change of pressure in the aortic arch so long as the pressure is above threshold for a given receptor. This finding suggests that the relative importance of recruitment of additional baroreceptor afferents as pressure increases is greater than that of increasing firing rates of individual fibers during the systolic phase. The results of Kardon et al. (3) demonstrate the importance of impulse number to aortic nerve reflex heart rate effects. These two studies, in combination with the present work, indicate that the important features of rabbit baroreceptor control of heart rate are the type and the number of fibers being activated over a given time period as well as their individual thresholds. If aortic C fibers have different pressure thresholds than do aortic A fibers, the reflex effect of C fiber recruitment on heart rate should differ from the reflex effect of recruitment of additional A fibers, as shown in this study. Therefore, increases in the frequency of whole aortic nerve activity in response to augmentation of arterial blood pressure must occur largely as a result of successive recruitment of nerve fibers having higher pressure thresholds rather than by increases in the firing rates of the individual nerve fibers involved. Finally, a previous study (2) has shown a progressive increase in reflex effects with increased recruitment of aortic nerve afferents: blood pressure effects are seen at the lowest stimulus intensities (rapidly conducting A fiber activa-
AORTIC AFFERENT HEART RATE CONTROL

...tion), but heart rate effects require recruitment of more slowly conducting myelinated fibers. Thus, it is inferred that the magnitude of the heart rate response can be augmented until all afferent C fibers are activated as well.

In combination with what has been shown in the present study to be the afferent-efferent specificity of the aortic baroreceptor pathways, the order of electrical activation of the afferent fibers from myelinated to unmyelinated fibers may illustrate the baroreceptor mechanisms normally used to modulate heart rate. If the A fiber pool with its combined vagal-stimulatory and sympathoinhibitory effects is activated at lower blood pressure thresholds, the normal baroreceptor control of heart rate would occur via their reciprocal action on vagal and sympathetic efferents at small increments in arterial blood pressure. More sustained increases in blood pressure would activate vagal efferents in larger proportion via C fiber afferents and would produce more profound heart rate effects.

Sympathetic regulation of heart rate in a predominantly high-frequency, low-threshold baroreceptor system suggests that these sympathetic efferents are modulated by subtle changes in arterial blood pressure. The known frequency-response characteristics of the sympathetic neuroeffector mechanism no doubt cause this subtle reflex bradycardia to occur over a longer time course than does that initiated by changes in vagal activity. Fluctuations in blood pressure which alter sympathetic influences via afferent A fibers would certainly cause rapid beat-to-beat changes in heart rate via vagal efferents. When blood pressure exceeds this range, the increased recruitment of C fiber afferents would cause the potential for beat-to-beat heart rate control to be progressively masked. Under these circumstances, heart rate would no doubt fall farther in response to continuous activation of both A and C fiber afferents and vary less in response to beat-to-beat blood pressure changes.

References

Reflex heart rate control via specific aortic nerve afferents in the rabbit.
M B Kardon, D F Peterson and V S Bishop

Circ Res. 1975;37:41-47
doi: 10.1161/01.RES.37.1.41

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/37/1/41